Czech Society for Structural Biology
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Book of abstracts

1st Student Conference in Structural Biology

Organized by the Czech society for Structural Biology
and the Institute of Biotechnology of the Czech Academy of Sciences

held online on

2-3 June 2021

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The recent years have witnessed an encouraging growth of high-quality research realized in the field of structural biology in the Czech Republic. Students and young scientists play an indispensable role in this. We realize that the new generation of young scientists needs, amongst others, support in the form of events such as this one – the 1st Student Conference in Structural Biology of the Czech Society for Structural Biology. The original impulse to initiate such a virtual meeting was based on the special situation in science in connection with the restrictions to physical meetings. Yet, the response of the scientific community came as a surprise, with the program being promptly filled with interesting and inspiring science. Twenty-two student presentations span from protein production to advanced structure analysis techniques, integrative approaches, computational analysis and software development. We would like to acknowledge the support of the Council of Scientific Societies of the Czech Republic to this Conference and in particular to the student competition.

Jan Dohnálek, Jarmila Dušková, Martin Malý and Michal Strnad

Czech Society for Structural Biology
Institute of Biotechnology of the Czech Academy of Sciences

Organized by the Czech Society for Structural Biology (Česká společnost pro strukturní biologii, z. s.), Vídeňská 1083, 14220 Prague, Czech Republic, https://cssb.structbio.org/, CSSB@structbio.org
with support from the Institute of Biotechnology of the Czech Academy of Sciences, Průmyslová 595, 25250 Vestec near Prague and the Council of Scientific Societies of the Czech Republic (Rada Vědeckých Společností ČR, z. s.), Národní 3, 11720 Prague

PROGRAMME

Wednesday, June 2

13:00 - 13:05 Introduction **Jan Dohnálek**

Chairperson: **Vladimír Sklenář**

13:05 - 13:20
Kristýna Adámková *(Institute of Biotechnology, Czech Academy of Sciences)*
Structures of S1 nuclease complexes at atomic resolution reveal details of RNA interaction with enzyme in spite of multiple crystal defects

13:20 - 13:35
Stefan Djukic *(Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences)*
Structure-assisted drug discovery: purine nucleoside phosphorylases

13:35 - 13:50
Barbora Kaščáková *(Institute of Chemistry, University of South Bohemia)*
Ixodes ricinus Serpins

13:50 - 14:05
Filip Melicher *(CEITEC, Masaryk University)*
Structural determination of lectins from *Photorhabdus spp.*

14:05 - 14:20
Pavel Pohl *(Institute of Physiology, Czech Academy of Sciences)*
Structural glimpse into 14-3-3 protein dependent regulation of ubiquitin ligase Nedd4-2

14:20 - 14:35
Elżbieta Wątor *(Małopolska Centre of Biotechnology, Jagiellonian University)*
Half way to hypusine. Structural characterization of human deoxyhypusine synthase

14:35 - 15:05 Break

Chairperson: **Jiří Pavlíček**

15:05 - 15:20
Darya Peramotava *(University of South Bohemia in v České Budějovice)*
Initial characterization of small protease inhibitors from *Ixodes ricinus*

15:20 - 15:35
Petra Havlíčková *(Faculty of Science, University of South Bohemia in České Budějovice)*
Production of TBEV NS5 protein for structural and functional studies

15:35 - 15:50
Katarína Hešková *(Slovak Academy of Sciences, Institute of Neuroimmunology)*
Flexible platform for production of proteins of a quality and quantity suitable for structural studies.

15:50 - 16:05
Aditya Chaudhari *(Institute of Biotechnology, Czech Academy of Sciences)*
In solution site-specific time-resolved structural biology of the light sensitive transcription factor EL222 monitored by infrared spectroscopy with vibrational probes

16:05 - 16:20
Raju Mandal *(Faculty of Science, Charles University)*
Structural basis of interaction between Forkhead box O4 (FOXO4) and p53

16:20 – 16:35
Karolína Honzejková *(Faculty of Science, Charles University)*
Role of thioredoxin in regulation of Apoptosis signal-regulating kinase 1 (ASK1)

Thursday, June 3

Chairperson: **Tomáš Obšil**

13:00 - 13:15
Martin Malý *(Institute of Biotechnology, Czech Academy of Sciences)*
Looking for the optimal resolution cutoff with PAIREF GUI

13:15 - 13:30
Ondřej Schindler *(Faculty of Science, Masaryk University)*
Partial atomic charges for proteins

13:30 - 13:45
Aliaksei Chareshneu *(CEITEC, Masaryk University)*
NAChRDB: Solving the puzzle of structure-function relationships to clarify the allostery of nicotinic acetylcholine receptors (nAChRs)

13:45 - 14:00
Zhengyue Zhang *(CEITEC, Masaryk University)*
Understanding Physical Effects of Phosphorothiation in RNA Context with Computational Methods

14:00 - 14:15
Marco Klepoch *(Institute of Biotechnology, Czech Academy of Sciences)*
Towards data standardization for biophysical data

14:15 - 14:30
Francesco L. Falginella *(CEITEC, Masaryk University)*
A multifaceted computational study to unravel the role of the C-terminal DEP domain of Dishevelled protein in Wnt signalling pathway

14:30 - 15:00 Break

Chairperson: **Jan Dohnálek**

15:00 - 15:15
Lucie Valentová *(CEITEC, Masaryk University)*
Structure of *Pseudomonas aeruginosa* infecting bacteriophage JBD30 solved by cryo-electron microscopy

15:15 - 15:30
Zuzana Trebichalská *(CEITEC, Masaryk University)*
Elucidating the life cycle of human enteroviruses *in situ*

15:30 - 15:45
Ján Bíňovský *(CEITEC, Masaryk University)*
Baseplate structure of bacteriophage phi812 reveals mechanism of cell wall binding and penetration

15:45 - 16:00
Marta Šiborová *(CEITEC, Masaryk University)*
Virion structure and mechanism of genome delivery of bacteriophage SU10 from the family *Podoviridae*

16:00 - 16:30 Break

16:30
Evaluation of student presentations, announcement of prizes
General assembly of CSSB

Structures of S1 nuclease complexes at atomic resolution reveal details of RNA interaction with enzyme in spite of multiple crystal defects

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Nucleases from the S1-P1 family are zinc-dependent mostly single-strand preferring phosphoesterases, which can be found in many species. They often cleave RNA and ssDNA with different rate and sometimes also in different pH optima. Crystal structures of four members of this family are already known as well as some of their complexes with products of DNA cleavage. These structures helped us understand substrate binding in the active site of S1-P1 nucleases and propose the mechanism of ssDNA cleavage, nevertheless, up to date there is no structural information about binding of RNA cleavage products [1].

