Posters

P1

EXPRESSION AND CHARACTERIZATION OF NOVEL S1/P1 NUCLEASE TbrN1 FROM PROTOZOAN PARASITE *TRYPANOSOMA BRUCEI*

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S1/P1 nucleases are zinc-dependent phosphodiesterases that are widely distributed among fungi, plants, bacteria and protozoan parasites. While the biological roles of plant and fungal representatives have been deciphered and they have been extensively characterised, structural and biophysical data for bacterial members has only recently emerged (studies of the Lpn1 nuclease from *Legionella pneumophila* and the highly active SmNuc1 nuclease from *Stenotrophomonas maltophilia*) and the biological function of bacterial members is still unclear [1, 2, 3].

A substantial number of S1/P1 genes have also been localised and identified in protozoan parasites, including species of *Leishmania*, *Plasmodium* and *Trypanosoma*. Several S1/P1 nucleases from *Leishmania* species have been characterised, contributing to purine salvage and potentially modulating host immune responses. However, no structural or biochemical data is currently available for homologues from *Trypanosoma* spp., the causative agent of human African trypanosomiasis. As trypanosomatids lack de novo purine biosynthesis, they rely entirely on salvage pathways. It is therefore assumed that S1/P1 nucleases act as extracellular nucleases, facilitating the uptake of purines from host nucleic acids.

Here, we present the first expression and purification of an S1/P1 nuclease from *Trypanosoma brucei gambiense* (TbrN1), as well as its initial biochemical characterisation. The recombinant enzyme was produced in a eukaryotic expression system and then purified and assayed for nuclease and 3'-nucleotidase activity. Preliminary assays indicate activity towards RNA, single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), and 3'-mononucleotides, with a slight preference for RNA as a substrate.

Given the absence of mammalian homologues and the essential role of nucleic acids metabolism in protozoan pathogens, TbrN1 is a promising candidate for mechanistic investigation and potential therapeutic targeting.

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P2

MUTATION-RESISTANT NEUTRALIZATION ANTIBODY AX677 RECOGNIZES SPECIFIC EPITOPE ON THE INNER FACE OF SARS-COV-2 SPIKE PROTEIN RBD

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SARS-CoV-2, a positive sense single stranded RNA virus has been responsible for Coronavirus disease 2019 (COVID-19), a respiratory illness and global pandemics. Therapeutic antibodies proved to be an efficient tool to stop SARS-CoV-2 infection in the hospitalized patients requiring oxygen support in a risk of respiratory failure, however, they suffered from loss of potency due to frequent mutations in new strains of virus which escaped from the antibody binding. The principal question for COVID-19 and perhaps also future viral threats is whether there exists a conserved epitope among virus variants, which can be targeted by an antibody with neutralising potential.

Humanized IgG1 chimeric monoclonal antibodies AX290 and AX677 with non-overlapping epitopes were developed after mice immunization with receptor binding domain (RBD) and S-protein, respectively [1]. Interest-

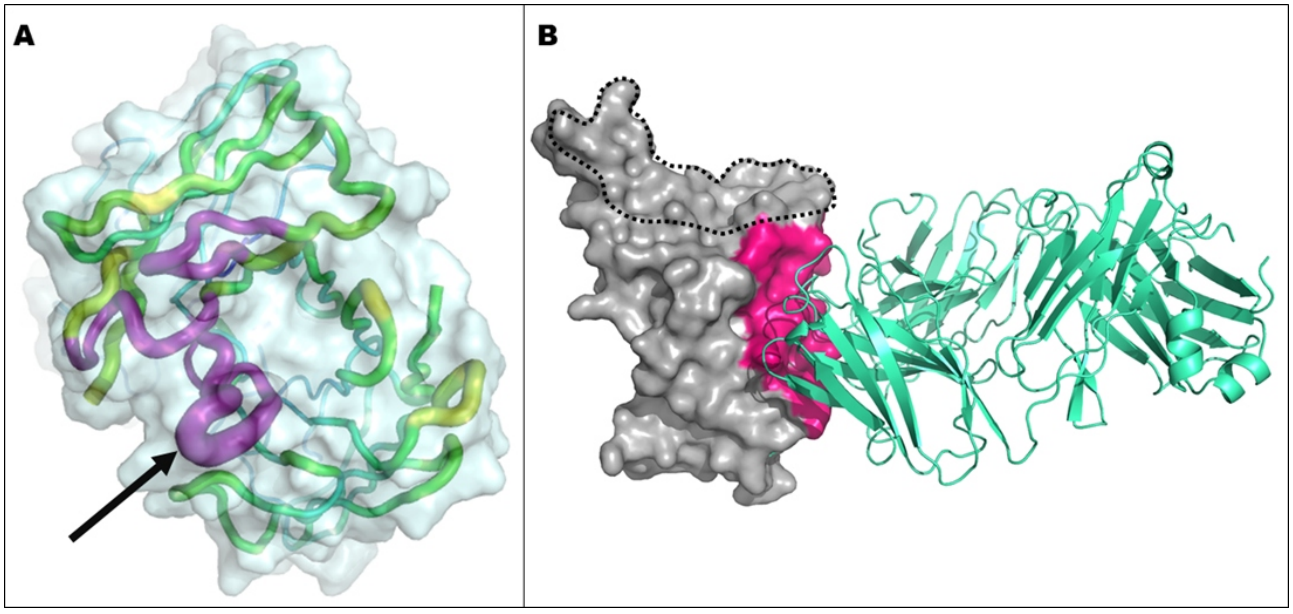

Figure 1. X-ray structures. A) The AX290 antibody. Tube thickness is proportional to B-factor, reflecting the flexibility; more flexible regions are thicker. The heavy chain CDRs are marked in purple, with an arrow pointing to CDRH3, the most flexible loop (CDR definition is according to IMGT). B) AX677-RBD complex. The RBD surface is grey with the ACE2 binding site outlined by a dotted line. AX677 is a cartoon model. The area recognized by AX677 is labelled by pink colour on the RBD inner face.

ingly, AX290 competed with ACE-2 binding to RBD, whereas AX677 did not. Both antibodies had a high neutralising potency and AX677 was insensitive to spike protein mutations in several virus variants of concern. Our aim was to understand the molecular basis of antibody action by describing the structures of AX290 and AX677 using X-ray crystallography.

The Fab fragments of AX290 and AX677 were crystallised alone and in the complex with the RBD of the spike protein. We collected complete data sets for AX290 Fab and AX677 Fab-RBD complex to 2.15Å and 2.7Å resolution, respectively. Structures were solved by molecular replacement in Phaser and refined by Refmac/Coot. The paratope of AX290 is very flexible namely in the CDRH3 (Fig. 1A). The structure of the AX677-RBD complex revealed localisation of antibody epitope on RBD. AX677 binds to the inner face of the RBD - a region known as the "escarpment"- which is outside of the ACE2-binding interface. The position of epitope correspond to the RBD-7c epitope community classification [2], however, AX677 retain a high neutralization potency against authentic virus, in contrast with the majority of RBD-7c antibodies. As analysed by PDBePISA server (https://www.ebi.ac.uk/pdbe/pisa/), AX677-RBD interface include RBD residues L368-Y369, S371-K378, G404-D405, V407, N437, V503-G504 and Y508 (Fig. 1B). As the inner face of RBD is conserved across the *Sarbecovirus* subgenus of *Betacoronavirus* , AX677 has a potential to be one of the antibodies with neutralization ability towards a large virus family and simultaneously insensitive to mutations.

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P3

A NEW ANTIBIOTIC RESISTANCE ENZYME FOR A CLASSIC TARGET: MrmA REDIRECTS RADICAL SAM METHYLATION TO 23S rRNA A2058

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The ongoing evolution and spread of antimicrobial resistance necessitate a deeper understanding of how resistance mechanisms emerge and diversify. The radical SAM methyltransferase family, exemplified by RlmN and Cfr, modifies the bacterial ribosome but with divergent outcomes: RlmN methylates C2 of 23S rRNA nucleotide A2503 as part of cellular physiology, while Cfr methylates C8 at the same position, conferring broad resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A ($PhLOPS_A$).

Here, through comparative genomics of *Clostridioides difficile*, we identify a novel radical SAM enzyme, MrmA, which represents a dramatic evolutionary shift in substrate specificity. MrmA methylates the nucleotide A2058 a canonical antibiotic-binding site whose dimethylation by Erm-family enzymes confers MLS_B resistance. Remarkably, MrmA is the first radical SAM enzyme shown to target this position. By installing a novel C2 methylation at A2058, MrmA specifically confers resistance to macrolides and streptogramin B but not to lincosamides.

The comparative analysis of MrmA, Cfr, and RlmN reveals how conserved enzyme scaffolds evolve novel functions. Together, these three enzymes provide a powerful model to elucidate the molecular determinants that dictate which nucleotide position is selected for methylation, offering crucial insights into the general mechanism of substrate selection by ribosomal methyltransferases and its role in the evolution of antibiotic resistance.

P4

C-TERMINAL DOMAIN OF DELTA, AN AUXILIARY RNA POLYMERASE SUBUNIT

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The RNA polymerase is an essential part of any living system, but the delta subunit is not. Only a select group of bacteria have it, including the opportunistic pathogen Staphylococcus aureus. Evidence suggests this delta subunit contributes to the survival of this bacterium in the host by increasing transcription specificity.

The N-terminal domain of delta is ordered and facilitates the binding to the beta prime subunit, while the C-terminal domain is intrinsically disordered and it's function has only been hinted at so far. We have combined NMR measurements, including PRE, with SAXS and we characterised this CTD as an ensemble of structures.

In an older article (Kubáň et al. 2019), an important structural element "K-tract", a patch of 7 lysine residues was identified in the *B. subtilis* delta and compared with it's mutant, which had the "K-tract" removed. In contrast, S. aureus lacks this element and we also studied it's mutant, that mimicks the "K-tract". Identifying the functional differences between the four aforementioned constructs will help us to understand the detailed function of the delta subunit.

Here we present structural differences between the four aforementioned constructs contributing to functional diversity of the delta subunit in different bacteria.

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P5

STRUCTURE-GUIDED HUMANIZATION AND ENGINEERING OF A HIGH-AFFINITY ANTI-PSMA ANTIBODY

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Prostate-specific membrane antigen (PSMA) is a clinically validated cell-surface target in prostate cancer, motivating development of antibody reagents with high affinity, strict specificity, and favorable developability. Using the Fab crystal structure of the murine anti-PSMA antibody 5D3, we performed structure-guided framework selection and CDR grafting to generate humanized variants and screened their physicochemical characteristics and affinity/specificity against human PSMA. One clone (5D3-6) consistently matched the parental antibody in target recognition and showed the most favorable overall characteristics. Structure-assisted mapping of 5D3/PSMA interface then guided CDR mutagenesis to remove prominent sequence liabilities while maintaining strong PSMA binding, yielding a humanized anti-PSMA antibody optimized for downstream translational formats such as imaging, conjugation, and therapeutic development.

P6

CRYSTAL STRUCTURES OF mRNA CAP-FORMING ENZYMES: TvRNMT1 AND Tv2'-O-MTASE *OF TRICHOMANAS VAGINALIS*

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Trichomoniasis is a sexually transmitted infection of the lower urogenital tract of women, and prostate and urethra of men caused by the anerobic protozoan *Trichomonas vaginalis* (Tv). Both sexes carrier the disease while men inhabited by Tv are usually asymptomatic. When considering other non-viral sexually transmitted infections trichomoniasis is the most prevalent worldwide [1]. Of concern is that trichomoniasis increases the risk of transmission of human immunodeficiency virus (HIV) in both women and men. In addition, the infection is also associated with adverse outcomes during pregnancy. The disease is underdiagnosed, and self-treatment of the infection by patients is sadly also common. Current treatment of trichomoniasis relies on administration of 5-nitroimidazole drugs. However, resistance has been increasingly recognized and may occur in up to 10% of infections [2].

An encouraging path represents targeting essential methyltransferases for parasite survival. The 5' cap mRNA (messenger RNA) is an essential feature of eukaryotic mRNA that is required for a stability and efficient translation. mRNA capping entails several enzymatic reactions where cap methylation, catalysed by RNA (guanine-N7) methyltransferase (RNMT) and conversion of a cap-0 to a cap-1 structure by 2'-O-Methyltransferase (2'-O-MTase) are common druggable targets [3]. As a result, successfully installed cap-1 structure ensures high translational efficiency and protects the mRNA of parasite from the host's innate immune system. For this reason, capping machinery is essential for parasite survival and disruption of mRNA modification is heavy desirable.

