Posters

P1

DESIGN AND EVALUATION OF RNA-DEPENDENT RNA POLYMERASE INHIBITORS OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2

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Quick spread of Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has struck the world out of the blue. At the beginning of this global pandemic, there was lack of reliable therapeutics and to this day, national authorities still cannot fully agree on the best approach. One of the possible approaches is to inhibit proteins involved in SARS-CoV-2 life cycle, mainly its key enzyme RNA-dependent RNA polymerase (RdRp). Therefore, we decided to develop new inhibitors of SARS-CoV-2 RdRp. Here we present a project, where we docked a large library of small molecules obtained from Maybridge database into several binding pockets of RdRp. Moreover, we managed to optimize method for production, isolation, and purification of recombinant wild-type RdRp, as well as its mutated forms, which were tailored based on docking studies. Furthermore, we plan to (i) evaluate inhibitory activity of candidate inhibitors on RdRp using quantitative polymerase chain reaction-based assay. (ii) Also, we plan to study conformational changes induction in RdRp structure by these inhibitors using hydrogen-deuterium exchange, and (iii) to evaluate their cytotoxicity in various mammalian cells. We believe that this project will give us much needed insight into how binding of small molecules changes the conformation of RdRp and its enzymatic activity and that it will help us to combat this virus.

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P2

STRUCTURAL VARIABILITY OF BASE PAIRS IN DNA

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Base pairing is an important feature of nucleic acid structure with fundamental implication for the flow of the genetic code. Base pairing is realized via distinct hydrogen bond edges and results in canonical and non-canonical geometries as described in Leontis-Westhof nomenclature [1]. We decided to analyze geometries of "mismatched" DNA base pairs, i.e. base pairs in non-Watson-Crick topologies by solving of crystal structures with mismatched pairs in 18-mer DNA duplexes. Previously crystallized 18-mer DNA oligonucleotide contained double thymine mismatch in the central positions [2], now we obtained nine additional variants covering both canonical and non-canonical base pairing. The refinement and validation stages of solving all our structures benefited from using the NtC dinucleotide classes (https://dnatco.datmos.org/) [3]. Firstly, we used NtCs as refinement targets and secondly for annotation of solved structures. We present a comprehensive structural annotation of the base pairs in the ten solved 18-mer duplexes.

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P3

IMMUNOPRECIPITATION PULL-DOWN ASSAY REVEALED BINDING BETWEEN HUMAN CD160 AND VIRAL UL144

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Human cytomegalovirus (HCMV) is a linear double-stranded DNA -herpesvirus controlled by a vigorous immune response so that infections are asymptomatic or symptoms are mild. If the immune system is compromised, HCMV can replicate to high levels and cause serious end organ disease [1]. On the other side, the immune receptors are in constant arm race with the viral proteins that specifically target them thus manipulate a wide range of immune responses. Therefore, accurately predicting their molecular interactions will be necessary for the development of safe and effective therapeutics to enhance immune responses and vaccination.

HCMV within its unique long (UL)/b' locus, encodes the key immunomodulatory proteins [2], such as UL144 which is highly orthologous to tumour necrosis factor receptor HVEM (TNFR/SF14, herpesvirus entry mediator) thus resembling some of its promiscuity on the cell surface. HVEM binds the TNF ligands, LIGHT and LT ; the immunoglobulin inhibitory receptor, B and T lymphocyte attenuator (BTLA); and the natural killer cell–activating receptor CD160. However, the initial studies shown that UL144 selectively binds only BTLA [3], while avoiding activation of inflammatory signalling initiated by CD160 in natural killer (NK) cells. This molecular network is quite well described, however, the engagement of CD160 by UL144 has not yet been satisfactorily studied. One study reports the viral glycoprotein UL144 from rhesus CMV can bind human and rhesus CD160 with low affinity [4]. This interaction likely represents a divergence between viral species as primate BTLA and CD160 are highly homologous. Moreover, wild-type (wt) UL144 maintains many glycosylation sites which are not present in other viral species thus suggest a direct involvement of N-glycans in receptor recognition on immune cell. Here, we focused on the characterization of CD160 binding to viral UL144, whose mutual engagement could lead to disrupted signalling under various glycosylation condition. We accessed the binding by co-immunoprecipitation assay (co-PI) using recombinant his-tagged proteins (dgUL144, rhUL144-wt, UL144-wt, UL144-N91) and CD160 fused to Fc. We observed binding between all UL144-glycosylation deficient variants to CD160. Moreover, considering that the ectodomain of UL144 is highly polymorphic across primate CMV we examined both human and rhesus CMV UL144 selectivity for its cellular receptor thus the binding of rhesus CMV UL144 to human CD160 was also observed. However, to fully understand the molecular basis of these interactions, further analyses have to be performed.

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P4

CRITICAL INTERACTIONS OF NEURONAL TRANSCRIPTION FACTOR REST WITH STABILIZER TRF2

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Glioblastoma is the most common and malignant brain tumor in adults. Glioblastoma is highly resistant to chemotherapy and radiotherapy. So far, there has been no successful treatment. Recent studies revealed a strong correlation between glioblastoma tumorigenicity and the aberrant expression of REST, the main repressor of neural stem cell differentiation [1]. The fate of the REST inside cells is mainly regulated by ubiquitylation. The primary protecting role is played by telomeric factor TRF2 that forms a complex with REST and protects it from ubiquitylation and therefore from proteasomal degradation [2]. TRF2 also forms the core of the shelterin complex that shields chromosome ends against unwanted end-joining and DNA repair machinery. REST indirectly regulates TRF2 expression; thus, it affects shelterin complex formation [3]. REST TRF2 complex disruption is a promising target of molecular therapy that will provide a dual effect on cancer stem cells.

Here, we have investigated in cell localization of REST and TRF2, and we have observed the formation of REST-TRF2 complex directly in the cell nucleus using Proximity ligation assay. We have determined the structure of transcription factor REST using cryo-electron microscopy single-particle reconstruction. A better understanding of the REST-TRF2 complex will provide valuable knowledge in development of drugs against glioblastoma.

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P5

BIOPHYSICAL RESEARCH FACILITIES AT CENTRE OF MOLECULAR STRUCTURE

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The biophysical research facility as a part of the Centre of molecular structure of the Institute of Biotechnology is a member of the Instruct-ERIC Research Infrastructure in the Czech Infrastructure for Integrative Structural Biology (CIISB) and also a member of the Molecular-Scale Biophysics Research Infrastructure (MOSBRI). The biophysical research facility provides shared resources of instruments and technologies for the determination of size, structure and stability of biomolecules, study of conformational changes and thermodynamics of temperature transitions and characterization of biomolecular interactions.

The following instruments and technologies are currently available: 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), microscale thermophoresis (Monolith NT.115 and NT.LabelFree), surface plasmon resonance (ProteOn XPR36) and bio-layer Interferometry (OCTET R8).

Users can apply for access to the biophysical instrumentation and expertise via CIISB (https://stigmator.ceitec.muni.cz/project_form/), Instruct (https://instruct-eric.eu/submit-proposal), MOSBRI (https://www.mosbri.eu/start-tna-proposal/).

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P6

CHANGES OF THE TRANSIENT SECONDARY STRUCTURE MOTIFS WITHIN Tau PROTEIN INDUCED BY ITS PHOSPHORYLATION

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Pathological conformational changes as well as hyperphosphorylation of microtubule-associated protein Tau are often connected with many neurodegenerative diseases (e.g., Alzheimer's disease). Due to the lack of tertiary structure Tau belongs to the group of intrinsically disordered proteins (IDPs). The widely used techniques Cryo-EM and X-ray crystallography are not sufficient for structural characterization of IDPs. On the other hand, nuclear magnetic resonance (NMR) can provide structural information at single residue resolution.

Due to Tau's intrinsically disordered character as well as the high content of prolines in its primary structure we employed 5D ^{13}C-directly detected multidimensional NMR experiments to assign the backbone and obtain structural characterization of Tau. With this approach, we have successfully assigned backbone and aliphatic side-chain chemical shifts (CS) of Tau protein in the native as well as phosphorylated (by PKA) form. Based on the CS we performed secondary structure propensity analysis X-Pro peptide bonds conformation prediction. Moreover, we monitored kinetics of PKA phosphorylation of Tau using time-resolved NMR experiment.

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P7

UNDERSTANDING THE STRUCTURAL BASIS OF INTERACTION BETWEEN ADENOVIRUS 5 TYPE C AND HOST RECEPTORS IN VIRAL ENTRY AND IMMUNE DEFENCE

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Human adenoviruses (HAdVs) frequently cause infections of the respiratory tract, gastrointestinal tract and eyes [1]. While CAR (coxsackie and adenovirus receptor) acts as receptor for most adenoviruses, entry of species C into polarized epithelial cells which lack CAR remained unknown. Recently it was discovered that HAdV-C5 blankets itself with lactoferrin (LF), an iron-binding innate immune protein, and exploits NCL, a natural receptor of lactoferrin, to infect host cells [1]. AdVs have been successfully utilised in vaccines against SARS-CoV2 and are of interests in the field of gene therapy. HAdV-C5 must bind to LF first via its major capsid protein, Hexon (AdV5H) in order to exploit NCL for entry into epithelial cells. Binding of AdV5 to NCL via LF is a 2-step process. LF has to bind to the virus first before the cellular receptor can be engaged. LF bound to NCL is unable to enhance viral entry via NCL. We aim to understand the mechanism of NCL mediated entry of HAdV-C5 into host cells via LF by determining the structure of HAdV-C5 Hexon: Lactoferrin: Nulceolin (AdV5H: LF: NCL) tripartite complex. Towards this aim, we first set out to prepare the AdV5H: LF, and HAdV-C5: LF complex and determine the cryo-EM structure. We employ mass spectrometry, microscale thermophoresis, and electron microscopy to characterise the structure of AdV5H: LF, and HAdV-C5: LF complex.

