Posters

P1

CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION ANALYSIS OF HUMAN CYTOMEGALOVIRUS UL144, AN HVEM ORTHOLOGUE

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Human cytomegalovirus (HCMV) is a -herpesvirus that has co-evolved with the host immune system to establish lifelong persistence. HCMV within its unique long (UL)/b' locus, encodes many immunomodulatory molecules, including the glycoprotein UL144 [1]. UL144 is a structural mimic of the tumor necrosis factor receptor superfamily member HVEM (TNFRSF14), which binds to the multiple cellular ligands, e.g, LIGHT, LT , BTLA, CD160, and gD [2, 3]. UL144, while being an HVEM orthologue, binds exclusively only BTLA, avoiding activation of inflammatory signaling initiated by CD160 in natural killer cells. Although, BTLA and CD160 cross-compete for HVEM, the structural basis for the UL144 ligand selectivity remains unclear [3].

The ectodomain of UL144 (wild-type, WT) contains a total of ten putative N-linked glycosylation sites. Many of them are not present in other viral species (e.g., Rhesus CMV UL144) suggesting interesting evolutionary consequences. However, due to high amounts of flexible N-linked glycans present in recombinant form of UL144-wt the glycosylation deficient mutant (UL144-DG) was generated and used for crystallization. Here, we report on UL144-DG crystallization by using standard vapor diffusion method. For the initial screening the high-throughput robotic system was set up with different commercial crystallization conditions (e.g., JCSG-plus, PACT premier or MIDAS plus). The protein solution of concentration > 3 mg/ml was mixed with precipitant in 1:1 or 2:1 ratio. The crystals suitable for X-ray diffraction measurement were observed in precipitant conditions containing 5-erythritol propoxylate or ethoxylate. Diffraction data were collected at macromolecular crystallography beamline P13 at DESY (Hamburg). The most promising UL144-DG crystals diffracted to 1.1 Å resolution.

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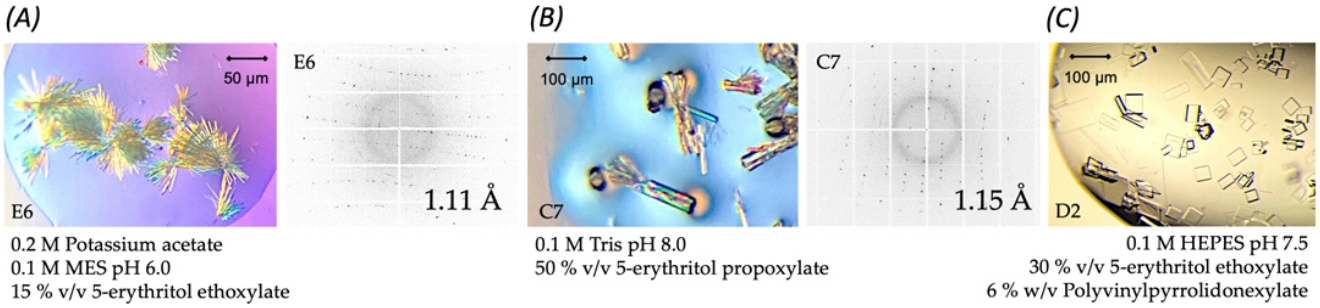

Figure 1. Crystals of HCMV UL144-DG (A-C) followed by X-ray diffraction images are shown. The crystals were obtained under above-mentioned crystallization conditions and tested for diffraction in DESY Hamburg.

P2

METHOD FOR ANALYSIS OF DNA INTERACTIONS USING PROBABILITY DENSITY MAPS

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A plethora of experimental and computational tools are used to improve the understanding of biomolecular structure-function relationships. At the intersection of theoretical and experimental approaches stand data analysis studies that use a large pool of structures available within structural databases, such as the PDB, to show patterns in large ensembles of macromolecular structures.

Here we plan to demonstrate how, using NtC's for local conformational description of nucleic acids [1,2], we can construct probability density maps for DNA fragments. Maps are constructed for relevant combinations of DNA building blocks and selected interacting atom/group [3]. These can be further examined or superposed to elucidate guiding patterns of interaction in biomolecular interfaces.

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P3

PREDICTION OF DNA HYDRATION BASED ON DATA MINING OF CRYSTALLOGRAPHIC STRUCTURES

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Water plays an important role in stabilizing DNA structure and in mediating its interactions. In our work, we utilize crystallographic data to compile the average hydration patterns around biomolecules - proteins [1,2,3] and nucleic acids [4,5,6]. Recently, we investigated hydration of DNA as a function of its conformation and sequence. We analyzed hydration of DNA dinucleotides from an ensemble of 2,727 non-redundant DNA chains containing 41,853 dinucleotides and 316,265 associated first-shell water molecules [6].

The dinucleotides were classified into categories based on their 16 sequences and the previously determined structural classes, so called nucleotide conformers (NtCs). The construction of hydrated dinucleotide building blocks allowed dinucleotide hydration to be calculated as probability of water density distributions. Peaks in the water densities - Hydration Sites (HSs) - uncovered the interplay between the base and the sugar- phosphate hydration in the context of sequence and structure.

Here, we present the overview of these results and the potential application of the the hydrated building blocks for the prediction of DNA hydration. The data for the hydrated building blocks and the predictions are available for browsing and visualization at the website watlas.datmos.org/watna.

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P4

CRYSTALLIZATION AND BINDING CHARACTERIZATION OF RECOMBINANT VARIANTS OF RHESUS CYTOMEGALOVIRUS GLYCOPROTEIN UL144

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Isoforms of CD160 interact with ligands through the immunoglobulin superfamily (IgSF) domain, including modest interactions with classical and nonclassical class I molecules of the major histocompatibility complex (MHC). Herpes virus entry mediator (HVEM), a known member of the tumour necrosis factor (TNF) receptor superfamily (TNFRSF), is one of the binding partners of CD160. CD160 and HVEM are the key regulators that exhibit multiple functional outcomes, including suppression of CD4+ T cell proliferation, interferon- (IFN-) production, increase cytokine production, promotion of lytic activity in NK cells [1, 2] and many others. Transmembrane glycoprotein UL144, encoded in the UL/b' genomic region of Human Cytomegalovirus (HCMV), is additionally homologous to HVEM. UL144 plays an important role in virus entry into the host cell using principles associated with HVEM. However, comparative studies revealed the ability of HVEM to activate NK cells to a higher degree than its viral counterpart UL144, which reflects the inability of UL144 to bind CD160. Interestingly, UL144 isolated from *Rhesus Cytomegalovirus* (RhCMV) interacts with both human and the rhesus CD160 with low affinity [3, 4] that represents evolutionary divergence between viral species.

We have further investigated into CD160 function and recombinantly prepared mutant UL144 that lacks all glycosylation sites. The binding to CD160 was observed with affinity comparable to that of soluble HVEM. It emphasizes the importance of post-translational modifications, including glycosylation. Moreover, the binding formation was observed by using co-immunoprecipitation assay (co-PI) between recombinant His-tagged UL144 variants (e.g., RhUL144-WT, UL144-WT, UL144-DG, UL144-N91) and human Fc-tagged CD160. To fully understand the molecular-structural basis of these interactions, more specific analyses will be completed in the future. In addition, we have performed the crystallization condition screening for all variants of UL144 alone (Fig. 1). Preliminary crystallization conditions were found and are being further optimized. The suitable crystals have been tested for X-ray diffraction. The structural data of all protein crystals are being optimized and processed for phasing.

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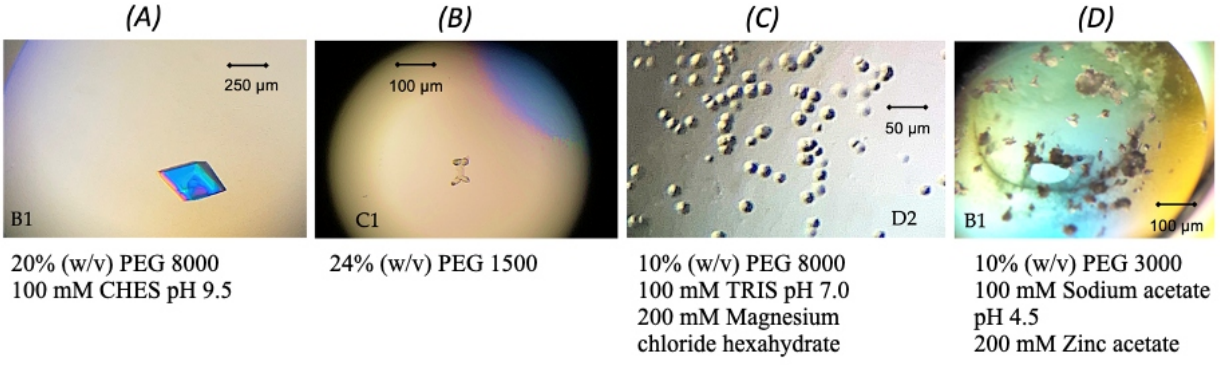

Figure 1. The crystals of RhCMV UL144-WT (A, B); HCMV UL144-DG (C) and HCMV UL144-N91 (D) were obtained under above-mentioned crystallization conditions with precipitant to protein ratio 1:2 in the drop.

P5

STRUCTURE OF phiKZ TAIL SHEATH AND TAIL TUBE

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The phage phiKZ is a jumbo bacteriophage that infects *Pseudomonas aeruginosa*. As in other myoviruses, its 200 nm-long tail is composed of an inner rigid tube and a contractile sheath. Upon adsorption to the bacterium, the tail sheath is contracted, pushing the tube against the bacterial wall. This movement makes a hole in the wall, which is used to eject the phage genome into the bacterium. Here, we present the structure of the tail sheath and tail tube a 3.5 Å resolution. They reveal a 6-fold and helical structure similar to other contractile-tailed phages. Each tail sheath protein has extensions that make contacts with 6 other copies, creating a mesh. The void space above and below each protein leave space for the contraction. In the tail tube, the arrangement of the protein in this rigid tube is more compact than in the tail sheath. Besides, each copy has long extensions that intertwined with the neighbouring copies. The structure of both the tail sheath and the tail tube protein is well conserved due to its importance in the phage cycle.