In order to reveal possible differences in binding and cleavage between RNA and DNA, we setup co-crystallization trials of S1 nuclease with various ligands. From this experiment we obtained two structures of S1 nuclease in complex with product-inhibitor bound in the active site: complex with 5′-mononucleotide cytidine-5′-monophosphate (S1-CMP) at 1.04 Å resolution and with mononucleoside uridine (S1-URI) at 1.06 Å resolution.

Due to the atomic resolution of both structures, we found suspicious peaks of electron density, uninterpretable as alternatives to model or molecules of solvent, which led to the detection of a multiple lattice translocation defect (LTD). LTD is a form of crystal disorder with well-ordered crystal layers being mutually displaced, manifested by strong non-origin peaks in Patterson map and by nonspecific peaks in electron density. Correction of LTD is based on the elimination of the effect of displaced layers as previously described in Wang *et al.* [2]. We extended this approach to correct for multiple dislocated layers in a single crystal. Precise data correction and atomic resolution of both structures enabled unambiguous interpretation of the fine details of ligand binding in the active site and comparison with already known complexes.

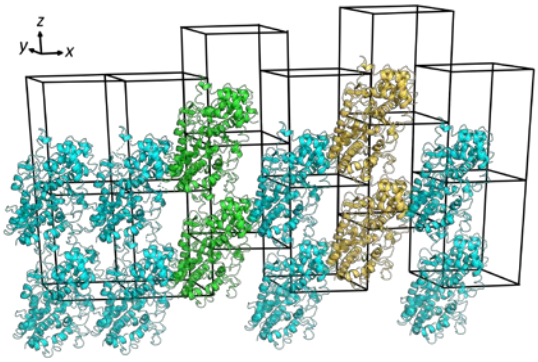

Figure 1 Demonstration of lattice translocation defect effect on S1-URI crystal structure. Random layers of the crystal are shifted (orange and green cartoon) with respect to the original structure (cyan cartoon) along the *z* axis of the unit cell.

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This work was supported by the Academy of Sciences of the Czech Republic (RVO: 86652036) and the European Regional Development Fund (Project CIISB4HEALTH, No. CZ.02.1.01/0.0/0.0/16_013/0001776 and ELIBIO, No. CZ.02.1.01/0.0/0.0/15_003/0000447) and also from specific university research (MSMT No 21-SVV/2020).

Structure-assisted drug discovery: purine nucleoside phosphorylases

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Nucleic acid synthesis and degradation are ongoing metabolic processes in most cells. The degradative processes lead to the release of free purines and the salvage pathway exists to recover them efficiently in a useful form [1].

Purine nucleoside phosphorylase (PNP) represents one of the key enzymes of the purine salvage pathway, which is considerably more energy-efficient than *de novo* pathway. Human PNP is overexpressed in T-cell leukemia, breast and colon cancer and during autoimmune diseases and PNP has been established as prospective target for drug design. Several hPNP inhibitors recently entered human clinical trials [2].

For many parasites and bacteria the purine salvage pathway is major or the only way to obtain purine nucleotides for the synthesis of nucleic acids. PNP enzymes from *Plasmodium falciparum* (*Pf*PNP) and *Mycobacterium tuberculosis* (*Mt*PNP) are thus potential targets for treatment of malaria and tuberculosis [3,4].

We are using X-ray crystallography in structure-assisted drug design of novel acyclic nucleotide analogues. Our goal is to design inhibitors with high affinity towards hPNP, *Pf*PNP and *Mt*PNP, respectively.

Enzymes were prepared by heterologous expression in *E. coli* and purified in high yields and purity necessary for crystallographic studies. Crystallization conditions for hPNP, *Pf*PNP and *Mt*PNP were identified through wide screening and optimization. Selected inhibitors were successfully co-crystallized with *Mt*PNP and hPNP, diffraction data have been collected on BL14.1 at the BESSY II electron storage ring operated by the Helmholtz-Zentrum Berlin and crystal structures were determined at high resolution. The knowledge of binding of these inhibitors to the enzymes will further help with the design of specific PNP inhibitors.

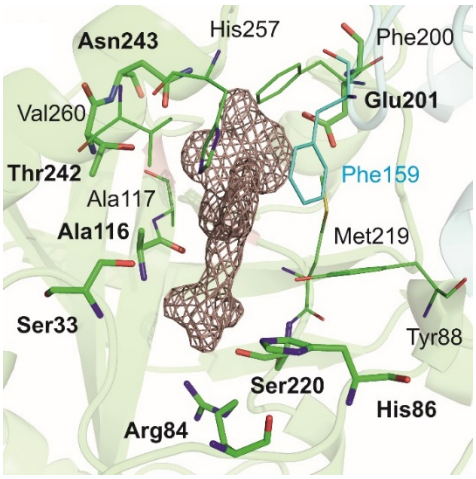

Figure1: Active site of the hPNP:inhibitor structure. Hydrogen-bond forming residues are represented with sticks and marked in bold, while other binding-interface-forming residues are represented as lines. Neighbouring subunit is represented with cyan. Ligand is represented with a mesh describing its 2Fo-Fc electron density map, contoured at 1.3 σ.

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Ixodes ricinus Serpins

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Serine protease inhibitors - serpins is a superfamily of structurally conserved proteins widely distributed in nature [1]. We focus our interest on *I. ricinus* serpins found in tick saliva. These serpins has primarily immunological and haemostatic functions, but their functions can vary according to their specificity. The tick serpins act as modulators of immune responses by using their anti-coagulation, anti-complementary functions and by playing important role in immunosuppression [2].