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P8

AHoJ: RAPID, TAILORED SEARCH AND RETRIEVAL OF APO AND HOLO PROTEIN STRUCTURES

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Studying protein – small molecule interactions can provide insights into diverse but fundamental topics in structural biology. Understanding the induced fit model, observing conformational shifts upon binding as well as exploring the promiscuity of a binding site are situations where both apo and holo snapshots of a protein are required. Machine learning applications that rely on structural information to predict such interactions, also stand to benefit from the availability of both bound and unbound states of the same protein.

There is currently no dataset or tool available, capable of generating a list of apo and holo conformations on demand, for a given protein structure.

Here we present Apo-Holo Juxtaposition (AHoJ), a web-based tool, that given a user-specified holo protein structure and one or more ligands that bind to it, identifies other structures that belong to the same protein, and classifies them as apo or holo, considering the users' preferences. A reverse search can also be conducted, in the case of starting with a structure that does not bind any ligands. The tool performs a search within the experimentally solved structures in the PDB and provides the user with a list of structures that are superimposed and visualised. The results are processed and displayed chain by chain, where each chain is annotated with a list of bound ligands and scored for its similarity with the query. AHoJ also features a multiple input mode, allowing it to generate customised apo-holo datasets.

P9

DETAILED ANALYSIS OF BINDING SITES IN THE PLL FAMILY

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Lectins, carbohydrate recognizing proteins, play an important role in various physiological and pathophysiological processes as well as both mutualistic and parasitic interactions between microorganisms and hosts [1]. In connection with the last-mentioned process, lectins from pathogenic bacteria can mediate the first step of infection and our goal is to investigate their specificity and suggest potential inhibitors.

The contribution is focused on the newly described PLL family, specifically on homologous lectins from the bacterium *Photorhabdus laumondii*. *Photorhabdus* spp is known for its complicated life cycle, including mutualism with microscopic nematode and pathogenicity towards insects [2]. Moreover, some species of *Photorhabdus* are also able to infect humans. Members of the PLL family share a seven-bladed beta-propeller fold and the presence of multiple binding sites within one protein domain [3]. Based on their structural characteristics, binding sites can be divided into two groups; hydrophobic (H) and polar (P). We solved multiple sets of X-ray structures of PLL family lectins in complex with different ligands. The main amino acids involved in the ligand-binding are highly conserved within the lectin family. An in-depth analysis of binding site occupancy was performed to better understand how the difference in amino acid composition within the binding site and its proximity influence the ligand binding.

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P10

^{19}F LABELLING OF DISORDERED AND HYBRID PROTEINS FOR ^{19}F NMR SPECTROSCOPY

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^{19}F NMR has been a useful complementary approach to traditional techniques - double labelling by ^{13}C and ^{15}N, especially due to the excellent magnetic NMR properties of the ^{19}F isotope. 1) It has a spin ″ and strong dipolar coupling, 2) High sensitivity (83% relative to ^{1}H) and broad chemical shift range (up to 400 ppm), 3) ^{19}F is 100% abundant in nature and virtually non-present in biologically relevant samples [1-3]. Selective ^{19}F isotopic labelling is therefore an outstanding technique for monitoring region-specific changes in protein structure thanks to minimal background signal [4,5].

Here, we present our progress in the preparation of hybrid and disordered protein samples, labelled with ^{19}F modified aromatic amino acids (AAs), for use in ^{19}F NMR spectroscopy measurements. When using identical protocols, different AAs proved to exhibit different incorporation efficiency rates. ^{19}F tryptophan was readily incorporated with 100% efficiency. The extent of incorporation of ^{19}F phenylalanine and tyrosine first ranged only between 30-50%, presumably due to similar biosynthetic pathways. Extensive optimisation of culture media, amount of labelled amino acid, wash and recovery period, and bacterial strains were utilised to increase the labelling rate of 14-3-3 and Tau proteins by the mentioned amino acids. In our efforts, we enhanced the labelling efficiency rate around twofold, as confirmed by MS/MS spectrometry and well-resolved 1D ^{19}F NMR spectra.

The optimized approaches will be used to study 14-3-3 PPIs and the *in vitro* formation of tau protein fibrils, a part of Alzheimer's disease pathology.

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P11

THIOREDOXIN INHIBITS ASK1 BY KEEPING IT IN REDUCED STATE

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ASK1 (apoptosis signal-regulating kinase 1) is a member of MAPKKK protein family, which directs cell towards inflammation or apoptosis by means of either p38 or JNK signalling pathway [1, 2]. ASK1 dysregulation has been associated with cardiovascular [3], tumour [4] and neurodegenerative [5] diseases and ASK1 thus represents a prospective target for therapeutic intervention. ASK1 regulation involves both oligomerization and interaction with multiple binding partners. To better understand the principle of ASK1 activation, we investigated the oligomeric behaviour of the N-terminal part under different redox conditions. Our results revealed that the N-terminal part of ASK1 forms dimers in solution and that this dimerization is affected by redox conditions. Moreover, we also identified regions that form the dimerization interface of the N-terminal part of ASK1. In addition, our data suggest that the interaction between ASK1 and its inhibitor thioredoxin is considerably weaker in strong reducing conditions. Altogether, our findings provide new insights into the mechanism of ASK1 inhibition by TRX.

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P12

METHODS FOR CHARACTERIZATION OF BIOMOLECULES AT BIC CORE FACILITY, CEITEC MU

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A biomolecular sample is a cornerstone of biochemical, biophysical, and structural analysis. The quality of such sample, especially protein, determines markedly the quality of subsequent measurements. It was suggested [1] that insufficient sample characterization may cause frequent problems in reproducing the published results. Based on the recommendations by ARBRE and P4EU associations [2], the basic characteristics of the protein sample (identity, purity, homogeneity, stability) should always be checked for improvement of scientific workflows.

At *Biomolecular Interactions and Crystallization Core Facility* (CF BIC) at CEITEC, we grant access to the state-of-art instrumentation for biomolecular sample analysis. The purity and homogeneity of the sample are typically assessed by UV/VIS spectrophotometry, analytical size exclusion chromatography (SEC-MALS), dynamic light scattering (DLS), or analytical ultracentrifugation (AUC). The thermal stability of the sample can be measured via differential scanning fluorimetry (nanoDSF), differential scanning calorimetry (DSC), or circular dichroism spectroscopy (CD). CD is also a first-choice method to determine protein and nucleic acid secondary structure. CF BIC ensures maintenance of machines and further develops the methodology, e.g., by designing a novel 48-well buffer screen for optimization of protein conditions [3]. Scientists benefit heavily from the presence of all the techniques in one core laboratory with personal support on site. The combination of various methods allows for detailed analysis of the sample in a short time and spares precious resources of the subsequent high-end analysis.

The further improvement of the services is connected to the facility's involvement in international networks such as ARBRE and Instruct-ERIC. The measurements for academic purposes are also financially supported by Czech Infrastructure for Integrative Structural Biology (CIISB), making the biomolecular characterization affordable to every scientist in the field.

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P13

STRUCTURAL AND BIOPHYSICAL ASPECTS OF LACTOFERRIN AND ITS INTERACTION WITH PLASMINOGEN

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Recently it was shown that lactoferrin (Lf), immunomodulatory protein of transferrin family of proteins interacts with plasminogen (Plg), protein of the fibrinolytic cascade [1]. The most probable mode of interaction is the contact of the N-terminal domain of Lf (corresponding to its peptide lactoferricin) with a mini-Plg region consisting of kringle 5 and a proteolytic domain.

Our aim is to further characterise the properties of Lf, comparing its recombinant and natural form. Unlike the native Lf, commercially available recombinant Lf produced in transgenic rice bears different type of glycosylation. Therefore, we aimed to prepare a recombinant Lf with human-like glycosylation by its expression in mammalian CHO cell line. Depending on the environment, Lf may oligomerise, losing its bactericidal and fungicidal immunomodulatory characteristics [2, 3]. Some properties of Lf can be changed in the presence of divalent ions (especially calcium ions [4]) inducing aggregation into higher oligomeric states. We used dynamic light scattering analysis to characterise Lf oligomerisation.

Previous results indicated that the functional part of Lf interaction with Plg consists of the peptide lactoferricin [1]. Our next goal was to optimise its production by pepsin digestion and its subsequent isolation. To determine the exact interaction of prepared lactoferricin with Plg, affinity determination by surface plasmon resonance will be used. Additionally to these experimental approaches, *in silico* molecular docking characterising the interaction sites will be applied.

