Posters

P1

PROTEIN PRODUCTION FACILITY – DNA & PROTEINS FOR YOUR RESEARCH

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

Our cloning services include both traditional cloning using restriction enzymes and restriction free (RF) methodologies. Customers can provide us with their templates, or we can order them from external companies. Subsequently, we can deliver and test several of our plasmids. Furthermore, we perform small-scale expression and solubility tests using various *Escherichia coli* strains under different conditions. We are expanding our services by introducing eukaryotic production. We can provide protein production in human embryonic kidney cells (HEK) or baculoviral expression system using Sf9 insect cells as an alternative to prokaryotic expression. Finally, we offer large-scale production and purification of target proteins.

In protein purification, we employ a range of steps, such as affinity chromatography (GST-tag purification, Strep-Tactin XT Sepharose or immobilized metal chelate affinity chromatography), performed either on an FPLC or in gravity flow setups. We also offer an ion-exchange chromatography, and size exclusion chromatography using Superdex columns (75 or 200, 10/300 increase, or HiLoad 16/600). Customers can request modifications to standardized protocols or provide us with established protocols for implementation.

In 2025, our team aims to organize the first workshop, expand and welcome new colleagues, customers, and collaborations.

The Biocev Protein Production core facility is a part of CMS operated by the Institute of Biotechnology, Czech Academy of Sciences as a member of the Czech Infrastructure for Integrative Structural Biology (ciisb.org). 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 by European Regional Development Fund-Project „Innovation of Czech Infrastructure for Integrative Structural Biology" (No. CZ.02.01.01/00/23_015/0008175).

Figure 1. Laboratory and services of the CF Protein Production at CMS.

P2

THE EFFECT OF THE CARDIAC-ASSOCIATED MUTATIONS ON THE BIOPHYSICAL PROPERTIES, FOLD AND STRUCTURE OF THE N-TERMINAL DOMAIN OF HUMAN RYANODINE RECEPTOR 2

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Calcium ions play a key role in physiological processes, such as the excitation-contraction process in muscle cells. The regulation in human cardiac muscle cells is primarily mediated by the human ryanodine receptor 2 (hRyR2). hRyR2 is a large calcium channel that mediates the transfer of calcium cations from the sarcoplasmic reticulum to the cytosol of cardiomyocytes. hRyR2 consists of four monomers, each composed of 10 domains. One of these domains is the N-terminal domain (NTD), where approximately 70 point mutations associated with severe arrhythmias have been identified. Mutations in the NTD cause several cardiologic disorders, primarily catecholaminergic polymorphic ventricular tachycardia 1 (CPVT1) and arrhythmogenic right ventricular dysplasia 2 (ARVC/D2).

In our study, we focused on the biophysical characterization and of hRyR2 NTD in its wild-type and mutant forms. We successfully expressed and purified wild-type and M81L and L433P mutant fragments of the hRyR2 NTD. We also verified their fold by CD spectroscopy, determined the influence of the mutation on the hRyR2 NTD size by FIDA analysis and determined their thermal stability using nanoDSF. We found that the M81L mutation increases the thermal stability of hRyR2 NTD. This mutant was also successfully crystallized. The L433P mutation reduces the solubility of hRyR2 NTD, affects its oligomeric state and alters the structure of this domain.

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P3

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

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

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P4

SOLUTION STRUCTURE OF cAMP-DEPENDENT PROTEIN KINASE RII SUBUNIT IN COMPLEX WITH MICROTUBULE ASSOCIATED PROTEIN 2c

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Intrinsically disordered proteins (IDPs) are a significant part of regulatory networks that control the cytoskeleton. Their interaction with kinases is key to understanding the mechanism of their function. Important IDPs in the brain include the MAP2/Tau family proteins. The ubiquitous cAMP-dependent protein kinase (PKA) also plays an important role in the function of brain neurons. Its localization is controled by A-kinase anchoring proteins (AKAPs), one of which is the microtubule associated protein 2c (MAP2c). The binding site for PKA on MAP2c was discovered a long time ago. However, structure of the complex had not been solved until now.

Here we present an atomic resolution structure of the interacting domains of the RII subunit of PKA in complex with MAP2c. The binding site was first confirmed using broadening of MAP2c NMR signals upon addition of PKA. Further relaxation and ensemble-based studies of free MAP2c indicated increased alpha-helical propensity for this region. ITC studies of the complex confirmed nanomolar binding affinity. For structure determination, the protein complex was prepared recombinantly with isotope labeling in both uniform and specific variants. NMR signals were assigned using tripple resonance and 3D NOESY spectra. Filtered 3D NOESY was used to probe intermolecular interactions in the complex. Residual dipolar couplings of NH pairs were measured using the Pf1 oriented phage. Distance restraints from 3D NOESY spectra were calculated using CYANA. The final structure was calculated using SCULPTOR-CNS.

We have solved the structure of MAP2c-PKA complex. The structure showed that intrinsically disordered protein MAP2c forms an -helix upon binding to PKA similarly to other AKAPs. However, the orientation the helix differs from the other known AKAP structures. This finding significantly extends our undersanding of the principles that govern specific recognition of AKAPs by regulatory subunits of PKA.

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P5

INHIBITION OF HISTONE DEACETYLASE 6 BY 1,2, 4-OXIADIAZOLES: STRUCTURAL AND BIOCHEMICAL CHARACTERIZATION

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Histone deacetylase (HDAC) inhibitors used in the clinic typically contain a hydroxamate zinc-binding group (ZBG). However, recent studies have shown that alternative ZBGs, including heterocyclic trifluoromethyl oxadiazoles (TMO), can confer greater isoenzyme selectivity and more favorable ADMET profiles. Here, we report the synthesis and biochemical and structural characterization of a series of HDAC inhibitors featuring 1,2,4-TMOs as ZBGs. Compared to 1,3,4-TMO inhibitors, incorporating the 1,2,4-TMO isomer into the inhibitory scaffold markedly alters selectivity, broadening activity across HDACs, including class IIa isoenzymes. Crystal structures of HDAC6-inhibitor complexes reveal that the 1,2,4-TMO ring of the parent oxadiazole undergoes hydrolysis within the enzyme's active site, yet the kinetics and catalytic mechanism differ from those observed for

1,3,4-TMO inhibitors. Overall, our findings provide compelling evidence that the choice of TMO warhead dictates not only HDAC isoform selectivity but also the transformation mechanism within target enzyme active sites.

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P6

TARGETING THE mRNA CAPPING MACHINERY OF *TRICHOMANAS VAGINALIS* AS THERAPEUTIC APPROACH IN TREATMENT OF TRICHOMONIASIS

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Trichomonas vaginalis is a member of flagellated protist that causes trichomoniasis, the most prevalent nonviral sexually transmitted human infection. Globally, the estimated annual incidence is more than 270 million cases [1]. The extracellular parasite resides in the urogenital tract of both sexes and can cause vaginitis in women and urethritis in men. Acute infections are associated with increased risk of human HIV-1 infection, cervical carcinoma, infertility, prostatic adenocarcinomas and adverse pregnancy outcomes. Current treatment of trichomoniasis relies on administration of antiprotozoal drugs. However, resistance has been increasingly recognized and may occur in up to 10% of infections [2].

A promising path represents targeting a mRNA (messenger RNA) capping machinery of this parasitic protozoan. The 5' cap is an essential feature of eukaryotic mRNA that is required for a stability and efficient translation. mRNA capping entails three enzymatic reactions and last step is the cap methylation reaction, catalyzed by RNA (guanine-N7) methyltransferase (RNMT). TRIV-RNMT is a 39.8 kDa protein with 347 aa, localized in nucleus and shares 33% sequence identity to a bigger human cap-methylating enzyme (Hcm1: 476 aa), however, the human cap methyltransferase contains nonessential N-terminal extensions that are missing in TRIV-RNMT. *T. vaginalis* homolog protein contains S-adenosylmethionine (SAM) binding motif, structurally resembling human Hcm1.

Here we present a newly discovered crystal structure of this protozoan methyltransferase with a high-resolution (~1.9 Å) view of the catalytic site, occupied by a co-factor competitive compounds: Sinefungin and SAH (S-adenosylhomocysteine). These preliminary crystal structures accompanied with high-throughput screening of small inhibitory molecules (unpublished data) provide a valuable structural insight for 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 that have been shown to be a high quality tool for non-linear dimensionality reduction and have many diverse applications. The architecture consists of two parts, an encoder, which transforms high dimensional input data onto a low-dimensional embedding, which is called latent space. Second part of the network, decoder, reconstructs these latent space values into the original high dimensional data.

In this work, we use structures of Tryptophan-cage miniprotein sampled from molecular dynamics (MD) simulation of high-temperature unfolding of the miniprotein, to train a simple autoencoder to be able to encode and decode miniprotein structures. Decoder part of the trained autoencoder can then be used to generate diverse protein structures based on input latent space values. The miniprotein structures generated this way include, but are not limited only to the structures present in the training dataset.

We show that a well-trained decoder can generate structures with displacement of individual particles so

small that, after a short potential energy minimization, the structures can be concatenated into a continuous trajectory based on Metropolis Criterion. 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. At the same time, structures sampled along the Monte-Carlo trajectory form an ensemble that follows Boltzmann distribution. Although the distribution of structures sampled this way differs slightly from one sampled from sufficiently long classical MD simulation, it is also provided at a tiny fraction of the computational cost of the MD simulation.

P8

STRUCTURAL INSIGHTS INTO 14-3-3-MEDIATED REGULATION OF HUMAN ASK1

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Apoptosis signal regulating kinase 1 (ASK1), also known as MAP3K5, is a widely expressed member and 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 stress stimuli appropriately. In unstressed condition, ASK1 is held in an inactive state through binding of negative regulators, such as thioredoxin and 14-3-3. Thioredoxin (Trx) is an oxidoreductase that participates in redox reactions and catalyzes dithio-disulfide exchange reactions whereas 14-3-3 is a highly conserved phosphoserine- and phosphothreonine-binding scaffold protein [2, 3]. Despite many years of intensive research, there is no atomic resolution structure of multi-domain ASK1 in complex with 14-3-3 or thioredoxin, which has hindered functional and mechanistic understanding of ASK1 regulation. Therefore, the main goal of this project is to unravel the structural and molecular basis of ASK1 regulation by the 14-3-3 protein 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).

The stability and stoichiometry of the ASK1:14-3-3 complex was investigated by SV-AUC. These experiments revealed that ASK1 and 14-3-3 form a complex with apparent dissociation constant (KD) of 90 ± 7 nM. The sedimentation coefficient distribution c(s) also suggested that the presence of 14-3-3 induced the tetramerization of ASK1, i.e. the formation of a complex with a stoichiometry of 4:4 (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 and cryo-EM. Cryo-EM reconstruction showed that each of the 14-3-3 dimers stabilizes the tetrameric arrangement of ASK1 by binding the C-terminal segments of ASK1 chains from opposite ASK1 dimers. This 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, thus explaining the inhibitory effect of 14-3-3 binding. Overall, our results provide the first insight into the structural basis of 14-3-3 protein-mediated regulation of ASK1 kinase.