P6

STRUCTURE OF BSCX AND BSCY OF THE BORDETELLA TYPE 3 SECRETION APPARATUS

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The type 3 secretion system (T3SS), also known as an injectosome, is a widespread macromolecular nanomachine that enables the delivery of bacterial effector proteins directly from bacterial cytosol into the cytosol of the host cells. Most T3SS subunits are genetically and structurally conserved among different Gram-negative bacteria, but there are some additional components that are distinct and species specific. In the genus *Bordetella*, these include the small T3SS protein subunits BscX and BscY, homologous to the *Yersinia* YscX and YscY proteins. These proteins appear to be a part of the export gate, orchestrating the secretion of early substrates, but their structure and function in the *Bordetella* T3SS apparatus remain unknown. Using nuclear magnetic resonance (NMR) spectroscopy, we determined the solution structure of the heterodimeric complex of BscX and BscY proteins, revealing that the six helical fold of BscY is wrapped by BscX made up by the N-terminal unstructured region and three C-terminal helices. Individual *bscX* or *bscY* deletion mutants of *B. bronchiseptica* did not exert any cytotoxic activity towards HeLa cells, indicating that presence of the BscX-BscY heterodimer is critical for proper function of the secretion apparatus. Moreover, removal of the first 22 amino acids from BscX rendered the bacterial mutant non-cytotoxic on HeLa cells, indicating that the N-terminal unstructured region of BscX is functionally important. In summary, the BscX and BscY proteins are required for function of the T3SS apparatus and delivery of the BteA effector of *B. bronchiseptica* into host cells.

P7

SHORT TRUNCATED TAU FRAGMENT 321-391 AGGREGATES IN THE PRESENCE OF HEPARIN DESPITE THE LACK OF VQIxxK SEQUENCE

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Microtubule associated protein tau is the main actor of tau hypothesis of Alzheimer´s disease (AD). Tau belongs to intrinsically disordered proteins (IDPs) which do not acquire any stable secondary nor tertiary structure. Under pathological conditions of tauopathy, tau dissociates from microtubules to form insoluble filaments with disease specific fold. It was previously shown that the presence of two hexapeptide sequences (VQIxxK) can trigger the protein aggregation [1]. Recently it was shown that the AD specific fold of tau filament can be recapitulated in vitro by aggregation of truncated tau 297-391 (dGAE) [2].

It was long believed that aggregation prone sequences were responsible for tau aggregation. It begins to be apparent that sequences in R' region that follow tau MTBR repeats play a crucial role in MT binding and potentially also in aggregation process [3]. Tau oligomers that spread the specific disease fold strain may be the pathological agent of disease and their structural features remain still elusive.

Recombinant truncated tau proteins tau306-391, tau316-391, tau321-391 and tau326-391 were aggregated under different conditions (presence of heparin and DTT). Results of aggregation were monitored using different techniques: ThT fluorescence, DLS, AFM, FTIR and capillary electrophoresis.

We have observed in vitro aggregation of several tau proteins, mainly tau 321-391 which lacks the aggregation prone sequence VQIVYK (PHF6 epitope). Early signs of oligomer formation were observed by measurements using capillary electrophoresis. The results will further widen the knowledge about pathological aggregation of tau proteins.

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P8

BIOPHYSICAL TECHNIQUES AT CENTRE OF MOLECULAR STRUCTURE OF BIOCEV

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The biophysical research facility as a part of the Centre of molecular structure of Institute of Biotechnology provides shared resources of instruments for the determination of size, molecular mass, structure and stability of biomolecules, study of conformational changes and thermodynamics of temperature transitions and 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).

Following techniques and instruments 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).

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

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P9

STRUCTURAL VARIABILITY OF THE RIBOSOME EXIT TUNNEL CONSTRICTION SITE

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Ribosomes are ribonucleoprotein particles responsible for synthesis of a nascent peptide, also known as translation, wherein a sequence of messenger ribonucleic acid (mRNA) is translated into a sequence of amino acids. They are essential in all three domains of life - Archaea, Bacteria, and Eukarya. Although the critical parts of ribosomes are conserved across the domains, still there are sites where ribosomes differ.

The nascent chain is released from the ribosome through an exit tunnel located within the large ribosomal subunit and some difference between bacteria and higher organisms lie here. Numerous interactions occur between the nascent peptide and the tunnel walls, with the narrowest part formed by extended loops of two ribosomal proteins, namely uL4 and uL22. Additionally, uL4 and uL22 have globular parts on the surface of the ribosome, through which they can interact with other proteins associated with the ribosomes [1].

The roles played by the two domains of ribosomal proteins contributing to the tunnel walls and why these proteins evolved into their shapes are not fully understood. In this study, we address these questions by analysing a set of experimental ribosome structures from the Protein Data Bank. Our analysis includes root-mean-square fluctuation analysis to reveal the flexible and rigid sections of the proteins. Principal component analysis of Cartesian coordinates suggests that some elements are structurally correlated. In addition, sequence alignment complements our analyses by offering insight into the conserved sections of the proteins.

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P10

VIRAL TRANSCRIPTION-TRANSLATION COUPLING IN MAMMALIAN CELLS

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The coupling of transcription and translation (CTT) controls the gene regulation in bacteria. Recent cryo-electron microscopy (cryo-EM) studies showed the physical coupling of these two processes [1,2]. Interestingly, the double-strand DNA Vaccinia virus (VACV) performs the viral genome replication, transcription, translation, and assembly of virions in infected mammalian cells within discrete cytoplasmic foci called viral factories [3-5]. The initial rounds of viral transcription and translation during the early phase of infection occur inside the host cytoplasm. In intermediate and late-phase of infection the viral gene expression is carried out inside the viral factories in close association with host ribosomes [5].

Here, we seek to uncover the potential regulation mechanism of viral gene expression via viral-host CTT. The primary approach is to reconstitute the CTT *in vitro* and use single particle cryo-EM to uncover the detailed view of the structural architecture of the viral-host CTT. We aim to employ cryo-electron tomography and correlated light and electron microscopy (CLEM) to directly visualize the viral factories in a near-native state at a sub-nanometer resolution to confirm the existence of viral-host CTT directly in VACV-infected cells.

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P11

STRUCTURAL BASIS FOR ALLOSTERIC REGULATION OF MYCOBACTERIAL GUANOSINE-5'-MONOPHOSPHATE REDUCTASE

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Guanosine-5´-monophosphate reductase (GMPR) catalyzes conversion of GMP to IMP, the hub metabolite for the biosynthesis of all purine nucleotides. This reaction enables mycobacteria and most other organisms to utilize guanine nucleotides in production of adenine nucleotides without the need of *de novo* synthesis. In our studies of purine metabolism in mycobacteria, we use *Mycobacterium smegmatis* (Msm) as a model organism. Although GMPR is not essential either for Msm or *Mycobacterium tuberculosis* (Mtb) under normal conditions, it contributes to the regulation of the purine nucleotide pool by recycling GMP to IMP. In our recently published study [1] we show that the enzymatic activity of Msm GMPR is allosterically regulated by ATP and GTP. Whereas ATP inhibits the activity of Msm GMPR, GTP blocks this inhibition, and thus restores the activity of Msm GMPR. Here, we present an explanation of allosteric regulation of Msm GMPR with ATP and GTP at the molecular level. It is based on crystal and cryoEM structures of Msm GMPR with ATP and GTP. Msm GMPR forms tetramers with four-fold axis which further assemble into octamers. The two tetramers in the octamer adopt either compressed or extended conformation [1]. The changes in the conformation induced by the compression or extension are transferred through various loops to the active site. These changes then can affect the Msm GMPR activity. Our results show that the ligands trap the Msm GMPR octamer in either active or inhibited conformation.

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P12

CRYSTAL STRUCTURE OF -CARBONIC ANHYDRASE FROM *CANDIDA PARAPSILOSIS*

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Pathogenic yeasts of the genus Candida represent the most prevalent cause of mycotic diseases worldwide. They behave as opportunistic pathogens, which means that they can live in human hosts as harmless commensals, being kept under the control by the host immune system. One of the key survival strategies of fungal pathogens is the ability to proliferate in different carbon dioxide (CO_2) concentrations. CO_2 is among the most important gases for living organisms. In nature, the concentration of CO_2 is balanced by an interconversion to hydrogen carbonate (HCO_3, bicarbonate through the spontaneous reaction $CO_2 + H_2O = HCO_3 + H^+$). Its average amount required by organism is much greater than the amount produced spontaneously from CO2, bicarbonate production requires fine tuned regulation. For this reason, a highly diverse family of enzymes has evolved that are able to accelerate the interconversion reaction up to 10 000-fold. The members of this family, carbonic anhydrases (CAs), are able to catalyze reversible hydration of CO_2 to give a $HCO_3 + H^+$. CAs evolved in all three domains of life, and are divided into eight, evolutionarily unrelated classes (, and) that independently arose from different precursors during convergent evolution. ß-CAs are present in many pathogenic microorganisms but not in the mammalian hosts and therefore represent possible target for drug development.

We determined the crystal structure of CA form Candida parapsilosis at 2.6 Å resolution. It assembles as a tetramer, with the active site located at the interface between two monomers. At the bottom of the substrate pocket, a zinc ion is coordinated by the three highly conserved residues Cys76, His131 and Cys134 in addition to a water molecule. Activity assays of full length and truncated versions of CpNce103 indicated that the N-terminal arm is indispensable for enzymatic activity and crystal packing.