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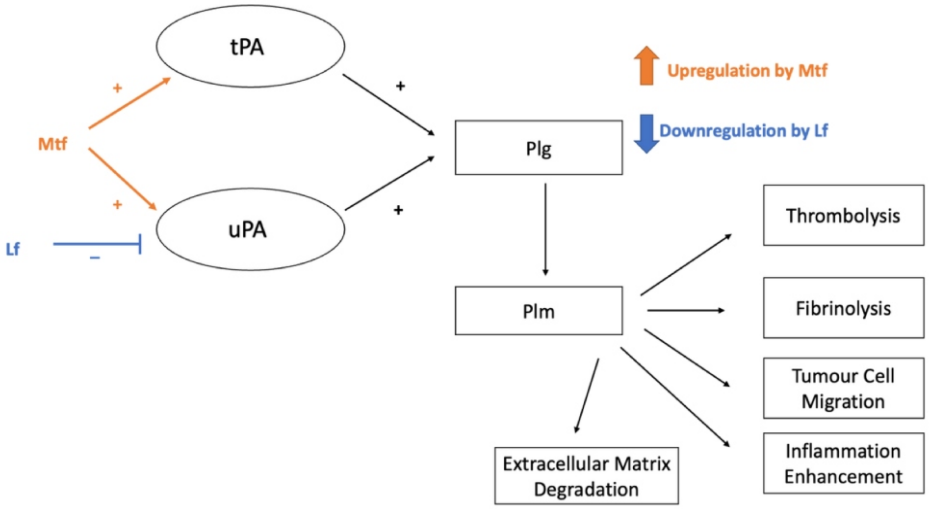

Figure 1: Plasminogen (Plg) activation to plasmin (Plm) by tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA) (modified from [5]) and its regulation by melanotransferrin (Mtf) and lactoferrin (Lf).

P14

Ni-REPLACEMENT IN Zn-DEPENDENT S1 NUCLEASE

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S1 nuclease from *Aspergillus oryzae* is a single-strand-specific endonuclease widely used for biochemical analysis of nucleic acids [1,2]. Its activity depends on presence of three Zn^{2+} ions in the active site composed of nine residues coordinating the zinc cluster. The cluster and its adjacent residues are conserved across the whole S1-P1 family of nucleases. Two of the Zn^{2+} ions of the cluster are buried on the bottom of the active site. The third Zn^{2+} ion is closer to the nuclease's surface.

A possibility of replacement of Zn^{2+} by Ni^{2+} was studied using biophysical assays and mainly the X-ray anomalous dispersion. Various molar ratios of S1 nuclease, chelating agent ethylenediaminetetraacetic acid (EDTA) and $NiCl_2$ were analysed. The mixture of S1:EDTA:$NiCl_2$ in molar ratio 1:5:10 was crystallized using a vapor diffusion method. The obtained crystals were of a good quality for the diffraction experiment on synchrotron radiation source Bessy II, Helmholtz Zentrum Berlin [3].

The diffraction data were collected at three different wavelengths in close proximity of the Ni- and Zn-absorption edges, respectively. The anomalous difference maps obtained from the collected data confirmed exchange of one Zn^{2+} ion by Ni^{2+}, whilst the remaining two Zn^{2+} ions remained unaffected. No structural changes of the surrounding residues were observed.

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P15

BIOCHEMICAL CHARACTERIZATION OF S1-P1 NUCLEASE FROM HUMAN OPPORTUNISTIC PATHOGEN *STENOTROPHOMONAS MALTOPHILIA*

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Stenotrophomonas maltophilia is an emerging multidrug-resistant opportunistic pathogen causing nosocomial infections of human respiratory tract. Its ability to form resistant biofilms further contributes to its spread in hospital environment [1]. As S1-P1 nucleases are not present in humans, but produced by several bacterial pathogenic species including *S. maltophilia*, they may represent potential drug or marker target [2] More knowledge of bacterial S1-P1 nucleases may lead to new therapeutic approaches in treatment of infections caused by multiresistant strains.

The S1-P1 nuclease from *S. maltophilia* SmNuc1 was recombinantly expressed in *E. coli* and following two-step purification process led to gain of the active nuclease in high yield and sufficient purity. Enzymatic characterization of SmNuc1 revealed several differences from already known S1-P1 nucleases. Structure of SmNuc1 was solved using X-ray crystallography. Current research is focused on ligand binding studies involving its potential inhibitors.

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ucation, Youth and Sports of the Czech Republic (LM2018127, support of BiocevCMS – core facilities Biophysical Methods, Crystallization of Proteins and Nucleic Acids and Structural Mass Spectrometry of CIISB, part of Instruct-ERIC).

P16

STRUCTURAL ARCHITECTURE OF NEIL3 GLYCOSYLASE IN ABASIC SITE DNA REPAIR

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DNA interstrand cross-links (ICLs) are very toxic DNA damage lesions covalently connecting the opposite strands. They form impenetrable barriers for the replication machinery during cell division. Abasic site ICLs (Ap-ICLs) are formed spontaneously when an abasic (Ap) site forms a covalent bond with a base located in the opposite DNA strand. This recruits NEIL3 glycosylase, an enzyme responsible for the removal of the Ap-ICLs, however, the molecular mechanism of this repair remains elusive [1,2].

NEIL3 contains three sets of zinc finger domains. First, located on the N-terminus is a catalytic Nei domain removing the Ap-ICL. Second, the ubiquitin-binding (NZF) domain contains a ubiquitin-binding zinc finger. Last, two adjacent Gly-Arg-Phe (GRF) domains are known for their ability to bind single-stranded DNA, but their role in the ICL repair is unclear [3].

We have investigated the recognition of a stalled DNA replication fork by NEIL3. We have solved the NMR structure of GRFs and shown how they recognise single-stranded DNA. We have further revealed how GRFs bind DNA forks with a slight preference for 5'-DNA overhangs. Along with GRFs inter-domain rigidity, our data outline how GRF recognises DNA forks suggesting their role in Ap-ICL repair.

Next, we have solved a crystal structure of mouse NEIL3 Nei domain in complex with the DNA reaction intermediate. With Nei's preference for a 3'-DNA overhang, we have outlined the interplay between Nei and GRFs in Ap-ICL repair. Our results suggest how NEIL3 recognises the structure of two collided replication forks, a DNA replication X-structure.

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P17

MODULATING FOXO3 TRANSCRIPTIONAL ACTIVITY BY SMALL, DBD-BINDING MOLECULES

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FOXO3 is a member of Forkhead Transcription Factor family. Forkhead proteins share an evolutionarily conserved winged-helix DNA-binding domain (DBD), which recognizes specific DNA sequence. Through interaction with target DNA, FOXO proteins modulate various biological processes, such as cell death, cell-cycle arrest, DNA repair and energy homeostasis [1]. Due to FOXO3 ability to induce cell cycle arrest, it is considered a tumour suppressor. However, in certain cases, it has been shown that FOXO3 can promote tumour development and

angiogenesis via maintaining cancer cell energy homeostasis. Moreover it also enhances tumour cell resistance to chemotherapeutic agents [2]. Therefore, targeting FOXO3 transcriptional activities by specific inhibitors can help to prevent drug resistance in cancer therapy.

A pharmacophore screening identified a low-molecular compound, named S9, that interacts with FOXO3-DBD and modulates FOXO3 transcriptional programme in human cells. The mode of interaction between S9 compound and FOXO3-DBD was characterized using NMR spectroscopy and docking studies [3]. This compound was further modified to increase its inhibitory potency. In this work we tested a group of newly designed S9 derivatives. Their inhibitory potency and interaction with FOXO3-DBD was tested using NMR spectroscopy and native electrophoresis. Furthermore, the effect of these compounds on FOXO3 transcriptional activity was evaluated in cell cultures. We have shown that these new derivatives are able not only to bind to FOXO3-DBD but also to inhibit its interaction with the target DNA.

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Figure 1. Graphical scheme of abstract

P18

CONSERVATION OF H3K36 DI- AND TRIMETHYLATED NUCLEOSOME RECOGNITION BY PWWP

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Eukaryotic transcription regulation is dependent on specific histone modifications. Their recognition by epigenetic reader domains triggers complex processes relying on coordinated association of transcription regulatory factors. Although, various modification states of a particular histone residue, e.g. mono-, di- or trimethylation, often lead to differential outcomes, it is not fully understood how they are discriminated at a molecular level. Lens Epithelium Derived Growth Factor/p75 (LEDGF/p75 or PSIP1) is a transcriptional co-activator responsible for tethering other factors to the regions of actively transcribed genes using its PWWP domain that specifically binds di- and trimethylated lysine 36 on histone 3 (H3K36me2/3). Cellular partners bound to its C-terminal integrase-binding domain (IBD) are thus drawn near the active chromatin. Through this interaction, LEDGF/p75 is associated with two distinct diseases, HIV infection and mixed-lineage (MLL) leukemia, and therefore becomes an attractive therapeutic target. Our cryo-EM data capture a LEDGF/p75 PWWP domain in complex with the H3K36 di- and trimethylated nucleosome, respectively. We show that both marks are recognized by the PWWP domain in a highly conserved manner that does not require linker DNA beyond the core nucleosome for stable complex formation even in the absence of chemical crosslinking. Using NMR spectroscopy, we reveal short DNA binding regions in the intrinsically disordered region of LEDGF downstream the PWWP domain that restrain the effective space for assembly of LEDGF-mediated transcriptional complexes. Our data demonstrate that the distinct transcriptional programs triggered by H3K36 di- and trimethylation marks are independent of the actual recognition mode by a cognate reader domain and might require an orthogonal regulatory mechanism.

P19

NON-NUCLEOTIDE RNA-DEPENDENT RNA POLYMERASE INHIBITORS THAT BLOCK SARS-CoV-2 AND FLAVIVIRAL REPLICATION

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Flaviviruses (family *Flaviviridae*) and coronaviruses (family *Coronaviridae*) both belong to single-stranded positive-sense RNA (+RNA) viruses. Unfortunately both of these families proved that they have a pandemic potential which was demonstrated recently by the ZIKV, MERS-CoV and SARS-CoV outbreaks. Therefore, effective treatment strategies are urgently needed to treat patients infected with flaviviruses [1, 2].