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P9

REPLICATION AND TRANSCRIPTION REGULATOR Rta OF EPSTEIN-BARR VIRUS - FUNCTIONAL AND STRUCTURAL IMPLICATIONS FOR NEW ANTIVIRAL STRATEGY

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The Epstein-Barr virus (EBV) is one of the most prevalent human viruses, infecting over 90% of the global population over the course of a lifetime. EBV is implicated in approximately 200 000 cancer cases annually and is associated with various premalignant lymphoproliferative disorders, including Hodgkin's lymphoma, gastric carcinoma, and nasopharyngeal carcinoma [1]. Beyond its oncogenic potential, EBV has been linked to infectious mononucleosis and multiple sclerosis [2,3].

The replication and transcription activator (Rta) is a key regulator of the EBV life cycle, as Rta mediates the transition from latency to the lytic phase. Rta functions as a transcriptional activator by binding to the Rta Response Element (RRE) on viral DNA, thereby initiating the expression of lytic genes, including the viral gene PAN [4]. Despite Rta functional significance, the structural properties of Rta remain uncharacterized, and no sequence homology has been identified between Rta and known DNA-binding or dimerization motifs [5].

In this study, we employed an interdisciplinary approach to elucidate the functional and structural properties of Rta. We characterized the biophysical attributes of Rta DNA-binding domain and examined Rta oligomerization. Additionally, we investigated the structural features of the DNA-binding domain and quantified Rta binding affinity for a DNA sequence containing the RRE motif. In parallel, we analyzed Rta function in a human cell line, focusing on Rta nuclear localization and sequestration from nucleoli.

Targeting Rta with small-molecule inhibitors represents a promising strategy for therapeutic intervention in EBV-associated diseases. Thus, a comprehensive understanding of Rta's structural organization and oligomeric state is crucial for the rational design of novel antiviral agents.

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P10

DYNAMIC ALLOSTERIC REGULATION OF MYCOBACTERIAL INOSINE MONOPHOSPHATE DEHYDROGENASE

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Inosine-5'-monophosphate dehydrogenase (IMPDH) is a key enzyme in purine metabolism and a promising drug target against mycobacterial infections. Despite its central role at the crossroads of multiple branches of the purine metabolism, the regulation of mycobacterial IMPDH remains poorly understood. Here, we describe the molecular mechanism by which allosteric regulators dynamically modulate IMPDH activity in *Mycobacterium smegmatis*. Using single-particle cryo-EM, we obtained a comprehensive structural series of IMPDH in complex with substrates and regulatory molecules, including ATP, GTP, and the bacterial alarmone ppGpp. Our cryo-EM data, complemented by HDX-MS and SAXS experiments, reveal that these effectors exploit a common mechanism to alter the intrinsic dynamics of the octameric assemblies. By stabilizing distinct conformational states, they effectively trap IMPDH in a compressed, inactive conformation. This mechanism highlights a complex allosteric regulatory system that extends beyond simple feedback regulation, offering potential insights for the design of selective antimycobacterial drugs targeting IMPDH.

P11

UNRAVELLING THE MYSTERIES OF NOVEL TWO-DOMAIN LECTINS FROM OPPORTUNISTIC HUMAN PATHOGENS

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LecB (PA-IIL) is one of two characterised lectins (saccharide-binding proteins) from the bacterium *Pseudomonas aeruginosa*. Both proteins (LecA and LecB) play a significant role in bacterial infection and biofilm formation in immunocompromised patients (e.g. cystic fibrosis patients) [1]. Several LecB homologs were described in the past, for example, lectins produced by *Burkholderia cenocepacia* [2]. Nevertheless, there are still uncharacterised LecB-like proteins in the pathogenic bacteria, some of which contain an additional domain of unknown function. Their characterisation could provide insights into the mechanism of infection and lead to the development of novel approaches for disease treatment.

The aim of this project is the functional and structural characterisation of three potential two-domain lectins containing a LecB-like domain with an emphasis on their binding properties. The genes encoding these hypothetical carbohydrate-specific proteins were identified by bioinformatic analysis, cloned into expression vectors, and expressed in *Escherichia coli*. In addition, new gene constructs were prepared to characterise each domain separately. A variety of methods were used to investigate thermostability (nanoDSF), homogeneity (DLS, AUC) and binding properties (ITC, AUC) of the purified proteins. Several crystallisation screens were performed to obtain the crystals of the separate domains. The initial hits for X-ray crystallography are currently being optimised to obtain well diffracting crystals. For the whole proteins, electron microscopy methods are planned because of the expected high dynamics of the whole system.

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P12

STRUCTURE OF DC11 FAB FRAGMENT SPECIFIC FOR THE PRE-AGGREGATION CONFORMATION OF IDP Tau

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

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

up the tau aggregation. The information about conformational epitopes of these antibodies are therefore of high significance.

To further uncover the binding mode of the conformational antibody DC11, we have performed NMR epitope mapping using ^{13}C, ^{15}N labelled tau321-391 and tau297-391 (dGAE) and recombinantly prepared Fab fragment of DC11 antibody. The overlay of HSQC spectra showed the region of tau between residues 370-390 to be affected by the binding of DC11, i.e. its C-terminal region.

We have solved the X-ray structure of DC11 Fab fragment to a resolution of 1.33 Å and deposited it into the PDB database with PDB ID 9H8H. We have further measured the synchrotron SAXS data to characterize the conformational ensembles of tau321-391 and tau297-391 in both batch and SEC-SAXS modes. We have also attempted to characterize the complexes between tau proteins and DC11 Fab fragment.

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

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P13

GENOME ANCHORING, RETENTION, AND RELEASE BY NECK PROTEINS OF *STAPHYLOCOCCUS* PHAGE 812

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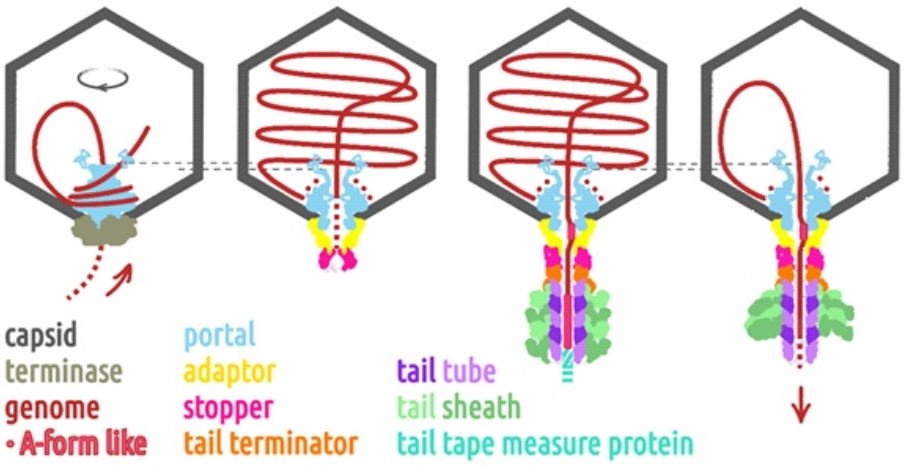
The virion of *Staphylococcus* phage 812 is formed by an icosahedral capsid and a contractile tail joined together by neck proteins. Despite the role of the neck proteins in virion assembly, DNA packaging, and regulation of genome release, their functions are not well characterized. Here we show that the neck of phage 812 consists of portal, adaptor, stopper, and tail terminator proteins decorated on the outside by two types of cement proteins. A dodecameric DNA-binding site on the portal complex anchors the genomic terminus inside the capsid, which may prevent an accidental escape of the DNA during the initial stages of genome packaging. The adaptor complex induces a local B-to-A form transition of the DNA in the neck channel that may serve to pause genome translocation. The gating loops of the stopper proteins prevent genome loss from fully packaged proheads by blocking the neck channel prior to the tail attachment. The binding of the tail terminator complex to the stopper complex induces opening of the gating loops and advancement of DNA into the tail. The structure of neck proteins is unchanged by tail sheath contraction. The expulsion of the tail tape measure protein rather than tail sheath rearrangement thus triggers genome release. Our results explain how the active interplay between neck proteins and the genome directs DNA packaging, prevents



premature genome release, and enables its ejection into the host cell.

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P14

BIOPHYSICAL CHARACTERIZATION OF BIOMOLECULES 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) techniques are available for the characterization of biomolecular interactions.

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

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

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P15

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

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Clostrioides difficile is one of the main causes of hospital-acquired diarrhea [1]. However, the accumulation of antimicrobial resistance in epidemic *C. difficile* lineages such as RT176 poses a significant risk for the spread of resistance determinants to other bacterial species targeted by these antibiotics. A comparative genomic analysis of one erythromycin-susceptible and six erythromycin-resistant *C. difficile* strains identified the novel resistance determinant carried by transposons Tn6110 and Tn7806 for which we propose the name mrmA (macrolide resistance methyltransferase A). We demonstrated that heterologous expression of the mrmA gene in *E. coli* confers resistance to erythromycin and to a lesser extent to streptogramin B, but not to other ribosome-targeting antibiotics.

MrmA encodes a putative SAM-radical 23S rRNA methyltransferase, similar to RlmN and Cfr [2]. RlmN is a housekeeping enzyme involved in translation fidelity and methylates nucleotide A2503 at carbon C23. Cfr-mediated methylation at the same A2503, but at carbon C8, confers resistance to antibiotics targeting the peptidyltransferase center (PTC) [3]: phenicol, lincosamide, oxazolidinone, pleuromutilin and streptogramin A, with no effect on erythromycin activity. In contrast, resistance to erythromycin is generally conferred by a different Erm-family of 23S rRNA methyltransferases [3]. These Erm enzymes dimethylate nucleotide A2058 in the exit tunnel, resulting in resistance to macrolides, lincosamides and streptogramins B antibiotics.

We hypothesize that MrmA methylates a different adenine residue on the 23S rRNA compared to Cfr and RlmN, which selectively affects erythromycin binding without affecting oxazolidinones or lincosamides [3]. To elucidate the exact site of modification, we will use *in vitro* biochemical and structural biology approaches, which we will present in detail.