The serpin inhibitory activity require rearrangement of the conformation. The typical secondary structure is made of 3 β-barrels, 9 α-helices and exposed, flexible reactive center loop (RCL) that contains proteinase recognition site. During crystallographic attempts, different types of conformation were solved and each of these structural rearrangements was important to understand the inhibitory pathway. The successful process of serine proteinase inhibition results in irreversible suicide substrate mechanism, by which serpin is covalently bound to target protease [3].

Here, we present the crystal structures of serpins found in tick saliva of Ixodes ricinus (IRS-1, Iripin-3, Iripin-4 and Iripin-5) compared to the structure of *Ixodes ricinus* serpin 2 (IRS-2) describe previously [4] (Fig. 1).

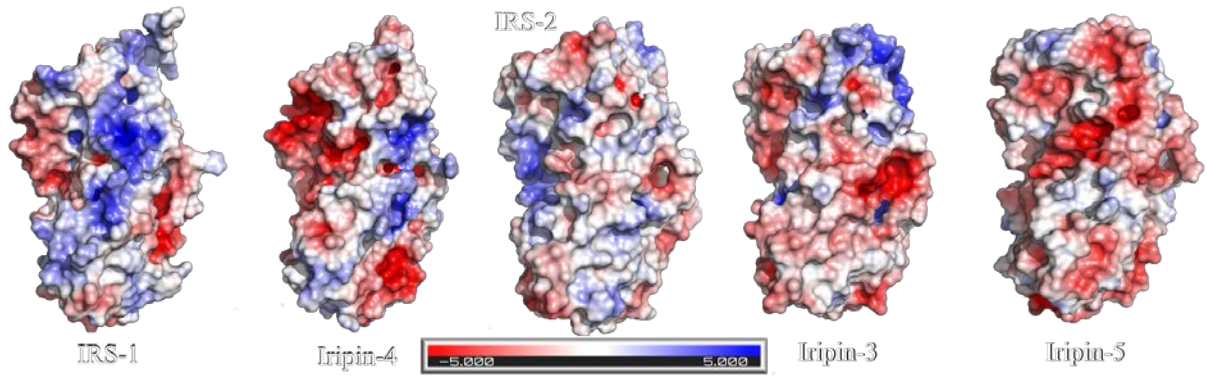

Figure 1. Comparison of surface electrostatic potentials of I. ricinus serpins known structures.

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This research is supported by GACR 19-14704Y, ERDF No. CZ.02.1.01/0.0/0.0/15_003/000041 and GAJU 04-017/2019/P.

Structural determination of lectins from *Photorhabdus spp.*

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Lectins are ubiquitous proteins with the ability to reversibly bind to the mono-, oligo- and polysaccharides with high specificity. These sugar-binding proteins can be found in most organisms, ranging from viruses and bacteria to plants and animals. They play an important role in many biological processes, such as cell-cell interaction or recognition of the host by a pathogen [1]. Lectins represent a heterogeneous group of proteins that vary in size, oligomeric state, and structure. Due to their importance, lectins are studied structurally and functionally to completely understand their role and mechanism of action.

Research is conducted on the lectins from gram-negative entomopathogenic bacteria *Photorhabdus asymbiotica*, which live in symbiosis with *Heterorhabditis* nematodes. This symbiotic complex can be found in soil, where it searches for the insect prey [2]. *Photorhabdus* genus is not a strict insect pathogen, but there are also clinical cases describing a human infection caused by *P. asymbiotica* [3]. Unusual dual behaviour and diversity in host selection make bacteria *Photorhabdus* compelling organisms and further study of biomolecules produced by these bacteria may reveal their importance in the pathogenic or symbiotic stage of life.

Beside functional characterization, structural information is essential for the discovery of the number of the binding sites, the key residues involved in interaction and the orientation of the binding partner. For this purpose, the protein crystallography was used to determine the 3D structure of lectins and their complexes with binding partners in atomic resolution.

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This work was supported by the Czech Science Foundation (21-29622S) and CIISB research infrastructure project LM2018127 funded by MEYS CR.

Structural glimpse into 14-3-3 protein dependent regulation of ubiquitin ligase Nedd4-2

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Neural precursor cell expressed developmentally down-regulated 4 ligase (Nedd4-2) is an E3 ubiquitin ligase playing a key role in human pathophysiology by regulating huge number of ion channels, membrane receptors, tumour suppressors and endocytic regulation proteins by targeting proteins for ubiquitination. The activity of Nedd4-2 is regulated by autoinhibition, calcium binding, oxidative stress, substrate binding through its WW domains and binding of 14-3-3 proteins in phosphorylation-dependent manner [1-3]. However, the structural basis of the 14-3-3-dependent Nedd4-2 regulation remains poorly understood. Here, we employed several techniques of integrative structural biology to characterize the Nedd4-2:14-3-3 complexes. The results of sedimentation velocity analysis and subsequent protein crystallography confirm the Ser^{342} and Ser^{448} as the key sites facilitating the 14-3-3-protein binding. Moreover, chemical crosslinking and SEC-SAXS based modelling suggest changes in position of the WW2 domain located between those sites after complex formation, leading to the more compact particle. Our findings demonstrate the first structural glimpse into the 14-3-3-regulated Nedd4-2 inhibition and reveal the potential therapeutic targets for Nedd4-2 associated diseases.

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This study was supported by the Czech Science Foundation (Project 20-00058S), the Czech Academy of Sciences (Research Projects RVO: 67985823 of the Institute of Physiology) and by Grant Agency of Charles University (Project 740119).

Half way to hypusine. Structural characterization of human deoxyhypusine synthase.