Flaviviruses are large group of viruses known for their ability to cause human infections. These viruses can cause diverse diseases, such as encephalitis, acute flaccid paralysis, hemorrhagic fevers or congenital abnormalities and fetal death [3].

SARS-CoV-2 has caused an extensive pandemic of COVID-19 all around the world. Key viral enzymes are suitable molecular targets for the development of new antivirals against SARS-CoV-2. With respect to its essential role in the replication of viral RNA, RNA-dependent RNA polymerase (RdRp) is one of the prime targets [4].

We demonstrate that various compounds inhibit RNA synthesis by viral RdRps. We use *in vitro* polymerase assays to show that these compounds interfere with RNA syntheses performed by the RdRps.

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P20

STRUCTURE OF RECOMBINANT Tau40 PROTEIN FIBRILS PREPARED WITHOUT ENHANCERS OF FIBRILIZATION

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Neurodegenerative diseases such as Alzheimer's disease remain a global issue with an increasing number of patients and without effective therapeutical solution. Tau protein fibrils are structured aggregates present in AD patient's brains. Accumulation of these Tau fibrils lead to neuronal cell death, decrease in brain density and progression of the AD. Under normal physiological conditions Tau binding to microtubules is responsible for stabilization of the microtubule network in axons. [1] This stabilization is regulated by posttranslational modifications. [2] However, some posttranslational modifications of Tau such as hyperphosphorylation and truncation are widely described as key pathological factors in formation of Tau fibrils with different modification resulting in structurally different filaments.

Recently cryo-EM showed that Tau fibrils isolated from brain of AD patients are present in two distinct fibril formations. [3] It was also shown that heparin induced recombinant Tau fibrils formed different types of filaments than filaments isolated from AD brains. [4] These results show the importance of origin and type of Tau fibrils while characterizing the fibril structure.

Our study focuses on structure of Tau filaments formed by recombinant full length Tau isoform (Tau40) prepared by fibrilization that does not require any enhancers. This isoform is the longest of all 6 isoforms[5] and considered to be the most abundant. We tested several different conditions of fibrilization based on type of buffer, temperature, presence or absence of agitation and additives. Fibrilization rate was quantified using Thioflavin T assay that allowed to detect the formation of fibrils by the increase in fluorescence response. Fibrils were visualized using negative staining, cryo-EM and atomic force microscopy (AFM). Based on our preliminary results, we found that the best fibril growth was

obtained with phosphate buffers at 37 degrees with 700 rpm agitation. These fibrils are being further analyzed by cryo-EM with a goal to reconstruct their atomic structure in order to test if our recombinant Tau fibrils prepared without fibrilization enhancers match the fibrils isolated from brains of AD patients.

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P21

STRUCTURAL STUDIES OF MutM AND ABASIC SITE INTERSTRAND CROSSLINK

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Bacterial MutM is a DNA repair glycosylase removing DNA damage generated form oxidative stress and preventing mutations and genomic instability. MutM belongs to the Fpg/Nei family of procaryotic enzymes sharing structural and functional similarities with their eukaryotic counterparts, for example, NEIL1-NEIL3. We present two crystal structure of MutM from pathogenic *Neisseria meningitidis*, MutM holoenzyme and MutM bound to DNA. The free enzyme exists in an open conformation, while upon binding to DNA, both the enzyme and DNA undergo substantial structural changes and domain rearrangement [1].

One of DNA lesion repairing by MutM is abasic site (Ap site) which if not repaired may spontaneously lead to creation of abasic interstrand crosslink (Ap-ICL) with an adjacent adenine in the opposite strand. NEIL3 glycosylase is known to remove Ap-ICL. With an array of different oligonucleotides, we have investigate the rates of formation, the yields, and the stability of Ap-ICL. Our findings point out how different bases in the vicinity of the AP site change crosslink formation *in vitro* [2].

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P22

RECOGNITION OF RNA POLYMERASE II C-TERMINAL DOMAIN BY RPRD2

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The largest subunit of human RNA Polymerase II contains highly flexible C-terminal domain (CTD) that is composed of 52 heptapeptide repeats (first half of repeats with consensus sequence YSPTSPS and second half largely degenerated in sequence). Several CTDs canonical and non-canonical residues can be subjects of post-translational modifications. Tyrosine, threonine, and serine residues undergo dynamic phosphorylation/ dephosphorylation resulting in specific phosphorylation patterns throughout different stages of transcription cycle. These phosphorylation patterns are recognized by various transcription and processing factors during the transcription cycle. Therefore, CTD plays an important role in the regulation of transcription and coupling of transcription to post-transcriptional processes such as mRNA processing.

In this study, we show that human transcription factor, RPRD2, recognizes specifically pSer2 or pThr4 phosphorylated forms of CTD via its CTD-interacting do-

main (CID) in a similar way to its yeast homologue, Rtt103. The interaction of RPRD2 CID with pSer2 phosphorylated CTD is further enhanced by additional phosphorylation on pSer7. To provide mechanistic details of the interaction between RPRD2 CID and pSer2,7 CTD, the solution structure was obtained using NMR spectroscopy. pSer 2 and pTh4 phosphomarks occur mainly during the late elongation and termination. RPRD2s preference for these two phosphomarks suggests possible involvement of RPRD2 in transcription termination.

P23

EFFECT OF A LOV PROTEIN MATRIX ON FLAVIN PHOTOCYCLE PROBED BY TRANSIENT RESONANCE RAMAN SPECTROSCOPY AND THEORETICAL CALCULATIONS

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In our previous work, we applied transient absorption and femtosecond stimulated Raman spectroscopy (FSRS) to free flavin mononucleotide (FMN), a biomolecule that functions as prosthetic group and cofactor in a myriad of biochemical processes. Time-resolved spectra were taken with time delays up to a few nanosecond after FMN photoexcitation and the observed Raman bands in the excited singlet S_1 and triplet T_1 states were assigned with the help of quantum chemistry calculations. Here in order to understand the influence of the protein environment on the photophysics of FMN, we measured FSRS of FMN bound to EL222, a photosensory receptor containing a light-oxygen-voltage (LOV) domain, with time delays from 100 femtosecond to 0.5 milliseconds. The displacement amplitude between the excited state potential energy surface (PES) minima was estimated using the displaced harmonic oscillator model. We propose a change in the topography of the potential energy surface in the S_1 state due to protein-mediated mixing of the bright * state and the dark n * state of FMN. The mixing is supported by the decrease of the corresponding transition dipole moment with a higher electronic state as a result of protein-chromophore interactions. We speculate that such a mixing may arise from non-symmetric hydrogen bonds between the O_4, atom of FMN and two surrounding asparagine residues present in the binding pocket. The mixing might also be favoured by a smaller energy gap between these two states due to favourable interactions between the FMN moiety and the LOV cage. In principle, the n * feature of the S_1 state will decrease the electron density of FMN N_5 atom and reduce its proton affinity. This provides a perspective to understand the primary photochemical reaction in the LOV domain that occurs on the T_1 state rather than the S_1 state.

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P24

SARS-CoV-2 AND MORE: HOW NEUTRON PROVIDE INSIGHTS INTO BIOLOGICAL QUESTIONS

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Biomolecules are both fascinating and inherently complex and thus need to be studied by an equally complex array of methods. Neutron scattering techniques are non-destructive, highly penetrating, do not require sample labelling tags and show strong differences in their sensitivity to hydrogen and its isotope deuterium. Thus, neutron scattering is a particularly suitable tool for the investigation of biological matter. Here, we discuss how neutrons - in particular small-angle neutron scattering (SANS) - at the Institut Laue-Langevin (ILL, Grenoble, France) contribute to biological sciences.

As an example, we use a recent effort by ILL scientists [1] geared towards obtaining a detailed understanding of SARS-CoV-2. Along with complementary lab methods, this study used neutron reflectometry, spectroscopy and SANS to investigate the roles of selected SARS-CoV-2 Spike fusion peptides (FPs) in cellular infection. Our study revealed a surprising difference in the biological significance of these peptides: the roles assumed by the FPs range from membrane disruption, dehydration of membrane lipids to fusogenic effects. Notably, this publication illustrates the importance of neutron scattering methods for biological questions, including those of fundamental, global importance.

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P25

MULTIPLE APPROACHES FOR PROTEIN PHOSPHORYLATION: A STORY OF 14-3-3

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Protein phosphorylation is a key regulatory mechanism involved in majority of biological processes [1]. In eukaryotes the dominantly phosphorylated residue is serine [2]. The phosphorylation of Ser58 has been observed for ubiquitous dimeric 14-3-3 proteins [3]. The 14-3-3 protein family represents a signalling hub, and its involvement has been confirmed in cancer progression and neurodegenerative diseases [4,5]. Since the Ser58 phosphorylation has been shown to induce monomerization, it has become a target of numerous studies to explore the properties of such monomer [3].

Unfortunately, the study of phosphorylation is often hindered by complicated sample preparation. This was the case of 14-3-3 as low or no phosphorylation has been achieved in pilot experiments, leading to the usage of so-called phosphomimicking mutants [6]. Since the reliability of phosphomimicking mutants is disputable [7], the goal of our work was to find a phosphorylation approach applicable for 14-3-3.