This work presents recently solved crystal structures of these protozoan methyltransferases with a view of the catalytic site, occupied by a co-factor competitive compound: Sinefungin and byproduct of methylation reaction SAH (S-adenosylhomocysteine). These preliminary crystal structures accompanied with high-throughput in vitro and in vivo screening of small inhibitory molecules (unpublished data) provide a valuable structural insight into consecutive rational drug design.

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P7

MONTE CARLO SIMULATIONS OF MINIPROTEIN FOLDING SAMPLED WITH AN AUTOENCODER

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Autoencoders are artificial neural networks used for non-linear dimensionality reduction and have many diverse applications. The architecture consists of an encoder transforming high dimensional inputs onto a low-dimensional embedding called latent space, and a decoder, which reconstructs these latent space values into the original high dimensional data.

In the work presented here, we use an autoencoder pretrained on diverse conformations of miniproteins for generating protein structures based on input latent space values. We sample from the structures generated by the network using Metropolis Criterion and we show that running such Monte-Carlo simulation of the miniprotein system provides a very computationally inexpensive way to visualize the structural behaviour of the studied miniprotein, including folding, unfolding and other events.

We show the results of this method applied to four model systems: Tryptophan Cage miniprotein, its non-folding variant, Villin headpiece and PDZ domain. We observe that although the distribution of structures sampled this way is slightly different from one sampled from sufficiently long classical molecular dynamics (MD) simulation, it is also provided at a tiny fraction of the computational cost of the MD simulation. We present the method as a potential alternative to classical simulations methods (like MD) that provide more accurate results, but require orders of magnitude longer wall times for the calculations.

P8

STRUCTURAL BASIS OF ASK1 REGULATION: FROM 14-3-3 MEDIATED INHIBITION TO MKK7 SUBSTRATE RECOGNITION

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Apoptosis signal regulating kinase 1 (ASK1), also known as MAP3K5, is a crucial stress sensor from the mitogen-activated protein kinase (MAPK) kinase kinase (MAP3K) family, directing cells toward apoptosis, differentiation, and senescence via the p38 and JNK signaling pathways [1]. Dysregulation of ASK1 has been associated with cancer, inflammatory, cardiovascular, and neurodegenerative diseases, among others. Hence, ASK1 activity has to be strictly regulated to respond to stress stimuli appropriately. In homeostatic condition ASK1 activity is negatively regulated by 14-3-3 adaptor proteins, suppressing the activation of the downstream MKK7/JNK axis [2,3]. Despite many years of intensive research, there is no high-resolution structure of multi-domain ASK1 in complex with 14-3-3 which has hindered functional and mechanistic understanding of ASK1 regulation. Elucidating the structural basis of 14-3-3-mediated ASK1 sequestration and the subsequent molecular recognition of the MKK7 effector is essential for understanding molecular mechanism of ASK1 regulation. Therefore, we aim to gain structural insights into the ASK1:14-3-3 and ASK1:MKK7 complexes using an integrated approach based on cryo-electron microscopy (cryo-EM), hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS), sedimentation velocity analytical ultracentrifugation (SV-AUC), and analytical size-exclusion chromatography (SEC).

In the present study, biophysical analyses by SV-AUC demonstrate that ASK1 undergoes homo-oligomerization, forming dimers (200 kDa) and higher-order oligomers up to tetramers. Upon 14-3-3 binding, the complex assemles with multiple stoichiometries that exist in equilibrium between higher to lower states with a clear preference for 4:4 assembly (two ASK1 dimers forming a tetramer stabilized by two 14-3-3 dimers). The formation of this 4:4 complex was subsequently confirmed by SEC-MALS and cryo-EM. The cryo-EM reconstruction showed that each of the 14-3-3 dimers stabilize the tetrameric arrangement of ASK1 by binding the C-terminal segments of ASK1 chains from opposite ASK1 dimers. The structure suggests that tetramerization of ASK1 causes steric hindrance of the catalytic centers of the kinase domains and presumably also interactions between the kinase domains and the MAP2K kinase substrate i.e. MKK7, thus explaining the inhibitory effect of 14-3-3 binding. In a preliminary cryo-EM structure of the ASK1:MKK7 complex, monomeric MKK7 engages a dimeric ASK1; distinct from the potentially

inhibited ASK1:14-3-3 complex which adopts a higher order oligomeric state. Together, these observations suggest that ASK1 oligomeric state may regulate the accessibility of the catalytic site and the substrate docking surface.

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P9

MURINE NKR-P1C, A KEY ACTIVATING RECEPTOR IN NK CELL IMMUNE SURVEILLANCE

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Natural Killer (NK) cells are a part of the vast repertoire of innate immune response agents in mammals. Amongst their primary functions is a process called immune surveillance. They use a variety of activating and inhibitory receptors on their surfaces to "scan" other cells in the organism to determine whether their target cells are healthy or infected, malignantly transformed, or otherwise negatively altered [1].

One such activating receptor is the murine Natural Killer Receptor – Protein 1C (mNKR-P1C). Despite being first described over 50 years ago, its structure and natural ligand are still unknown [2]. A lack of knowledge about this activating receptor limits our understanding of its precise role in immune responses and regulation, which is essential, as mice serve as model organisms and their NK cell receptor repertoire differs from that of humans.

This project focuses on the recombinant production of mNKR-P1C in two types of mammalian cell lines with distinct N-glycosylation patterns. This allows mNKR-P1C to be studied using several biophysical methods that could help paint a comprehensive picture of the receptor's structure and function. While the HEK293T cell line provides wild-type human N-glycans, the HEK293S GnTI$^-$ cell line lacks N-acetylglucosaminyltransferase I, which means all proteins are uniformly glycosylated with a simple mannose motif, supporting their homogeneity and crystallizability. Initially, we expressed a construct encompassing the entire extracellular domain of mNKR-P1C in a HEK293S GnTI$^-$ stable cell line, providing excellent yields; however, this construct never crystallized. Next, we prepared a truncated version of the extracellular domain of NKR-P1C lacking the flexible hinge region. The second construct was transiently produced in both HEK293T and HEK293S GnTI$^-$ cell lines. Based on previous structural studies of homologous receptors, such as a human NKR-P1, we believe that truncating or removing the hinge can enhance protein stability and crystallizability [3]. The oligomeric state, glycosylation, and disulfide bond patterns were assessed by analytical ultracentrifugation and mass spectrometry.

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P10

MOLECULAR BASIS OF DNA BINDING AND OLIGOMERIZATION BY REPLICATION AND TRANSCRIPTION REGULATOR RTA OF EPSTEIN-BARR VIRUS

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Epstein–Barr virus (EBV) infects over 90% of the global population and contributes to approximately 200,000 malignancies annually. Reactivation from latency into the lytic cycle is initiated by the viral replication and transcription activator (Rta), which binds Rta response elements (RREs) and induces early lytic gene expression. Despite Rta's central role, the structural organization and oligomeric state of Rta have remained insufficiently defined.

Here, we combined biophysical, single-molecule, and cellular approaches to characterize Rta structure and function. We demonstrate that Rta comprises a structured -helical DNA-binding domain and an intrinsically disordered transactivation domain. Biophysical analyses indicate that Rta predominantly forms dimers in solution, whereas single-molecule AFM reveals a population of tetrameric assemblies, suggesting dynamic higher-order oligomerization. Quantitative binding assays show that Rta binds RRE-containing DNA with affinities in the nanomolar range. Targeted mutations within the DNA-binding domain disrupt Rta-DNA interaction, reducing affinity to the micromolar range and indicating that mutated residues are critical for specific recognition.

In EBV-positive P3HR1 cells undergoing chemically induced lytic reactivation, Rta forms discrete nuclear foci, including accumulations at the nuclear periphery, consistent with spatially regulated lytic activation.

Together, these findings define the domain architecture, oligomeric behavior, and DNA-binding properties of Rta, providing a structural framework for understanding EBV lytic reactivation and informing efforts to target Rta function therapeutically.

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P11

BIOPHYSICAL CHARACTERIZATION OF PROTEINS AND PROTEIN COMPLEXES BY SEDIMENTATION VELOCITY ANALYTICAL ULTRACENTRIFUGATION

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Sedimentation Velocity Analytical Ultracentrifugation (SV-AUC) is a powerful solution-based technique for analyzing the hydrodynamic and thermodynamic properties of biomolecules. During ultracentrifugation, macromolecules sediment in a high gravitational field and are monitored in real time using UV/Vis absorption, fluorescence, or interference optics. The resulting sedimentation profiles are analyzed using mathematical modeling to determine molecular weight, shape, size distribution, oligomeric states, and binding interactions.

We applied SV-AUC to characterize formation of the ternary complex between CDK16, pCCNY, and 14-3-3 and determined its apparent dissociation constant. Combined with site-directed mutagenesis and kinase activity assays, we identified key residues required for stable ternary complex formation, which is essential for CDK16 activation.

This work demonstrates the utility of SV-AUC for quantitative characterization of protein–protein interactions and multicomponent complexes.

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P12

WHEN LecB IS NOT ALONE: STRUCTURAL AND FUNCTIONAL ANALYSIS OF A TWO-DOMAIN LECTIN

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LecB (PA-IIL) is one of two well-characterized lectins from the opportunistic pathogen *Pseudomonas aeruginosa* and plays a key role in host colonization and biofilm formation, particularly in immunocompromised patients such as those with cystic fibrosis [1]. Homologous lectins have also been identified in other pathogenic bacteria, including *Burkholderia cenocepacia* [2]. Notably, several uncharacterized LecB-like proteins contain an additional N-terminal domain of unknown function, suggesting potential functional diversification within this lectin family.

This project aims to structurally and functionally characterize a putative two-domain lectin comprising a canonical LecB-like carbohydrate-binding domain and an N-terminal accessory domain. A candidate gene encoding such a protein from an insect pathogen was identified through bioinformatic analysis, cloned into expression vectors, and expressed in *E. coli*. In addition to full-length constructs, individual domains were expressed separately to enable domain-specific characterization.

Purified proteins are being analyzed using biophysical methods to assess oligomeric state, thermostability, and sample homogeneity, and their carbohydrate-binding properties are under investigation. Extensive crystallization screening was performed for both full-length proteins and isolated domains. The structure of the N-terminal domain has been largely determined by molecular replacement and is currently undergoing refinement. Crystallization of the LecB-like domain is still being optimized. However, comparative analysis of predicted structures with canonical LecB suggests potential differences in ligand-binding architecture that may reflect altered specificity or regulatory function.

These findings provide new insight into the structural diversity of LecB-like lectins and contribute to understanding bacterial adhesion mechanisms in pathogenic bacteria.

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P13

PREPARATION AND CHARACTERIZATION OF OLIGOMERS FORMED BY TRUNCATED tau PROTEINS *IN VITRO* AND *IN SILICO*

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Soluble oligomers are thought to be the toxic agents of several neurodegenerative diseases and not the relatively inert mature filaments. Oligomers can propagate between neurons and could be isolated by in vivo microdialysis from brain interstitial fluid. Tau oligomers can be induced *in vitro* by seeding with amyloid- (A) oligomers or using tau with modified cysteines [1,2]. However, intermediate filaments with shorter amyloid interface compared to mature filaments were observed early in the course of tau aggregation [3].

Previously we have summarized structural data about oligomers formed by amyloid- , -synuclein, tau a prion protein, that show substantial lack of structural information about tau protein oligomers [4].

Isolated *in vitro* tau oligomers prepared from truncated tau were characterized by ion mobility mass spectrometry, size exclusion chromatography, dot blot and western blot using oligomer specific antibodies T22, A11 and TOMA-1.

Moreover, representative structures of tau dimers and trimers were obtained after clustering of coarse-grained MD simulations. Set of previously reported small molecules with potential to inhibit tau aggregation was docked in the presumed binding pockets identified in these structures. The inhibition was probed by measuring the ThT signal of the aggregation reaction in the presence of smallmolecules.

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P14

BIOPHYSICAL TECHNIQUES AT THE CENTRE OF MOLECULAR STRUCTURE

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Biophysical research facility of the Centre of molecular structure is a shared resource for the characterization of biomolecules 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), switchSENSE (Helix^{+}) techniques are available for the characterization of biomolecular interactions.

Facility is a member of Instruct-ERIC and Czech Infrastructure for Integrative Structural Biology (CIISB).

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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P15

STRUCTURAL BASIS OF ATP-MEDIATED INHIBITION OF MYCOBACTERIAL GMP REDUCTASE

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

In our studies of purine metabolism in mycobacteria, we use *Mycobacterium smegmatis* (Msm) as a model for the infectious *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis in humans.

In a previously published study [1], we demonstrated that the enzymatic activity of *Msm*GMPR is allosterically regulated by ATP and GTP. While ATP inhibits the enzymatic activity of *Msm*GMPR, GTP counteracts this inhibition, and thus restores the enzymatic activity.