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P16

STRUCTURAL STUDIES OF BIOLOGICALLY RELEVANT PROTEIN VARIANT OF CANCER-RELATED CARBONIC ANHYDRASE IX

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During tumor development cancer cells express various proteins to proliferate, overcome the unfavorable conditions within the tumor environment and further progress through the body. Each of these processes is enhanced by overexpression of enzyme carbonic anhydrase IX (CA IX) on cellular surface, making it an attractive target for the development of anticancer therapy. However, the discovery of specific drug compound has been hindered by existence of other fourteen CA isoforms within the human body and the fact that CA IX high-yield production and reproducible crystallization has been challenging. For that, number of protein variants have been established to ease the process of structure-based drug design, though some of them have lost the CA IX's property of dimer formation. Here, we describe a prospective workflow of heterologous production of recombinant CA IX extracellular region from *Escherichia coli*. Importantly, the biophysical characterization and crystal structure revealed that the biologically relevant dimeric arrangement is preserved and disclosed residues crucial for maintaining the dimeric interface. This CA IX variant thus represents valuable tools to reproducibly produce and crystallize biologically relevant dimeric form of CA IX.

P17

INFLUENCE OF THE REPARAMETRISATION OF THE PROTEIN-WATER INTERACTIONS ON THE CONFORMATION ENSEMBLE OF Tau(210-240)

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Tau is a well-known intrinsically disordered protein (IDP), regulating the assembly and maintenance of the microtubules. [1] However, under pathological conditions, tau undergoes various hyperphosphorylations. Those modified tau species detach from the microtubules, fibrillise and assemble into neurofibrillary tangles, the main pathologicalhallmarks of Alzheimer's disease (AD) in human brains. [2]

Understanding the mechanism of tau fibrillation is the key to understand the neurodegenerative process of AD. Molecular dynamics (MD) simulations can provide insights on the time-evolution of tau fibril formation. Coarse-grained (CG) simulations allow to extend the lengths and time scale of the simulations by orders of magnitudes compared to the all-atom resolution. [3] Accurate CG force fields are necessary for the description of Tau proteins in MD simulations. However, CG force fields tend to underestimate the global flexibility of IDPs and result in conformations, that are too collapsed. [3]

The accuracy of the CG force fields SIRAH 2 [4] and Martini 3 [5] for the description of the monomeric ensemble of Tau(210-240) is investigated. The accuracy of the CG force fields is evaluated by comparison with atomistic simulations. Furthermore, experimental results, such as

NMR data and the radius of gyration is used to validate the accuracy of the CG force fields. The unmodified SIRAH 2 and Martini 3 force fields are resulting in too collapsed structures. The collapsing nature of the CG force fields is shown to be overcome by strengthening the water-protein interactions. The reparametrised CG force fields give a fair description of the conformational ensemble of Tau(210-240) and can accurately reproduce experimental data.

The computational resources of IT4Innovations were granted by the Ministry of Education, Youth and Sports of the Czech Republic through the e-INFRA CZ (ID:90254). This project was supported by the Brno Ph.D. Talent Scholarship, funded by the Brno City Municipality and by the European Union's Horizon Europe 2020 program under the grant agreement No. 101087124 - ADDIT-CE.

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P18

INVESTIGATING NUCLEOSOME SPACING AS AN EPIGENETIC REGULATORY MECHANISM

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Nucleosomes are complexes of histone proteins and DNA formed by eukaryotic cells whose function is to prevent non-specific aggregation of DNA by allowing for controlled condensation in the form of chromatin as well as regulation of transcription and other processes by either physically blocking parts of DNA or, depending on its posttranslational modifications (PTMs), recruiting the transcription machinery [1]. In chromatin, nucleosomes typically form arrays in which the distance between neighbouring nucleosomes (the average length of histone-free DNA linker) is relatively constant. While the presence of regular arrays is a feature of chromatin conserved though all eukaryotic organisms, the average length of DNA linkers differ between organisms, and even between different cell types of one organism [2]. Although several factors are known to affect the positioning of nucleosomes, the biological function of their differential spacing remains poorly understood [3].

Previous research has shown that linker length and even spacing in chromatin influence the formation of higher-order chromatin structures. These structural variations can affect the accessibility of transcription machinery and even contribute to the formation of phase-separated condensates [4]. Additionally, several enzymes have been identified with a preference for binding di-nucleosomes over mononucleosomes, where the length of the DNA linker affects their activity [2]. Based on these findings, we hypothesize that linker DNA serves as an additional layer of epigenetic regulation, with its length acting as a discriminating factor for the binding of chromatin-associated factors.

We have prepared a DNA construct library with variable linker lengths covering all the typical10n+5 as well as less common 10n options and ranging from 10 to 35 bp. Upon reconstitution of the nucleosomes and immobilization on streptavidin beads, these constructs will be used to pull down protein binders specific for each linker length from nuclear lysates. The bound proteins will be identified by mass spectrometry based proteomics and selected binder will be selected for further structural characterization.

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P19

STRUCTURAL, SOLVENT, AND TEMPERATURE EFFECTS ON PROTEIN JUNCTION CONDUCTANCE

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Electronic conductance of redox proteins in their native environment is typically facilitated by the organometallic cofactors, like hemes in cytochromes, which can be reversibly reduced / oxidized. The electrons thus flow through such proteins by sequential hopping that can be well described by the Marcus theory. However, when these proteins are immobilized on metal surfaces, and their conductance is probed by a scanning tunneling microscope (STM) or its electrochemical variant (EC-STM), magnitudes, shapes, and temperature dependencies of the measured current-voltage curves suggest coherent tunneling as the undergoing transport mechanism rather than hopping. [1-3] To elucidate these data and investigate the factors affecting charge transport in biomolecular junctions, we developed a computational procedure based on multiscale modeling involving classical molecular dynamics (MD), electronic-state calculations within density functional theory (DFT), and electronic coupling calculations. [4-6] Here, we demonstrate its feasibility in the study of single-heme cytochrome b_{562}, [7] for which the EC-STM data were previously reported in the literature.

Computational resources were supplied by the project "e-Infrastruktura CZ" (e-INFRA LM2018140) provided within the program Projects of Large Research, Development and Innovations Infrastructure.

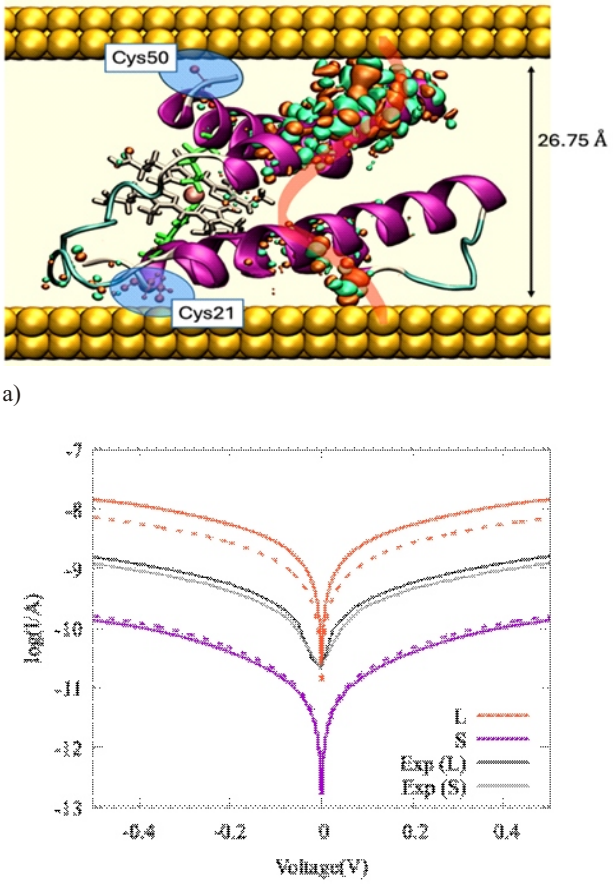

Figure 1: Cytochrome b_{562} junction: (a) representative structure with highlighted chemisorbed cysteines and highlighted conduction channel; (b) computed tunneling current curves for different structures with indicated solvent-screening effects.

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P20

ASYMMETRIC PARTICLES OF TBEV PROVIDE INSIGHT INTO MECHANISMS OF FLAVIVIRUS ASSEMBLY AND MATURATION

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

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

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

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P21

STRUCTURAL CHARACTERIZATION OF AIRE AND ITS INTERACTIONS

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The Autoimmune Regulator (AIRE) is essential for maintaining immune tolerance by promoting the expression of tissue-specific antigens (TSAs) in thymic medullary epithelial cells [1]. Mutations in AIRE are linked to APECED (Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy), a severe multi-organ autoimmune disorder [2].

AIRE consists of multiple domains interspersed with intrinsically disordered regions (IDRs), which together facilitate its interactions with chromatin and transcriptional regulators. While significant progress has been made in understanding AIRE's physiological role, the molecular mechanisms underlying its transcriptional regulation function remain unclear.

To investigate AIRE's interactions, we prepared a panel of AIREs constucts. These constructs will be used in pull-down assays followed by mass spectrometry to identify interacting partners. We will further characterize these interactions structurally and biophysically to gain deeper insights into the molecular basis of AIRE function.

A more detailed understanding of the interactions between AIRE and its partners will provide valuable insights into the molecular mechanisms of transcriptional regulation in eukaryotes and immune tolerance.

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P22

SEARCH FOR PATHOLOGY-INDUCING *IN VITRO* Tau FILAMENTS

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Tau protein, predominantly found in the central nervous system, plays diverse roles in neurons, including microtubule regulation, signal transduction, and fast axonal transport. However, misregulated tau forms pathological filaments, which are a hallmark of neurodegenerative diseases like Alzheimer's disease. Aggregation and accumulation of misregulated tau is toxic to the neurons. Despite intense efforts of scientific community, the underlying mechanisms of tau pathology are still not well understood [1].

Advances in amyloid cryo-EM helical reconstruction have revealed distinct structural differences in tau filaments among various diseases [2]. However, the mechanism of tau aggregation remains unknown, and the reason behind conformational diversity is not yet clear.

Currently, disease-like tau filaments can be prepared in vitro using dGAE (297-391) tau fragment and phosphomimicking tau constructs [3-5]. They have higher aggregation propensity and readily form filaments without aggregation inducers. Interestingly, their conformation highly depends on buffer conditions.

Our goal is to understand the factors influencing and driving tau aggregation and to prepare in vitro recombinant tau filaments. We are preparing tau filaments using tau isoform 2N4R and dGAE tau fragment in different buffer conditions (salt content, pH). Filaments are analysed using negative stain EM, and cryo-EM. Here we present our preliminary results of the ongoing study and discuss the aggregation protocol, morphological differences, and biological relevance of the prepared filaments.

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P23

UNDERSTANDING THE PROTEIN CRYSTALLIZATION

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Current Practice of Protein Crystallization

Already a half century, thousands of laboratories around the world are routinely using the protein crystallization as the most accurate method for experimental determination of the structure of proteins and their complexes. The diffraction methods on the small-molecular organic crystals allow the structure determination in subatomic resolution and often offering the observation of atom orbitals. But the accuracy in protein crystallography is pretty much worse mostly because a very low quality of protein crystals. In addition, a number of mysteries without any reasonable explanation surrounds the protein crystallization.