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P13

JOSEF DADOK NATIONAL NMR CENTRE

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Core Facility of High Field NMR Spectroscopy provides access to NMR spectrometers in the range of proton frequencies from 500 MHz to 950 MHz. The equipment is suited mainly to the studies of structure, dynamics, and interactions of biomolecules, i.e., proteins, nucleic acids, and carbohydrates. However, the instrumentation is flexible enough to cover also various research needs in material science, organic and inorganic chemistry, biochemistry, biology, and biophysics.

Available is the following instrumentation:

- NMR Spectrometer Bruker AVANCE NEO 500 MHz – available with a 5 mm nitrogen-cooled multinuclear cryoprobe (Prodigy), room temperature 5 mm dual broad-band probe (^{1}H, ^{15}N - ^{19}F), triple-resonance (^{1}H, ^{13}C, ^{15}N) 5 mm probe, 10 mm dual (^{1}H, ^{13}C) probe, and 4.0 mm solid-state dual CP/MAS probe.
- NMR Spectrometer Bruker AVANCE NEO 600 MHz equipped with a quadruple-resonance (^{1}H/^{19}F, ^{13}C, ^{15}N, ^{31}P) cryoprobe with -40 to 150°C temperature range.
- NMR Spectrometer Bruker AVANCE NEO 700 MHz for biomolecular applications, equipped with a triple-resonance (^{1}H/^{19}F, ^{13}C, ^{15}N) cryoprobe with -40 to 150°C temperature range and a triple-resonance (^{1}H, ^{13}C, ^{15}N) cryoprobe optimized for detection of ^{13}C, -40 to 80°C temperature range.
- NMR Spectrometer Bruker AVANCE NEO 700 MHz for multinuclear applications, equipped with a 5 mm dual broad-band probe, 5 mm dual inverse broad-band probe, 1.7 mm triple resonance (^{1}H, ^{13}C, ^{15}N) probe, 5 mm dual broad-band probe for diffusion measurements, 3.2 mm high-resolution solid-state triple-resonance (^{1}H, ^{13}C, ^{15}N) MAS probe, and 4.0 mm solid-state dual CP/MAS probe.
- NMR Spectrometer Bruker AVANCE NEO 850 MHz equipped with a triple-resonance (^{1}H/^{19}F, ^{13}C, ^{15}N) cryoprobe with 0 to 135°C temperature range and a triple-resonance (^{1}H, ^{13}C, ^{15}N) cryoprobe optimized for detection of ^{13}C, -40 to 150°C temperature range.
- NMR Spectrometer Bruker AVANCE NEO 950 MHz equipped with a triple-resonance (^{1}H/^{19}F, ^{13}C, ^{15}N) cryoprobe, -40 to 150°C temperature range.
- X-Band (9,75 GHz) EPR spectrometer Bruker EMX nano.
- Bioreactor system for NMR studies of proteins/nucleic acids in living mammalian cells

Services provided:

- Measuring of NMR spectra at magnetic fields from 11.75 T to 22.32 T (corresponding to proton frequencies from 500 MHz to 950 MHz) for compounds identification and quality control.
- Consultations concerning the choice and setup of multidimensional NMR experiments, data processing and spectra evaluation according to the users' needs.
- Structure analysis of organic compounds by NMR spectroscopy.
- Studies of structure and dynamics of proteins and nucleic acids by NMR including measurement and processing of multidimensional spectra of proteins with up to 5 dimensions using non-linear sampling.
- Mapping of minor conformations (invisible states) by relaxation dispersion.
- Studies of interactions using STD (Saturation Transfer Difference) spectroscopy.
- *In vivo* conformational studies of biomolecules in cell.
- Investigation of material structure by solid state NMR spectroscopy of ^{13}C, ^{31}P, ^{29}Si, and ^{27}Al.
- The centre is available to academic user from around the world for free or a fraction of the operation cost through CIISB, iNEXT-Discovery, instruct ERIC, and Alliance4Life grants. The project proposals can be submitted through the web pages of the respective grants.

https://www.ciisb.org/open-access/proposal-submission
https://inext-discovery.eu/submit-proposal/
https://instruct-eric.org/
https://alliance4life.ceitec.cz/core-facility-database-and-proposal-submission/

Industrial users can access the services of the Centre on commercial bases.

P14

ELECTRON TRANSPORT MECHANISMS IN PROTEIN JUNCTIONS INVESTIGATED BY COMPUTATIONAL TECHNIQUES BASED ON DENSITY FUNCTIONAL THEORY

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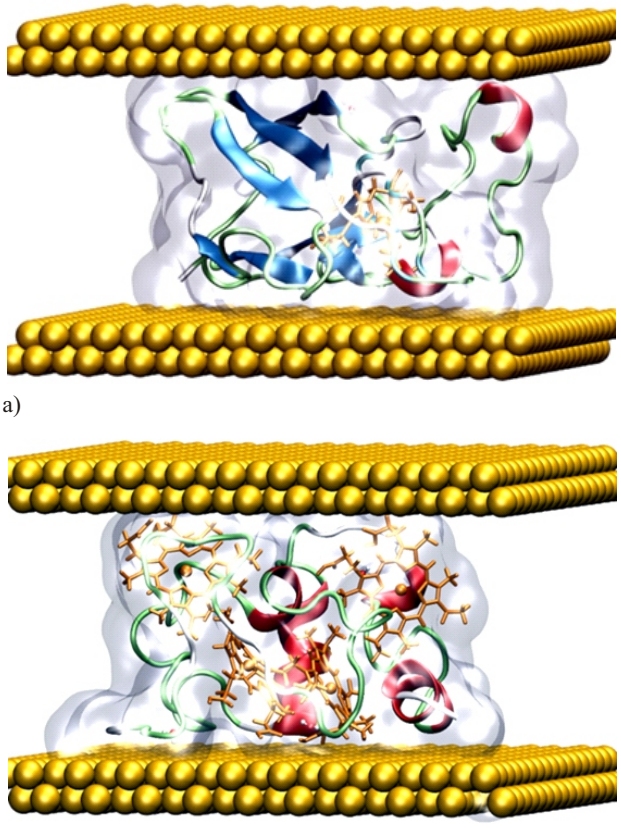
Electron transfer facilitated by redox-active proteins is utilized in various biological processes, including photosynthesis, respiration cycle, or denitrification reactions. Blue copper proteins such as Plastocyanin or Azurin and the heme-containing cytochromes often participate in these redox cascades. Recently, these proteins started to be utilized in nanobioelectronic devices due to their suitable electron-transfer properties. However, non-expected physical phenomena were observed when the proteins were incorporated between metal contacts or electrodes. While in a native aqueous environment, the electron flow through the system of redox sites proceeds by the thermally activated hopping mechanism, the temperature-independent currents of relatively high magnitudes were detected on protein/metal junctions [1, 2]. These data suggest that the electrons on the bio/metallic interfaces and junctions are transferred by the coherent tunneling mechanism, independently of the redox-active states.

We investigate these electron-transport phenomena by means of computer simulations based on classical molecular dynamics (MD) as well as the first-principles description within the framework of density functional theory (DFT) [3, 4]. While the incoherent hopping could be studied by combined quantum-mechanical/molecular-mechanical (QM/MM) techniques [5], the coherent tunneling requires a quantum description of the whole interface models. Recently, we applied these methodologies on Azurin blue-copper protein and on small tetraheme cytochrome (STC), which were previously studied experimentally. We showed that the transport mechanism in both Azurin and STC junctions between gold electrodes is the coherent tunneling facilitated by valence-band states of the proteins. In contrast to their redox properties in solution, the presence of the metal cations in the protein structures is not essential for their conductivity on the metal interfaces. The reason for this drastically different behavior in solution and on the metal interfaces is the significant electronic-level misalignment between the protein and metallic states [6].



a)

b)

Figure 1. Structure of (a) the blue-copper protein Azurin, and (b) STC gold junctions.

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P15

STRUCTURE OF TICK-BORNE ENCEPHALITIS VIRUS IMMATURE PARTICLE SOLVED BY CRYO-ELECTRON MICROSCOPY AND SUB-TOMOGRAM AVERAGING

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Tick-borne encephalitis virus (TBEV) is an enveloped virus belonging to the family *Flaviviridae*. It is mainly transmitted by ticks and causes severe disease of central nervous system in humans. Virion surface is covered by envelope proteins (E-protein), that are together with the membrane proteins (M-protein) anchored in virus lipid bilayer. During the viral life cycle, the immature non-infectious virus undergoes a maturation process. This process includes proteolytic cleavage of prM and major reorganization of the envelope proteins on the viral surface.

To determine the structure of immature TBEV particles, we purified them from infected tissue culture cells and used cryo-electron microscopy for visualization. In comparison with smooth mature TBEV particles, the immature particles have "spiky" surface formed by the E-protein-prM-protein complex. We combined cryo-electron tomography and sub-tomogram averaging with single-particle analysis methods using localized reconstruction of the surface "spikes", to determine the high-resolution structure of the immature E-protein complexes and their interaction with the prM-protein. The organization of the particle surface indicates that the TBEV maturation mechanism involves a complex reorganization of the envelope proteins on the viral surface.

The results show more detailed insight in the viral maturation process which may be targeted by specific antiviral drugs.

P16

STRUCTURES OF LA JOLLA VIRUS AND MOTTS MILL VIRUS INFECTING *DROSOPHILA SUZUKII*

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Drosophila suzukii is an invasive insect pest causing severe economic damage to fruit crops. Chemical pesticides are inefficient in controlling the spread of *D. suzukii*, therefore other strategies need to be deployed. One promising alternative is the utilization of entomopathogenic viruses. Advantage of viruses as a biopesticide is that they are host specific and have limited impact on non-targeted organisms.