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

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

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P16

FROM GENE TO PROTEIN: LAUNCHING AN INTEGRATED PROTEIN PRODUCTION SERVICE AT CEITEC MU

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The efficient production of recombinant proteins is essential for structural biology, biochemistry and biomedical research. The Biomolecular Interaction and Crystallography Core Facility (CF BIC) at CEITEC Masaryk University has established new protein production services utilising both prokaryotic and eukaryotic expression systems, tailored to the specific requirements of diverse target proteins. Our services cover the full protein production workflow, including construct design, cloning, expression screening, scaling up and purification, all of which are supported by standardised quality control. The *E.coli* platform enables the rapid and cost-effective production of simple, high-yield proteins, while the insect cell system (Sf9/High Five) is ideal for producing complex eukaryotic proteins that require proper folding, disulfide bond formation or post-translational modifications. Combining these complementary expression systems allows us to adapt flexibly to challenging targets and increases the overall success rate of protein production projects. CF BIC currently offer a complete portfolio of services for protein-based research, accessible to both the internal and external scientific communities.

P17

STRUCTURAL COMPLEXITY IN BACTERIAL TRANSCRIPTION REGULATION

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The process of gene expression comprises number of steps, including transcription of DNA into RNA, translation of RNA into protein, and protein posttranslational modification. In bacteria, the regulation of the expression represents the major response to environmental changes and is therefore critical for adaption and cell survival. Based on the availability of different nutrients, bacteria turn on and off different genes encoding for proteins of the respective metabolic pathways. The whole metabolic machinery can thus be regulated at the very beginning of the expression, namely the initiation of transcription. For that, bacteria use numerous transcription repressors belonging to various protein families. Members of the GntR family, present across diverse bacteria species, are mainly responsible for regulation of enzymes utilizing catabolism of various carbon sources (e.g. sugars). The family is defined by a conserved N-terminal DNA-binding domain (DBD) with a winged-helix-turn-helix motif. The C-terminal effector binding domain (EBD) distinguishes these regulators into five subfamilies. When an effector binds the EBD, the transcription repressor loses its ability to bind DNA, which consequently leads to activation of transcription. Although there are thousands of such repressors across most bacterial species, their mechanism of action is poorly understood on the structural level. Here we present the preliminary structural biology data on the prototypic member GntR, from the model organism of gram-positive bacteria *Bacillus subtilis*. By the method of cryogenic electron microscopy (cryo-EM), we try to understand the molecular mechanism behind the protein-DNA complex formation, the oligomeric arrangements, and their changes upon effector binding.

P18

FORCE-FIELD DEPENDENT CHANGES IN THE CONFORMATIONAL ENSEMBLE OF Tau(210–240) UNDER TUNED PROTEIN–WATER INTERACTIONS

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Tau is a prominent intrinsically disordered protein (IDP) that regulates mikrotubule assembly and stability. [1] Under pathological conditions, hyperphosphorylated tau detaches from microtubules, aggregates into fibrils, and forms neurofibrillary tangles, a primary hallmark of Alzheimer's disease (AD) in the human brain. [2] Elucidating tau fibril formation mechanisms is crucial for understanding AD neurodegeneration. Molecular dynamics (MD) simulations offer insights into the temporal evolution of tau fibrillization, with coarse-grained (CG) models extending accessible length and time scales far beyond all-atom simulations. However, standard CG force fields often fail to capture IDP flexibility, yielding overly compact conformations. [3]

This study assesses the accuracy of SIRAH 2 [4] and Martini 3 [5] force fields in modeling the monomeric conformational ensemble of Tau(210-240). Validation incorporates experimental NMR data, including chemical shifts and 3J coupling constants, alongside radius of gyration measurements. Both unmodified force fields produce excessively collapsed structures, an issue counteracted by enhancing water-protein interactions via reparameterization. SIRAH 2 demands more extensive reparameterization than Martini 3, yielding fair reproduction of global properties like radius of gyration for SIRAH, while Martini 3 shows severe deviations from experiment. Despite global improvements, local structural properties remain less changed. The reparameterized models better describe the Tau(210-240) ensemble, aligning with experimental observables.

Computational resources were provided by IT4Innovations, funded by the Ministry of Education, Youth and Sports of the Czech Republic via the e-INFRA CZ project (ID: 90254). This work was further supported by the Brno Ph.D. Talent Scholarship from the Brno City. Municipality and by the European Union's Horizon Europe program under grant agreement No. 101087124 (ADDIT-CE).

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P19

ExTraMD A SERVICE FOR EFFICIENT EXTENSION OF MOLECULAR DYNAMICS SIMULATIONS IN LATENT SPACE

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Molecular dynamics (MD) simulations provide detailed insight into biomolecular systems but are often limited by the high computational cost of long trajectories. Recent advances in latent space modeling demonstrate that molecular dynamics can be approximated in a low-dimensional latent representation, enabling the generation of long trajectories from relatively short simulations. We present ExTraMD, a planned service that implements latent space simulation techniques based on the Molecular Latent Space Simulators framework and makes them available as an easy-to-use, automated tool. ExTraMD will allow users to upload MD trajectories, train latent dynamical models, and generate statistically consistent extended trajectories with minimal manual intervention. The service is being designed with a focus on scalability, GPU acceleration, and integration into existing molecular dynamics workflows. ExTraMD will be integrated into the MD Dashboard environment and connected to trajectory search and data repositories, enabling seamless use within established simulation pipelines. The goal is to lower the barrier for applying latent MD modeling and to support efficient exploration of slow and rare biomolecular processes.

P20

CHARACTERIZATION OF THE AIRE INTERACTOME IN TRANSCRIPTIONAL REGULATION

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The Autoimmune Regulator (AIRE) is a transcription factor for maintaining central immune tolerance. It functions by driving the expression of tissue specific antigens (TSAs) within medullary thymic epithelial cells, a process essential for the clonal deletion of self-reactive T cells. Mutations in the AIRE gene result in Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APECED).

Despite its known physiological importance, the precise molecular mechanisms by which AIRE coordinates transcriptional regulation remain poorly defined. In this study, we developed a systematic panel of AIRE truncation and domain-specific constructs to map its interactome. Using pull-down assays coupled with mass spectrometry, we aim to identify novel interacting partners across different AIRE domains.

These findings will advance our understanding of eukaryotic transcriptional regulation and the fundamental mechanisms of immune tolerance.

P21

MICROTUBULE-ASSOCIATED PROTEIN TPPP1 EXPANDS THE MICROTUBULE LATTICE

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Tubulin Polymerization Promoting Protein 1 (TPPP1) is a microtubule-associated protein implicated in microtubule organization and stability. Microtubules are structurally and chemically heterogeneous polymers defined by their lattice conformation, nucleotide state, post-translational modifications (PTMs), or tubulin isotype composition. How microtubule-associated proteins interpret and alter this multilayered identity remains largely unclear. Here, we show that TPPP1 is a state-dependent reader and regulator of microtubule identity. We show that TPPP1 binds cooperatively to compacted microtubule lattice, inducing local lattice expansion. In addition TPPP1 displays differential binding to microtubules distinguished by PTM patterns and tubulin isotype composition, suggesting that TPPP1 can act as a selective expander of distinct microtubule subset in the cell. Together, our findings support a model in which TPPP1 integrates structural and biochemical signals to selectively remodel specific microtubules, potentially influencing their dynamic behavior.

P22

EFFECT OF 14-3-3 PROTEIN FAMILY ON Tau PROTEIN FIBRE FORMATION

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Tau protein aggregation into fibres is a hallmark of Alzheimer's disease and related tauopathies. The 14-3-3 protein family, highly abundant in the brain, modulates Tau phosphorylation, microtubule binding, and aggregation propensity. Recent studies demonstrate that the 14-3-3 isoform significantly inhibits fibrillization of GSK3 -phosphorylated full-length Tau (2N4R), as evidenced by Thioflavin T assays, electron microscopy, AFM, chemical crosslinking, and NMR showing direct interactions.[1] Similarly, stoichiometric binding of 14-3-3 dimers promotes dissociation of phosphorylated Tau from microtubules, enhances cytosolic solubility, suppresses liquid-liquid phase separation (LLPS) and amyloid-like aggregation, thereby preventing pathological seed formation.[2]

This study aims to gain deeper insights into the interaction between GSK3 -phosphorylated full-length Tau (2N4R) and the 14-3-3 protein.

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P23

STRUCTURAL CHARACTERIZATION OF THE 16S rRNA METHYLTRANSFERASE RsmH FROM *STAPHYLOCOCCUS AUREUS*

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Post-transcriptional methylation of 16S rRNA is a conserved bacterial modification that regulates ribosomal function and facilitates adaptation to environmental stress. RsmH is a SAM-dependent cytosine-N4-methyltransferase that catalyzes methylation of C1402 within the decoding center of 16S rRNA, a modification associated with virulence and stress tolerance in *Staphylococcus aureus*. In this study, the RsmH gene from *S. aureus* was cloned into a recombinant expression vector for heterologous production. The recombinant protein was purified to homogeneity, and subsequent crystallization trials were performed in the presence of sinefungin, a SAM analog. Analysis of high-resolution X-ray diffraction data allowed us to determine the previously unresolved crystal structure of RsmH from *S. aureus*. These findings provide a structural framework for mechanistic investigations and may support future structure-based drug development strategies targeting methicillin-resistant *S. aureus* (MRSA).

P24

DETECTION OF NEUROCHEMICAL BRAIN PROFILES IN RATS USING ąH MRS

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Proton magnetic resonance spectroscopy (^{1}H MRS) is a non-invasive method that enables *in vivo* characterization of brain neurochemistry at the molecular level, linking metabolite composition to cellular structure and function. In this study, ^{1}H MRS was applied to detect region-specific neurochemical profiles in the rat brain and to assess metabolic alterations induced by disease models and pharmacological interventions.

9.4 T high-field ^{1}H MRS allowed reliable quantification of key brain metabolites, including *N*-acetylaspartate (NAA), choline-containing compounds (Cho), total creatine (tCr), glutamate (Glu), glutamine (Gln), *myo*-inositol (mIns), and taurine (Tau). These metabolites reflect neuronal viability, membrane turnover, energy metabolism, neurotransmission, and astroglial function, providing insight into molecular structure-function relation- ships relevant to structural biology.

In the olfactory bulbectomy rat model of depression, significant neurochemical alterations were detected in the hippocampus and cortical regions, indicating impaired neuronal metabolism, astroglial dysfunction, and altered osmoregulation. Specifically, changes in NAA and choline levels suggested neuronal dysfunction and membrane remodeling, while reduced taurine levels reflected impaired modulation of neurotransmitter activity.

Importantly, ^{1}H MRS was sensitive to both chronic and acute pharmacological manipulation. Chronic treatment with citalopram induced distinct metabolic changes predominantly in the hippocampus, whereas acute administration of methamphetamine resulted in rapid neurochemical alterations in cortical regions. These findings demonstrate the ability of ^{1}H MRS to capture dynamic neurochemical responses to pharmacological interventions *in vivo*.
Our results highlight ^{1}H MRS as a valuable tool bridging structural biology and neuroscience by enabling the detection of subtle molecular changes related to brain dysfunction and therapeutic modulation, with potential applica-b ility in identifying biomarkers of neurodegeneration.

P25

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF IRIPIN-7 FROM *IXODES RICINUS* REVEALS DETERMINANTS OF PROTEASE SPECIFICITY

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Ticks secrete serine protease inhibitors (serpins) via their saliva to modulate host inflammatory and hemostatic responses during blood feeding. Although serpins share a conserved fold and inhibitory mechanism, subtle variations within the reactive centre loop (RCL) can profoundly influence protease specificity [1].

Here, we present a structural and functional characterization of the tick serpin Iripin-7. To investigate determinants of specificity, Iripin-7 was compared with the homologous serpin iripin-2, previously characterized as IRS-2 in [2]. Sequence alignment revealed conservation of the RCL required for inhibitory activity, with differences observed in the predicted P1 site.

Protease inhibition assays demonstrated significant functional divergence despite overall sequence similarity. Iripin-2 exhibits a narrow specificity, primarily targeting chymotrypsin-like proteases [2]. In contrast, Iripin-7 inhibits a broader spectrum of enzymes, with strongest effects observed for inflammatory proteases and related targets.

Together, these findings show that high RCL similarity does not necessarily translate into equivalent protease specificity. Instead, subtle structural features within the recognition region contribute to target selection and functional diversification. This work provides structural insight into how closely related serpins evolve distinct biological roles in modulating host defense pathways.