Practical crystallization is de facto the method of trials and errors. Crystallization robots set thousands of crystallization conditions that have been proven useful in the past for some other proteins and their complexes. Theoretical works describing protein crystallization using only classical thermodynamics slip usually into clarification of the precipitation rate. They do not explain well the function of so-called crystallization additives that were experimentally proved as necessary for a success of crystallization. Namely, the classical theory also do not solve clearly the fundamental problem of crystallographers, i.e. why the precipitation results in a crystalline phase or in a non-diffracting amorphous precipitate. Our approach adds some

new aspects including the kinetics of molecules in the supersaturated crystallization solution important for the error-free stacking of protein molecules into the growing crystal.

Dynamic Theory of Protein Crystallization

Large surface of protein molecule offers many possible adhesion modes between molecules. The adhesion modes mutually incompatible in a single crystal form lead to stacking faults disabling a growth of the regular crystal. Any successful method of protein crystallization can be explained by a reduction of the incompatible adhesion modes. Understanding how it works requires an analysis of physical processes acting during temporal clustering of molecules in the molecularly overcrowded crystallization solution close to saturation. An important role plays the orientation of the protein-additive clusters during their motion in the saturated crystallization solution before their deposition on the crystal surface. The additives can also block the deposition of protein molecules in the incompatible adhesion modes. The molecules of additives are usually pressed out of the growing crystal. However sometimes, they are preserved in their interaction with protein in the crystals serving thus as an excellent experimental proof of our theory [1-4].

As far as the initiation of crystallization by the hetero-surfaces immersed in solution, it is explained by formation of very stable error-free crystal-seeds on the hetero-surface. The stable seeds can survive and can grow even under the conditions where the spontaneously formed irregular-crystal-seeds in the bulk solution dissolve.

The differences in comparison with the classical theory include:

- formation of temporal (meta-stable) clusters of protein molecules with other molecules in crystallization solution (additives).
- changes of adhesive patches on the protein surface temporarily induced by the clustering.
- recognizing the mechanical forces accompanying the molecular motion of protein clusters in the solution close to saturation.
- recognizing the preferred orientation of supramolecular clusters during their movement in the molecularly overcrowded crystallization solution close to saturation.

Our dynamic theory of protein crystallization explains all common problems and mysterious behavior of uncountable number of crystallization experiments. It analyses formation of temporary supramolecular adducts and their motion in the saturated crystallization solution. This new approach uses the physical forces that are not commonly used in chemical and biochemical sciences. However, the formation of the supramolecular adducts is experimentally well confirmed by thousands of experiments in the PDB, as it is shown for example in [1-4]. The validity of models describing their kinetics is seen in that they fully explain thousands of experimental crystallization experiments.

Conclusion

Rational approach to crystallization based on the dynamic theory of protein crystallization and on the theory of adhesion modes can be used:

- for design of optimal crystallization screens tailored to a specific protein,
- to increase the quality of crystals leading thus to a better resolution of structure determination,
- to increase the efficiency of crystallization screens and lower consumption of protein sample,
- to explain the function of the protein crystal catalysers.

The work was supported by GAČR 25-17546S.

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P24

WHEN SIGNALING GOES ASTRAY: UNDERSTANDING MAP2K1 MUTATIONS IN NEURODEVELOPMENTAL DISORDERS

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The RAS/MAPK signalling pathway is one of the most extensively studied pathways, mainly due to its fundamental role in the regulation of cell cycle, proliferation, or senescence. Mutations in RAS/MAPK are primary drivers of cancer [1]. They also contribute to developmental syndromes known as RASopathies. These syndromes are associated with body malformations of different severity and with impaired cognitive function. Individuals with these syndromes often experience intellectual disability (ID) and autism spectrum disorder (ASD) [2, 3]. In this study, we investigate *de novo* recurrent single-point missense mutations in *MAP2K1* gene (encoding MEK1 protein), found in individuals with ID and ASD. They are currently classified as variants of unknown significance (VUS) [4]. It is crucial to investigate whether and how they impact the protein function, as some variants in RASopathies are known to increase or decreas the kinase activity.

The variants were selected from large sequencing studies [4] using an initial dataset of all missense variants found in ID/ASD individuals. We considered the number of affected individuals with each variant and the presence of secondary mutations in other genes. Further, the variants were cross-validated for their association with ID or ASD with ClinVar Miner (https://clinvarminer.genetics.utah.edu/), SysNDD (https://sysndd.dbmr.unibe.ch/) and SFARI database (https://gene.sfari.org/).

We cloned the wild-type MEK1 (MEK1wt), and the corresponding VUS-containing variants. We express and purify the proteins for assessment of kinase activity and for investigating the effect of VUS on 3D structure with X-ray crystallography. This study aims to elucidate the pathogenicity of the novel mutations and the molecular mechanisms by which they lead to ID/ASD. This will help to improve diagnostics and find tailored treatments targets.

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P25

NEW KIDS ON THE BLOCK: UPDATE ON EQUIPMENT INSTALLED AT BIC CORE FACILITY, CEITEC, BRNO

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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 and nucleic acids, being visited by almost a hundred of scientists and students every year. Since the methodology in the field of biomacromolecular studies develops rapidly, it is necessary to perform regular upgrades of the instrumentation as well as acquiring new machines. Over the last year, several new pieces of equipment have been installed and are now available to the users.

Mass photometer TwoMP (Refeyn) is designed to precisely determine molecular mass of proteins, nucleic acids and other macromolecules in a broad range of sizes. The machine is equipped with a recently developed fluidics

system, allowing to analyze both high- and low-stability complexes across various concentrations down to nM range. Modular spectrofluorometer Fluorolog QM (Horriba) enables various fluorescence measurements including fluorescence anisotropy and time-resolved anisotropy with time-correlated single photon counting. CD spectrometer Chirascan V100 (Applied Photophysics) is essential for investigating the 2D structure and stability of proteins, nucleic acids, and chiral drugs. It replaced the previous system, providing higher signal to noise ratio and sensitivity. The analytical SEC instrument OmniSEC (Malvern Panalytical) has been equipped with a fraction collector, allowing for subsequent analysis of the samples using highly sensitive techniques, e.g. mass spectrometry or electron microscopy.

Measurements at CF Biomolecular Interactions and Crystallography of CIISB, Instruct-CZ Centre are supported by MEYS CR (LM2023042) and European Regional Development Fund-Project „UP CIISB“ (No. CZ.02.1.01/0.0/0.0/18_046/0015974).

P26

STRUCTURAL VARIABILITY OF PEPTIDE DEFORMYLASE

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The first enzyme encountered by bacterial proteins after synthesis is peptide deformylase (PDF). PDF binds to the ribosomal surface and removes the formyl group from the N terminus of the nascent protein as it emerges from the exit tunnel. Based on structural and sequence similarity, PDFs are divided into Type I, II, and III, with Type I being further divided into subgroups IA and IB. Type I PDFs feature a C-terminal -helix that serves as the connection point between the PDF's catalytic domain and the ribosome's surface. Conversely, Type II PDFs exhibit an intrinsically disordered C-terminal region and the mechanism by which Type II PDFs bind to the ribosome is unknown. Due to sequence divergence in otherwise conserved motifs of Type I and II, Type III PDFs are presumed to be inactive. Until recently, PDFs were thought to exist only in bacteria. However, eukaryotic PDF analogues have since been identified in plant and mammalian cells. Nevertheless, the role of PDF in mammalian cells remains unclear.

In our study, we investigate the folding behaviour of the C-terminal region by conducting all-atom molecular dynamics simulations of PDFs derived from various organisms. We selected five representatives of prokaryotic PDFs and two eukaryotic PDFs: a plastidial representative and an enzyme that was synthesized to resemble the human mitochondrial PDF. Our results suggest a high degree of similarity between bacterial and eukaryotic PDFs, particularly in classification, C-terminal flexibility, and structural resemblance.

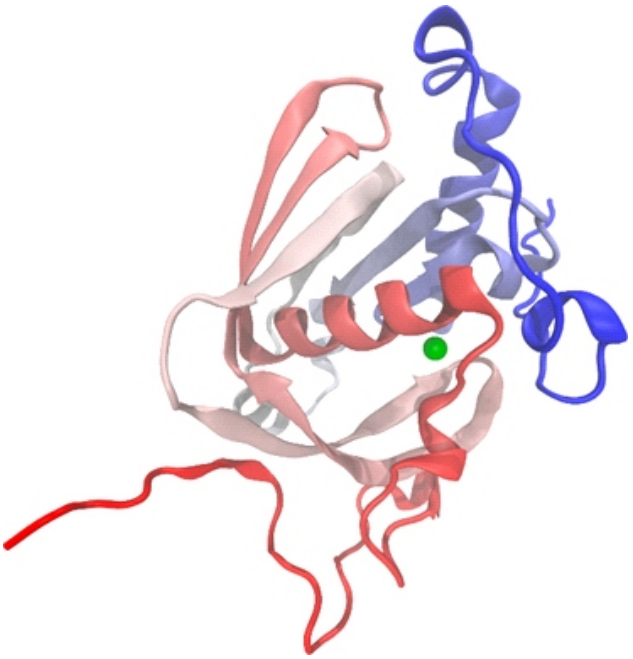

Figure 1. Human mitochondrial PDF analogue, 4JE6.

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P27

LEAKING WATER INTO A TEM: METHODS TO OVERCOME PREFERRED ORIENTATION IN CRYO-EM

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Preferred orientation is a frequently encountered problem in current single particle cryo-electron microscopy (cryo-EM) [1]. Proteins often adsorb to the air-water interface in a limited number of orientations, which subsequently results in sparsely populated or completely missing views during imaging in the electron microscope. Consequently, obtaining high resolution reconstructions may be difficult or impossible altogether [2].

Previously, we observed a reduction of preferred orientation after rapid laser flash melting and revitrification of samples containing highly symmetric and large particles [3-5]. Here, we expand this observation on varying systems, including T20S proteasome, 50S ribosomal subunit and HIV-1 Envelope protein.

Cryo-EM samples are locally flash-melted using a laser pulse, which exerts small forces on the protein particles, detaching them from the air-water interface and scrambling their orientations. When the laser is switched off, the sample rapidly revitrifies, trapping the particles in their newly adopted orientations [6, 7].

Our experiments show that the changes of angular distributions result from two competing processes. First, particles must be detached from the interface to even allow for their free rotation and hence adopt the new orientations. Simultaneously though, particles may diffuse back to the interface where they can once again settle into their preferred orientation.

An experiment involving *in-situ* deposition of amorphous ice onto the cryo-EM specimen prior to laser flash melting allows us to separate these processes [8]. We deposit 20 nm of amorphous ice by dosing water vapor into the specimen region inside a modified transmission electron microscope. When the sample is subsequently flash melted, particles find themselves not attached to the interface, but surrounded by liquid from all sides. Therefore, the resulting angular distribution reflects only the result of their free diffusion.