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Deoxyhypusine synthase (DHS) is a transferase catalysing the formation of deoxyhypusine, which is the first, rate-limiting step of unique post-translational modification: hypusination. DHS catalyzes the transfer of 4-aminobutyl moiety of spermidine to a specific lysine of eIF5A precursor in an NAD-dependent manner. This modification occurs exclusively on only one protein: eukaryotic initiation factor 5A (eIF5A) and it is essential for cell proliferation [1]. Malfunctions of the hypusination pathway, including those caused by mutations within the DHS encoding gene, are associated with such conditions as cancer or neurodegeneration [2].

The presented study aimed to investigate substrate specificity of the first step of hypusination using macromolecular crystallography as the main tool and additionally to assess the impact of newly recognized pathological mutations in DHS encoding gene on protein stability, activity and structure.

Human DHS wild type and its two mutants were expressed, purified and crystallized. Our attempts lead to six high-resolution crystal structures of DHS wt in apo form and complexes with natural substrates. Additionally, 2 crystal structures of N173S DHS were determined allowing for detailed analysis and comparison of the apo form and physiologically relevant mutant. Based on crystal structures and activity tests it was shown that despite almost identical binding of spermidine and spermine, probably only spermidine can serve as a proper substrate of deoxyhypusine formation. Furthermore, it was shown that against the previous studies, no conformational changes occur in the DHS structure upon spermidine-binding [3]. Additionally, we proposed four different possible loss-of-function mechanisms for identified pathological DHS variants.

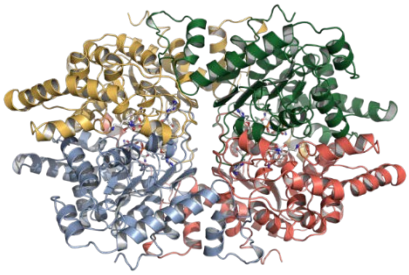

Figure 1. Crystal structure of human deoxyhypusine synthase.

Availability of high-quality structural data will aid the design of novel DHS inhibitors for potential applications in cancer therapy and can significantly advance our understanding of newly recognized genetic DHS deficiency.

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The research has been supported by National Science Centre (NCN, Poland) research grant no. 2019/33/B/NZ1/01839 to P.G and 2019/35/N/NZ1/02805 to E.W.

Initial characterization of small protease inhibitors from *Ixodes ricinus*

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Ticks are obligate blood-sucking ectoparasitic arachnids that are common throughout. They are parasites of vertebrates such as animals and humans. The hard tick *Ixodes ricinus* is the most important pathogenic vector in Europe [1]. Tick saliva affects complement activation, blood clotting and immune response, cytokine production, and host cell maturation. Ticks also facilitate the transmission of *Borrelia, Ehrlichia, Anaplasma, Francisella tularensis, Rickettsia,* including various viruses and protozoa for both humans and animals. The most important adaptation of ticks to an ectoparasitic lifestyle is their ability to bypass innate immunity and inflammation, as well as tissue repair and acquired antigen-specific immunity. In this regard, their saliva contains an extremely complex and varied mixture of pharmacologically active components, including protease inhibitors that act as anticoagulants, aggregation inhibitors, vasodilators and suppressors of inflammation and immunity.

In this regard, their saliva contains an extremely complex and varied mixture of pharmacologically active components, including protease inhibitors that act as anticoagulants, aggregation inhibitors, vasodilators and suppressors of inflammation and immunity.

Several low molecular weight protease inhibitors 32-cathelicidin, 84-ranamicin, 31-cathelicidin and elastofilin were purified and used for the initial crystallization screening to identify the suitable conditions for the 3D crystal grows. The crystals will be used for the structure determination processes later on. Structural studies of these inhibitors are of great importance, since some of them can serve as antigens in the creation of vaccines against ticks [3] or biomolecules that can be used in the future as medicinal compounds [4,5]. Also, identifying the structure of these proteins will enable a better understanding of the interactions between ticks and their hosts during the tick feeding process and their role in the transmission of tick-borne microorganisms.

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Production of TBEV NS5 protein for structural and functional studies

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Tick-borne encephalitis virus (TBEV) is a major human pathogen, transmitted by ticks from family *Ixodidae* [1, 2]. TBEV is an enveloped virus with a ~ 11 kb positive-sense single-strand RNA genome that encodes a single 375 kDa polyprotein. During the infection in the host cells, the polyprotein is cleaved by cellular and viral enzymes into three structural (capsid (C), pre-membrane (prM) and envelope (E)) and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins [3]. While structural proteins are involved in the architecture of new virions [4], non-structural proteins are responsible for the virus replication, forming replication complex [5].

Non-structural protein NS5 is a large bi-functional protein comprising of two domains connected by highly flexible 10aa linker, which is important for RdRp activity as well as for the overall shape of the protein. N-terminal methyltransferase (MTase) domain is involved in the capping process. C-terminal part of the protein displays RNA-dependent RNA polymerase (RdRp) activity, crucial for virus replication [6].

This project focuses on structural studies of TBEV NS5 protein. Various constructs of TBEV NS5 protein were designed: a – full length NS5 full length including both domains, b – RdRp domain, c– MTase domain. Expression and purification of individual constructs was optimized and pure samples were used for initial crystallization screening applying various commercially available crystallization screens (Molecular Dimensions, Hampton Research) as well as for cryo-EM analysis.

So far, we have obtained cryo-EM data for RdRp domain. Data collection was performed on Titan Krios using Falcon 4 camera and Relion processing pipeline yielded a reconstruction of 6 Å resolution. This data will be further refined. Needle shaped protein crystals of RdRp domain grew in several crystallization conditions. Their protein origin was verified by UVEX imaging system and these conditions will be used for further optimization in order to grow more suitable protein crystals for X-ray diffraction analysis. Functional experiments are currently being and will be discussed as well.

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Flexible platform for production of proteins of a quality and quantity suitable for structural studies

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Monoclonal antibodies (mAbs) play important role in structural biology studies, where they are used as chaperones in co-crystalization with hardly crystallizable targets, structural probes for capturing the transient conformations of flexible proteins, and as significant therapeutic molecules whose structure reveals the mechanism of action [1]. mAbs, mainly their Fabs, can perform all these tasks because they meet the necessary criteria for a stable, homogenous, and well-defined material needed [2]. Reproducible preparation of mAbs is essential for not only structural, but also biophysical, functional, and preclinical studies calling for fast and abundant delivery of proteins of interests. However, traditional means of producing Fabs by partial papain cleavage of some immunoglobulins result with only 50% successfully cleaved product even after 24 hours [3]. Recombinant technologies can provide higher yields and pure and homogenous product.