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P26

CHEMILUMINESCENT DEOXYRIBOZYME SENSORS FOR DNA-EDITING ENZYMES

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DNA editing enzymes such as those in the APOBEC family of cytidine deaminasesplay important roles in both normal and pathogenic function, while engineered enzymes offer exciting new possibilities for genome editing. Despite their importance, widely-used assays for DNA editing enzymes are time consuming and expensive. Here we describe a new assay for DNA editing enzymes in which the substrate in the reaction is a chemiluminescent deoxyribozyme called Supernova. Editing alters the sequence of Supernova, which results in a change in catalytic activity and light production. By analyzing a dataset of Supernova variants previously identified by selection and high-throughput sequencing, it was possible to generate sensors with a wide range of specificities. Sensors were also developed for APOBEC3A, a cytidine deaminase which converts C to U in single-stranded DNA and RNA. These include a turn-off sensor that produces light 14-fold slower after incubation with recombinant APOBEC3A than in its absence, and a turn-on sensor that generates light 10-fold faster after incubation with APOBEC3A than in its absence. Assays that use these sensors are faster and less expensive than existing ones, and should be particularly useful for applications such as high-throughput screening.

P27

PROTEIN PRODUCTION FACILITY – DNA & PROTEINS FOR YOUR RESEARCH

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The Protein Production core facility at the Centre of Molecular Structure (CMS) of the Czech Infrastructure for Integrative Structural Biology (CIISB), part of Instruct-ERIC centre, offers comprehensive services covering every step of protein production from DNA to the purified protein. These include gene cloning into expression vectors, site-directed mutagenesis, and expression, followed by protein purification.

Our cloning services include both traditional cloning using restriction enzymes and restriction free (RF) methodologies. Furthermore, we perform small-scale expression and solubility tests using various *Escherichia coli* strains under different conditions. As an alternative to prokaryotic expression, we can provide protein production in human embryonic kidney cells (HEK293T, Expi293, and Expi293F GnTI-) or in baculoviral expression systems using Sf9 and High5 insect cells. Finally, we offer large-scale production and purification of target proteins.

For protein purification, we employ a range of steps, such as Strep-Tactin XT and immobilized metal chelate affinity chromatography (IMAC), both on FPLC or in gravity flow setups. We also provide ion-exchange chromatography and size exclusion chromatography using Superdex 75 and Superdex 200 columns (10/300 Increase or HiLoad 16/600). Customers may request adaptations of standardized protocols or provide us with established protocols. All services can be ordered individually or as a single end-to-end package.

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) and OP JAK project "Innovation of Czech Infrastructure for Integrative Structural Biology" (no. CZ.02.01.01/00/23_015/0008175).

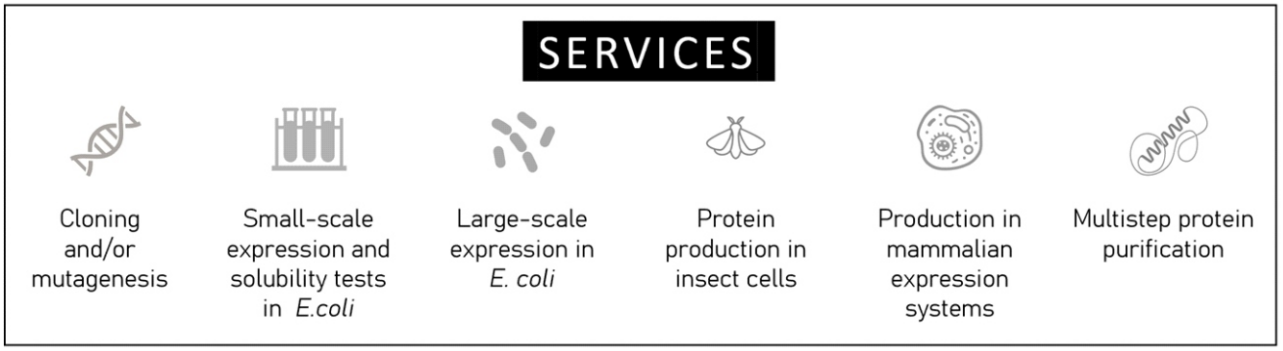

Figure 1. Services of the Protein Production core facility in CMS.

P28

CORRECTION FOR MULTI-LATTICE TRANSLOCATION DEFECTS IN DIFFRACTION DATA FROM CRYSTALS OF ZINC-DEPENDENT NUCLEASES

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Crystallization of biological macromolecules is one of the key methods in structural biology. The growth of well-diffracting crystals is essential for structure determination using crystallographic methods. For the majority of experiments, crystallization still represents a trial-and-error approach. Crystals are usually evaluated according to their physical appearance, for example their size, visual defects, or intergrowth of multiple crystals. However, visual

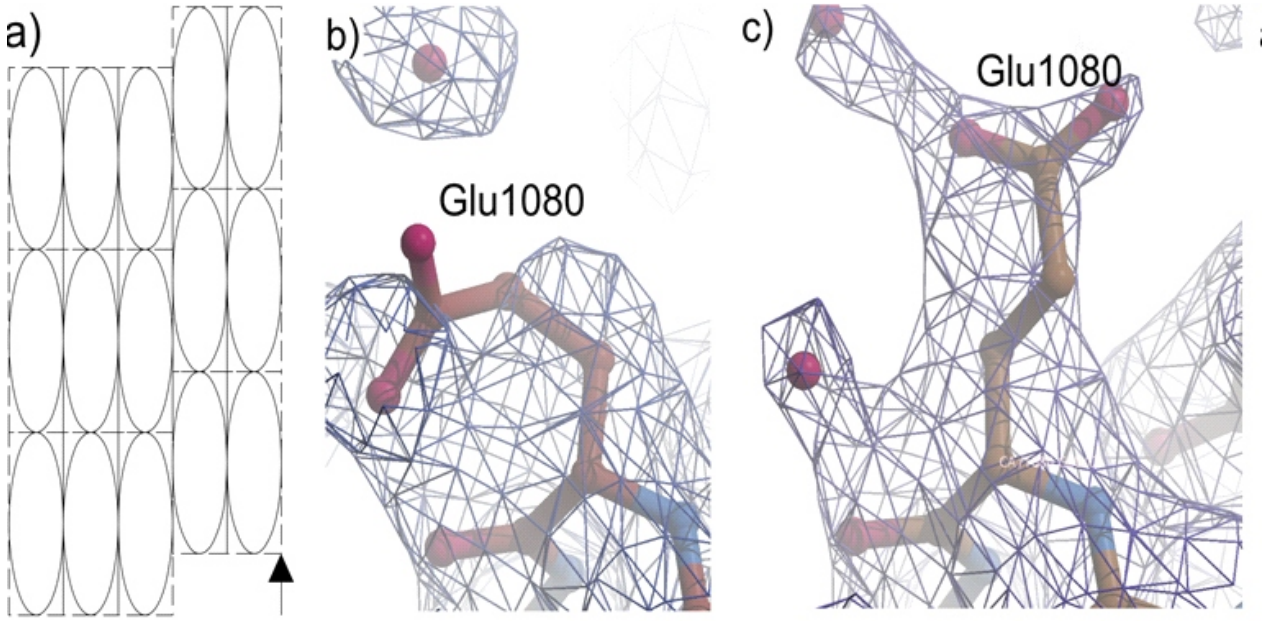

Figure 1. a) Scheme of lattice translocation defect. Unit cells (dashed line) containing a macromolecule (ellipse) of a crystal domain are translocated by a translocation vector (arrow). b) Uncorrected observed electron density contoured at the level of 1 is shown for the residue Glu1080 of the FtsK motor domain (PDB code 2IUS). c) Observed electron density contoured at the level of 1 after correction for LTD is shown for the same residue as in b). Apparently, correction for LTDs may play a crucial role and affect fine structural details.

inspection does not prevent internal disorder or other structural properties that may become apparent only after exposure to X-rays, such as twinning, diffraction anisotropy, or lattice translocation defects (LTDs). Moreover, some of these effects are difficult to detect even from visual inspection of diffraction images, for example merohedral twinning or LTDs.

LTD represents a situation in which a crystal contains more than one identical lattice domain that is translationally shifted by a translocation vector. LTDs modify the profiles of a subset of reflections, generate off-origin peaks in the native Patterson map, and distort the observed electron density [1]. If not taken into account, this phenomenon may significantly affect the success of the structure-determination process. Fortunately, it can be easily corrected by applying several calculation procedures.

Crystals of zinc-dependent nucleases S1 from *Aspergillus species* and SmNuc1 from *Stenotrophomonas maltophilia* frequently suffer from LTDs. Correction for LTDs was crucial for the precise interpretation of atomic resolution studies of complexes of both nucleases with ribonucleotides [1, 2]. Similar observations have been made in diffraction data from other complexes of the nucleases that have not been published yet.

Recently, we developed a Python 3 module named *TRANSLOCATION*. The module is easy to install using the 'pip' command and supports various multiple file formats, including MTZ, mmCIF, SCA, HKL, and hkl. We tested the module on a set of structures deposited in the PDB. In all cases, we achieved a decrease in R values, R-gap ($R_{free} - R_{work}$), and a significant improvement in the calculated electron density maps, which facilitated the interpretation of experimental observations. For example, the corrected electron density for the residue Glu1080 in crystal structure of FtsK motor domain indicates an alternative rotameric state (PDB code 2IUS, Figure 1). Although there are more methods of correction for LTDs, our approach is the only one that enables correction for more than one translocation within a crystal. The method is also ready for implementation in other automated pipelines.

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P29

CRYO-EM TOWARDS VISUALIZATION OF SMALL MOLECULES

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The Cryo-EM core facility at IOCB Prague has established a state-of-the-art infrastructure to advance structural biology, with a particular emphasis on the visualization of protein complexes bound to small-molecule ligands. The facility provides comprehensive end-to-end services, including sample preparation and characterization, grid screening, high-resolution data acquisition, and single-particle analysis, supported by a dedicated high-performance computing cluster.

The purpose-built underground facility is engineered to ensure exceptional environmental stability. It incorporates a passive multilevel antivibration foundation, an integrated in-wall cooling system, and an automated liquid nitrogen distribution network. The instrumentation portfolio includes a 300 kV Thermo Fisher Scientific Krios G4, a 200 kV Thermo Fisher Scientific Glacios, and a JEOL JEM2100plus transmission electron microscope.

The platform is designed to enable high-resolution structural studies approaching sub-2Å resolution. A key strategic objective is also implementation of three-dimensional (3D) electron diffraction methodologies, facilitating structural characterization of small molecules, including products of chemical synthesis and natural compounds.

Together, this integrated cryo-EM and 3D electron diffraction infrastructure will provide a powerful framework for structural analysis of biologically relevant macromolecules and small-molecule systems, thereby accelerating translational research in chemical biology and drug discovery.

P30

BUILDING LEGOS: PURIFICATION AND REASSOCIATION OF *BACILLUS SUBTILIS* RNA POLYMERASE INITIATION COMPLEX

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RNA polymerase (RNAP) is the central enzyme of the transcription machinery, responsible for synthesizing RNA from a DNA template. In Gram-positive bacteria such as *Bacillus subtilis*, RNAP consists of multiple subunits and regulatory components, including factors that direct promoter recognition and initiation of transcription. The primary factor ^{A} controls transcription of housekeeping genes during vegetative growth, whereas the alternative ó factor ^{B} governs the general stress response.

This work focuses on the recombinant expression, purification, and reassociation of *B. subtilis* RNAP core subunits together with ^{A} and ^{B} to assemble transcription initiation complexes on synthetic DNA scaffolds. Focus of this work is placed on optimizing purification to obtain pure and stable protein components suitable for the complex formation. The resulting reassociated initiation complexes will provide a foundation for structural and functional studies of factor–dependent transcription initiation in *B. subtilis* using cryo-electron microscopy (cryo-EM) and nuclear magnetic resonance (NMR) spectroscopy.

P31

PROBING THE SPECIFICITY OF FLUORESCENT DEOXYRIBOZYMES USING SINGLE-STEP SELECTIONS AND MACHINE LEARNING

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The ability of proteins and nucleic acids to form specific binding sites for ligands is critical for biological function, and methods to modulate biochemical specificity are important for fields such as enzyme engineering and drug design. Here we systematically investigated the specificities of self-phosphorylating deoxyribozymes that convert the coumarin substrate 4-MUP into a fluorescent product using biochemical assays, single-step selections, and machine learning. Activity assays using a panel of 20 catalytic motifs and 10 substrates that generate different types of signals when they are dephosphorylated revealed that these deoxyribozymes are extremely specific for 4-MUP. To identify mutations that change specificity, we constructed a library based on a self-phosphorylating fluorescent deoxyribozyme called Aurora. A series of single-step selections yielded variants that react with 4-MUP and the structurally similar substrate diFMUP, but not with the more distinct substrates pNPP and ELF. Pairwise analysis of sequences in the 4-MUP and diFMUP datasets revealed four mutations that modulate Aurora specificity. The effects of these mutations were confirmed using biochemical assays, and could be predicted using models developed by machine learning. Taken together, our results show how single-step selections can be used to identify mutations that change the specificity of a deoxyribozyme. They also highlight how machine learning can be used to model complex datasets from in vitro selection experiments.