These experiments provide a set of tools to change and reduce preferred orientation of a wide range of systems, allowing for more reliable reconstructions as well as, in some cases, improvement of resolution. Importantly, the method does not require any extensive changes to the sample preparation and can be integrated into the existing workflows.

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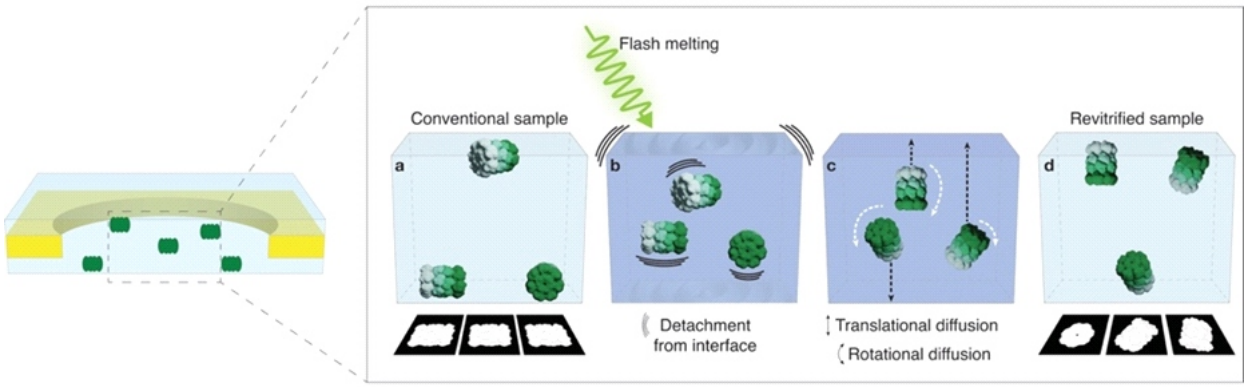

Figure 1. Mechanism of protein reorientation after laser flash melting and revitrification. T20S proteasome (PDB ID: 6BDF) is shown as an example. **(a)** In a conventional sample, particles adsorb to air-water interface, leading to limited viewing angles during imaging. **(b)** Laser flash melting detaches the particles from the interface and scrambles their orientation. **(c)** While the sample is kept liquid, particles are free to rotate and diffuse, adopting broader range of orientations. **(d)** Particles diffuse back to the interface over the time scale of our experiment, where they adopt new orientations. After revitrification, the particles are trapped in these orientations, resulting in more diverse viewing angles during imaging.

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P28

ENHANCING PETASE EFFICIENCY: A UNIFIED APPROACH TO PRODUCTION AND ACTIVITY ASSESSMENT

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The enzymatic degradation of polyethylene terephthalate (PET) has gained increasing attention as a promising strategy for plastic waste management. PETases, first discovered in *Ideonella sakaiensis*, have since been optimized through both natural evolution and protein engineering to enhance their efficiency and stability. However, comparing PETase variants across studies remains challenging due to inconsistencies in enzyme production, reaction conditions, and analytical methods.

To address this issue, we have developed a standardized protocol for PETase production, including expression, purification, and quality control steps. Building upon this standardized production protocol, we have systematically benchmarked the activity of five PETase variants with different thermal stabilities under a range of experimental conditions. Our study evaluates key factors such as enzyme concentration, reaction conditions, and analytical methodologies to establish a robust framework for assessing PET-degrading enzymes. Additionally, we employed deep learning-based tools trained on hyperthermophilic proteins to design a novel thermostable PETase variant. This AI-generated enzyme is currently undergoing experimental validation to determine its catalytic efficiency and stability compared to existing PETases.

By combining standardized production methods, systematic benchmarking, and AI-driven enzyme design, our work provides a comprehensive strategy to advance plastic degradation technologies. This approach lays the foundation for accelerating research in enzymatic PET degradation and its applications in plastic waste management.

P29

TARGETING 3Cpro: A NOVEL STRATEGY FOR EFFECTIVE ENTEROVIRUS D68 INHIBITION

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Enterovirus D68 (EV-D68), a member of the *Picornaviridae* family, has emerged as a significant pathogen associated with severe respiratory illnesses and neurological complications, particularly in children. Notably, EV-D68 has been linked to acute flaccid myelitis (AFM), a condition affecting the gray matter of the spinal cord and resulting in polio-like neurological symptoms such as muscle weakness and paralysis. A critical component of the EV-D68 life cycle is the 3C protease (3Cpro), which processes the viral polyprotein into functional units essential for viral replication and maturation [1, 2], making it an attractive target for antiviral drug development. Both covalent and non-covalent inhibitors of 3Cpro have shown promise in preclinical studies, though challenges related to resistance, specificity, and drug delivery remain [3, 4].

In this study, we present the high-resolution crystal structure of the EV-D68 3Cpro in complex with a novel inhibitor, RHCDS1a, resolved at 1.81 Å resolution. The gene encoding 3Cpro was cloned, overexpressed in *E. coli*, and purified using affinity and size-exclusion chromatography to obtain a stable and highly pure protein sample. Biochemical characterization revealed the inhibitory efficacy of RHCDS1a, with an IC_{50} of 550 ± 43 nM as determined by fluorometric protease inhibition assays. Surface plasmon resonance (SPR) analysis confirmed the strong binding af-

finity between RHCDS1a and 3Cpro, with a dissociation constant (K_D) of 766 ± 2 nM.

Structural analysis of the 3Cpro-RHCDS1a complex revealed that the inhibitor binds to the active site of 3Cpro, interacting with key residues within the catalytic triad (Cys147, His40, and Glu71) and the substrate-binding pocket. These interactions effectively disrupt protease activity. The inhibitor's imidazole ring exhibits two alternative conformations, suggesting opportunities for further optimization to enhance its potency and selectivity.

Our findings provide a detailed structural basis for the development of RHCDS1a as a therapeutic candidate against EV-D68. The insights gained from this study offer valuable guidance for the rational design of next-generation antivirals targeting 3Cpro, highlighting the importance of structure-based drug design in combating emerging viral pathogens.

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P30

STRUCTURAL CHARACTERIZATION OF THE INTERACTION BETWEEN BRCA1-BARD1 AND RNA POLYMERASE II

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Transcription competes with other DNA-dependent processes, such as DNA repair and replication, for access to its substrate, DNA. However, the principles governing the interplay between these processes remain poorly understood. Evidence suggests that the BRCA1-BARD1 complex, a key player in the DNA damage response, may act as a mediator of this crosstalk. The BRCA1-BARD1 complex is involved in multiple aspects of double-strand break (DSB) repair, protection of stalled replication forks, and the prevention of transcription-replication conflicts. Notably, BRCA1-BARD1 has been reported to interact with RNA polymerase II (RNAPII), yet the functional significance of this interaction remains unclear.

In our study, we investigated the molecular mechanism of the interaction between RNA polymerase II (RNAPII) and the BRCA1-BARD1 complex, as well as its functional consequence. Our data suggest BRCA1-BARD1 directly interacts with RNAPII through the binding of its BRCT domains to the phosphorylated C-terminal domain (CTD) of RNAPII. Moreover, we show that this interaction is critical for the organization of RNAPII into condensates with liquid-like properties. Analysis of disease-associated variants within the BRCT repeats further supports the biological relevance of this condensation process.

Collectively, these findings suggest that BRCA1-BARD1 may function as a molecular bridge between transcription and DNA repair pathways, facilitating crosstalk between these processes at sites of DNA damage and transcription-replication conflicts. The formation of liquid-like condensates may represent the underlying mechanism through which BRCA1-BARD1 mediates this crucial role.

P31

USING MASS PHOTOMETRY FOR SORTING AND SELECTION OF NATIVELY PURIFIED PROTEIN COMPLEXES

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Mass photometry (MP) is a relatively new biophysical technique based on the principles of the interference reflection microscopy. It measures the interferometric scattering signal of individual particles (macromolecules). It is perfectly suited for the direct molecular weight measurement of biological macromolecules in the solution without any labeling or prior immobilization in a wide interval of masses (from ~ 50 kDa to 5 MDa). The measurement itself is relatively inexpensive, fast and user-friendly. The main applications of MP are 1. Testing of samples for purity and monodispersity. 2. Analysis of oligomerization and formation of complexes. 3. Quality and stability control (e.g. aggregation in the sample over time) [1]. MP can also be used for the analysis of biomolecular interactions including the estimation of K_D, but these applications require special conditions and more considerations [2].

In our studies of the bacterial transcription machinery, we often isolate complexes of RNA polymerase (RNAP) directly from the native source (e.g. *B. subtilis*). Naturally, such samples contain complexes of RNAP with various transcription factors and also nucleic acids. Based on the growth conditions and processing procedures, we can to some extent control the enrichment of the sample by the complexes of our interest, nevertheless, these solutions contain several different "species" of RNAP even after multi-step purification, including size exclusion chromatography. We have found that the analysis of the post-purification fractions using MP helps us to select fractions with the highest possible uniformity and thus prepare the most suitable samples for follow-up structure-function and biophysical analyses.

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P32

STRUCTURAL BASES OF "COPY-OUT-PASTE-IN" TRANSPOSITION

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The copy-out-paste-in transposition mechanism is a major replicative pathway used by many prokaryotic transposable elements. In contrast to the classical replicative mechanism involving Shapiro intermediate and a resolution step, this pathway is characterized by the formation of a single-stranded circular DNA intermediate as a product of a strand-transfer reaction between terminal inverted repeats (TIRs) of the transposon as a very first step. The circular DNA thus carries a junction of TIRs with a few base pairs long linker originating from a transposon flanking sequence. The DNA circle is then repaired by host factors and integrated in a targeted or random manner as a second step. Both steps depend on a poorly characterized transposase featuring a DDE type of catalytic domain with a high potential for mobilization of various genetic elements including antibiotic resistance genes and different promoters. We determined X-ray structures of a dimeric transposase *from ISCth4* transposon from *C. thermocellum* in a complex with DNA intermediates in the copy-out- paste-in pathway. The structures show how the transposase recognizes its TIRs in a bipartite manner and suggest conformational changes that control the position of DNA flanking sequences in the active site.

P33

PROTEIN PRODUCTION IN MAMMALIAN CELLS VS *E. COLI* – IMPACT ON Tau PROTEIN PROPERTIES

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Hyperphosphorylated and abnormally post-translationally modified Tau protein forms aggregates called neurofibrillary tangles (NFTs), which are typical hallmark of Alzheimer's disease (AD) in the human brain [1]. As the pattern of post-translational modifications (PTMs) within Tau is complex [2], AD-relevant Tau protein is not easily obtainable by recombinant production in *E. coli* and subsequent modification by individual enzymes *in vitro*. To prepare Tau protein with naturally occurring PTMs, we employed mammalian cell culture (HEK293). We aimed to identify the PTMs incorporated in the HEK293 cells, to compare them with published data from AD-patients and afterwards to study the impact of the PTMs on Tau properties in comparison to Tau from *E. coli*.