Here we present cryo-EM structures of two such candidate biopesticides, La Jolla virus (LJV) and Motts Mill virus (MMV). Both, LJV and MMV, have small non-enveloped icosahedral capsids enclosing +ssRNA genomes. We have reconstructed virion and empty particle of LJV with respective resolutions 3.0 Å and 3.3 Å, and virion of MMV with resolution 2.7 Å.

LJV has been preliminary classified as an iflavirus and its overall virion structure follows the typical picorna-virus-like pattern. The three capsid proteins VP1, VP2 and VP3 form a pseudo-T3 icosahedral shell with the diameter ~300 Å. Unlike the previously characterized iflaviruses of honeybees, LJV capsid does not contain surface domains or decoration proteins. The interpentamer contacts are stabilized by the N-termini of VP1 and VP2. These N-termini were not resolved in the empty particle resulting in the reduction of interpentamer contacts and expansion of the particle.

MMV is related to solemoviruses and tombusviruses. The capsid is arranged with icosahedral T3 quasi-symmetry with three copies of the coat protein forming the icosahedral asymmetric unit. The architecture of the virion is stabilized by the bivalent cation-mediated interactions of the coat proteins.

P17

RimM AND RsfS RIBOSOMAL FACTORS REGULATE THE TRANSLATION IN BACTERIA

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It is crucial for bacteria to save energy when stressed or the access to nutrients is limited. Since synthesis of proteins is an important energy draining source, bacteria rely on slowing down the protein synthesis in harsh conditions [1]. The ribosomal silencing factor (RsfS) has a significant role in stalling the protein synthesis by binding the 50S ribosomal subunit and inhibiting the assembly of 70S ribosome [2]. Meanwhile, the ribosomal maturation factor RimM is vital for the maturation of the 30S small ribosomal subunit. Moreover, during stress RimM is highly enriched in the hibernating 100S dimerized ribosome to stall the protein synthesis and preserve energy [3]. In this work, we use a combination of biochemical studies and cryo-electron microscopy to elucidate the close relationship between RimM and RsfS, and their regulation capabilities on translation.

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This study was supported by LL2008 project with financial support from MEYS CR as a part of the ERC CZ program (to G.D.).

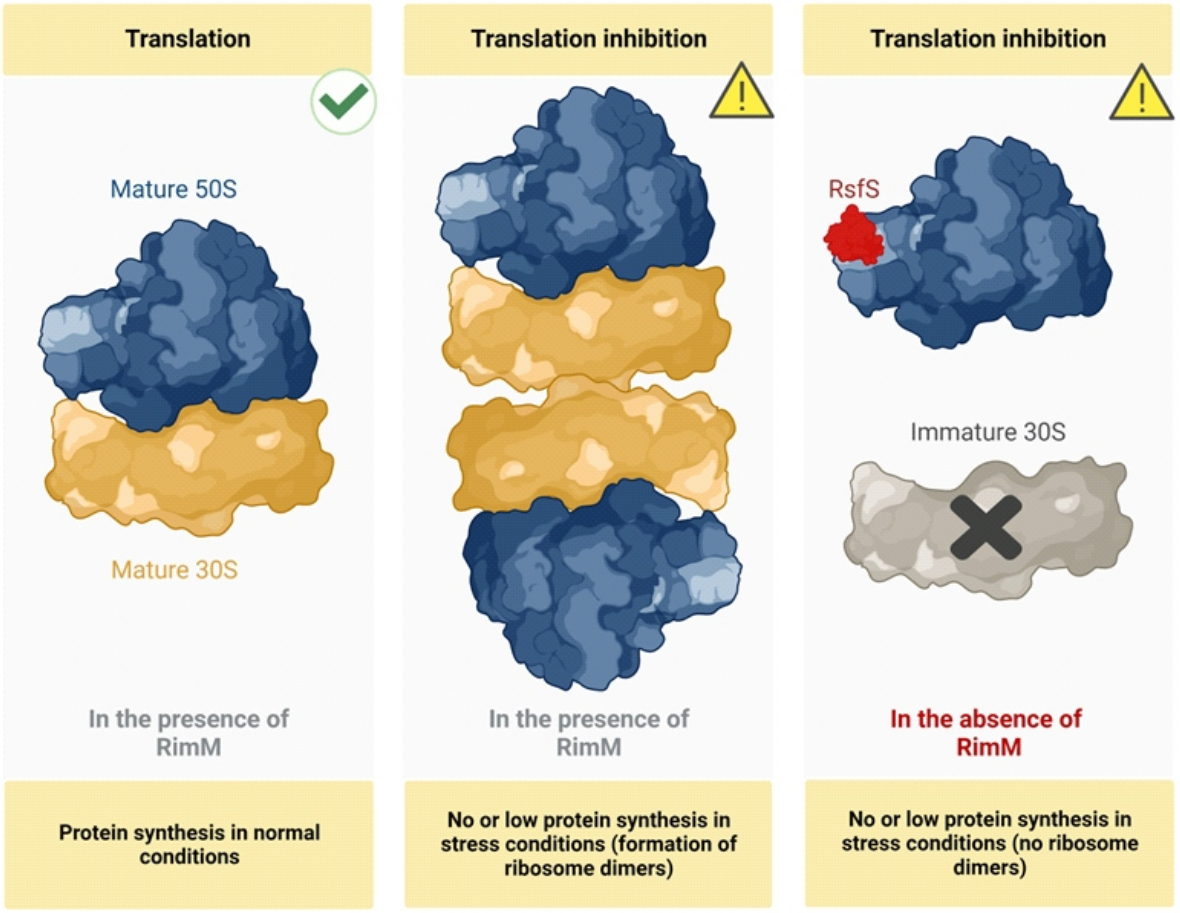

Figure 1. In normal conditions, bacteria with intact 70S ribosomes perform regular protein synthesis. Under stress or nutrients deprived conditions, bacterial 70S ribosomes in presence of RimM dimerize to 100S to stall protein synthesis. Deletion of RimM results in accumulation of RsfS to inhibit the assembly of 70S ribosome.

P18

CONFORMATIONAL CHANGES UPON PHOSPHORYLATION OF PROLINE RICH REGION OF TAU(210-240) PEPTIDE USING MOLECULAR DYNAMIC SIMULATION

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The conformational dynamics of intrinsically disordered proteins (IDPs) regulated by post-translational modifications (PTMs) such as phosphorylation is challenging to elucidate. A well-known IDP Tau is found hyper-phosphorylated in Alzheimer's disease (AD) in humans [1]. The proline-rich motif of tau(210-240) peptide directly interacts with proteins such as BIN1, 14-3-3 etc. Microsecond time scale, all atoms molecular dynamic (MD) simulation studies have been performed for apo and four phosphorylated (212^{P}Thr, 217^{P}Thr, 231^{P}Thr, 235^{P}Ser) tau(210-240) peptide using three different temperature variants (278K, 298K and 310K) and two different force field parameters (AMBER99SB-ILDN and CHARMM36m) with TIP4PD water model as these force fields parameters combine with water model worked the better for IDPs found from our group previous studies [2, 3]. These four-phosphorylations cause increase in compactness of the peptide. The binding of associated proteins like BIN1 with tau may alter by the strong salt bridges, forming nearby lysine and arginine due to the phosphorylation [4]. Phosphorylation induces a strong structural transition, with tau(210-240) favouring a bent conformation. The MD simulation results were verified using NMR experimental parameters like chemical shift and ^{3}J-coupling. The experimental part has been carried out by our collaborator Prof. Isabelle Landrieu.

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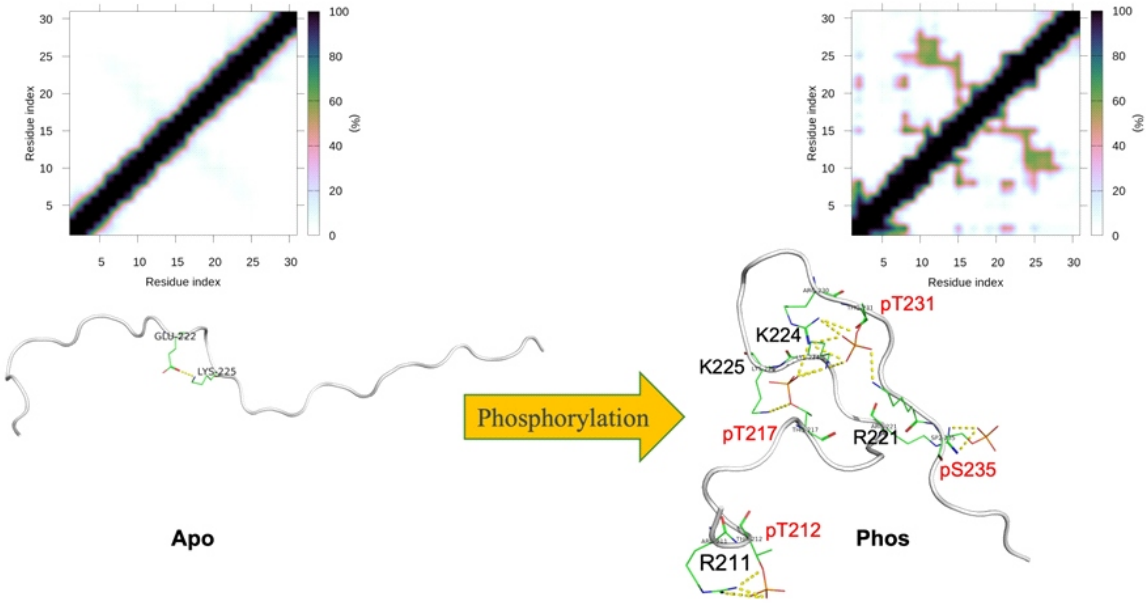

P19

THE ROLE OF CALCIUM AND 14-3-3 IN THE REGULATION OF HUMAN UBIQUITIN LIGASE Nedd4-2

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Human neuronal precursor cell-expressed developmentally down-regulated 4-2 (Nedd4-2) ubiquitin ligase is a ubiquitous member of the family of HECT ubiquitin ligases that targets and selects different proteins for endocytosis and/or proteasome degradation. Its targets are mostly integral membrane proteins – ion channels or receptors. Any defects in this enzyme cause the development of different pathophysiological conditions and among them mostly investigated are Liddle syndrome, electrolytic imbalance, hypertension, respiratory distress, cardiovascular conditions and cystic fibrosis. This HECT ubiquitin ligase has three different types of domains: N-terminal C2 domain, four WW domains and the C-terminal HECT domain, together connected with unstructured flexible regions. Described manners of regulation have been cytosolic levels of calcium and 14-3-3 proteins, but the specific mechanism and circumstances are not completely clear. In the phosphorylated state, the modified amino acids of Nedd4-2 act as a binding site to different inhibitors and activators. In our previous research, we found that in the case of the adaptor protein 14-3-3, the phosphorylated residues are pSer342, pThr367 and pSer448, all of which are surrounding the WW2 domain [1]. When Nedd4-2 is forming the complex with 14-3-3, WW3 and WW4 domains are more rigidly bound, while the WW1 domain (adjacent to the C2 domain) and the HECT domain have shown increased mobility, which may facilitate catalytic activity [2].