Here we present four distinct methods for preparation of 14-3-3 phosphorylated at Ser58: *in vitro* phosphorylation by catalytic subunit of protein kinase A (PKA), co-expression of 14-3-3 and PKA in *E. coli*, *in vivo* phosphorylation by PKA covalently linked to the 14-3-3 (i.e., chimeric construct) and a direct incorporation of phosphoserine via expanded genetic code. We have tested and compared the methods based on their efficiency, yield and simplicity. Moreover, we offer direct comparison with the phosphomimicking mutants and show the shortcomings of their employment.

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P26

MULTIAPPROACH DOCKING STUDY FOR BINDING OF INTRINSICALLY DISORDERED TAU PEPTIDES TO MONOCLONAL ANTIBODIES

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Microtubule associated protein tau is the main actor of tau hypothesis of Alzheimer´s disease origin [1]. Under pathological conditions, by hyperphosphorylation of amino acid residues of the tau polypeptide chain, or upon truncation of tau protein, tau dissociates from microtubules. At the same time disintegration of microtubule and aggregation of tau monomers occur. Both of these events subsequently lead to nerve cell damage. Although it has not yet been confirmed whether this process is the trigger for Alzheimer's disease, the presence of insoluble aggregates of tau has been shown to be a hallmark of AD [2]. Therefore, tau protein appears to be a potential molecular target in the AD cure search process, whereas the main idea would be to design an inhibitor of tau aggregation to prevent disease progression. But we must first resolve the structural features of tau protein. Because of its intrinsically disordered character, tau doesn´t acquire any stable secondary structure. Monoclonal antibodies appear to be a useful tool to address the structural issue of tau, mainly their use as crystallization chaperones or in our case as docking and molecular simulation partners [3,5]. In addition to crystallization of tau complex with monoclonal antibody, *in silico* methods were implemented to obtain the transient tau structure.

Monoclonal antibody DC11 discriminates very strictly between physiological tau proteins and truncated tau peptides. This indicates the presence of a conformational epitope which carries the pathological function of tau and which is recognized by the mentioned antibody [3,4]. Therefore, we focused on the crystallization of the Fab fragment of DC11 antibody with $tau_{321-391}$ to determine the conformational epitope of tau and to approximate the transition of tau from physiological to pathological conformation. Crystals of DC11Fab alone gave diffraction up to 1.4 Å. We also plan to dock the most populated clusters of tau conformations observed during MD simulations into the DC11 apo Fab structure.

A pan-tau monoclonal antibody DC25 recognizes tau epitope Lys347-Lys353 [5]. Using FlexPepDock and CABS-dock webservers we performed docking of flexible peptide $tau_{347-353}$ into DC25 antibody structure with modest flexibility in CDR regions. From the resulting modelled complexes we have observed also the helical propensity of tau which was proposed also by the FELLS predictor.

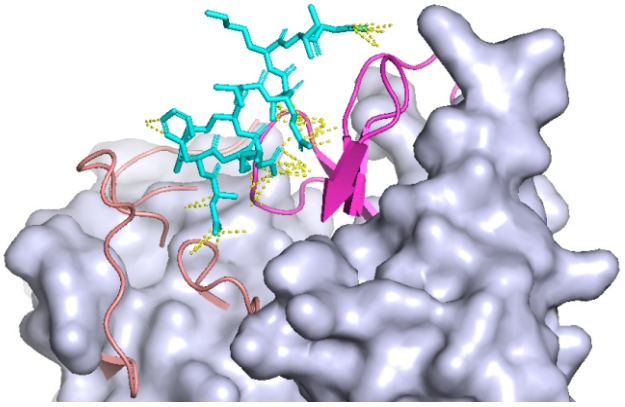

Figure 1: Docking result of $tau_{347-353}$ with DC25 Fab from CABS-dock. The CDR regions of DC25 light chain are indicated as salmon cartoon and heavy chain CDR regions as magenta cartoon, tau is shown as cyan sticks, Fab framework regions as grey surface and contacts between chains within 3.0 Å are indicated in yellow (http://biocomp.chem.uw.edu.pl/CABSdock).

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P27

PROSTATE CANCER: DEVELOPMENT OF PSMA-DIRECTED ANTIBODY-BASED MOLECULES INTENDED FOR IMMUNOTHERAPY

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Prostate cancer stands for very frequent death-causing malignancy in man population especially in countries with high standard of living. High mortality in progressed stages of disease triggers development of new reagents and medical treatments that aim to target prostate tumor and particularly tumor-derived metastases with high efficiency and specificity. Prostate-specific membrane antigen (PSMA) represents prominent biomarker of prostatic tumors since the level of its expression is highly correlated with progression of prostate cancer. Antibody-based molecules directed to PSMA have high potential to specifically target cancer cells with minor toxicity in non-target tissues.

Previously we developed and characterized PSMA-specific antibody, named 5D3, that reveals high specificity and affinity to native PSMA. Moreover, we proved suitability of 5D3 as well as its fragments for *in vivo* application using mouse model of xenografted prostate cancer. Here we show the strategy for protein engineering of bispecific molecules based on 5D3 that would engage host immune cells in the site of cancer. Bispecific molecules are intended to trigger specific anti-tumor activity of host immune system in close proximity to tumor cells resulting in their elimination. Accordingly, our pilot *in vitro* study shows that 5D3 fragment fused to anti-CD3 fragment has potential to trigger cell death in cancer cells via activity of associated T cells. Moreover, 5D3 fragment linked to cyclic peptide 33 is capable of enhancing production of reactive oxygen species by activated monocytes when localized in close proximity to PSMA antigen. Detailed characterization as well as testing of 5D3-derived molecules under *in vivo* conditions is currently on the run.

P28

IMMUNOMODULATORY CATHEPSIN B FROM THE HOUSE DUST MITE *DERMATOPHAGOIDES FARINAE*: FUNCTIONAL AND STRUCTURAL CHARACTERIZATION

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Mites are a major source of allergens and contributor to the rising incidence of allergic diseases, including bronchial asthma, rhinitis, and atopic dermatitis. Digestive enzymes produced by mites and released into the environment are potent allergens and a target for the treatment of allergic hypersensitivity.

We performed the first detailed profiling of digestive proteolytic enzymes in the house dust mite *Dermatophagoides farinae* using functional proteomics and identified cathepsin B (DfCB) as a new major component protease. Recombinant DfCB was produced in the yeast expression system and enzymologically characterized. Furthermore, the purified DfCB was crystallized, and its preliminary 3D structure was solved by X-ray crystallography.

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P29

SYNERGISTIC ANTIMICROBIAL ACTIVITY OF MAGAININ 2 AND PGLa REVISITED

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Emerging bacterial resistance against current antibiotics is a growing concern of the 21st century [1]. Antimicrobial peptides (AMPs), compounds commonly found within all living organisms, are considered promising candidates to fight these resistant pathogens. Many strategies for their optimization have been investigated, one of them being the use of a combination of multiple AMPs, leading to a synergistic effect. Magainin 2 and PGLa are AMPs present in the skin of African clawed frog *Xenopus laevis* and were shown to exhibit this synergistic activity against various Gram-negative bacteria [2].

The original studies, both experimental and computational, lead to the conclusion that the mechanism of action by magainin 2 with PGLa was based solely on the formation of toroidal pores in the bacterial membranes [2-4]. Here we present recent evidence showing the two peptides promote adhesion and aggregation of lipid membranes. We achieved the results via a combination of modern techniques: coarse-grained computer simulations on model lipid bilayers, cryogenic electron microscopy, small angle X-ray scattering, and fluorescence confocal microscopy using lipid vesicles [5]. Additionally, we performed super-resolution lattice structured illumination microscopy on *E.coli*.

Our results do not fit with the current model of the synergistic action by magainin 2 and PGLa, represented by a formation of toroidal transmembrane pores. A novel model will be required to explain the previous observations of membrane disruption, specific peptide alignment from nuclear magnetic resonance spectroscopy, together with the membrane adhesion and fusion we observed.

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P30

NEW INSTRUMENTATION AVAILABLE IN CENTRE OF MOLECULAR STRUCTURE, INSTITUTE OF BIOTECHNOLOGY CAS (BIOCEV)

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The core facilities for structural biology in BIOCEV are organized under the Centre of Molecular Structure, run by the Institute of Biotechnology, Czech Academy of Sciences and belong to the Czech Infrastructure for Integrative Structural Biology. The Centre of Molecular Structure (CMS) encompasses several laboratories providing a complex approach to studies of three-dimensional structure, function and biophysical properties of biological molecules.

The BIOCEV building is located just outside of Prague. It can be easily reached by car or public transport.

The range of instruments offered by CMS has recently been extended to include several other important techniques, like SONICC (Formulatrix), timsTOF pro (Bruker Daltonics), or Vitrobot (Thermo Scientific). SONICC enables detection of extremely thin crystals, microcrystals smaller than 1 m, and crystals obscured in birefringent LCP. New mass spectrometer timsTOF can be used for high throughput shotgun proteomics, hydrogen-deuterium exchange, covalent labelling experiments, and native mass spectrometry with ion mobility separation. Vitrobot is a new tool for preparation of samples suitable for electron diffraction and cryoEM experiments.

The services of CMS are offered in open access regime and users can apply for access via https://www.ciisb.org/ and https://instruct-eric.eu/centre/biocev/.

The Centre of Molecular Structure is supported by: MEYS CR (LM2018127); 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), and ELIBIO (CZ.02.1.01/0.0/0.0/15_003/0000447).