We optimized the expression and purification protocols yielding sufficient amount of HEK-Tau for its characterization. Using LC-MS/MS, we identified around 20 phosphorylation sites with diverse extent of phosphorylation. The detected phosphorylation patterns were similar to those found in the brains of AD-patients. Afterwards, we performed interaction study between HEK-Tau and 14-3-3 protein, which provided surprising result compared to Tau expressed in *E. coli* and phosphorylated *in vitro* [3]. Finally, we used Thioflavin T assay and negative stain EM to obtain insight into aggregation propensities of HEK-Tau.

The protein production in HEK293 cells was performed within an Industrial PhD programme in the biotech company BioVendor.

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P34

STRUCTURAL CHARACTERISATION OF PROTEINS INVOLVED IN THE METABOLISM OF R-LOOPS

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During transcription, RNA polymerases (RNAP) may pause, creating obstacles on the DNA, which increase the risk of collision with other DNA-centred processes such as DNA replication. If these conflicts are left unresolved, they may result in replication fork stalling and subsequent double-strand DNA breaks. Pausing of RNAP may also lead to the formation of R-loops – tripartite nucleic acid structures in which nascent RNA hybridises with its complementary DNA strand, leaving the non-template strand unpaired. Persistent R-loops may be responsible for DNA damage due to the exposure of fragile single-strand DNA.

Helicase senataxin (SETX) is a member of the SF1B-family of helicases, which, in contrast to its yeast counterpart – Sen1 – translocates preferentially on RNA [1]. Fittingly, SETX's preferred substrate is an R-loop [1]. Additionally, SETX terminates transcription in a species-specific manner [1], suggesting that specific, hitherto unknown, sequence features have evolved to accommodate

this feature. These properties of SETX place it as a transcription termination factor specialised in R-loop metabolism and control, which is essential for the resolution of transcription-replication collisions, crucial for the maintenance of genome stability. Since the fundamental functional differences between SETX and Sen1 are likely based on distinct structural features and no experimental structural data is yet available on SETX, we resort to methods of structural biology to mechanistically investigate the functions of SETX.

In our poster, we present our latest results, which bring first insights into the molecular mechanism(s) behind the functions of SETX.

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P35

STRUCTURAL INSIGHTS INTO FATTY ACYL DESATURASES AND REDUCTASES IN INSECT PHEROMONE BIOSYNTHESIS

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Fatty acyl desaturases (FADs) and reductases (FARs) are key enzymes in insect pheromone biosynthesis, catalyzing the modification of fatty acyl-CoAs into species-specific pheromone precursors or components. The specificity of these enzymes is determined by structural features within the substrate cavity, which influence substrate binding and catalysis. Understanding these structural features is essential for elucidating the molecular basis of pheromone divergence, and for engineering enzymes with tailored specificities.

To identify residues modulating enzyme specificity, we use AlphaFold 2 and 3 to predict FAD and FAR models. Such models facilitate mutant design, enabling us to probe the functional role of specific residues in substrate recognition and regiospecificity. Engineered enzymes are expressed in yeast, followed by lipid analysis using gas chromatography. This approach has revealed critical residues that influence chain-length specificity and regiospecificity without impairing catalytic activity.

Using structure prediction, we can efficiently identify candidates with selected specific characteristics. Our findings provide a framework for accelerating specificity prediction and engineering enzymes to tailor product properties. This approach has broad applications in biotechnology, e.g., pheromone-based pest control.

P36

STRUCTURE OF Tau FILAMENT CORE FRAGMENT dGAE WITH MN423 ANTIBODY

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Intrinsically disordered proteins, tau protein being our focus, lack stable tertiary structure, but form a conformational ensemble of molecular states exhibiting dynamic and complex network of intramolecular interactions. In a group of human diseases called tauopathies tau protein aggregates and propagates the pathology. To uncover specific conformation of tau aggregates, we performed X-ray crystallography investigation of the tau filament core fragment dGAE (tau297-391) complexed with filament conformation specific antibody MN423.

Tau fragment and antibody Fab were prepared by recombinant expression in *E.coli* and CHO cell line, respectively, purified to homogeneity and the complex was isolated by size exclusion chromatography of the mixture of both proteins. Crystals of complex were obtained from hanging drops, fished out with nylon loop and flash-cooled in liquid nitrogen. Diffraction data were collected at beamline PXI (SLS, PSI, Villigen, Switzerland). Diffractions were indexed in *P*1, integrated and scaled in XDS package and data were merged in TRUNCATE [1-3]. Diffractions were indexed in *P*1, integrated and scaled in XDS package and data were merged in TRUNCATE [1-3].

Molecular replacement was employed to solve the phase problem using the structure of MN423 Fab as a model (PDB ID 2v17). Asymmetric unit of crystal contained six molecules of Fab; we used variable and constant domain of antibody Fab separately as models to overcome the possible difference in elbow angles in the model molecule. Using REFMAC/COOT for macromolecular refinement we further refined the structure. Despite the

satisfactory fit of the model into electron density, we are not able to lower substantially R-factors (Figure 1).

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This work was supported by the H2020-MSCA-RISE-2019 Grant number 873127. This work was also funded by the grants APVV 21-0479, Vega 2/0125/23 and 2/0141/23.

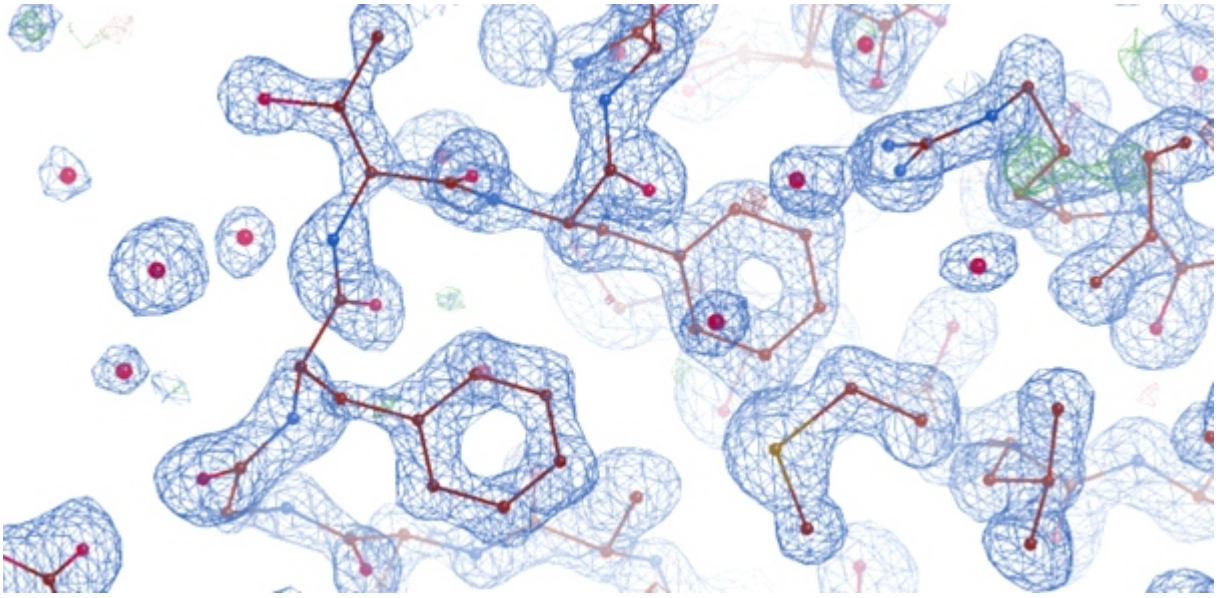

Figure 1. Modelled structure fit to electron density. 2Fo-Fc electron density map (blue) is contoured at 1,5 sigma.

P37

DISCOVERING STRUCTURAL SECRETS OF A JACALIN-RELATED LECTIN: A FUN GUY AMONG THE FUNGI

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This study focuses on the investigation of the properties and structural analysis of the lectin, a saccharide binding protein, derived from the mushroom *Calocera viscosa* (CalVL). Mushroom lectins have been extensively studied over the decades for their potential applications in biomedicine and diagnostics. Through their glycan-binding abilities, these lectins demonstrate significant biological activities, including antiproliferative, antimicrobial and mitogenic effects [1].

Lectin CalVL was determined to adopt a -prism I fold, a structural characteristic shared by all the members of the jacalin-related lectins (JRLs) family. JRLs are typically divided into two groups based on their saccharide preference: gJRLs and mJRLs, preferring D-galactose and D-mannose, respectively [2].

CalVL was produced in the *Escherichia coli* expression system and subsequently purified through affinity chromatography using a mannose-agarose resin. Agglutination assays demonstrated that CalVL can agglutinate both yeast cells and human erythrocytes due to the interaction with their surface saccharides. Further analysis of binding properties by a glycan array resulted in narrow range of biantennary complex *N*-glycans which are likely its optimal binding partner. CalVL was successfully crystallized

in various conditions using vapour diffusion method, particularly the sitting drop technique. X-ray diffraction data were collected at the synchrotrons PETRA III in Hamburg and BESSY II in Berlin, Germany. Preliminary structures of CalVL in apoform, with D-mannose and *N*-acetylglucosamine are nearing the completion of the refinement process. The phase problem was solved through molecular replacement, employing a CalVL model predicted by AlphaFold2. To the best of our knowledge, CalVL represents the first structurally characterized fungal JRL among the animal and plant members of this family.

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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 measurements at the CF Biomolecular Interactions and Crystallography.

P38

STRUCTURES OF THE GCPII AND PSMA-617 AND ITS DERIVATIVES WITH MODIFIED LIPOPHILIC LINKER REGIONS COMPLEXES

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PSMA-617 is widely recognized as a benchmark ligand for prostate-specific membrane antigen (PSMA) due to its extensive use in prostate cancer (PCa) targeted radionuclide therapy. This poster explores the structure of PSMA-617 alongside two novel analogs with modified linker regions. In compounds P17 and P18, the 2-naphthyl-L-Ala moiety was substituted with a less lipophilic 3-styryl-L-Ala moiety, while P18 also features a phenyl group in place of the cyclohexyl ring. The first-ever crystal structure of the PSMA/PSMA-617 complex reported here revealed a folded conformation of the PSMA-617 linker. In contrast, the PSMA/P17 and PSMA/P18 complexes exhibited extended linker orientations, demonstrating linker flexibility within the PSMA cavity an insight that can be leveraged for structure-guided design of PSMA-targeting agents. Despite their structural modifications, the analogs retained strong PSMA inhibition potency, cellular binding, and internalization. These findings, together with the structure–activity relationships and *in vivo* biodistribution studies discussed in the manuscript under review, provide a strategic framework for the rational design of PSMA ligands, paving the way for the development of next-generation theranostic agents for PCa.