P32

MOLECULAR DYNAMICS BEYOND COMMANDLINE

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Nowadays, handling MD simulations requires far more than running a simulation with a set of magic commands, analysing results, and publishing a paper. Both better user experience and ubiquitous FAIR principles are required. We introduce an ecosystem of services developed in e-Infra CZ focused on molecular dynamics.

MDDB is a European-wide activity which provides distributed infrastructure of repositories specialized on MD data. We aim at provisioning CZ national node built on top of National Repository Platform services/capacities allowing CZ users to publish large-scale MD data using the aligned interfaces.

MDFind extends successful AlphaFind (3D structure based search in Alphafold database) with the capabilities to search in MD trajectories at three levels: metadata, geometrical similarity of trajectory frames with a query structure, and similarities of essential characteristics of whole trajectories.

MDDash provides generic environment for running MD simulations and analyses. The simulation protocol, in the form of Jupyter notebook, is recorded thoroughly for further reuse. Simulation hyperparameters (multiprocessing, domain decomposition, GPU use...) are tuned for optimal performance semi-automatically. MD data repositories are interfaced for both down- and upload. Integration of advanced functionalities (like ExTraMD, ML technique to extend MD trajectories considerably) is planned.

P33

AUTOMATED HIGH-RESOLUTION CRYO-FIB-SEM VOLUME EM ENABLES SUB-VOLUME AVERAGING OF CELLULAR STRUCTURES

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Conventional volume electron microscopy (volume EM) provides three-dimensional views of cellular organization but commonly relies on chemical fixation, dehydration, and heavy-metal staining. These preparative procedures can perturb native ultrastructure and impose fundamental limits on achievable resolution and interpretability. Cryo-volume electron microscopy (CVEM) overcomes these limitations by enabling three-dimensional imaging of vitrified cells and tissues in a near-native state, thereby preserving biological architecture and molecular context.

Here, we present an integrated experimental and computational workflow for high-fidelity CVEM that systematically addresses imaging geometry, ion species selection, and data-processing strategies. This optimized approach enables robust acquisition of volumetric datasets with high structural preservation and contrast. Using CVEM, we achieve isotropic resolutions of approximately 15–20 nm in vitrified cellular and tissue samples. Notably, the resulting data quality is sufficient to enable, to our knowledge, the first application of sub-volume averaging in unstained volume EM samples.

To promote accessibility and reproducibility, we further provide an open-source software implementation of the complete workflow. Together, these advances establish CVEM as a powerful platform for high-resolution, near-native three-dimensional cellular imaging and expand its applicability for quantitative structural analysis.

P34

MASS PHOTOMETRY AS A CONVENIENT TOOL IN SQ ASSESSMENT ROUTINE

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The Core Facility Biomolecular Interactions and Crystallography at CEITEC MU in Brno serves as a central hub for biophysical analysis of proteins, nucleic acids and other macromolecules. It provides access to advanced instrumentation, focusing on the biophysical techniques for sample characterization and interaction studies. The combination of various methods allows for detailed analysis of the sample in a short time and spares precious resources of the subsequent high-end techniques. Since the methodology in the field of biomacromolecular studies develops rapidly, it is necessary to perform regular upgrades of the instrumentation. At the end of 2024, Mass photometer TwoMP completed with MassFluidix HC add-on device has been installed and successfully used in numerous projects.

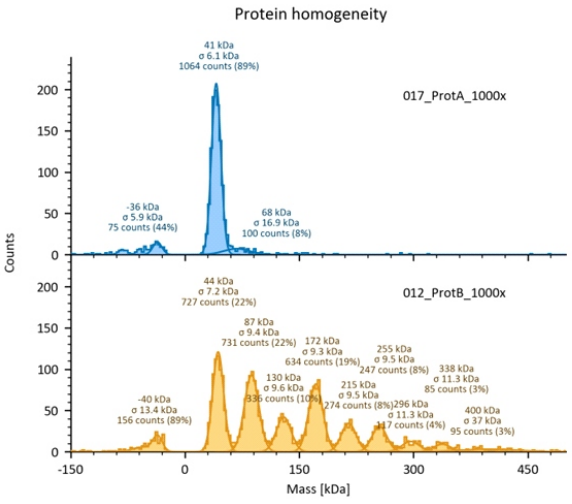

Figure 1. Protein homogeneity check.

Mass photometry (TwoMP with MassFluidix HC) enables the measurement of various types of biomolecules across a broad mass range (30 kDa–5 MDa). With only a very small amount of sample (ng), the method provides precise characterization of native samples under different buffer conditions. It enables the detection of high-affinity interactions, and when combined with the MassFluidix HC add-on device can also be extended to medium-affinity interactions. Thanks to its easy handling and rapid workflow**,** the method has strong potential to become a standard tool not only for quality control in bio-project workflows, but also for gaining insight into interaction and stability studies.

„CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042 and European Regional Development Fund-Project „Innovation of Czech Infrastructure for Integrative Structural Biology“ (No. CZ.02.01.01/00/23_015/0008175), is gratefully acknowledged for the financial support of the CF Biomolecular Interactions and Crystallography.“

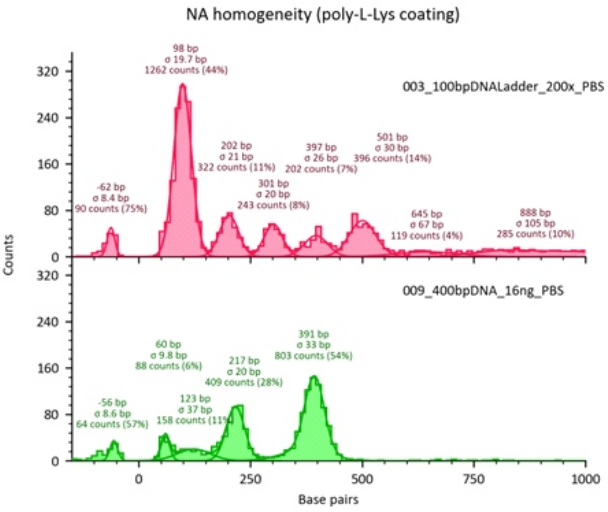

Figure 2. DNA homogeneity check

P35

USE OF BIOINFORMATICS IN THE DEVELOPMENT AND OPTIMIZATION OF CATALYTICALLY ACTIVE DNA

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In vitro selection is a powerful tool for exploring the functional potential of nucleic acids. Large pools of randomly synthesized DNA molecules (on the order of 107 -1015 sequences) are incubated with potential substrates. Active molecules are separated from inactive ones, the active fraction is amplified by PCR and the process is repeated until activity is detected. Next gen sequencing of the reaction product provides valuable information about selected functional motifs. As the patterns hidden in the data can be very convoluted, we are developing new methods to understand these datasets and use these results to complement our research in the wet-lab. One of our recent advances focuses on the optimization of a deoxyribozyme from our lab called Aurora. We use the idea of secondary structure libraries and machine learning to model and further explore the sequence space encoded by our ibraries to search for new, more active variants of Aurora.

P36

SIMULATION MODEL OF THE MITOCHONDRIAL RIBOSOME

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University of Chemistry and Technology, Prague

Mitochondrial ribosomes (mitoribosomes) are biomacromolecular complexes located within mitochondria. A mitochondrion is a cellular organelle responsible for several crucial biochemical pathways. One of the most important is the respiratory chain followed by oxidative phosphorylation (OxPHOS), which is essential for the production of cellular energy in the form of ATP. The reactions of the respiratory chain are carried out by respiratory chain complexes (RCCs). The genetic information for the synthesis of several essential RCC subunits is encoded in

the mitochondrial genome (mtDNA). Mitoribosomes are responsible for performing the final step of mtDNA expression - mitochondrial translation. Taken together, proper mitoribosomal function directly affects one of the central processes of cellular metabolism. Therefore, a detailed understanding of mitoribosomal behaviour is of great importance. This project aims to build an all-atom simulation model of the complete mitoribosome in the presence of ions and explicit solvent, to perform molecular dynamics (MD) simulations, and to analyse the obtained trajectories. Special attention will be paid to selected specific regions of the mitoribosome in order to describe their structural dynamics. To the best of our knowledge, this would represent the first ever all-atom simulation model of the complete mitoribosome under such conditions. In the future, this project may contribute, for example, to the development of more efficient antibiotics. Mitoribosomes evolved from bacterial ribosomes and share many structural similarities with them. Therefore, describing the differences in antibiotic interactions between bacterial ribosomes and mitoribosomes at the atomic level is essential for achieving a breakthrough in this field.

P37

DEVELOPMENT AND APPLICATION OF CATALYTIC DNA MOLECULES

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The double helix is well-suited for the storage of genetic information. However, the functional potential of DNA is far greater than this canonical role might suggest. Our group uses a method called artificial evolution to learn more about the interesting and useful things that DNA can do. This involves purifying rare molecules with a desired property (such as the ability to bind a ligand or catalyze a reaction) from large random sequence libraries. Here we provide an overview of DNA enzymes recently discovered in our group that generate chemiluminescent, fluorescent and colorimetric products. These DNAzymes generate signals using a fast and easy workflow, can be engineered to only generate signals in the presence of specific inputs, and can be used for applications like high-throughput screening. We also describe the development of new methods to explore sequence space using structured libraries and single-step selections. Our work highlights the potential of catalytic DNA and shows how it can be used to solve real world problems.

P38

IDENTIFICATION OF KEY REGIONS IN AGGREGATION-PRONE dGAE FRAGMENT OF Tau

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Intrinsically disordered protein (IDP) tau is a driving force of the progression of neurodegenerative tauopathies. In the physiological state, it binds microtubules, regulates axonal transport, and neurite outgrowth; while in its pathological state it undergoes conformational transitions, which promote assembly into fibrils. Tau's aggregation-prone dGAE fragment (tau 297–391) retains the ability to assemble into fibrils and seed the aggregation of full-length tau [1,2].

Sidechain Propelled Rings (SPuRs) are small, 9-15 membered ring elements of protein secondary structure. Derived from observations in crystal structures and supported by molecular simulations, these SPuRs may modulate conformational behavior, as well as influence the recognition of dGAE by monoclonal antibodies [3,4]. Two distinct SPuRs located at positions S305 and D387 were revealed by previously solved crystal structures of antibody-tau complexes: PDB ID 2V17 (Fab MN423 bound to the PHF core C-terminus) and PDB ID 5MO3 (Fab DC8E8 bound to a 14-mer tau peptide) [5,6].

To elucidate the structural and functional contribution of these SPuRs, we designed dGAE mutants modifying the native SPuR architecture. Substitutions S305A and D387A avoid SPuR formation, whereas D387N is expected to preserve ring formation due to preservation of hydrogen-bonding capacity (Fig. 1A). Double-mutant constructs (S305A/D387A and S305A/D387N) targeting both sites

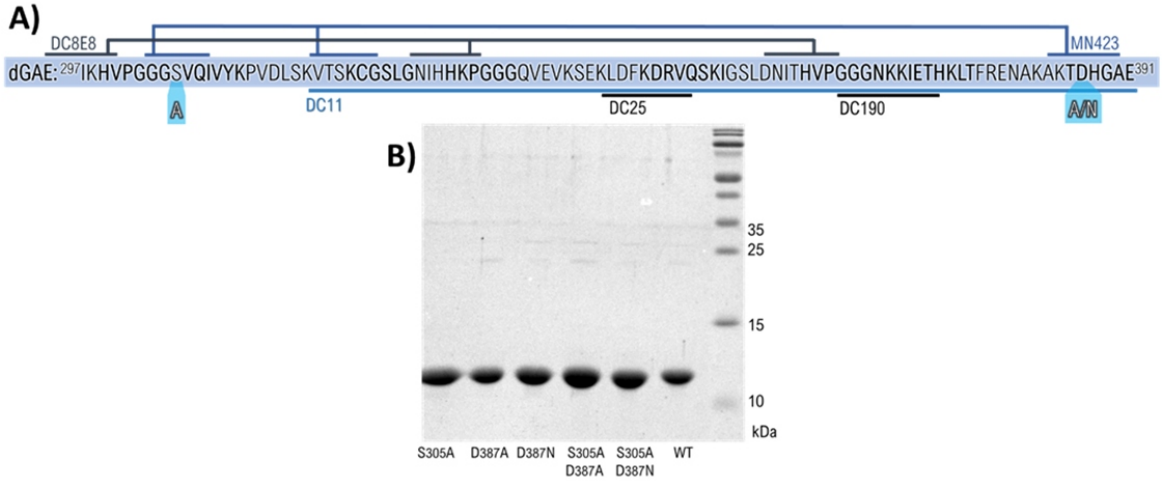

Figure 1. A) Schematic representation of the tau dGAE fragment showing the positions of engineered substitutions (S305A, D387A, D387N) and antibody epitopes recognized by DC8E8, DC11, DC25, DC190, and MN423 used for affinity measurements, B) SDS–PAGE analysis of five purified dGAE mutants and wild type protein.

were generated to assess potential cooperativity between these transient structural elements.