P31

14-3-3 DIRECTLY INTERACTS WITH THE KINASE DOMAIN OF CaMKK1 AND INHIBITS CALMODULIN BINDING

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The calcium/calmodulin-dependent kinases (CaMK) are of great interest due to their important functions in calcium signalling and especially in neuronal development [1]. Calcium/calmodulin-dependent kinase kinase 1 (CaMKK1) is a member of the CaMK family and it was shown that the phosphorylation at Thr^{108} and Ser^{458} residues by the cAMP-dependent protein kinase (PKA) directly inhibits its activity, whereas phosphorylation at Ser^{74} and Ser^{475} induces binding to the 14-3-3 protein [2,3]. The 14-3-3 is a family of regulatory proteins, which bind to other proteins in a phosphorylation dependent manner [4]. The binding of 14-3-3 to CaMKK1 via PKA phosphorylation is known to suppress CaMKK1 activity [2,3], but the underlying mechanism has not yet been described. Our research aimed to unravel this mechanism.

We analysed the structure of a complex formed between CaMKK1, phosphorylated at four different PKA sites, and a C-terminally-truncated version of the ă isoform of the 14-3-3 protein (14-3-3 C). Using hydrogen/deuterium exchange coupled to mass spectrometry, we show that 14-3-3 C binding alters the protein structure of the phosphorylated CaMKK1 in multiple regions. First, the the N- and C-terminal segments, which contain phosphorylation sites responsible for 14-3-3 binding. Second, the N-lobe of the kinase domain, which is in close proximity to the CaMKK1 active site. Third, the region around the calcium binding domain. Additional small angle X-ray scattering (SAXS) and chemical cross-linking analyses revealed that the CaMKK1:14-3-3 C complex is dynamic and conformationally heterogeneous.

These findings suggest that the 14-3-3 protein-dependent inhibition of CaMKK1 may be explained by structural changes in both the catalytic and calcium binding domains. The 14-3-3:CaMKK1 complex formation might interfere with the access of Ca^{2+}/CaM to the CaMKK1 calmodulin-binding domain and/or promote interaction be-

tween the autoinhibitory domain and the catalytic domain, thus inhibiting CaMKK1. To test the former hypothesis, we performed fluorescence anisotropy measurements using dansyl-tagged calmodulin. We revealed that while CaMKK1 binds to dansyl-calmodulin with a K_D of 2 M, no binding is observed in the CaMKK1:14-3-3 C complex.

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P32

14-3-3-PROTEIN REGULATES Nedd4-2 BY MODULATING INTERACTIONS BETWEEN HECT AND WW DOMAINS

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Neural precursor cell expressed developmentally down-regulated 4 ligase (Nedd4-2) is an E3 ubiquitin ligase that targets proteins for ubiquitination and endocytosis, thereby regulating numerous ion channels, membrane receptors and tumor suppressors. Nedd4-2 activity is regulated by autoinhibition, calcium binding, oxidative stress, substrate binding, phosphorylation and 14-3-3 protein binding . However, the structural basis of 14-3-3-mediated Nedd4-2 regulation remains poorly understood. Here, we combined several techniques of integrative structural biology to characterize Nedd4-2 and its complex with 14-3-3. We demonstrate that phosphorylated Ser342 and Ser448 are the key residues that facilitate 14-3-3 protein binding to Nedd4-2 and that 14-3-3 protein binding induces a structural rearrangement of Nedd4-2 by inhibiting interactions between its structured domains. Overall, our findings provide the structural glimpse into the 14-3-3-mediated Nedd4-2 regulation and highlight the potential of the Nedd4-2:14-3-3 complex as a pharmacological target for Nedd4-2-associated diseases such as hypertension, epilepsy, kidney disease and cancer [4-5].

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P33

STRIKINGLY DIFFERENT ROLES OF SARS-CoV-2 FUSION PEPTIDES UNCOVERED BY NEUTRON SCATTERING

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SARS-CoV-2 is an encapsulated virus responsible for a lethal respiratory illness since its outbreak at the end of 2019. It consists of a lipid envelope and a set of structural membrane proteins that include the envelope, membrane and *spike* (S) proteins, which are responsible for virion assembly. Interestingly, the *fusion domain of the spike protein* triggers the fusion between viral and host membranes, initializing the infection. However, the molecular mechanism regulating this process is not deeply understood. Our approach [1] has been to study the interaction of several putative *fusion peptides (FPs)* at or near the N-terminus of S2 subunit with model membranes in the form of monolayers, bilayers and small unilamellar vesicles, composed of both synthetic lipids and natural lipids extracted from yeast cells. The multi-technique approach exploited in this work implied the use of spectroscopic, interfacial and scattering techniques, particularly neutron reflectometry (NR). NR revealed that FPs assume different functions in the initiation of viral infection. The results obtained here especially shed light on the critical role of FP1 (the N-terminus of the Spike fusion domain), which is able to fully penetrate membranes, in a Calcium-dependent manner (**Figure 1**), and FP4, whose high binding affinity (**Figure 2**) enables it to work as a bridge between membranes. Moreover, this work also provides a powerful interdisciplinary framework for future investigations of eukaryotic and viral membranes fusion mechanism.

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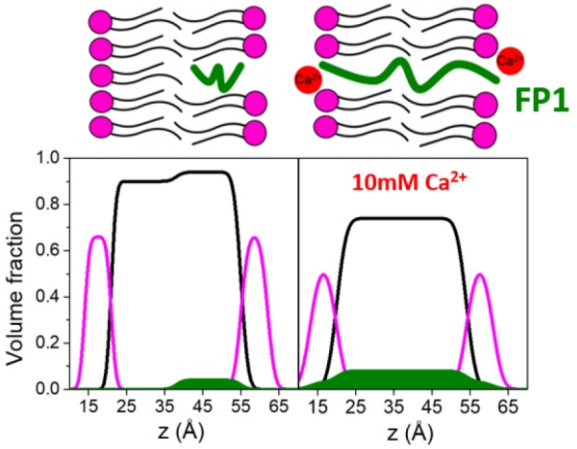

Figure 1. Sketches and volume fraction profiles, from NR, showing FP1 inserted in the membrane.

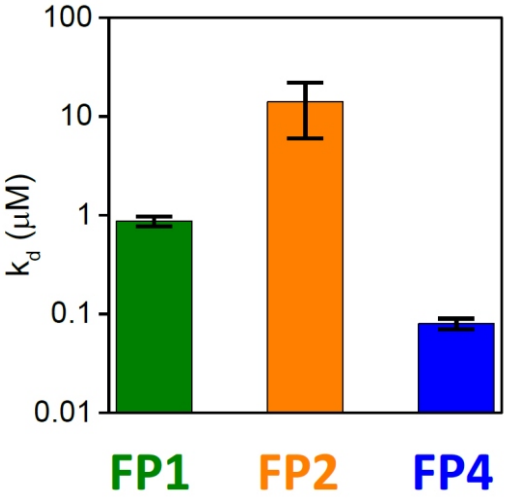

Figure 2. Values of dissociation constant of FPs to an *in vitro* plasma membrane-mimicking system.

P34

RAMAN OPTICAL ACTIVITY OF NUCLEOTIDES – THEORETICAL AND EXPERIMENTAL STUDY

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Biological activity of nucleotides is strongly dependent on their conformation. For example, chemically modified (oligo)nucleotides are widely applied as therapeutical agents and alternation in the structure impacts their conformational dynamics.[1] Raman optical activity (ROA) can be conveniently used to examine the conformation of biomolecules in aqueous solutions. However, ROA applications to nucleotides are rather scarce due to the complexity of the experiment and calculations. To investigate the potential of this spectroscopy, Raman and ROA spectra of common mononucleotides (rAMP, rGMP, rCMP and dTMP) were measured and interpreted on the basis of molecular dynamics combined with density functional theory. It was shown, for example, that the sugar puckering in all nucleotides is strongly dependent on intramolecular H-bonding. Further analysis of theoretical free energy surfaces as well as simulated and experimental spectra revealed details of the conformer distribution.

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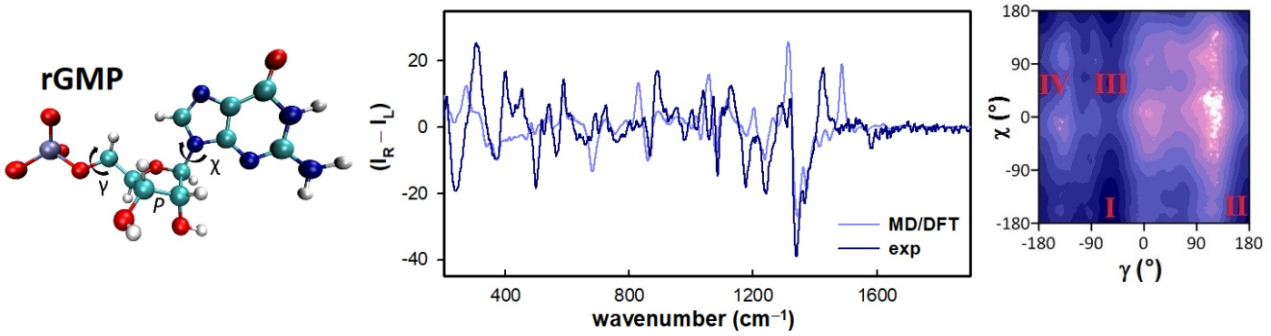

Figure 1. Structure of rGMP (left), calculated and experimental ROA spectra (middle) and dependence of the free energy on the and torsion angles as obtained from molecular dynamics (right).