The neglected part of this enzyme is the C2 domain, a domain activated by binding cytosolic calcium ions and responsible for binding to different lipids, for example those present in the cell membrane or vesicles. In that way, this interaction enables the other parts of this protein, especially the WW domains, to get near to different target molecules. What our focus was in this study is to demonstrate how 14-3-3 has an inhibitory effect on the function of C2 domain, by using the hydrogen-deuterium exchange and liposome-binding assays. Additionally, by combining aforementioned results with the data obtained from analytical ultracentrifugation, small angle X-ray scattering and mass photometry, we were also able to on some basic level structurally characterize the mechanism of inhibition. In total, this is proof that 14-3-3 has another way to influence not only the activity but also the subcellular localization of this protein. What we hope to achieve next is to try to switch the focus on only structural characterization of this complex, by either crystallography or cryo-EM, which would provide a better and more refined understanding of its regulation.

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P20

AVIAN ORTHOREOVIRUS NON-STRUCTURAL PROTEINS NS AND NS - STRUCTURE AND FUNCTION

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The fusogenic avian Orthoreovirus (ARV) infection can cause considerable economic losses in the poultry industry, mostly infecting young chickens. The ARV has been associated with various disease conditions in poultry (enteric and respiratory diseases, myocarditis, hepatitis, stunting-malabsorption syndrome, and the most important one viral arthritis/tenosynovitis) [1]. The ARV are non-enveloped icosahedral particles of 85 nm external diameter with 10 dsRNA genomic segments (23.5 kb) encased within two concentric protein shells, forming the outer capsid and the core [2].

Reoviruses' RNA replication and morphogenesis occur exclusively within cytoplasmic inclusion bodies, also known as 'viroplasms' or viral factories (VF). VF are globular, dynamic, phase-dense, cytoplasmic inclusions lacking membranes or cellular organelles. VFs are formed by abundant viral non-structural (NS) proteins and structural proteins recruited into VF by interaction with NS proteins. NS proteins are expressed inside the infected cells but are not part of the mature virion. The two most abundant VF proteins are NS and NS. NS is a 70 kDa protein that is forming viroplasm inside infected cells and attracts and associates with other viral proteins including 41 kDa RNA chaperone NS [3]. NS is causing specific RNA-RNA interaction between all 10 genomic segments specifically by destabilizing of RNAs helical regions [4]. There is missing information about a fashion of coupling of NS with NS protein needed for the understanding of viroplasm formation mechanism.

Only a low-resolution structure of NS has been established while no structural information is available for NS. This is chiefly due to the poor solubility of NS and polydispersity of NS which forms oligomers ranging dimers to octamers and RNA containing filaments [4]. To tackle these problems and obtain structural information we have generated various fusion constructs for expression, purification, and further structural study by X-ray crystallography and cryo-electron microscopy. Due to their multi-facet role in virus biology, the detailed knowledge of their structure is necessary for a better understanding of their functions and could provide the rationale for the development of new antiviral drugs.

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P21

PAIRED REFINEMENT IN CCP4i2

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The final decision on the high-resolution diffraction limit can be based on results from paired refinement [1]. This routine is computationally demanding because it involves a lot of refinement cycles and multiple analyses of agreement between multiple models and data. The method was implemented in the program *PAIREF* [2,3]. Recently, we developed an algorithm that suggests the verdict on high-resolution diffraction limit of the data.

Program *PAIREF* is distributed within the *CCP4* software suite [4]. It can be launched from the command line. This option provides the possibility to use two refinement engines: *REFMAC5* [5] and *phenix.refine* [6]. The users of the *CCP4* can launch the jobs in the *i2* graphical interface (see Figure 1). The interface provides access to a comprehensive log file in HTML form.

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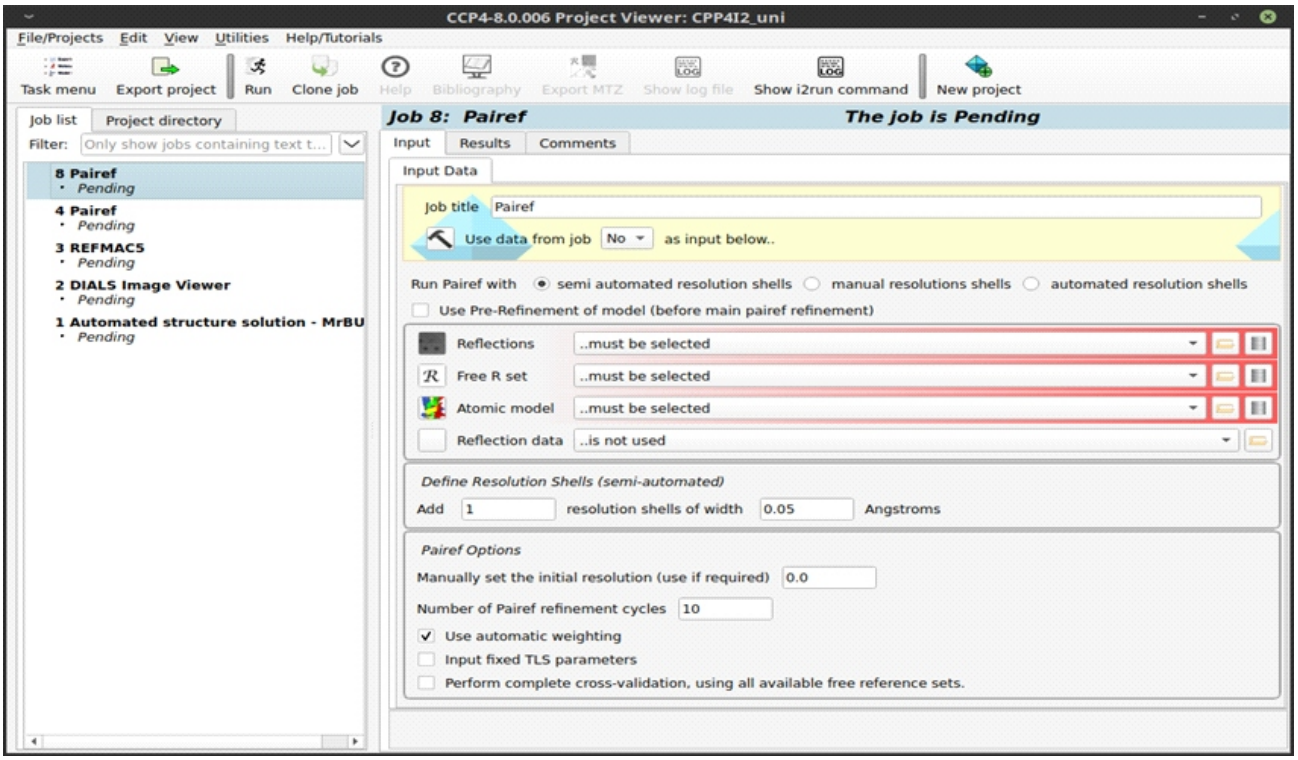

Figure 1. The view of the *PAIREF* project window in *CCP4i2*.

P22

STRUCTURE DETERMINATION OF ASK1 USING CRYO-EM

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ASK1 (apoptosis signal-regulating kinase 1) is a member of the protein family MAP3K which triggers p38 or JNK dependent signaling pathways leading to inflamation or apoptosis [1,2]. ASK1 dysfunction has been implicated in neurodegenerative, cardiovascular and oncogenic diseases [3,4,5] and, thus, ASK1 represents a prospective drug target. However, key aspects of its activation mechanism remains unclear. To better understand the principle of ASK1 activation, we investigated the structure and oligomeric behavior of N-terminal part of ASK1. Here, we present the structure determination of this N-terminal part of ASK1 using cryo-EM including sample optimization, strategy to manage the preferred orientation of particle, data analysis with local 3D classification and refinement to overcome the heterogeneity of the complex.

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P23

MOLECULAR AND STRUCTURAL STUDY OF FERRITIN II SECRETED FROM *IXODES RICINUS*

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Ferritin is a ubiquitous protein with a crucial role in tick biology. Ticks digest large amounts of host blood and are exposed to an enormous amount of free iron, which has to be treated properly to avoid its toxicity. Two types of ferritin were discovered in the tick *Ixodes ricinus* – tagged as ferritin I and ferritin II [1]. Ferritin I is a globular protein composing 24 subunits (25kDa each) and forming a hollow-sphere complex. [2; 3]. Ferritin I functions as an intracellular scavenger of potentially toxic free iron and is capable to sequester up to 4 500 iron atoms [2]. The function of ferritin II is not entirely clear, but it probably plays a role in the transport of non-heme iron between the tick gut and the peripheral tissues. Silencing of ferritin II using RNA interference had a detrimental effect on tick development and reproduction [1]. The vaccination of mammalian hosts with recombinant ferritin II revealed its promising potential as an efficient anti-tick vaccine [4].