Mutated dGAE were prepared by site-directed mutagenesis, wild-type and mutant dGAE variants were expressed in E. coli and purified by cation exchange chromatography followed by size-exclusion chromatography (Fig. 1B). We tested the effect of mutations on binding to antibodies DC8E8, DC25, DC190, DC11 and MN423 by SPR and their impact on tau aggregation in vitro.

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P39

ULTRAFAST PHOTOPHYSICS OF AXITINIB BY TRANSIENT ABSORPTION SPECTROSCOPY AND FEMTOSECOND STIMULATED RAMAN SPECTROSCOPY

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Photopharmacology has emerged as a promising strategy for locally inhibiting the activity of enzymes, thereby enabling spatially confined targeting of specific enzymes and minimizing systemic side effects. Among several such initiatives axitinib ,a tyrosine kinase inhibitor, has been demonstrated to be optically switchable between active and inactive forms via light-induced isomerization. Although its steady-state optical properties have been characterized, an in-depth understanding of the underlying photophysical processes, particularly on ultrafast timescales, remains lacking. In this work, we investigate the ultrafast photophysics of the cis–trans isomerization of axitinib. Using femtosecond time-resolved spectroscopy, we identify two distinct kinetic processes associated with the cis-to-trans isomerization, with characteristic time constants of X and Y, and three kinetic processes for the trans-to-cis isomerization, with time constants of A, B, and C. These results provide a detailed mechanistic insight into the photoisomerization dynamics of axitinib, with implications for its rational design and optimization as a photopharmacological agent.

P40

SURFACE-ENRICHED HCMV UL141 IS ENDOH-RESISTANT IN VIRIONS AND ENGAGES SOLUBLE RECEPTORS BY SPR

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Human cytomegalovirus (HCMV) encodes an extensive repertoire of immunomodulators that counter diverse host defenses and thereby support lifelong persistence and reinfection. The glycoprotein UL141 is a particularly versatile factor: it restricts surface display of the DNAM-1/CD96 ligands CD155 and CD112 and inhibits TRAIL death receptors, and recent evidence links UL141 to virion entry complexes. In our preliminary analyses, UL141 was the most abundant viral protein detected at the infected-cell plasma membrane, approaching gB levels, and it was present from early times and maintained across infection, consistent with rapid turnover and recycling at the cell surface. Virion-associated UL141 was full-length and predominantly EndoH-resistant, indicating maturation through the secretory pathway prior to incorporation. Using surface plasmon resonance with intact virions, we further observed that virion-displayed UL141 engages soluble receptors but with detectable binding restrictions, suggesting a regulated, context-dependent interaction landscape on the particle. To rationalize UL141 multireceptor capacity, Structure-guided interface prediction on the UL141–TRAIL-R2 structure highlighted two high-probability protein–protein interfaces: an accessible region on the N-terminal Ig-like domain and a surface on the back of the C-terminal domain, both characterized by exposed -strands/ -helices and permissive despite glycan shielding. In parallel, ITC with an engineered clinical anti-TIGIT antibody supports that UL141 recapitulates key TIGIT-like structural determinants relevant to CD226-axis ligand recognition, while preserving a distinct mode for TRAIL-R2 engagement (Figure 1). Together, these data position UL141 as a virion-displayed, receptor-competent immunoevasin with separable binding surfaces, strengthening its promise as a therapeutic target and motivating systematic dissection of UL141 cognate interactions on virions.

Financial supports provided by the Slovak Research and Development Agency (APVV-24-0351) and the Scientific Grant Agency of the Slovak Republic (VEGA-02/0049/26) are gratefully acknowledged.

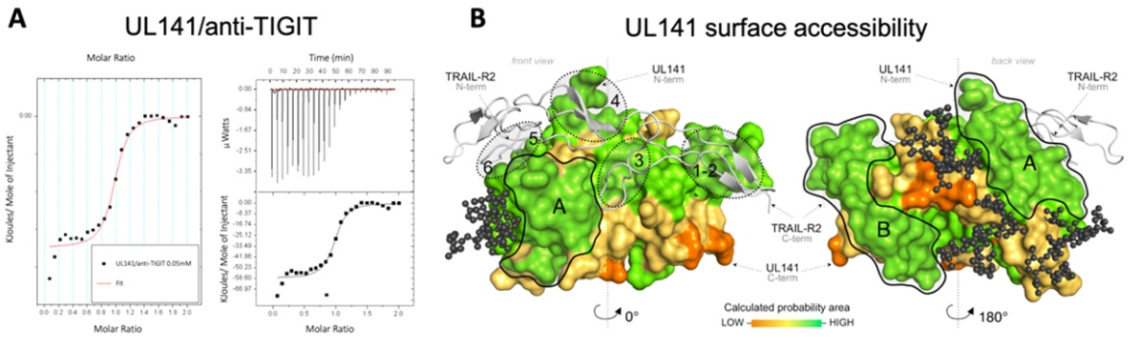

Figure 1. (**A**) Measurement of interaction between UL141 and engineered clinical anti-TIGIT antibody using ITC. (**B**) UL141 surface accessibility for receptor binding. Structure of UL141 (surface) in complex with TRAIL-R2 (cartoon). N-glycosylation sites were modeled with a high mannose glycans (black). Area A and B indicate available and accessible protein binding sites on UL141.

P41

UNCOVERING THE ASSEMBLY OF THE RISC-LOADING COMPLEX

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Argonaute and Dicer proteins are key components of RNA-dependent gene silencing. In mammals, Argonaute-2 (Ago2) functions as the primary effector of microRNA (miRNA)-mediated gene regulation. Following Dicer-mediated processing of precursor miRNAs (premiRNAs), Ago2 is selectively loaded with mature miRNA guides to form the RNA-induced silencing complex (RISC). Despite more than two decades of extensive research, the molecular rules and structural basis governing guide-strand selection and loading remain only partially understood. Notably, multiple studies suggest that Dicer and its cofactor TARBP2 (or PACT) play important roles in facilitating the accurate and efficient loading of Ago2. Here, we present our preliminary efforts to characterize the molecular interactions between Ago2 and the Dicer–TARBP2 complex, as well as the specific contributions of each component during the RISC-loading process. Our structural biology approach provides insights into the roles of the individual components and sheds light on the overall mechanism of RISC assembly.

P42

METHYLTRANSFERASES Rv2847c (CysG), Rv0470c (PcaA), AND Rv2954c AS POTENTIAL ANTI-TUBERCULOSIS DRUG TARGETS

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, remains one of the leading causes of death from infectious disease worldwide. The emergence of multidrug-resistant and extensively drug-resistant strains highlights the urgent need for novel therapeutic targets that are mechanistically distinct from currently used antibiotics. S-adenosyl-L-methionine (SAM)-dependent methyltransferases represent an underexplored yet promising class of drug targets due to their essential roles in bacterial metabolism, cell wall biosynthesis and stress adaptation.

This work focuses on the structural and biochemical characterization of three Mtb methyltransferases: Rv2847c (CysG), Rv0470c (PcaA), and Rv2954c. Rv2847c encodes a multifunctional uroporphyrinogen III methyltransferase involved in tetrapyrrole biosynthesis, contributing to siroheme and cobalamin production, pathways important for redox metabolism and intracellular survival [1]. Rv0470c (PcaA) is a cyclopropane mycolic acid synthase that modifies mycolic acids in the mycobacterial cell wall, directly influencing cell wall architecture, virulence and persistence within macrophages [2]. Rv2954c is predicted to participate in lipid-associated methylation processes linked to complex cell envelope components, which are critical for host-pathogen interactions and immune modulation [3].

This study is aimed at identifying and enabling the development of novel potential inhibitors targeting these enzymes. By characterizing their catalytic mechanisms, cofactor-binding pockets and structural determinants of substrate recognition, this work establishes a foundation for structure-based inhibitor discovery and contributes to expanding the pipeline of candidate antitubercular agents.

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P43

COMPARISON OF 5-FLUOROTRYPTOPHANE AND 6-FLUOROTRYPTOPHANE LABELLING IN 14-3-3 PROTEINS

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^{19}F nuclear magnetic resonance (NMR) spectroscopy is a powerful approach for studying biomacromolecules and their interactions. The ^{19}F isotope is 100% naturally abundant, magnetically active, and possesses a gyromagnetic ratio approximately 83% that of ^{1}H, providing high sensitivity without isotopic enrichment. [1, 2, 3, 4] Because fluorine is absent from native biomacromolecules, ^{19}F NMR enables background-free detection and highly sensitive monitoring of local structural changes. [3,5]

Site-specific incorporation of fluorinated amino acid analogues offers a versatile strategy for introducing ^{19}F probes into proteins. A cost-effective strategy for protein labelling, particularly for fluorination of tryptophan residues, is supplementation with fluorinated amino acid precursor such as fluoroindole. [1,3] Tryptophan's indole ring can be selectively fluorinated at positions 4, 5, 6, or 7. [1] This unique tool allows us to study interactions between proteins and their binding partners as well as conformational changes of the proteins such as folding and unfolding with a simplified view whilst keeping the protein and/or its partners intact. [1, 6, 7]

Here, we compare incorporation of 5-fluorotryptophan and 6-fluorotryptophan into the 14-3-3 protein. We assess protein yield, labelling efficiency, and key biophysical characteristics relative to the non-fluorinated protein. Furthermore, we analyse the resulting 1D ^{19}F NMR spectra to evaluate sensitivity and suitability for monitoring conformational changes and ligand interactions.

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P44

PHL2: A BLACK SHEEP OF THE PLL LECTIN FAMILY

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Lectins are a well known saccharide recognizing proteins with an irreplaceable role in many fundamental natural processes. They mediate cell-cell interactions on molecular level and play an important role in interactions between microorganisms and hosts [1]. Our research is focused on studying a PLL lectin family: seven-bladed beta propeller lectins from entomopathogenic bacterium *Photorhabdus* spp. *Photorhabdus* bacteria are known for complicated life-cycle, including mutualism with a nematode host and pathogenicity towards insects [2]. On top of that, *P. asymbiotica* is also a potent emerging human pathogen [3].

This contribution is focused on the PHL2 lectin, the newest member of the PLL family [4]. All members of the PLL family are highly similar on the sequence level (sequence identity 56-81%) and they share common structural fold (seven-bladed beta propeller). The recombinant PHL2 was produced in *E. coli* and purified by affinity chromatography on the D-mannose agarose resin. PHL2 saccharide specificity was screened by glycan-array and top ligands were further analysed by isothermal titrational calorimetry. Suprisingly, PHL2 prefers D-glucose and glucosylated ligands over sugars containing L-fucose. This is unique within the PLL family, where all other members prefer to bind fucosylated saccharides. Detailed study of potential PHL2 binding sites and comparison with other family members will be presented and possible reasons for different saccharide specificity discussed.

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P45

CORE FACILITY FOR CRYSTALLIZATION AND DIFFRACTION OF PROTEINS AND NUCLEIC ACIDS, CENTRE OF MOLECULAR STRUCTURE, IBT CAS, BIOCEV

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The Centre of Molecular Structure at IBT CAS (BIOCEV, Vestec, Czech Republic) is a sophisticated complex of scientific core facilities specializing in structural biology. Services are provided via the Czech Infrastructure for Integrative Structural Biology (CIISB) and the European infrastructure for structural biology Instruct-ERIC. Its crystallization and diffraction core facility uses among other equipment also automated robotic systems for high-throughput crystallization experiments and specialized crystallization hotels with comprehensive imaging capabilities. Diffraction part of the facility provides services for characterization of protein structure using single crystal X-ray diffraction and small-angle X ray scattering for analysis of liquid samples. The quality of high-intense X-ray beams is enabled by using MetalJet sources (Excillum).

A notable technological feature is the SONICC instrument (Formulatrix) integrated with the RI1000 crystallization hotel. This system employs Second Harmonic Generation (SHG) and Ultraviolet Two-Photon Excited Fluorescence (UV-TPEF) techniques, enabling unprecedented detection of micro- and nanocrystals. Such precise screening is crucial for advanced methodologies, allowing researchers to analyze crystallization experiments with exceptional sensitivity and precision.

The crystallization robots have recently been upgraded, enabling the robotic setting of experiments at either 20 °C or 10 °C. The crystallization hotel's software environment

was also upgraded to ensure long-term sustainability and automated scoring using multiple evaluation algorithms.