In our work, we aimed to develop a platform consisting of a plasmid for universal cloning of Fabs of various monoclonal antibodies, their eukaryotic expression in CHO cell line and a one-step purification. Using the backbone of the commercially available pCMV-Script vector we prepared new vector allowing flexible cloning of antibodies and other proteins for eukaryotic expression. This vector is derived from the high copy plasmid pUC, expression in mammalian cells is driven by the human cytomegalovirus (CMV) promoter, and the kanamycin resistance gene allows for selection in bacteria. Newly created pCMV_3´UTR vector further contains an insert storing the Kozak sequence, the initiation codon, the IGKV3-11 signal peptide, multiple cloning sites and the 3´untranslated region of the EF1 alpha gene. The presence of the 3'UTR regulates the stability, localization and translation of the mRNA [4]. The signal peptide sequence drives the secretion of produced protein to the culture medium. Silent mutation at the end of the signal peptide sequence created the site for the AgeI enzyme at a position that allows seamless cloning of sequence of protein of interest, that is, without the addition of amino acids and in the correct reading frame. The sequences of proteins of interest are cloned through the AgeI and XhoI sites (Fig. 1).

We transfected this plasmid into ExpiCHO-S cells and produced proteins during a 14-day cultivation in 30-50 ml cell cultures. In an optimized flexible antibody production system, we were able to prepare 10-15 mg of purified antibody Fabs, with which we managed to prepare crystals that are relatively large, single crystals with probably proteinaceous nature because they contained 100% purified Fab DC39N1 and tau constructs of purity higher than 95%.

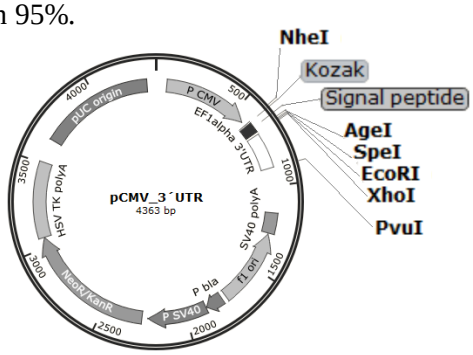

Figure. 1. Map of plasmid pCMV_3´UTR with marked restriction endonuclease cleavage sites.

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In solution site-specific time-resolved structural biology of the light sensitive transcription factor EL222 monitored by infrared spectroscopy with vibrational probes

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EL222 is a blue light sensitive transcription factor from the bacteria *Erythrobacter litoralis.* In the dark, the flavin-binding light-oxygen-voltage (LOV) domain is tightly packed to the DNA-binding helix-turn-helix (HTH) domain. Upon blue light excitation by the embedded flavin mononucleotide (FMN) chromophore, EL222 undergoes structural changes ultimately leading to protein oligomerization and association with DNA. Our goal is to elucidate the conformational changes that occur when EL222 is photoactivated and its recovery in the dark.

Fourier transform infrared (FTIR) spectroscopy is an established technique to investigate protein secondary structure in aqueous solutions. However, the similar absorption frequencies of many protein and cofactor groups causes spectral congestion making it difficult to assign the observed bands to particular bonds. Here, we use the nitrile group (C≡N) as a site-specific vibrational reporter that absorbs IR radiation in a region spectrally isolated from the protein signal. EL222 variants containing each a single non-canonical amino acid p-cyanophenylalanine (CNF) in different locations across the protein were prepared by genetic code expansion (amber suppression) technology. Screening of a large set (more than forty) of labelled positions was done by recording the steady-state FTIR difference spectra between light and dark states. Some residues showed a clear shift in the position of the CN stretching vibration, suggesting a distinct local environment around the probe. Subsequently, EL222 mutants with multiple CNF probes (up to three) at desired locations were investigated by rapid-scan time-resolved IR spectroscopy with millisecond time resolution. Time constants and assignment of each individual CNF tag was determined by global kinetic analysis. We found that for most labelled positions the CNF recovery time was similar to the backbone (Amide I/II IR bands) and FMN (UV-Visible absorption band) lifetimes. Strikingly, few labelled positions, particularly those belonging to residues in intrinsically disordered regions, equilibrated back to the dark state at a slower rate than the backbone and FMN sites.

In summary, we were able to track the lit-to-dark transition of EL222 residue-by-residue and did not detect any intermediate states. Our integrative FTIR/UV-Vis spectroscopy approach in combination with protein engineering tools, helps to understand the mechanism and kinetics of EL222 photocycle, and may be applied to other proteins and light-initiated reactions.

Structural basis of interaction between Forkhead box O4 (FOXO4) and p53

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Forkhead box O (FOXO) and p53 transcription factors control multiple cellular processes including cell cycle regulation, metabolism, tumour suppression and apoptosis [1-3]. FOXOs are classified into four members: FOXO1, FOXO3, FOXO4 and FOXO6 which shares a highly conserved DNA binding domain (DBD) throughout evolution in mammals, while their function is tightly regulated by post-translational modification (phosphorylation, acetylation and ubiquitination) and interaction with other proteins [4]. A recent finding suggests that FOXO4 interacts with p53, which promotes cellular senescence over apoptosis and maintains senescence cells viability [5]. As a result, senescence cell accelerates the ageing process and other age-related diseases. However, many aspects of FOXO4:p53 interaction remains unknown. Therefore, our main goal is to elucidate the structural details of the FOXO4:p53 complex. We designed various truncated FOXO4, p53 constructs to perform sedimentation velocity analytical ultracentrifugation (SV-AUC), 2D ^{1}H-^{15}N HSQC NMR, chemical cross-linking coupled to mass spectroscopy and molecular docking study. We found that both proteins are interacting with the affinity (K_D) in the micromolar range. NMR analysis suggests that the transactivation domain (TAD) of p53 mainly interacts with the N-terminal segment of FOXO4-DBD but several other regions of FOXO4 and p53 are also involved in the complex formation.