This study focuses on the molecular, biochemical and structural characterization of ferritin II from *Ixodes ricinus.* We have cloned ferritin II into three *E. coli* expression vectors (pET100, pET-SUMO and pASK-37+), and optimized its production in various expression cells and conditions (e.g., temperature, times and concentrations of inducer). To obtain enough amount of pure recombinant ferritin II for

following structural studies, we will concentrate on improving protein isolation and purification.

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P24

FINE TUNING OF THE MULTICOPPER OXIDASE ACTIVITY THROUGH THE PRECISE MUTATION OF ITS ACTIVE SITE VICINITY

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Enzymes belonging to blue multicopper oxidase family use four copper ions localized in two spatially separated sites to oxidize many different natural and artificial substrates and transfer electrons to oxygen. Due to this ability, multicopper oxidases are intensively studied and also used for many applications in biotechnology. For example, laccases are utilized in food industry for color enhancement, cork modification or brewing [1].

Our research is focused on bilirubin oxidase (BOX) from *Myrothecium verrucaria* (EC: 1.3.3.5) which is already used in medical diagnostics (e.g. level of bilirubin in blood). For its high redox potential and its ability to, based on reaction conditions, selectively oxidize chemically distinct types of substrates BOX is in the focus of the development of biosensors [2] and biofuel cells [3].

In our previous study, we kinetically characterized BOX activity towards four different substrates (ferrocyanide, ABTS, bilirubin and 2,6-dimethoxyphenol). We obtained the structure of BOX in complex with ferricyanide which is one of its products (PDB ID: 6I3J) and explained the role of the unique tryptophan – histidine covalent adduct present in its active site [4]. Based on these results and also on two new structures of complexes with BOX inhibitors we designed, expressed and enzymatically characterized several functional mutants of BOX targeting the vicinity of the active site. Interestingly, different point mutations have diverse effects on the activity of BOX toward the aforementioned substrates with some of them leading to an increase of activity. These recent results present a solid base for further optimization of BOX activity through mutagenesis and its future possible utilization in biotechnology.

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P25

SERIAL CRYO-FIB-SEM MICROSCOPY OF BIOLOGICAL VOLUMES

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FIB-SEM serial block face milling (SBFM) and imaging is a volumetric technique for 3D reconstruction of cells and tissue at the ultrastructural resolution. The sample preparation for standard SBFM requires embedding of the specimen into resin block which is a lengthy multi-step process. In addition, the procedure involves sample dehydration and staining with heavy metal ions which may adversely affect the structure of the biological samples. Direct imaging of the cryo-fixed samples (prepared by e.g. high-pressure freezing) [1, 2] provides an alternative for sample image at near-native state which offers simple sample preparation workflow and does not involve sample processing steps which would influence the sample structure. Thanks to the recent developments in the field of high-resolution SEM imaging, the cryoFIB-SEM imaging of high-pressure frozen biological specimens can be used for 3D volume reconstruction at ultrastructural resolution. Here, we demonstrate the potential of cryo-FIB/SEM block face milling for reconstruction of the whole cells.

The cells were cultured, and resuspended in cultivation medium. The suspension was vitrified by high pressure freezing on 3mm carrier. Subsequently it was transferred to FIB-SEM microscope (Helios Hydra 5 CX, ThermoFisher Scinetific) equipped with cryo-stage. For the data acquisition the sample was milled out in the shallow angle 38 degrees with respect to the sample plane. This geometry increases detection rate of secondary electrons and improves image contrast. Milling was done by Oxygen plasma ions (30kV, 1,7nA). Oxygen ions create milling face with low surface distortion (curtaining). For imaging we used immersion mode with deceleration voltage that results of electrons landing energy 2kV. The selected voltage balances the charging artefacts visible on higher voltages and insufficient contrast induced by lower voltage. The consecutive milling and imaging was orchestrated by Auto Slice & View 5 (ThermoFisher Scientific). Images were processed by line-by-line high pass filter, histogram normalization and the dataset where drift corrected by AMST algorithm [3].

We have acquired data for the reconstruction of the whole INS-1E cell (volume 6,786um^3 , voxel size 10x10x10nm) in telophase and carried out volume segmentation to analyse the level of detail which can be obtained from the data.

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P26

PHAGES EXPRESSING FLUORESCENT PROTEINS AS TOOLS TO UNVEIL THE MYSTERIOUS PHAGE-BIOFILM INTERACTION

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One of the major worldwide health concerns is the increasing occurrence of antibiotic-resistant bacteria. Furthermore, bacteria have the ability to form biofilm, a multicellular structure embedded in a polymer matrix. Biofilm enhances bacterial resistance to the immune system and antibiotics. Biofilm eradication is a challenge which opens an opportunity to develop new approaches such as phage therapy. [1,2]

We focus on visualisation of infection of *Staphylococcus aureus* biofilm by bacteriophage phi812. The objective is to genetically modify phage phi812 so that it expresses fluorescent proteins in infected cells for the purpose of observation by fluorescence microscopy.

The strategy of the genetic modification lies in the homologous recombination of the phage genome with a vector containing a coding sequence for a fluorescent protein. Bacterial defence mechanism CRISPR-Cas10 will be used for targeting the unmodified regions of the phage genome.[3]

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P27

EXPLORING ISOFORM-SELECTIVE INHIBITION OF CARBONIC ANHYDRASES WITH MOLECULAR DOCKING

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Carbonic anhydrase (CA) is a zinc-containing metalloenzyme that catalyses the reversible hydration of carbon dioxide to bicarbonate and proton. The human genome contains 15 isoforms of carbonic anhydrase [1]. Unlike other CA isoforms essential for cellular and tissue homeostasis, CA IX features mainly in cancer, making it an interesting target for developing new anti-cancer drugs [2]. Although a wide variety of carbonic anhydrase inhibitors is commercially available, selective inhibition of individual isoforms remains challenging due to the conservation of catalytic domain and high structural similarity among different isoforms [3]. In our study, we used computational chemistry methods to identify potential selective ligands to target CA IX specifically. We screened various small molecules against the binding site of CA IX using molecular docking and analysed the binding energies and interactions. The identified compounds have the potential to serve as lead molecules for the development of new drugs that selectively target CA IX in cancer cells.

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P28

FUNCTIONAL IMPACT OF SPECIFIC N-TERMINAL PHOSPHORYLATION ON TRPC5 ION CHANNEL ACTIVITY

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The Transient Receptor Potential Canonical 5 (TRPC5) receptor is a non-selective calcium permeable ion channel that functions as a polymodal cellular sensor highly expressed in sensory neurons, kidney, and brain [1]. TRPC5 activation is associated with inflammatory, mechanical, and spontaneous pain. Recently, it has been shown that selective inhibition of TRPC5 in mice results in pain relief [2], making TRPC5 a potential target for developing new pain treatments. However, the TRPC5 mechanism of activation in the context of cellular processes is not well understood. Although it is known that post-translational modifications are important for TRPC5 channel gating and plasma membrane trafficking [3], only a few have been reported so far. Here, we focused on two phosphorylation sites at the membrane-proximal part of the N-terminus that were previously identified by mass spectrometry, but their functional impact on channel gating is unknown. The phosphorylation state was mimicked by substitution of serine with aspartate (phospho-mimicking) or alanine (phospho-null). Using the patch-clamp technique in the whole-cell configuration, we evaluated the membrane currents induced by voltage and agonist. Individual substitu-

tion of both serines with aspartate significantly slowed the onset of agonist response compared with wild-type (WT) TRPC5. Alanine substitution of S193 caused a gain-of-function phenotype whereas the S195A mutation did not significantly alter the channel response. These findings indicate that phosphorylation of these sites mediates the attenuation of TRPC5 channel activity. We performed molecular dynamics simulations of WT and the phosphorylated state of the channel and observed that phosphorylation at S193 alters the interactions with neighbouring subunit in close proximity to this residue. Taken together, our results suggest that the N-terminus of TRPC5 contains a previously unrecognized site of functional regulation by post-translational modification.

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P29

OXADIAZOLE-BASED INHIBITORS OF HISTONE DEACETYLASE 6 ARE HYDROLYZED UPON ENZYME ENGAGEMENT

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Zinc-dependent histone deacetylases (HDACs) play critical roles in numerous (patho)physiological processes and thus serve as targets of therapeutic interventions in cancers and neurological diseases. HDAC inhibitors used in clinic typically contain a hydroxamate zinc-binding group (ZBG) yet the use of alternative ZBGs, including heterocyclic oxadiazoles, can confer to higher isoenzyme selectivity and more favorable pharmacological profiles. We co-crystallized HDAC6 and an oxadiazole-based inhibitor to shed light onto inhibitor recognition by the enzyme. Surprisingly, the crystal structure of the HDAC6/inhibitor complex revealed unexpected oxadiazole ring hydrolysis converting the parent oxadiazole into an acylhydrazide that chelates the active-site zinc ion of the enzyme. An identical cleavage pattern was also observed *in vitro* using the purified HDAC6 and QM/MM calculations corroborated kinetic in-solution experiments and provided the detailed reaction scheme of the hydrolysis, including structures of all intermediates and corresponding energy barriers. Overall, our data provide evidence that oxadiazole warheads can be efficiently transformed in active sites of target metallohydrolases into compounds with distinct selectivity and inhibition profiles.

P30

ANALYSIS OF INSULIN IN PANCREATIC CELL BY CRYO-CORRELATIVE LIGHT-ELECTRON MICROSCOPY

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Insulin is a peptide hormone produced in pancreatic beta cells which is responsible for glucose metabolism regulation in mammals.