The facility's equipment represents the latest technology in structural biology research, facilitating complex crystallization and diffraction experiments, as well as detailed structural analysis of biomacromolecular complexes.

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) and OP JAK project „Innovation of Czech Infrastructure for Integrative Structural Biology" (No. CZ.02.01.01/00/23_015/0008175).

P46

THE ODD ONE OUT: INVESTIGATION OF THE 14-3-3 THERMOSTABILITY

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The 14-3-3 proteins are a ubiquitously expressed family of eukaryotic regulators with multiple isoforms in most organisms: two in *Saccharomyces cerevisiae*, two in *Drosophila melanogaster*, twelve in *Arabidopsis thaliana*, and seven in mammals including humans [1]. The seven mammalian isoforms are named and . They form a complex cellular regulatory hub. Despite high sequence conservation, the 14-3-3 isoforms differ in interactomes, expression levels in various tissues, biophysical properties, and preferred oligomeric states [2-4].

This work focuses on the biophysical properties of human 14-3-3 , which we have found to have a significantly lower melting temperature than other human 14-3-3 isoforms. We confirmed this using differential scanning calorimetry (DSC) and further studied human 14-3-3 phosphorylated at the Ser59 residue. Previous work of our colleagues showed that Ser58 phosphorylation in human 14-3-3 induces monomerization of the isoform and lowers the melting temperature by ~10 °C [5, 6]. We observed similar behaviour for Ser59 phosphorylated 14-3-3 , with an unfolding onset at 39 °C. These findings highlight isoform-specific differences that may relate to their distinct roles in physiological and pathological processes.

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P47

PLASMA X-RAY SOURCE FOR TIME-RESOLVED DIFFRACTION IN ELI

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We present a pulsed X-ray source delivering sub-picosecond pulses at a repetition rate of 1 kHz. The radiation is generated by focusing an intense laser onto a copper target, producing characteristic 8 keV emission suitable for time-resolved diffraction experiments. The broadband bremsstrahlung component enables X-ray absorption measurements.

Comprehensive characterization of the plasma X-ray source (PXS) performance, driving laser parameters, and available end-station capabilities is provided [1]. We report the first powder diffraction snapshot of the organic compound azobenzene.

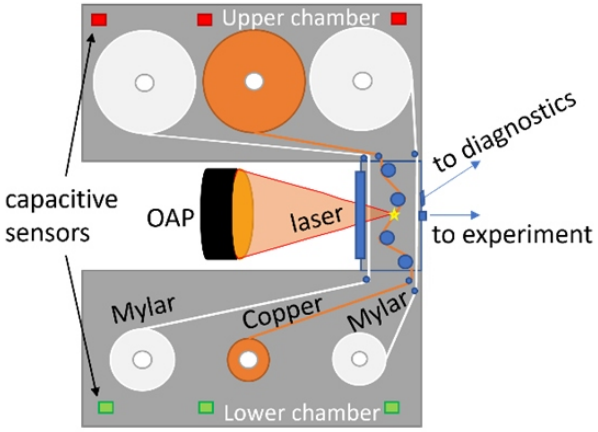

Figure 1. PXS layout.

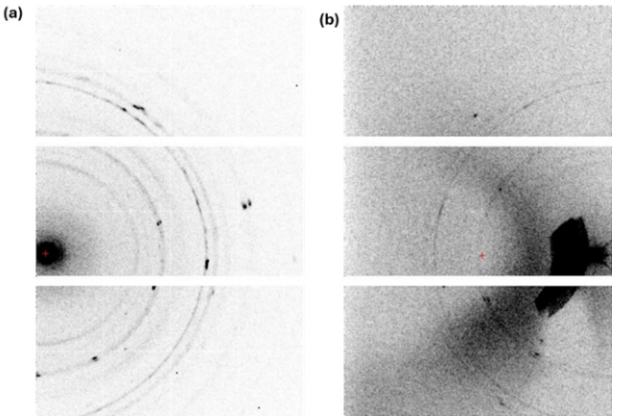

Figure 2. Two-dimensional X-ray powder diffraction from 4-Phenylazophenol (a) collected with a continuous X-ray tube source

The PXS system is equipped with a Jungfrau detector, enabling precise shot-to-shot flux normalization and improved signal-to-noise ratio. The implementation of a pump–probe setup, planned for 2026, is also presented.

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P48

IDENTIFICATION OF INHIBITORS OF THE POLYMERASE DOMAIN OF THE DENGUE VIRUS

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Dengue virus is a globally significant pathogen responsible for hundreds of millions of infections worldwide each year, yet no specific antiviral therapy is currently available. The viral RNA-dependent RNA polymerase (RdRp), the C-terminal domain of the NS5 protein, is essential for genome replication and represents an attractive antiviral target. This study aimed to evaluate synthetically modified nucleotides as potential inhibitors of the RdRp domain of dengue virus type 2. Recombinant RdRp was expressed in E. coli and purified using affinity, ion-exchange, and size-exclusion chromatography. Its activity was analyzed in vitro using an RNA template, and reaction products were resolved by denaturing PAGE. Several tested compounds inhibited RdRp activity. For the most active compounds, IC_{50} values in the single to low tens of micromolar range

were determined, indicating moderate inhibitory potency and providing a basis for further optimization.

P49

REGULATION OF Nedd4-2 BY NDFIP ADAPTOR PROTEINS

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Nedd4-2 ubiquitin ligase is a member of the Nedd4 family of HECT-type E3 ligases, comprising a calciumdependent C2 domain, four WW domains mediating protein-protein interactions, and a HECT domain with catalytic activity. Nedd4-2 is the last enzyme in the ubiquitination cascade, where it is responsible for recognizing the target substrate and transferring ubiquitin molecules to its lysine residues. The reason for its intensive research is the fact that this E3 ubiquitin ligase is associated with many pathophysiological phenomena, including Liddle syndrome, which involves abnormally high activity of epithelial sodium channels (ENaC) caused by dysfunctional Nedd4-2 [1]. Possibilities for regulating the function of Nedd4-2 include autoinhibition caused by intramolecular interactions between the C2 domain and WW3-4 - with the HECT domain [2], activation of the locked state by calcium ions [2], and intermolecular interaction with proteins through WW domains [3-4].

In our work, we investigate how Ndfip adaptor proteins modulate Nedd4-2 in vivo in order to understand how the regulatory mechanism works. We propose the hypothesis that Ndfip adaptor proteins are able to loosen the locked structure of Nedd4-2 (regardless of the presence of calcium ions) by binding PY motifs to WW domains, thereby significantly accelerating the transfer of ubiquitin to the target substrate. To test this hypothesis, we used analytical gel filtration, analytical ultracentrifugation, ubiquitination assays with liposome, and, in the future, we plan to use hydrogen deuterium exchange coupled with mass spectrometry (HDX-MS). As part of the project, it was necessary to purify Ndfip constructs (Ndfip1 and Ndfip2) without a transmembrane domain together with the entire Nedd4-2 in *E. coli*. Furthermore, analytical gel filtration chromatography was used to demonstrate that Ndfip proteins do indeed interact with Nedd4-2. Analytical ultracentrifugation was used to determine the dissociation constant of these complexes at the submicromolecular level, which will enable us to perform HDX-MS to elucidate the structural mechanism of this experiment. The positive effect of Ndfip proteins on the kinetics of Nedd4-2 autoubiquitination and on the ubiquitination of the protein substrate ASK1 (apoptosis signal-regulating kinase 1) was also confirmed.

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P50

DESIGN OF PROTEINS BY METADYNAMICS IN THE SEQUENCE SPACE

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Protein design has become a viable alternative to the prospection of natural proteins (enzymes, binding proteins, etc.) or protein engineering. Recently, this field has been revolutionized by the introduction of machine learning. One of protein design strategies is to sample amino acid sequences and score them using structure prediction tools such as ESMfold. Monte Carlo simulated annealing has been used as a sampling algorithm. Here we replace simulated annealing with more efficient parallel tempering metadynamics. Metadynamics can be used to disfavor sampling of previously sampled protein designs, thus accelerating sampling of various properties of designed proteins,

e.g. secondary structure composition, net charge, or hydrophobicity. The approach can be used to evaluate the designability of proteins that differ in these properties.

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P51

NATIVE PURIFICATION AND STRUCTURAL CHARACTERISATION OF RNA POLYMERASE COMPLEXES FROM *BACILLUS SUBTILIS*

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The bacterial RNA polymerase (RNAP) is a dynamic multi-subunit molecular machine. Its activity and regulation depend on temporal interactions with additional factors [1]. Structural studies of these assemblies are frequently challenging, mainly because of the difficulty of isolating intact, native complexes in sufficient quantities and with consistent stoichiometry. This challenge is particularly pronounced in some Gram-positive organisms, as RNAP interacts with species-specific regulatory proteins such as the subunit [2].

In this contribution, we present a workflow for the native purification of RNAP complexes from *Bacillus subtilis*. The strategy combines affinity capture followed by chromatographic steps to preserve unstable interactions while removing contaminants and partially associated assemblies.

The resulting samples were analysed by biochemical and biophysical methods and used for structural characterisation using cryogenic electron microscopy. Our data provide insight into the architecture of the *B. subtilis* RNAP core (subunits) [1] and its association with the subunit.

By successfully isolating the -associated RNAP complex, we provide evidence that these interactions persist under *in vivo* conditions. This highlights developing native purification, despite the difficulties, as an indispensable approach for structural studies.

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P52

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 orga-

nized 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.

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P53

PHOSPHORYLATION OF MICROTUBULE-ASSOCIATED PROTEIN 2c BY SERINE/THREONINE KINASES AND THE EFFECT OF PHOSPHORYLATION ON ITS INTERACTIONS

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Cytoskeletal microtubule-associated proteins (MAPs) regulate the stability and dynamics of the microtubules. Inside the neurons, occurrence of various MAPs differs. Tau, associated with the pathology of the Alzheimer's disease, is present mostly in axons, whereas microtubule-associated protein 2 (MAP2) is found in dendrites. Both Tau and MAP2 are classified as intrinsically disordered proteins (IDPs) and play a vital role in neuronal development. They are subjected to frequent post-translational modifications (PTMs), in particular phosphorylation by various protein kinases. Phosphorylation is the key regulatory mechanism that has been shown to have a significant impact on their function, interactions with their binding partners and involvement in neuropathologies.

Utilizing NMR spectroscopy, we investigated the phosphorylation of the juvenile form of MAP2, MAP2c, by Ser/Thr kinases cAMP-dependent protein kinase A (PKA), extracellular signal-regulated kinase 2 (ERK2) and glycogen synthase kinase 3 (GSK3). We identified the phosphorylated residues, monitored the phosphorylation kinetics and observed the influence of phosphorylation on the interactions of MAP2c with physiologically relevant binding partners, e.g., the regulatory proteins 14-3-3 and SH3 domains of tyrosine kinases.

Notable differences were observed in phosphorylation rates and patterns, as well as mutual cross-talks between the respective protein kinases. Priming of GSK3 by PKA resulted in an enhanced GSK3 activity and an increased phosphorylation rate of the residues close to the main PKA phosphorylation site Ser435. On the other hand, prior phosphorylation of MAP2c by ERK2 substantially decreased the phosphorylation of Ser435 by PKA.

Phosphorylation of MAP2c by PKA increased the binding affinity of 14-3-3 to MAP2c. This can be seen by considerable decrease of the intensities upon binding of 14-3-3 around PKA's main phosphorylation sites Ser184, Ser189, Thr220 and Ser435. ERK2 mediated phosphorylation of MAP2c resulted in markedly weaker binding of the SH3 domains of protein kinases Fyn, Abl and the adaptor protein Grb2.

All in all, our study shows that MAP2c is substantially phosphorylated by PKA, ERK2 and GSK3 . Site-specific phosphorylation by these kinases regulates its ability to interact with the physiologically relevant binding partners. Mutual influence of the respective kinases modulates site preferences and rate of phosphorylation of individual residues. These findings may suggest that phosphorylation of MAP2c by these protein kinases possibly plays an important role in regulation of its activity *in vivo* and may induce direct downstream effects.

P54

STRUCTURE OF THE LE3 PHAGE INFECTING *LEPTOSPIRA BIFLEXA*

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Phage therapy based on lytic bacteriophages complements conventional treatment approaches to combat increasing antimicrobial resistance. Reduced antibiotic susceptibility has also been reported in *Leptospira*, a genus of Gram-negative spirochetes responsible for leptospirosis in humans and animals. *Leptospira biflexa* serves as a non-pathogenic bacterial model to study leptospiral biology and phage infection. Bacteriophage LE3 is one of the few described lytic phages infecting *L. biflexa*.