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Role of thioredoxin in regulation of Apoptosis signal-regulating kinase 1 (ASK1)

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Apoptosis signal-regulating kinase 1 (ASK1), a member of mitogen-activated protein (MAP) kinase kinase kinase family, is an apical kinase, which upon its activation by stress stimuli phosphorylates and thereby activates downstream targets from MAPK pathway [1]. This results in the activation of p38 kinase and C-jun-N-terminal kinase, which promote apoptosis and cell death [2]. The dysregulation of ASK1 is known to trigger the pathogenesis of various diseases and for this reason, it is necessary that this protein is tightly regulated [3, 4]. It is known that the regulation of ASK1 activity is mediated through oligomerization and protein-protein interactions, but the precise molecular mechanism is unclear [5]. Also, despite the structures of individual domains, we are missing a more complex view of the full ASK1 itself, whether in complex with its important regulatory binding partners or in the context of the assumed dimer. To better understand the principle of ASK1 activation, we investigated the oligomeric behaviour of the N-terminal part of ASK1 under different redox conditions. Our results revealed that indeed, the N-terminal half of ASK1 forms dimers in solution and that this dimerization is affected by redox conditions. Moreover, we also identified the regions that form the dimerization interface of the N-terminal half of ASK1. In addition, our data also suggest that the interaction between ASK1 and its inhibitor TRX is considerably weaker in strong reducing conditions and that TRX does not prevent ASK1 dimerization.

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Looking for the optimal resolution cutoff with PAIREF GUI

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The *paired refinement* protocol is currently considered the optimal approach for the high-resolution limit estimation. Besides the obsolete criteria based on the indicators of data quality, it allows linking the data and structure model quality [1]. Recently, we published a software tool *PAIREF* that executes all the protocol routines automatically and shows a comprehensive analysis for the decision on the optimal cutoff [2].

The freely available multiplatform program is written in Python [3] and uses software from the *CCP4 software suite* [4] or *PHENIX* [5]. Newly, a simple graphical user interface (GUI) in the Qt framework has been developed. All the protocol parameters available in the command line can be set interactively in the application window (Fig. 1). The current implementation makes the program user-friendly and improves the user's control under the executed calculations. Further details can be found at our website *https://pairef.fjfi.cvut.cz*.

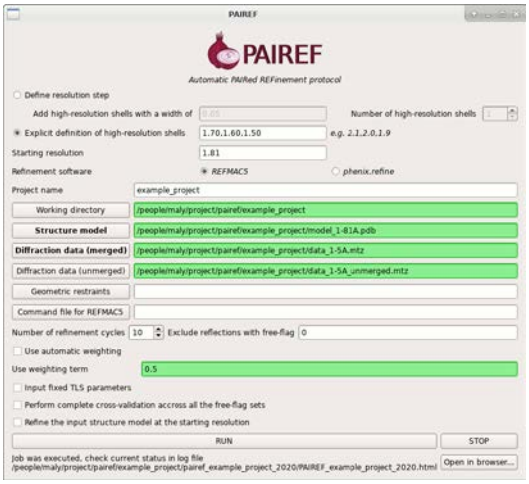

Figure 1. Window of the *PAIREF* graphical user interface with set job parameters.

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Partial atomic charges for proteins

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The distribution of the electron density determines many characteristics of the molecule. Electron density is difficult to represent; however, it can be approximated by the partial atomic charges, which are real numbers describing what portion of electron density belongs to each atom. These charges are often used in computational chemistry, chemo- and bioinformatics. Unfortunately, finding the electron density for large proteins is not possible. For this reason, faster but still reasonably accurate empirical methods must be used. These methods try to replicate the results of quantum mechanical (QM) calculations. Nevertheless, the calculation of partial atomic charges for proteins is not yet fully solved.

Recently, we have developed the empirical method *Split Charges Equilibration with Initial Parameterized Charges* (SQE+qp) to tackle this issue. Additionally, we have suggested a new definition of the atomic types (i.e., grouping the atoms into the classes). In particular, each atom is assigned the type based on the elements of the bonded atoms. This fine-grained division ensures the precise assignment of the initial charges. However, such a model has many more parameters than the previous ones, so it was also necessary to design a faster parameterization technique [1].

To demonstrate our results, Figure 1 shows the strong correlation between the reference QM charges and charges of the parameterized SQE+qp model on the dataset of 60 proteins and 60 peptides. We currently work to extend this methodology to support the calculation of partial atomic charges for metalloproteins which we believe is the crucial step to calculate charges for the majority of proteins in the PDB database. The implementation of SQE+qp is included in *Atomic Charge Calculator II* [2], a web application available freely at https://acc2.ncbr.muni.cz/.

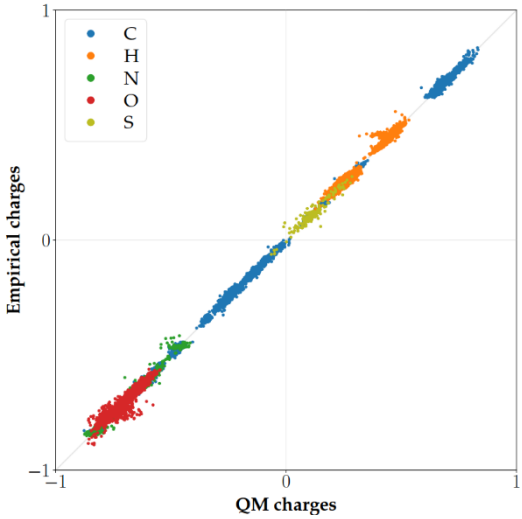

Figure 1. Correlation graph for 24 test structures between B3LYP/6-31G*/NPA and SQE+qp charges.