P39

ROLE OF NON-CANONICAL NUCLEOTIDES IN PROTORIBOSOME

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Our group has a long-standing interest in the molecular details of protein synthesis. This key cellular process occurs within large protein-RNA complexes called ribosomes. The part of the ribosome that surrounds the peptidyl transferase center (PTC), which is responsible for peptide bond formation, is known as the protoribosome [1]. Many of the details about the role of non-canonical nucleotides in the protoribosome remain unknown. Molecular dynamics simulations, using the software GROMACS were used to study differences in the behaviour of protoribosome with non-canonical bases and protoribosome with non-canonical bases replaced by canonical ones. This work may shed

some light on the reasons why non-canonical bases remain in the ribosome and are not replaced by canonical bases.

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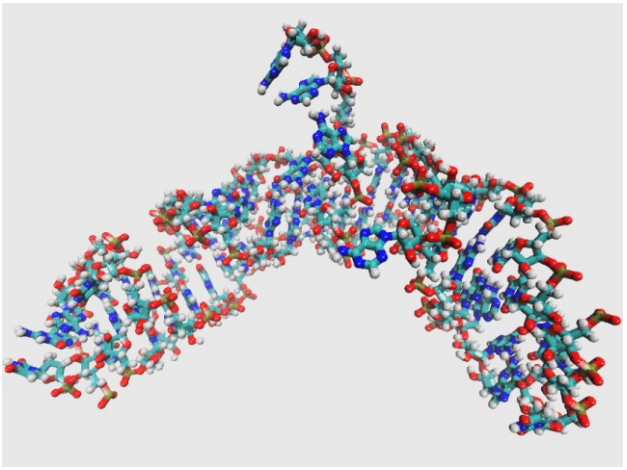

Figure 1. Model of protoribosome

P40

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

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The Centre of Molecular Structure at IBT CAS (BIOCEV, Vestec, Czech Republic) is a sophisticated completels with comprehensive imaging capabilities.

A notable technological advancement 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 software environment of crystallization hotels is currently undergoing a major upgrade to ensure long-term sustainability and autox of scientific core facilities specializing in structural biology. Its crystallization core facility uses among other equipment also automated robotic systems for high-throughput crystallization experiments and specialized crystallization homated scoring using multiple evaluation algorithms.

The facility's equipment represents cutting-edge technology in structural biology research, facilitating complex crystallization experiments and detailed structural analysis of biomacromolecular complexes.

P41

VALIDATION OF SMALL STRUCTURE MOTIFS IN DISORDERED PROTEINS BY MOLECULAR DYNAMICS SIMULATIONS

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Local structure motifs in the shape of monocyclic or polycyclic, low-membered rings stabilized by main chain-main chain or main chain-side chain hydrogen bonds are frequent in globular proteins. They often contribute to the formation of helical or flat ribbon structures. It has been proposed that these ring structures may exist at least transiently also in intrinsically disordered proteins (IDPs) intimately imprinting their conformational ensemble [1]. Our hypothesis is that these motifs are determinants of physiological and pathophysiological properties of IDPs. The implication of these motifs for IDP tau is in the process of amyloid formation during tau aggregation. Amyloids are characterized by highly ordered cross- structures and we suppose the role of these motifs in regulation of misfolding and aggregation. Interestingly, several motifs can be identified on existing crystal structures of tau protein complexes [1, 2]. To investigate the effect of these motifs on tau protein, we can use site-directed mutagenesis following the changes of tau aggregation *in vitro* and alteration of affinity to tau interaction partners. Another tool for characteriza-

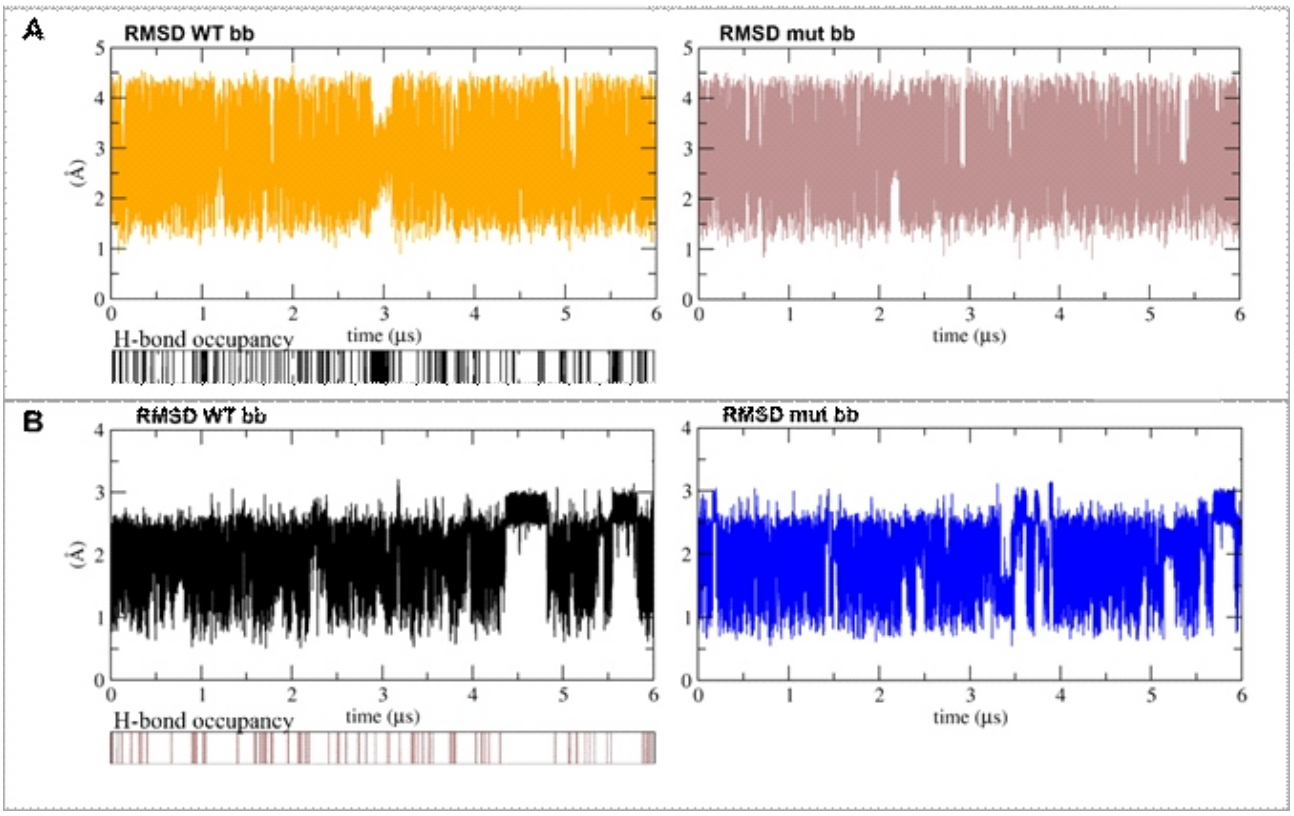

Figure 1. Molecular dynamics of tau peptides. (A) 298KHVPGGGSVQIVYK311, (B) 382AKAKTDHGAEIVY394. Both peptides were simulated as WT (left) and with mutated residue creating small motif in WT (right) revealing the influence of small motif for the conformational ensemble of disordered peptide. RMSD analysis were performed against backbone atoms of X-ray structures involving the peptide – 5MO3 in (A) and 2V17 in (B).

tion of these small structural motifs are molecular dynamics methods (MD).

We used MD to observe the formation of the motifs on tau fragments in solution – without the constraint of interaction partners as for the crystal structures. We simulated three fragments encompassing following two motifs identified on tau protein: *-turn* formed by interaction of main-chain carbonyl of Gly302 with main-chain amide of Ser305 in the peptide tau298-311 and *Asx-turn* formed by interaction of side chain carboxyl of Asp387 with main chain amide of Gly389 in two peptides – tau381-391 and tau382-394. We validated the existence of the motifs based on H-bond analysis. We also compared the effect of these motifs on conformation ensemble by mutation of Ser305Ala for the first peptide and Asp387Ala for the second and third peptide. The -turn induced formation of metastable populations that were stabilized by the motifs and in accordance to low radius of gyration values as these conformations were at least partially folded. For Asx-turn and 382-394 fragment, both WT and mut fragments adopted hairpin conformation stabilized by salt bridges. Interestingly, even though the Asx-turn was not highly populated and no major effect was observed on formation of metastable populations, the hairpin conformation was shifted towards C-terminus for the mutant fragment. Our next steps consist of *in-vitro* part – to biophysically characterize these short peptides and then to assess the effect of mutations of selected motifs on aggregation of tau filament core fragment tau297-391 (dGAE).

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P42

LASER-DRIVEN PLASMA X-RAY SOURCE AT ELI BEAMLINES

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ELI Beamlines

We report on experimentally measured characteristics of a kHz laser-driven Cu plasma X-ray source that was recently commissioned at ELI Beamlines facility. The source is powered by a 50-fs TW laser, producing X-ray pulses that enable sub-ps resolution for time-resolved experiments. The X-ray source parameters with the two driving lasers are compared, providing photon flux up to the order of 10^{13} photons/4 /s. Besides the X-ray beam characteristics, the experimental platforms for ultrafast X-ray diffraction and X-ray absorption and emission spectroscopy are described to outline the possible application experiments, as the system will be operated in a user-based access mode.

P43

PhiKZ BASEPLATE STRUCTURE

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PhiKZ is a bacteriophage that infects *Pseudomonas aeruginosa*, an opportunistic human pathogen. The phage phiKZ is known for its large genome and complex structure, making it a notable subject for structural biology studies. Among all its parts, the baseplate is the most complex. It adheres to the phage prey and triggers the genome ejection. The signal that triggers the ejection travels from the tail fibres to the tail through the baseplate. Here, we used cryo-Electron Microscopy to visualise the structure of the phiKZ tail and the baseplate at high resolution, the first one of a jumbo bacteriophage. Our results reveal an intricate network of proteins organised in six-fold symmetry. Structural comparisons with related systems highlight the universal conservation observed in contractile injection systems. This sheds light on phiKZ's baseplate specificities and suggests a potential mode of action. Its structural analysis enhances our understanding of phiKZ and contributes with valuable knowledge to the broader field of myovirus biology.

P44

IMPROVED VALIDATION AND REFINEMENT OF BIOMOLECULAR STRUCTURES

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We present a further development of datasets and methods available in our laboratory for improved validation and refinement of biomolecular structures. We are building on synergies of data and tools involving structural alphabets [1-3] and flexibility of biomolecules [4,5].

The conformation- and location-dependent flexibility and solvation behavior derived from high quality non-redundant biomolecular structural data was employed for improvement of protocols for validation and refinement of biomolecular structures.