P35

CRYSTAL STRUCTURE OF HUMAN NATURAL KILLER CELL RECEPTOR NKP30 IN COMPLEX WITH ITS TUMOR LIGAND B7-H6

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NKp30 is an activating receptor on the surface of human natural killer (NK) cells. Its crystal structure is known since 2011, published and deposited by Joyce *et al*. [1] with PDB code 3NOI. B7-H6 is an activating immunoligand expressed by some tumor cells. Crystal structure of its complex with NKp30 has been published and deposited by Li *et al*. [2], PDB code 3PV6.

Here we present a new crystal structure of NKp30:B7-H6 at resolution 3.1 Å. While NKp30 in previous studies originated from bacterial productions, this is the first structure of the complex where both components come from eukaryotic cell lines. Both proteins are homogenously glycosylated and were produced in HEK293S GnTI$^-$ cells. For the structural study, NKp30

was used with complete glycosylation, while B7-H6 was deglycosylated after the first GlcNAc for better crystallization.

The new structure showed the same NKp30:B7-H6 interaction interface as observed by Li *et al*. (3PV6). Similarly as in the structure of Joyce *et al*. (3NOI), NKp30 form dimers; However, the dimers of glycosylated NKp30 are different (the glycan presence hinders the formation of the dimers observed in PDB 3NOI), and according to the PISA server validation, the new dimers are more likely biologically relevant. Furthermore, the asymmetric unit of the new crystal structure contains a dimer of NKp30 placed among two B7-H6 molecules (contacts of chains A-C and B-D_{symm}). This arrangement may indicate possibility of binding of the NKp30 dimer between two B7-H6 ligands even during the contact of the NK cell and the tumor cell.

The structure has been deposited in the Protein Data Bank under code 6YJP and published [3].

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P36

MECHANISM OF AGGREGATION OF TAU PROTEIN FORMS ASSOCIATED WITH ALZHEIMER'S DISEASE AND INFLUENCE OF THE LOCAL STRUCTURAL MOTIF ON TAU FUNCTIONS

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A natively unfolded protein tau, which is encoded by the MAPT gene, regulates axonal transport and stabilizes microtubules in the human brain. Stabilization and binding to microtubules are enabled by the repeat domain and proline-rich domain of tau. On the other hand, pathologically modified tau causes tauopathies, which include Alzheimer´s disease (AD). The post-translation modifications – hyperphosphorylation and truncation lead to filament formation – paired helical and straight filaments are present in AD – that further build up tau neurofibrillary tangles. The presence of hexapeptides ^{275}VQIINK^{280} and ^{306}VQIVYK^{311} is known to cause protein aggregation [1]. However, the sequence ^{225}KVAVVRT^{231} in the proline-rich domain, which contains hydrophobic residues, is homologous to the mentioned sequences and could represent another sequence that promotes the formation of aggregates [2].

In the present work, we investigated the mechanism and kinetics of in-vitro aggregation of different forms of tau – tau297-391, tau306-391, tau316-391, and tau321-391 induced by heparin but also without its addition. Dynamic Light Scattering and Thioflavin T fluorescence were used to confirm the presence of aggregates and to monitor the kinetics of this process. The results of the experiment illustrate the time dependence and the influence of different conditions like the addition of heparin and DTT on the formation of aggregates. We found that forms tau316-391 and tau321-391 are able to aggregate despite the fact that they are lacking the presence of hexapeptides ^{275}VQIINK^{280} or ^{306}VQIVYK^{311} in their sequence. Furthermore, this project should provide morphological data of the obtained filaments using microscopic methods such as Atomic Force Microscopy and Electron Microscopy. We hypothesize a possible formation of the steric zipper like interfaces that could be formed by sequences ^{337}VEVKSE^{342}, ^{375}KLDF^{378} and ^{357}LDNITH^{362}. These sequences are forming interfaces in the structure of filaments isolated from patients with corticobasal degeneration [3, 4].

The project also focuses on the influence of the previously identified T-motif (^{220}TRE^{222}), which is located before the sequence ^{225}KVAVVRT^{231}, and its presence could affect the conformation of this sequence. It could have an impact on tau-microtubule binding as well [5]. To achieve our goals, we used the neuroblastoma cell line SH-SY5Y, into which we inserted a vector with mutation T220A that ensures the production of tau bound to a green fluorescent protein. Fluorescence Recovery After Photobleaching will be used to monitor the behavior of GFP-tau. The results of our work will further clarify the physiological function of the tau protein.

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P37

VISUALIZATION OF PHAGE PROPAGATION IN A *STAPHYLOCOCCUS AUREUS* BIOFILM

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Biofilm is a three-dimensional structure formed by cells embedded in the extracellular matrix. Bacteria in a biofilm are more resistant to antibiotics, proteases released by host defence cells, and other environmental stress factors. *Staphylococcus aureus* is a major human pathogen causing a wide range of diseases including hospital-acquired infections. The persistence of chronic and medical device-related infections caused by *S. aureus* is related to its ability to form biofilm. Phage therapy is an alternative approach for the treatment of infections caused by antibiotic-resistant bacteria. However, the details of how the phage propagates through the structured biofilm are largely unknown.

We use light-sheet fluorescent microscopy (LSFM) with an integrated microfluidic system. To visualize biofilm-forming cells we labelled *S. aureus* by red fluorescent protein mCherry constitutively expressed from a plasmid. The components of the biofilm matrix are labelled by dyes fluorescent in non-overlapping spectra.

To study the propagation of phage in a biofilm we utilise different approaches to induce fluorescence in phage-infected cells. First, we cloned reporter plasmids containing a gene for green fluorescent protein (GFP) controlled by phage late promoters into *S. aureus* cells. Second, we are trying to modify phage genomes using CRISPR-Cas10 targeting specific parts of phage genome and recombination with a template containing GFP gene. Furthermore, we are also attempting to directly label the phage P68 capsid by a fusion of GFP to its head fibre protein. Taken together, we will be able to monitor the propagation of the phage through *S. aureus* biofilms in great detail using LSFM.

P38

METADYNAMICS DRIVEN BY ALPHAFOLD OUTPUT

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AlphaFold is a novel method for 3D structure prediction based on deep learning. It uses a multiple sequence alignment of the modeled protein with its homologues for which amino acid sequences are known. These multiple sequence alignments intrinsically contain information about the 3D structure, in particular residue-residue distances. They are predicted and further converted into a structure model by AlphaFold. However, despite its success, AlphaFold has a limited capability to model the outcome of mutations, conformational changes, interactions with other types of molecules and other important phenomena. Here we used the output of AlphaFold in the form of inter-residue distance probability profiles to guide biomolecular simulations by metadynamics method. This approach was tested on folding of a mini-protein tryptophan cage. With parallel tempering metadynamics we were able to simulate multiple folding and unfolding events and to predict the temperature-dependent free energy profile in agreement with biophysical studies and reference simulations.

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P39

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 and crystal dehydration, SAXS is equipped with in-situ UV-Vis spectroscopy and a liquid chromatography system for SEC-SAXS. The setups enable easy access and fast turn-around of samples under different conditions, but also collection of high quality end-state data without further need for synchrotron data collection in many cases. CF Diffraction Techniques provides services in synergy with the other CFs on-site, therefore scientific questions can be quickly answered as they emerge from the experiments.

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P40

TO EACH THEIR OWN: OVERCOMING CHALLENGES IN STRUCTURAL CHARACTERISATION OF CLOSELY RELATED PROTEIN-PROTEIN COMPLEXES USING SINGLE PARTICLE CRYO-EM

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With an increasing demand for structural characterisation of large protein-protein complexes, single-particle cryo-EM has been gaining popularity over the recent years. Due to widespread access to high powered microscopes and the continuous development of user-friendly data processing software, the main bottlenecks limiting the success of structure determination by cryo-EM, are now sample preparation and vitrification. Here we describe the technical challenges we faced during the structural characterisation of two closely-related protein-protein complexes, both entailing the same trypanosome surface protein but different naturally occurring variants of human complement factor 3.

P41

TO HOMODIMERIZE OR TO HETERODIMERIZE: STORY OF 14-3-3 PROTEIN DIMER FORMATION

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14-3-3 is highly evolutionary conserved eukaryotic protein family, ubiquitously expressed in mammalian tissues. Importance of 14-3-3 protein in cell cycle and metabolism is supported by the number of interaction partners as over 1200 of them were discovered. [1] For the proper function of this rigid and highly helical protein its dimeric state is essential. [2]

7 mammalian isoforms of 14-3-3 are known: , and . These isoforms differ in their sequences, interactoms and expression levels in various tissues, whereas their structure and general biophysical properties are similar. It is known that isoforms can form hetero- and homodimers, but propensities of isoforms to dimerise were not quantified yet. In addition, information about chemical nature of this process is ambiguous across the literature. [3, 4] Characterization of mutual affinities of 14-3-3 isoforms is target of this work.

To study the isoform dimerization, we used fluorimetric experiments based on fluorescence resonance energy transfer (FRET), designed previously in our group. Such measurements provide insight into kinetics of dimerization including rate and dissociation constants. [5] Previously, our colleagues were able to characterise dimerization of isoform. In this study, we extended the knowledge to homodimerization of and heterodimerization of and . We focused on dimerization properties at different temperatures and NaCl concentrations. From the dependency of rate constant on temperature we determined Arrhenius parameters. On the other hand,we did not observe significant trend in the dependency of dimerization parameters on NaCl concentration, indicating low effect of ionic strength on dimer formation.