Insulin molecules are stored in 200-300 nm single membrane granules inside cytoplasm of beta cell. As we and others previously demonstrated [1, 2], insulin molecules in secretory granules are organized into crystalline particles.

We have used correlative light-electron microscopy workflow under cryogenic conditions to characterize insulin granules in the cellular context and under near native conditions.

Rat beta cells INS 1E were seeded onto golden electron microscopy grid with carbon foil and incubated overnight to allow them to adhere on the grid surface. Adhered cells were fluorescently stained with live cell imaging dyes (nucleus, insulin granules and cell membrane) and plunge

frozen into the liquid ethane. Vitrified cells were transferred into the cryo-plasma focused ion beam scanning electron microscope (PFIB-SEM) with integrated light microscope. Optical data of the cell of interest were obtained and correlated with SEM data.

Cellular lamella in the region of insulin fluorescent signal was prepared by xenon plasma FIB to the final thickness 200 nm. Optical data in the polished lamella were collected to verify the presence of insulin granules. Grids with lamellae were subsequently tranferred into transmission electron cryo-microscope operating for cryo-electron tomography. Collected electron tomography data confirm crystalline of the secretory granules.

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P31

PATHOLOGICAL TAU PROTEIN FIBRILS – STRUCTURE AND CELLULAR CONTEXT

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Tau protein fibrils are aggregated polymers of pathological forms of tau proteins present in the brains of Alzheimer's disease (AD) patients and patients suffering from other types of neurodegenerative tauopathies. Tau pathology spreads between neurons and propagates misfolding in a prion-like manner throughout the brain, leading to significant neuronal death. Despite the advancements in understanding tau pathology, the relationships between initial tau misfolding, the formation of fibrils, pathology propagation across connected neurons, and subsequent cytotoxicity on the level of individual neurons remain unclear.

Recently cryo-EM showed disease-specific structures of tau fibrils isolated from patients' brains depending on the type of tauopathy [1, 2]. Moreover, heparin-induced recombinant tau fibrils, frequently used as model filaments for AD research, formed different types of filaments [3]. These findings show that tau protein can adopt structurally distinct conformations depending not only on the tau protein variant (isoform, phosphorylation, truncation) but also on buffer conditions (ionic inducers, pH).

This project addresses the question of tau fibril structure on two levels; *in-vitro* and *in-situ*. We try to understand what tau modifications and external conditions cause tau protein fibrilization, what kind of structure these fibrils adopt, and which conditions lead to the formation of AD-specific fibril topology. We also investigate the ultrastructural aspects of the intake and seeding of various tau fibril variants directly inside neurons.

In this poster, we present an ongoing systematic screening of full-length 2N4N tau isoform fibrilization conditions (type of buffer, temperature, presence or absence of agitation, and additives) monitored by Thioflavin T assay, negative stain EM, AFM, and cryo-EM. We also show preliminary data from *in-situ* cryo-ET of AD-tau fibril intake by neurons.

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P32

CF PROTEIN PRODUCTION IN CENTRE OF MOLECULAR STRUCTURE: PROTEINS FOR YOUR RESEARCH

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The Protein Production core facility (CF) of the Centre of Molecular Structure (CMS) provides all steps of protein production. These include the cloning of target genes in expression vectors, their possible site-directed mutagenesis and heterologous expression in *Escherichia coli* expression systems, as well as the subsequent purification of the corresponding proteins (Figure 1).

We offer both traditional cloning using restriction enzymes, or restriction free (RF) methodologies. You can provide us with your templates or we can order them from external companies. Subsequently we can deliver and test a number of our plasmids. Furthermore, we perform small scale expression and solubility tests with more than twenty *E. coli* strains under different conditions. It is also possible to order large scale production and purification for your protein, expression of which was already optimised.

For protein purifications we use various kinds of affinity techniques (Strep-Tactin XT, immobilized metal chelate affinity chromatography - IMAC, both on FPLC or in gravity flow setup) and chromatography techniques (ion-exchange chromatography and/or size exclusion chromatography using Superdex 75 or 200, 10/300 increase or HiLoad 16/600 columns).

Last but not least we offer optimization of expression or purification protocols and protein identity and purity testing. Customizations of our standardized protocols are possible and preparations according to your established protocols are welcome.

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

Figure 1. Laboratory of CF Protein Production in CMS.

P33

CLOUDIFICATION OF THE RAW CRYO-EM DATA AND METADATA USING iRODS

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Service cryo-EM facilities are responsible for acquisition of a considerable portion of the single particle and cryo-electron tomography data generated worldwide. Each cryo-EM experiment usually produces at minimum 0.5 – 2 TB of raw data that first need to be made available to the researcher (the data owner) and afterward should eventually be made publicly available with proper annotation. In addition, it is nowadays a standard practice that both single particle and cryo-ET data are pre-processed on-the-fly to gather information about data quality and fasten the downstream data analysis. The service facilities thus need to invest and maintain additional computational and storage resources apart from the electron microscopy instrumentation. We have developed a workflow that facilitates raw data management and runs the on-the-fly data analysis on remote high-performance computing (HPC) resources. Our workflow is based on an engine accessible directly from the microscope computer via a web browser, which harvests the metadata and starts the data transfer and analysis in parallel with the initiation of the data acquisition. A federated cloud solution based on iRODS was selected to carry out the data transfer to the storage close to an HPC center, where the data are submitted to Relion, CryoSparc, or Scipion pipeline. The results of the data analysis are collected to update the data acquisition parameters if necessary. Subsequently, the data are made discoverable to the owner for the subsequent data analysis or the raw data are later transferred to different storage within the iRODS zone for archival. In parallel, the metadata can be made publicly available through EUDAT.

P34

MOLECULAR MECHANISM OF GENE EXPRESSION INDUCED BY LINCOSAMIDE ANTIBIOTICS TARGETING THE 50S RIBOSOMAL SUBUNIT

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Bacterial Antibiotic REsistance ABC ATPases F (ARE ABC-F) are cytosolic proteins conferring resistance to 50S ribosomal subunit binding antibiotics via target protection mechanism. Expression of all previously characterised ARE ABC-F genes that confer resistance to peptidyl transferase centre targeting antibiotics lincosamides, streptogramins A and pleuromutilins (LS_AP) is regulated by ribosome-mediated attenuation in response to these antibiotics, however the molecular mechanism is lacking. Considering that antibiotic-driven attenuation generally requires sequence-dependent translational arrest, we would expect the same also for LS_AP-driven attenuation. The aim of our research is to shed light on the mechanism of LS_AP-induced translation inhibition using ribosome profiling techniques (toeprinting and high-throughput inverse toeprinting) and single particle cryo-EM and based on these data, to clarify the expression regulation of ARE ABC-Fs in pathogens and antibiotic producers.

P35

DUAL SPECIFICITY OF PLL2 LECTIN

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Lectins are proteins able to reversibly bind glycoconjugates with high specificity. Lectins mediate cell-cell interactions on molecular level and play an important role in various biological processes, including interactions between microorganisms and hosts [1]. Our research is focused on studying lectins from entomopathogenic bacterium *Photorhabdus laumondii*, which is known for complicated life-cycle, including mutualism and pathogenicity towards two different invertebrate hosts [2].

This contribution is focused on the PLL2 lectin. PLL2 is a member of structuraly related PLL lectin family with shared seven-bladed beta-propeller fold. Multiple binding sites are present within PLL2 monomer and are situated in between the blades. Based on their structural characteristics, binding sites can be divided into two groups; hydrophobic (H) and polar (P) [3]. We determined a set of X-ray structures of PLL2 in complex with various saccharide ligands. The analyses showed different ligand specificity of H and P sites. Dual specificity is not very common in lectins, and the existence of two sets of sites with different specificity within lectin monomer is rather unique and was not described outside the PLL lectin family.

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P36

CRYSTALLIZATION OF PROTEINS AND NUCLEIC ACIDS AT THE CENTRE OF MOLECULAR STRUCTURE, IBT CAS

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

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

P37

REGULATION OF IL-24/IL-20R2 COMPLEX ASSEMBLY VIA PHOTOXENOPROTEIN ENGINEERING

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Many biological processes depend on protein-protein interactions (PPI) and thus the on-demand regulation of the binding affinity between interacting partners offers exciting possibilities in both basic and applied research. Human Interleukin 24 (IL-24) is a multifunctional cytokine that associates with cell membrane receptors and plays critical roles in oncogenesis, immune response, host defense and tissue homeostasis. Since the optical control of PPI is advantageous over other approaches, particularly in terms of temporal and spatial resolution, we have developed a strategy based on genetic code expansion technology for the OFF-to-ON switch of the binding between IL-24 and one of its receptors, IL-20R2, by light. Introduction of the photocaged non-canonical amino acid ortho-nitrobenzyl tyrosine at selected positions of IL-20R2 largely inhibits heterocomplex formation as determined by microscale thermophoresis and yeast display. Irradiation with UV light (365 nm), which removes the caging group and reconstitutes the canonical tyrosines, restores the native binding strength between IL-24 and IL-20R2. We envision that photocaged IL-20R2 may become a useful tool for the photo-control of the JAK/STAT signalling cascade.

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P39

TOWARDS STRUCTURAL STUDIES OF hnRNP PROTEIN COMPLEXES

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HnRNP (heterogeneous nuclear ribonucleoprotein) proteins are a group of RNA-binding proteins that play a crucial role in the processing, transport, and translation of mRNA in eukaryotic cells. They bind to pre-mRNA and facilitate the splicing of introns, the processing of 3' and 5' ends, and the assembly of mRNA-protein complexes. HnRNP proteins can form higher order complexes, such as hnRNP:RNA complex sedimenting at 40S, which was identified already almost 60 years ago. The 40S particle is primarily composed of hnRNP C1/2, hnRNP A1/B2, and hnRNP B1/A2 proteins and was hypothesized to play a role in stabilization of pre-mRNA transcripts representing a functional analog of DNA nucleosome. Nevertheless, the exact function and structural arrangement of the 40S still remains elusive. Our aim is to structurally characterize the 40S particle using combination electron cryo-microscopy (cryo-EM) and mass spectrometry data. We have optimized protocols for the purification of A1, C1 and C2 hnRNPs from the bacterial expression system to assemble 40S sub-complexes, particularly the hnRNP C1/C2 tetramer. We have also prepared HEK293 cell lines stably producing labeled hnRNP proteins for purification of the whole 40S particles.