We extracted normalized B factor values of protein residues derived from high resolution structural data and collected distributions for protein residues in the interior and residues at the surface, either not found in contacts or involved in direct or water mediated contacts with biomolecular binding partners.

For structure validation we compared how well the B factor distribution in a model follows the corresponding reference distribution. The expected reference distribution from high-resolution structures can be also used prior to structure refinement replacing the currently used uniform B factor values. This can improve the convergence as well as accuracy of the structure refinement.

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P45

FUNCTIONAL CHARACTERISATION OF THE LUMINOUS APPARATUS OF THE SEA PEN *PENNATULA PHOSPHOREA*

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Bioluminescence is the production of visible light by living organisms. It occurs through the oxidation of specific luciferin substrates catalysed by luciferase enzymes. Auxiliary proteins, such as fluorescent proteins and coelenterazine-binding proteins, can modulate the wavelength of emitted light or stabilise reactive luciferin molecules. Bioluminescent organisms offer a variety of light-emitting enzymes and photoproteins with immense potential for bioengineering applications, from biosensors to zero-electricity lighting solutions. However, despite the abundance of marine bioluminescent species, only a few systems have been biochemically and structurally characterised. Among anthozoans, the *Renilla*-type bioluminescence is the most studied [1,2], relying on a coelenterazine -dependent luciferase, a calcium-dependent coelenterazine- binding protein, and a green fluorescent protein.

Recently, transcriptomic analyses identified putative luciferase and auxiliary protein sequences responsible for bioluminescence in the sea pen *Pennatula phosphorea* [3]. Here, we perform biochemical reconstitution and functional characterisation of the *Pennatula*-encoded luciferase, providing insight into its light-emitting properties.

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P46

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

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

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

The Centre of Molecular Structure is supported by: MEYS CR (LM2023042); project Czech Infrastructure for Integrative Structural Biology for Human Health (CZ.02.1.01/0.0/0.0/16_013/0001776) from the ERDF; UP CIISB (CZ.02.1.01/0.0/0.0/18_046/0015974), ELIBIO (CZ.02.1.01/0.0/0.0/15_003/0000447), and MOSBRI from EU Horizon 2020 (No. 101004806).

P47

MAVISp: A MODULAR STRUCTURE-BASED FRAMEWORK FOR PROTEIN VARIANT EFFECTS

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The role of genomic variants in diseases, including cancer, continues to expand thanks to the advent of advanced sequencing techniques integrated into clinical practice [1]. The rapid growth in the identification of genomic variants has led to the classification of many variants as Variants of Uncertain Significance (VUS) or those with conflicting evidence, posing challenges in their interpretation and application. Additionally, current methods for predicting pathogenic variants do not necessarily provide information on the mechanisms underlying pathogenicity [2-4]. MAVISp (Multi-layered Assessment of VarIants by Structure for proteins), a modular structural framework for variant effects, to handle high-throughput saturation variant analysis with a standardised workflow, integrating results with various pathogenicity predictors [5]. Currently, MAVISp offers analyses for 500 different proteins, encompassing more than 3 million variants. The framework facilitates the analysis of variant effects at the protein level and has the potential to advance the understanding and application of mutational data in disease research. In this context, we propose an additional module based on an Adversarial Autoencoder [6] to enhance the systematic understanding of mutational effects and to establish a foundation for further investigations that remain unexplored, thereby broadening the scope of future research in this domain. The application was carried out in the case study of p53.

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P48

CRYSTAL STRUCTURE OF HUMAN NK CELL ACTIVATION RECEPTOR NKp80

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Natural Killer (NK) cells, a subset of effector lymphocytes, can spontaneously destroy target cells, such as infected, damaged, or malignant cells. NK cell cytotoxicity is mediated by activating receptors on their surface, such as NKp80 (Natural Killer protein 80 kDa) [1]. The human receptor NKp80 stimulates cytotoxicity through its interaction with its ligand AICL (Activation Induced C-type Lectin), which is constitutively expressed by all myeloid cells. In pathological conditions, such as cancerous or damaged cells, AICL is often upregulated, resulting in the lysis of these cells by NK cells expressing NKp80 [2]. This interaction is thus a promising immunotherapeutic target for treating myeloid leukaemia.

However, the structures of both proteins have remained elusive. Hence, we have focused on successfully producing the extracellular domain of NKp80 in sufficient quality and quantity. Here, we introduced a series of mutations in the stalk region to study their effect on the production, stability, and homodimer formation. Using stably transfected HEK293S GnTI$^-$ cells, we produced in total seven variants of NKp80, replacing cysteines with serines, and the proteins were analysed using techniques such as size-exclusion chromatography, differential scanning fluorimetry, and mass spectrometry.

Our findings demonstrate a substantial improvement in the production yield for six of the seven NKp80 mutants, with some variants exhibiting up to a fivefold increase compared to the wild-type NKp80 extracellular domain. Consequently, we have selected the two most successful variants for large-scale production to enable crystallization trials. The trials resulted in the elucidation of the hitherto unknown structure of NKp80 homodimer at a resolution of 2.9 Å. The NKp80 homodimer adopts an overall conformation similar to that of the homodimer of human NKR-P1 [3], with helices 1 forming the dimerisation interface. The stalk region included in the expression construct was not observed in the crystal structure; in fact, the only NKp80 construct/preparation to crystallize was the one that showed limited proteolysis upon enzymatic deglycosylation resulting in a loss of most of the stalk region, as confirmed by N-terminal protein sequencing. Thus, the NKp80 stalk is probably unstructured or very flexible, at least when the extracellular part of NKp80 is produced in a soluble form and is not tethered to the transmembrane part and the rest of the whole receptor.

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NMR STRUCTURAL AND INTERACTIONAL CHARACTERIZATION OF HUMAN Tau PROTEIN

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Tau protein is a microtubule-associated protein localized and expressed in human brain cells, predominantly in axons of neurons. The function of this protein is imperative for correct axonal microtubule formation and function. Tau protein has been found to form aggregates and filamentous structures in the brains of patients suffering from Alzheimer's disease or other neurodegenerative diseases. However, the exact molecular mechanism of neurodegeneration remains unknown. Tau protein belongs to the group of intrinsically disordered proteins, which makes NMR a valuable tool in analysis of its structural properties and interactions [1, 2].

In this project, several advanced NMR techniques were utilized to study the properties of Tau protein. Firstly, temperature effects on secondary structure propensities of full-length tau protein (tau isoform 2N4R) were studied. Secondly, we studied the effect of phosphorylation by GSK-3 beta kinase on the interaction of Tau protein with microtubules. Additionally, the expression of Tau protein in E. coli cells was carried out, with the intention to optimise the expression protocol for effective incorporation of fluorinated amino acids, with further goals to use this construct for in-cell NMR measurements.

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STRUCTURE AND DYNAMIC PROPERTIES OF PORPHYRIN AGGREGATES IN SOLUTION

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Multiple factors influence the aggregation behavior of porphyrins in solution; however, obtaining detailed structural and dynamic insights remains challenging. Motivated by intriguing yet puzzling experimental data from UV/VIS and NMR spectroscopies, we investigated the aggregation of 3,4,5-TEG-TPP porphyrins. We analyzed the stability and dynamic properties of these molecules in solution using various computational methods. We developed detailed models of solvated porphyrin monomers, dimers, and larger aggregates, which underwent relaxation before being propagated through molecular dynamics simulations. From the sampled trajectories, we computed optical and magnetic spectra and compared them with experimental results. This approach provides valuable insights into the mechanisms of porphyrin aggregation in aqueous envi-

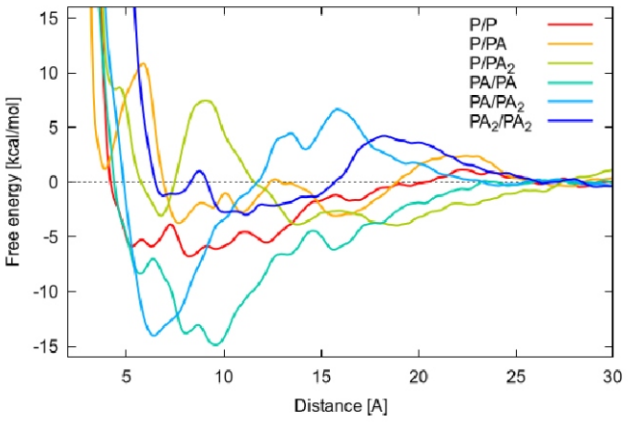

Figure 1. Free energy profile of dimers with different charge.

ronments and enhances our understanding of their structural stability.

Computational resources were provided by the e-INFRA CZ project (ID:90254), supported by the Ministry of Education, Youth and Sports of the Czech Republic.

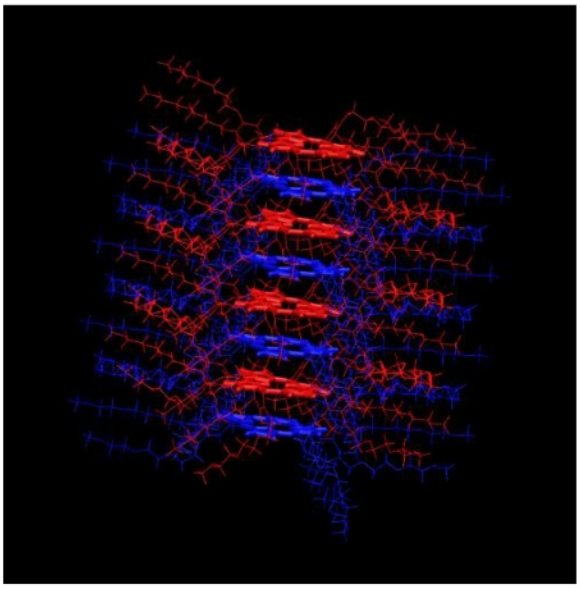

Figure 2. Model of aggregate.

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A pY READER OF ARID1A FACILITATES SIGNAL-DEPENDENT cBAF ACTIVITY

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ARID1A, a key component of the cBAF chromatin-remodelling complex, is essential for maintaining cBAF functionality and structural integrity [1].

Here, we reveal that specific phosphotyrosine (pY) residues within the intrinsically disordered region (IDR) of ARID1A play a pivotal role in modulating its function and localization.

Using a combination of NMR spectroscopy, mass spectrometry and confocal fluorescence microscopy, we identify a novel pY reader specific to these phosphotyrosines, which interacts with ARID1A in a phosphorylation-dependent manner. Importantly, phosphorylation of these tyrosine residues alters the cellular distribution of the pY reader, relocalizing it to specialized nuclear compartments. This regulatory mechanism underscores a phosphorylation-sensitive interaction network, potentially controlling ARID1A's recruitment and activity within chromatin remodelling processes. These findings highlight a new layer of regulatory complexity in cBAF-mediated chromatin dynamics and suggest a broader role for post-translational modifications in directing subnuclear compartmentalization.

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