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P42

VIRAL CAPSIDS AS TOOLS FOR STRUCTURAL BIOLOGY

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The goal of structural biology is to elucidate the structure of bio-macromolecules in atomic detail. Until now, the method of choice was X-ray crystallography. However, its bottleneck is the preparation of diffraction quality crystals. Recently, a new method was developed, the cryo-electron microscopy (cryoEM) that relies on imaging individual molecules using electrons [1]. CryoEM can reach the atomic resolution by combining imagines of thousands of molecules, however, it is itself limited by the size of the sample analyzed. Too small (<100 kDa) bio-molecules are difficult to align to access the atomic resolution. We plan to prepare virus like particles (VLPs) and pentamers of viral major capsid protein (VP1) derived from mouse polyomavirus that would harbor target small proteins. In order to produce a versatile system, such a small protein would be an antibody fragment such as the cameloid nanobody that would be targeted against the protein of interest. Prepared VLPs and pentamers will be analyzed using cryoEM and protein crystallography to demonstrate their ability as a useful tool for structural analysis of small proteins in cryoEM. Up until now, we were able to design, optimize and crystallize a stable protein complex of VP1 pentamer with CFP fused to truncated VP3.

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P43

SEARCHING FOR THE SPECIFIC INHIBITOR OF S1-P1 NUCLEASE USING FRAGMENT SCREENING

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S1-P1 nucleases, hydrolases that cleave phosphodiester bonds of nucleic acids, are found in plants, fungi, bacteria and trypanosomatid [1]. They are dependent on divalent metals such as zinc, calcium or magnesium. These metals are usually found in the active site and form the trinuclear cluster. Another important part of the active site (NSB1) is responsible for binding the nucleobase and sugar group of the substrates [2]. It is theoretically possible to use structure-based inhibitor design in order to synthetize highly specific inhibitors of S1-P1 nucleases.

In order to study the binding of ligands to the S1-P1 nuclease active site the crystallographic fragment screening method was used as it has abundant applications in the discovery of suitable inhibitors and subsequent drug development. S1-P1 nuclease crystals were soaked in ligand solutions prepared using Frag Xtal Screen (Jena Bioscience) and then vitrified. These crystals were then subjected to X-ray structural analysis at the BESSY II synchrotron radiation source in Berlin [3]. The diffraction data were subsequently processed and the structures of the complexes were determined by molecular replacement. Binding of different fragments in the active site of the nuclease was analysed and compared to the known binding of native products.

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FRAGMENT-BASED CHARACTERIZATION OF SUBSTRATE FOR NOVEL FAD-DEPENDENT OXIDOREDUCTASE FROM *CHAETOMIUM THERMOPHILUM*

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Novel FAD-dependent oxidoreductase from lignocellulose-degrading fungus *Chaetomium thermophilum* (*Ct*FDO) has a potential for industrial fields processing the lignocellulosic biomass, the most abundant material in the world. *Ct*FDO is a member of glucose-methanol-choline (GMC) superfamily of oxidoreductases which act on hydroxyl groups of non-activated alcohols, carbohydrates and sterols. They share a two-domain character, conserved FAD-binding GxGxxG motif, and usually His-His or His-Asn catalytic pair in the active site accessible via a narrow tunnel or covered by a flexible loop [1].

The *Ct*FDO crystal structure reveals a unique His-Ser active-site pair in a large wide-open pocket further extended beyond the pyrimidine moiety of FAD. These features indicate a different type of substrate than what is common for GMC oxidoreductases. This was confirmed by *Ct*FDO enzymatic activity tests which, however, also excluded small lignin moieties and about 950 other compounds. To define the nature of the substrate, the crystallographic fragment-screening approach has been utilized. A series of six complexes led to identification of five binding subsites inside the active-site pocket with high preference for binding of aromatic moieties (Fig. 1). The conformational flexibility of interacting amino acids allows binding of compounds with a molecular weight greater than 500 Da. Our results indicate a complex polyaromatic nature of putative substrate, possibly with the character of larger lignin-building units [2] (Fig. 1).

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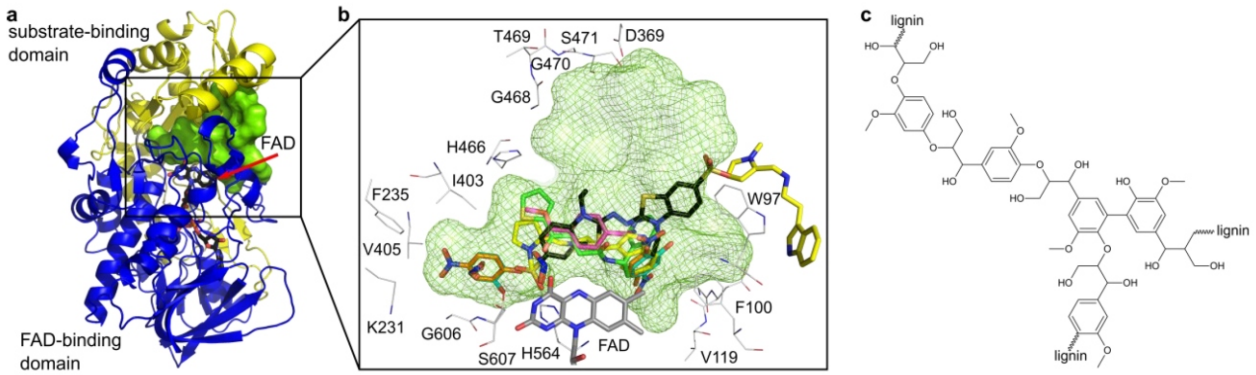

Figure 1. (a) Crystal structure of *Ct*FDO with color-coded substrate-binding (yellow) and the FAD-binding (blue) domains. FAD is shown as sticks with black C atoms and the active-site pocket with green surface. (b) Three-dimensional superposition of active sites of the *Ct*FDO complexes. Residues surrounding the pocket (green mesh) and FAD are shown as sticks with gray C atoms and the ligands with orange, cyan, black, yellow, green, and magenta C atoms. Graphics were created using PyMOL (Schrödinger). (c) Chemical structure of lignin.

P45

A HUNTING STRATEGY AND VIRION STRUCTURE OF *P. AERUGINOSA* BACTERIOPHAGE JBD30 REVEALED BY CRYO-ELECTRON MICROSCOPY

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Pseudomonas aeruginosa is a human pathogen, whose treatment is complicated by its frequent antibiotic-resistance. *Siphoviridae* bacteriophage JBD30 infects and kills bacterium *P. aeruginosa*, which makes it a potential agent for phage therapy. Here we present the structure of bacteriophage JBD30 virion and its replication strategy, revealed by the combination of cryo-electron microscopy analysis techniques and cryo-electron tomography.

The virion of bacteriophage JBD30 is composed of non-enveloped icosahedral capsid, long flexible non-contractile tail and baseplate decorated with tail fibers. The capsid with a diameter of 60 nm is built from major capsid protein organised in T = 7 icosahedral lattice and decorated on three-fold and pseudo-threefold axis with trimers of minor capsid protein. In one vertex of the capsid, the penton of major capsid protein is replaced by dodecameric portal. The portal complex forms an interface between the capsid and 180 nm long tail. The tail is built from 44 hexameric discs of major tail protein. Distal tail protein trimer follows-up the last tail disc and forms an attachment site for the long tail fibers. The baseplate is terminated with a tripod complex of receptor binding protein trimers.

Using cryo-electron tomography we followed the infection process of *P. aeruginosa* by JBD30 phage from attachment to bacterial cell, to the production of new phage progeny and host cell lysis. Bacteriophage JBD30 uses its long tail fibres for binding to *P. aeruginosa* pili type IV. After attachment to pili, the virion either diffuses or is pulled towards the cellular surface, where it irreversibly binds by its receptor binding proteins. Afterwards, the phage punctures the outer cellular membrane, degrades the peptidoglycan layer and injects its DNA into host cell. New phage progeny is released approximately after 80 minutes post infection.

The combination of cryo-electron microscopy methods allowed us, to propose the mechanism of key stages of phage infection and describe it at molecular level.

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CRYO-EM REFINEMENT AND MODEL BUILDING OF PROTEIN-RNA COMPLEXES

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Cryo-electron microscopy has proven to be a powerful and innovative technique in the field of structural biology. It has gained well-deserved popularity among the scientific community due to the relative ease of sample preparation, the ability to capture the molecules under study in different conformational states, and the ability to reconstruct their structure in a matter of days. Thus, it is not surprising that the cryo-EM field and technology are growing rapidly. Many different programs can be used in the workflow of the cryo-EM data processing and the researchers should be able to choose and use them correctly according to the unique requirements of the collected dataset. In presented work, we have studied mouse RNase III in complex with double-stranded RNA molecule using cryo-EM. The cryo-EM density maps have been obtained in relatively high resolution (locally up to 3.5Å resolution) thanks to the advanced workflow of data processing. The refinement software cryoSPARC and RELION were used to reconstruct the electron map as 3D heterogeneous refinement and local refinement of individual protein domains had a significant effect on the final resolution of the electron map. To build protein-RNA models, we used software Coot and the final structural refinement was performed using programs PHENIX and Isolde. My poster presentation highlights the latest advances in cryo-EM data processing and their direct usage in refinement of protein-RNA complexes.