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NAChRDB: Solving the puzzle of structure-function relationships to clarify the allostery of nicotinic acetylcholine receptors (nAChRs)

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Nicotinic acetylcholine receptor (nAChR) is an evolutionary ancient allosteric membrane protein mediating synaptic transmission [1]. These prototypic pentameric ligand-gated ion channels (pLGICs) are expressed in many tissues & species, being involved in neuromuscular transmission, cognition, energy metabolism, and many pathologies (snake envenomation, myasthenia gravis, Parkinson & Alzheimer diseases, addictions, possibly COVID-19 [2]). Since nAChR isolation in 1970 [3], extensive studies resulted in ~5000 publications with huge amounts of structural-functional data.

However, the cumulative residue-level knowledge on nAChRs *is not systematically accessible*. Scatteredness of literature data, aliases & diverse terminology, various receptor types & residue numbering schemes make it harder to summarize the current knowledge and apply it efficiently to promote the further discoveries. There is no single resource providing access to & visualization of such vast information. NAChRDB [4] (https://crocodile.ncbr.muni.cz/Apps/NAChRDB/) fills this gap by: *a)* delivering relevant & systematized structural-functional residue-level information collected from the literature and *b)* facilitating its interpretation via mapping onto interactive 3D visualization & sequence alignment. In addition, literature data is supplemented by the computational predictions of potentially allosterically important residues based on the charge profile analysis.

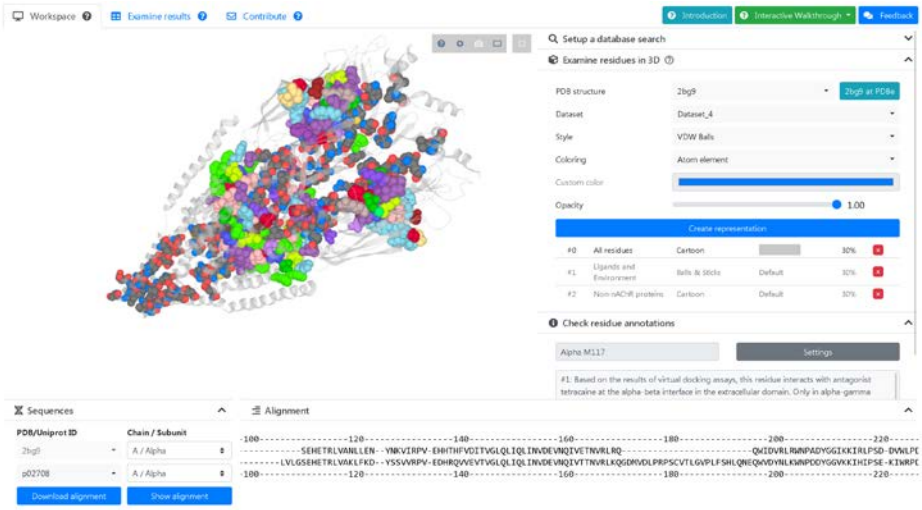

Figure 1. NAChRDB workspace.

By providing quick & easy access to the structural-functional knowledge via a user-friendly interface (Fig. 1), accompanied by interactive tutorials and case studies, NAChRDB can both serve as an educational resource and a practical tool, helping to study allosteric regulation, reveal gaps in the current knowledge, and guide the further studies in the fields of nAChRs and pLGICs.

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Understanding Physical Effects of Phosphorothiation in RNA Context with Computational Methods

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Phosphorothioates (PTs) are important chemical modifications of the RNA backbone where a single nonbridging oxygen of the phosphate is replaced with a sulfur atom. PT can stabilize RNAs by protecting them from hydrolysis and is commonly used as a tool to explore their function [1,2]. It is, however, unclear what basic physical effects PT has on RNA stability and electronic structure. *Here, we present molecular dynamics (MD) simulations and quantum mechanical (QM) calculations exploring the effects of PT modifications in the structural context of the neomycin-sensing riboswitch (NSR).* The NSR is the smallest biologically functional riboswitch with a well-defined structure stabilized by a U-turn motif. Three of the signature interactions of the U-turn: an H-bond, an anion−π interaction, and a potassium binding site; are formed by RNA phosphates, making the NSR an ideal model for studying how PT affects RNA structure and dynamics (Fig. 1) [2]. By comparing with high-level QM calculations, we reveal the distinct physical properties of the individual interactions facilitated by the PT. The sulfur substitution, besides weakening the direct H-bond interaction, reduces the directionality of H-bonding while increasing its dispersion and induction components. It also reduces the induction and increases the dispersion component of the anion−π stacking. The sulfur force-field parameters commonly employed in the literature do not reflect these distinctions, leading to the unsatisfactory description of PT in simulations of the NSR. We show that it is not possible to accurately describe the PT interactions using one universal set of van der Waals sulfur parameters and provide suggestions for improving the force-field performance.

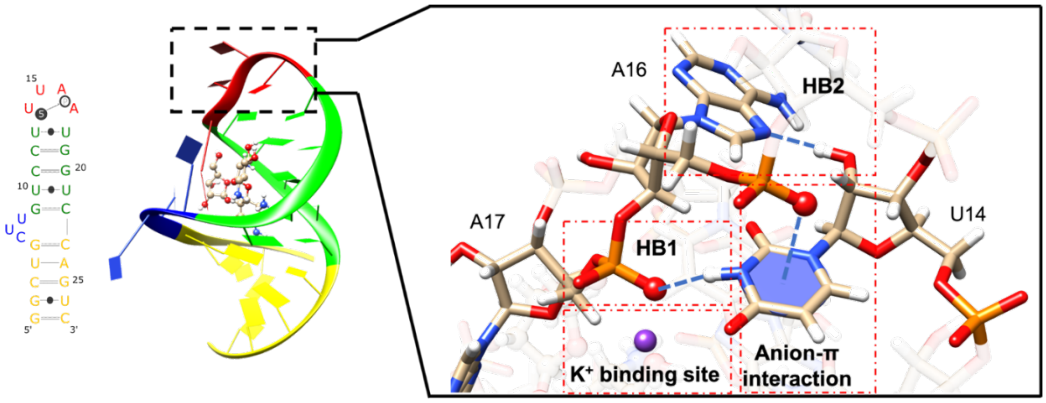

Figure 1. Secondary structure of the NSR and its 3D structure with the bound ribostamycin shown in CPK representation. The A-RNA helical segments are shown in green and yellow, respectively, separated by the flexible bulge in blue. The U-turn loop is in red. Its two signature H-bonds (termed as HB1 and HB2, respectively), the anion-π interaction, and the potassium binding site are shown in detail. The interaction involved oxygen atoms of the A16 and A17 phosphate groups are highlighted as spheres.