In our study, we determined the structure of phage LE3 using cryo-electron microscopy. The virion exhibits a typical tailed bacteriophage morphology, comprising a 65 nm icosahedral capsid, a contractile tail 65–80 nm in length, and a 30 nm baseplate. Furthermore, the thin, electron-transparent morphology of *L. biflexa* allows direct visualization of phage particles within cells and facilitates structural analysis of phage assembly intermediates.

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P55

MOLECULAR INSIGHT INTO 5’ RNA CAPPING WITH Np$_n$Ns BY BACTERIAL RNA POLYMERASE

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RNA capping with dinucleoside polyphosphates (Np$_n$Ns) has recently been found in both prokaryotic and eukaryotic cells. Dinucleoside polyphosphates, also known as alarmones, are directly incorporated into RNA transcripts by RNA polymerase, which can utilize them as non-canonical initiating nucleotides [1, 2]. We explored by cryo-EM how exactly these compounds bind into the active site of RNA polymerase during transcription initiation, using open-bubble DNA templates in combination with dinucleoside polyphosphates and *Thermus thermophilus* RNA polymerase. We found that both nucleobase moieties of dinucleoside polyphosphates can pair with the DNA template (Figure 1). While the proximal nucleobase pairs with the template in a canonical Watson-Crick manner, the dis-

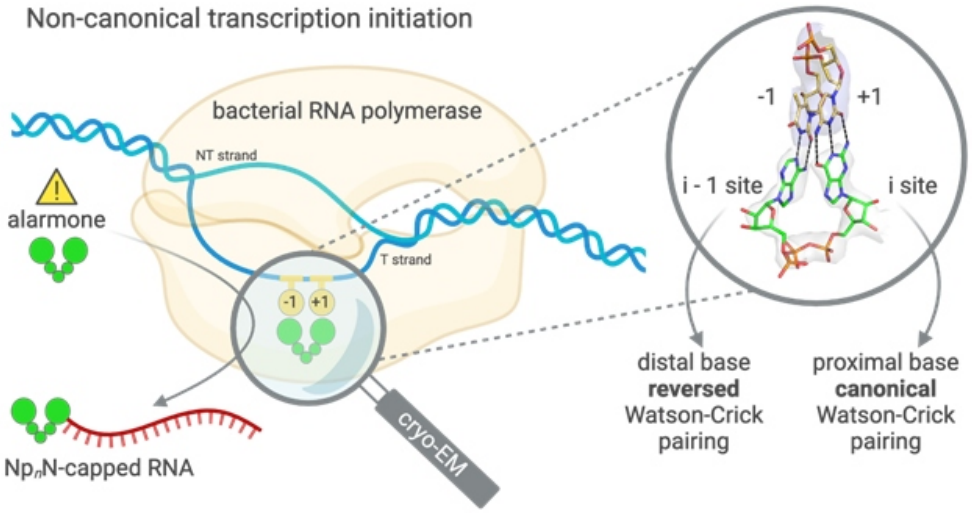

Figure 1. Structural analysis of transcription initiation with dinucleoside polyphosphates (Np$_n$Ns).

tal nucleobase pairs non-canonically in a reverse Watson-Crick manner. Our work sheds light on the molecular mechanism of transcriptional 5' capping with dinucleoside polyphosphates [3].

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P56

CONTRIBUTION OF THE INSTITUTE OF NEUROIMMUNOLOGY SAS, BRATISLAVA TO THE ADVANCED PROTEIN BIOTECHNOLOGY CONSORTIUM

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The Advanced Protein Biotechnology Consortium (APBC) is a unique scientific and innovation project, the aim of which is to strengthen cooperation between academia and the biotechnology industry, support the emergence of innovative and cutting-edge solutions in the field of protein biotechnology and contribute to the development of the regional economy. The project is designed to have a significant regional, national and international impact – it will support economic growth by the development of the start-up ecosystem and a competitive high-tech sector, but above all it aims to create new possibilities for talented Slovak and foreign young scientists and to support the return of Slovak experts from abroad.

The APBC is leaded by the University of Pavel Jozef Safarik in Kosice, Slovakia, comprises other two institutes of Slovak Academy of Sciences and four international partners including research institutions from Czechia, Germany, Netherland and United Kingdom. Two non-academic partners from Slovakia and Czechia complement the project team.

Institute of Neuroimmunology's contribution to the APBC consists in building and supplying a wide portfolio of eukaryotic (*E. coli*) and prokaryotic (insect cells, CHO and HEK cell lines) expression systems, protein purification platforms, start-of-the-art biophysical methods for protein characterization (light scattering and calorimetry methods, CD and FTIR spectrometry, high-throughput SPR and BLI instruments, stopped-flow methods) and X-ray crystallography. The Institute also offers immunogenicity testing of candidate therapeutics developed by Consortium. We will present the results achieved by the Institute in the first year of project implementation.

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P57

CVFORMER

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Molecular Dynamics simulations provide insights on the biomolecular motion, however it is intrinsically limited by timescale separation. Rare events such as folding, conformational transitions, or allosteric rearrangements often occur on timescales that exceed those accessible to standard MD. Enhancing sampling methods, such as Metadynamics, addresses this limitation by enhancing sampling along a set of Collective Variables (CVs), which act as low-dimensional descriptors of the system's slow degrees of freedom. However, the effectiveness of metadynamics critically depends on the quality of the chosen CVs, suboptimal variables can lead to inefficient, or incomplete, exploration of the free-energy surface. The identification of physically meaningful and dynamically relevant CVs therefore remains a central challenge in enhanced sampling.

CVFormer is a Transformer-based autoencoder for the extraction of CVs from Molecular Dynamics trajectories. The model compress conformational information into a low-dimensional latent space (typically 2D), suitable for driving enhanced sampling simulations such as metadynamics. By analyzing this reduced representation, we can not only characterize metastable states and transition pathways, but also trace back which structural degrees of freedom are responsible for shaping the learned collective variables.

A key aspect of the approach is the fully data-driven selection of CVs. Instead of relying on handcrafted descriptors, the model learns representations directly from molecular dynamics trajectories. Attention weights provide residue-level importance scores, enabling the identification of structural regions that contribute most strongly to the learned collective variables. This interpretability is complemented by mutual information analysis within the latent coordinates, allowing a quantitative assessment of dependency and redundancy.

The methodology is demonstrated on the Trp-cage mini-protein, a well-established benchmark for folding and conformational studies. CVFormer successfully learns a low-dimensional representation that captures the essential folding landscape and separate metastable states.

The analysis highlights specific residues with dominant contributions to the learned CVs, in agreement with known structural determinants of Trp-cage stability.

The selected CVs were subsequently used to bias metadynamics simulations, leading to efficient enhancement of conformational transitions and accelerated crossing of free-energy barriers.

P58

BEYOND IC_{50}: A RE-ENGINEERED STOPPED-FLOW KINETIC FRAMEWORK FOR HUMAN CARBONIC ANHYDRASE INHIBITION STUDIES

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Carbonic Anhydrases (CAs) comprise a ubiquitous and diverse enzyme family that regulates inorganic carbon homeostasis in tissues, cells and subcellular compartments. As such, CAs are involved in a wide range of cellular processes, including energy metabolism, photosynthesis, membrane transport, or cell adhesion. The human genome encodes 15 homologous CA isozymes that differ in expression patterns, localization, oligomeric state and catalytic activity. Among these, isozymes IX and XII receive greater attention due to their extensive expression in many solid tumours relative to healthy tissues. [1]

A distinctive branch of CA research focuses on the development of isozyme-selective inhibitors targeting CA IX and XII. [2] The inhibition assay most commonly employed in these studies measures initial reaction rates for conventional IC_{50} determination. Its core principle, established by *Khalifah* in 1971 [3], involves detection of protons released during the enzymatic reaction via pH-indicator absorbance in a stopped-flow setup. As contemporary studies often report dozens of compounds with activities and selectivities within an order of magnitude, the methodology becomes increasingly insufficient in accuracy, reproducibility, and throughput for cross-comparative or structure-activity relationship (SAR) studies. Furthermore, the exact protocol is poorly documented and concerns have been raised regarding the experimental and publication practices involving a substantial part of the existing literature. [4]

In this poster, a re-engineered version of the CA assay is presented, based on the original 1971 article. The revised approach incorporates explicit signal-to-substrate concentration conversion, internal referencing to spontaneous and uninhibited reaction rate referencing, and differential kinetic modeling. Inhibition constants (K_i) are derived directly from fitted kinetic parameters, rather than converted from IC_{50} values, and are complemented by statisti-

cally-derived error estimates. Properly annotated, the new methodology is capable of both greater accuracy and throughput, and should improve quality of data (and life) in the field.

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P59

STRUCTURAL CHARACTERIZATION OF METTL21A INHIBITION BY NOVEL SMALL MOLECULES

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Methyltransferase-like protein 21A (METTL21A) is a S-adenosyl-L-methionine (SAM)–dependent lysine methyltransferase that modifies molecular chaperones, including Hsp70 family proteins and the co-chaperone BAG3. These post-translational modifications enhance protein stability and reduce degradation. Recent studies link METTL21A activity to hepatocellular carcinoma progression and metastasis, highlighting its emerging disease relevance; however, no selective small-molecule inhibitors have yet been reported.

Here, we designed and synthesized small-molecule inhibitors of METTL21A and evaluated their biochemical activity. Recombinant METTL21A was expressed and purified for structure-based studies, and co-crystallization experiments were conducted with selected compounds. We determined and refined a high-resolution crystal structure of METTL21A in complex with a representative inhibitor, revealing key features of ligand binding to the SAM-binding site. These findings provide a framework for inhibitor optimization and support METTL21A as a potential therapeutic target.

P60

SIMULATION OF ELECTRON TRANSFER ON BIOMOLECULAR BRIDGES BY NON-ADIABATIC MOLECULAR DYNAMICS

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Molecular electronics utilizes (bio)organic molecules and organometallic complexes as parts of electronic devices to manufacture, for example, highly sensitive biocompatible sensors. Tuning and further development of such devices require an understanding of electron transport mechanisms and processes, as well as structural details. These could be provided by computer simulations based on quantum calculations and molecular dynamics techniques. However, the theoretical description of the (bio)molecular junctions remains challenging due to the interplay between discrete molecular electronic states and quasi-continuous electronic states of the metallic contacts and electrodes. Moreover, the soft-matter nature of the organic compounds requires the involvement of thermal motion, vibrations, and non-adiabatic electron-transfer events. Therefore, an explicit treatment of coupled electronic and nuclear dynamics is needed.

In this work, we employ non-adiabatic molecular dynamics (NAMD) with the Fewest Switches Surface Hopping (FSSH) method [1] to simulate electron-transfer processes in model molecular bridges. The simulations are performed using the open-access LIBRA modular library [2], which enables not only the simultaneous propagation of nuclear motion and the population of the related electronic states, but also state-of-the-art modifications and developments needed for the treatment of heterogeneous molecule/metal interfaces. We demonstrate the feasibility of these calculations on several model systems, ranging from Tully's models [1] to small rigid molecules treated under the neglect-of-back-reaction approximation (NBRA) to atomistic junction models based on benzene-1,4-diammine (BDA) and benzene-1,4-dithiolate (BDT) molecules. These methodology benchmarks and developments are presented in the context of intended applications on complex biomolecular electronic devices based on redox proteins that are investigated in our group.

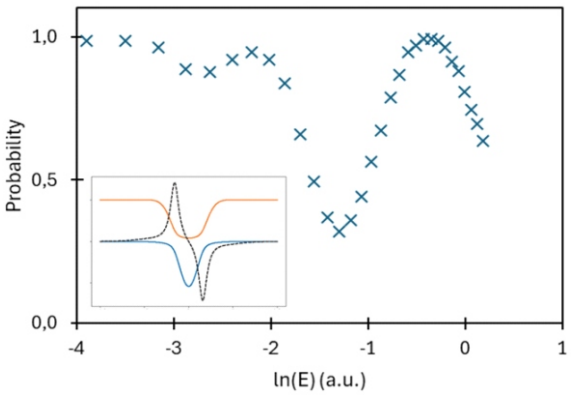

Figure 1. Computed transmission obtained by FSSH on Tully's DAC model.

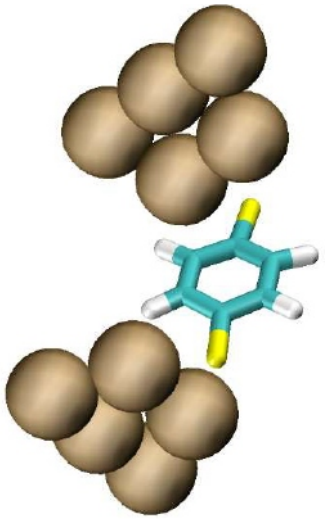

Figure 2. Atomistic model of BDT.

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