P40

MASS SPECTROMETRY FOR PROTEIN STRUCTURE ANALYSIS

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Structural mass spectrometry (MS3D) is a fast growing field on analytical chemistry representing a new approach for protein structural studies. In CMS, the tools of structural mass spectrometry, including native mass spectrometry, hydrogen-deuterium exchange, chemical cross-linking and other labelling method, are well established and allow to look beyond the edge of traditional structural techniques. The structural mass spectrometry core facility is equipped with state-of-the-art instrumentation such 15T FT-ICR, timsToF Pro, timsToF CSP, automation system for HDX, UPLC and nanoUPLC systems. Besides the MS3D, the core facility offers other services including identification and quantification of proteins, precise determination of protein molecular mass, characterization of various posttranslational modifications (phosphorylation, glycosylation, acetylation …).

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P41

STRUCTURE OF THE PHAGE LUZ19 INFECTING MULTI-ANTIBIOTIC RESISTANT *PSEUDOMONAS AERUGINOSA*

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The challenging treatment of infections caused by multi-antibiotic-resistant bacteria is an increasingly pressing health concern. Phage therapy is a promising alternative to conventional antibiotics. Phages are naturally occurring viruses that specifically target and lyse bacteria.

We used cryo-electron microscopy to gain insights into the process of phage LUZ19 infection of a clinically relevant *P. aeruginosa* strain. The phage LUZ19 virion consists of a 43,5 kbp dsDNA genome packaged into a 640 Å wide icosahedral capsid. The capsid is composed of major capsid protein, head cement protein, and flexible head decoration proteins. The internal core, portal, and tail occupy one of the capsid vertices and serve for genome delivery into the bacterial cell. A dodecameric portal complex medi-

ates the symmetry mismatch between the five-fold symmetric capsid vertex and the four-fold symmetric core complex. The tail has 280 Å in length and is differentiated into a dodecameric adaptor and a hexameric tail nozzle. It is further decorated by six flexible tail fibers. [ZC1] The whole tail has a channel passing through it that is employed to transfer the genome from the head. The tail fibers consist of a proximal arm, elbow, and distal arm facing the capsid. The research on the phage LUZ19 structure broadens our understanding of phage infection, to fight *P. aeruginosa* infections.

The research of the phage LUZ19 structure broadens our understanding of phage infection, to fight *Pseudomonas aeruginosa* infections.

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

P42

JUMPCOUNT: ESTIMATION OF UNCERTAINTIES IN BIOMOLECULAR SIMULATIONS

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Increasing number of biomolecular simulations provide sufficiently long simulations to predict equilibrium constants and associated free energies of simulated processes such as protein folding or protein-ligand binding. The equilibrium constant of a process of a transition from the state A to state B can be estimated as the fraction of times spent in B and A. Here we present a simple, yet exact, method to calculate the errors of the free energy estimations, which is based solely on temperature and the number of transitions from A to B and B to A. The 95% confidence interval of free energy calculated from a trajectory with one A to B and one B to A is the value ± 9.1 kJ/mol (2.2 kcal/mol) at 300 K. For two A to B and B to A transitions it is the value ± 5.7 kJ/mol (1.4 kcal/mol). Markovianity of the process is the prerequisite of the method. The error can be calculated online by our tool at jumpcount.cz.

The work was supported by Czech Science Foundation (22-29667S and 19-16857S) and the Ministry of Education, Youth and Sports of the Czech Republic (LM2018140, LM2018131).

P43

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.

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P44

IMAGING PROCESS OF PHAGE INFECTION OF *S. AUREUS* BIOFILM BY LIGHT SHEET FLUORESCENCE MICROSCOPY

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Staphylococcus aureus is a major human pathogen causing wide-range of diseases including infections acquired in hospitals. The persistence of *S. aureus* infections is related to its ability to form biofilms. Bacteria in biofilm are more resistant to antibiotics and to the host immune system [1]. Extracellular matrix components play an important role in unique lifestyle and virulence of biofilms [2]. Phage therapy is alternative approach to treat infections caused by antibiotic-resistant bacteria.

We used light-sheet fluorescent microscope with an integrated microfluidic system to study the formation of *S. aureus* biofilm and its infection by phages. To visualize the biofilm-forming cells, we modified *S. aureus* to stably express red fluorescent protein mCherry. The main components of biofilm matrix, such as extracellular DNA and polysaccharide intercellular adhesins, were labelled by specific fluorescent dyes. We introduced different phages into the mature biofilm and used time-lapse monitoring to detect their effect on biofilm disintegration.

Light-sheet fluorescent microscope with microfluidic system and time-lapse monitoring enabled us to detect the distinct stages of biofilm formation and the impact of phage infection on the biofilm development.

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P45

ANALYSIS AND SAMPLING OF MOLECULAR SIMULATIONS BY ADVERSARIAL AUTOENCODERS

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This research is motivated by acceleration. Molecular simulations make true possibility to simulate the motion from small molecules to big proteins and their combinations in drug-target complexes. It let us to predict their changing confirmation, their stability and a plenty of other properties thanks to the evolution of molecular structure. However, application of molecular simulations is affected by the large computational costs in computing steps that must be in order of femtoseconds, to assure numerical stability to integrate Newton equation of motion. Taking into account this limitation, a typical molecular dynamics simulation is capable to sample only a small fraction of the states available to the simulated system, with the likely catch or unlikely loss of some slow or rarely occurring processes, where likelihood depends on the simulation time. There are numerous techniques to address this limitation and to speed up simulations. Metadynamics is an enhancing method based on biasing Hamiltonian of the system that helps to cross barriers and go head through new unexplored free energy surface areas, thanks to some selected internal coordinates, so called collective variables. Choosing correct collective variables to make metadynamics successful is not a trivial task and it depends first of all on a knowledge and expertise of the user. In last few years there are emerging opportunities for machine learning and artificial neural networks in this field. We decided to develop an adversarial autoencoder as a tool to analyse simulation data and to support user to derive good collective variables to enhance molecular dynamics simulation. The potential of this platform is demonstrated on Trp-cage folding.

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P46

IN-SITU CRYO-ELECTRON TOMOGRAPHY OF ENTEROVIRUS REPLICATION

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Enteroviruses, a large group of non-enveloped picornaviruses, cause a range of human diseases, from the common cold to poliomyelitis. The molecular mechanisms of enterovirus replication and virion assembly are not well understood.

Here, we present the cryo-FIB milling and cryo-electron tomography of Echovirus 18-infected cells to characterize the late stages of enterovirus infection *in situ*. We describe rearrangements of cellular membranes that enable the formation of viral replication factories. Furthermore, we have identified different virus particle assembly intermediates – capsid segments, open and complete capsids, and progeny virions. Some of the virus particles and assembly intermediates were localized in the cytoplasm, whereas others were bound to membranes. We have employed template matching using the structures of the whole virus capsid and a capsid missing three pentamers to identify the complete genome-containing and empty virus particles and virus assembly intermediates, respectively. The particles have been further analysed by subtomogram averaging and classification.

Our results provide insight into the virus arrangement during the late stages of the replication cycle of enteroviruses. Additionally, the employed subtomogram averaging pipeline presents a further analysis of viruses and large molecular complexes *in situ*.

We acknowledge the Cryo-electron microscopy and Tomography Core Facility of CEITEC MU for their support in data collection and analysis.

P47

STRUCTURE OF THE PRE-REACTION COMPLEX OF FUCT

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Helicobacter pylori is a severe human pathogen associated with several gastrointestinal disorders, including gastric inflammation, ulcers, and cancer. However, its primary treatment using antibiotics has become increasingly more challenging because of the emergence of antibiotic resistance. As a result, there is a growing need for novel antibacterial agents [1]. In order to design such new compounds, it is necessary to thoroughly understand the fundamental biochemical processes that could serve as therapeutic targets. The purpose of this study is to elucidate the enzyme structure of *H. pylori* glycosyltransferase FucT. FucT is an essential enzyme in the biosynthesis of type II blood group antigens that are thought to mask the bacteria from the host's immune system. It consists of an N-terminal domain with the glycosyltransferase activity and a C-terminus that is responsible for dimerisation and, presumably, acceptor binding. Unfortunately, current experimental structures are monomeric and lack much of the carboxyl terminus which was removed as it hindered crystallisation efforts for X-ray studies. As a result, the crystal structures retained only 20 % of the original enzyme activity. The experimental structure of the fully active dimer complex remains unknown [2].

To resolve this limitation, we have decided to utilise a computational approach using Alphafold2. Alphafold2 is an *ab initio* structure prediction program capable of predicting several possible protein structures based on its primary sequence. While the program provides an internal ranking of these structures, it remains unclear if the arrangement of the polypeptide chains is optimal even in the best-ranking prediction. Using Alphafold2, we have obtained the protein dimer predictions for 3 sequences of FucT differing in their C-terminus length. We have performed molecular mechanics simulations on every structure prediction for each of the three sequences to select the most optimal structure for each one. Next, we studied the effect of the C-terminal length on the dimer complex stability.

The best of these structures will be used in the future for state-of-the-art hybrid QM/MM simulations. The aim is to determine the transition state geometry of the reaction which could become the starting point for the rational drug design of transition state mimetics for new *H. pylori* treatment options.

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