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Towards data standardization for biophysical data

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Usage of an information technology is on the rise in every field and the field of biophysics is no exception. The task we decided to take care of in this situation is establishing data format standardization.

The suggested standard could serve for storing data locally and in web repositories or databases. It will also improve exchange of data from different instruments and institutions among scientists; which is compliant with the FAIR data principles.

Biophysical data come in a variety of formats and with varied amounts and completeness of metadata describing the experiment. The standard format should be able to encapsulate both data and metadata. Text-based formats have many advantages and disadvantages, but in general, for complex data and metadata there are alternative, more convenient formats. One of them is a format on the rise - Hierarchical Data Format (HDF) [1], a binary format becoming widely used in other scientific fields and communities.

Possibilities of the current conversion tools from used format, both text based and binary, to possible standard format are limited. For every method a new tool need to be programmed. A much more effort needs to be put into simplifying of this process.

1. Hierarchy Data Format Group, www.hdfgroup.org

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A multifaceted computational study to unravel the role of the C-terminal DEP domain of Dishevelled protein in Wnt signalling pathway

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Wnt signaling is a group of evolutionary conserved pathways crucial for tissue development and homeostasis, and defects in its physiological function are related to numerous diseases, including cancer [1]. The signal transduction begins with the binding of Wnt protein to Frizzled transmembrane receptor, which then relays the signal to Dishevelled protein (DVL). DVL acts as a branch point and selectively initiates one of various intracellular pathways by a yet unknown mechanism [2]. It has been hypothesized that the dynamic changes in DVL intracellular localization are associated with the activation of the specific signaling branches [3-4].

By combining classic and biased all-atom Molecular Dynamics and coarse-grained Monte Carlo simulations, we provide new insights into the interactions regulating the relocalization of DVL between the cytoplasm and the plasma membrane. In particular, we focused on the role of the membrane binding C-terminal DEP domain of DVL. Our results suggest that the binding of DEP domain to anionic phospholipid membranes is primarily driven by non-specific electrostatic attraction, further optimized to preferentially interact with phosphatidic acid. However, phosphorylation modifications altering the charge of the domain did not abolish DEP-membrane binding due to local cation-mediated interactions. We believe that the improved molecular understanding of the membrane binding function of DEP domain will help us decipher the early cytoplasmic stages of Wnt signaling.

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Structure of *Pseudomonas aeruginosa* infecting bacteriophage JBD30 solved by cryo-electron microscopy

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Pseudomonas aeruginosa is an opportunistic human pathogen that causes acute and chronic infections, which can lead to life-threating septic shock. Treatment of these infections is further complicated by the ability of these bacteria to form biofilm. Biofilm serves as a shield through which the antibiotics cannot pass. Unlike antibiotics, bacteriophages are able to penetrate through biofilm, infect and kill bacterial cells.

Here we present the structure of a bacteriophage JBD30 virion resolved by cryo-electron microscopy. Bacteriophage JBD30 belongs to the family *Siphoviridae*, order *Caudovirales.* Its virion is composed of non-enveloped icosahedral head of 60 nm in diameter connected via dodecameric portal with a non-contractile 180 nm long flexible tail. JBD30 tail is terminated with a baseplate embellished with side-tail fibres. Bacteriophage head is built from 415 copies of HK-97 like major capsid protein and further decorated on three-fold and quasi-three-fold axis with trimers of minor capsid protein.

Data from cryo-electron tomography revealed that bacteriophage JBD30 uses the baseplate side-tail fibers for the attachment to its prey – bacterium *Pseudomonas aeruginosa*. *P. aeruginosa* pili type IV, that grow only from bacterial cell pole [1], serve as a primary receptor for the bacteriophage JBD30. After binding to pili, phage is pulled towards the cell surface by pili retraction [2], injects its nucleic acid into the bacterium and starts its replication cycle.

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Elucidating the life cycle of human enteroviruses *in situ*

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Human echovirus 18 is a member of the Enterovirus B species. It has diverse tissue tropism: the cells of the nervous system, lower respiratory tract, and gastrointestinal system. The long-term effects of viral encephalitis are acquired brain injury, memory loss, and altered consciousness [1]. Furthermore, it has been reported that this virus is connected to chronic gastrointestinal diseases, particularly, to Crohn's disease [2].

In this project, we characterize the interactions of cell and Echovirus 18 particles and the genome delivery *in situ*. We study the replication cycle to determine the mechanisms of recombination that cause the emergence of new viral strains. By observation of the virion assembly, we identify the mechanism of genome packaging.

We use the *focused-ion beam milling* (FIBM) to prepare lamellae of the infected cells and image them using *cryo-electron microscopy*. The thin (200 to 300 nm) lamellae are used to record *tomographic tilt series* [3, 4]. The image alignment is followed by the *3D reconstruction* of cell sections [5]. The reconstructed parts of cells provide information about the positioning and interactions of the macromolecular complexes of the cell with the virion assemblies and their intermediate states. Since enteroviruses are a homogeneous group of viruses, the data for human enterovirus 18 will be relevant also as a reference for further experiments on other enterovirus species.

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Baseplate structure of bacteriophage phi812 reveals mechanism of cell wall binding and penetration

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Antibiotic-resistant strains of *Staphylococcus aureus* cause human infections that are difficult to treat and can lead to death [1]. Bacteriophage (phage) phi812K1/420 from the family Myoviridae infects 95% of clinical isolates of *S. aureus* and therefore is a promising candidate for a phage therapy agent [2]. As the native phage particle approaches its host cell, phage receptor-binding proteins make a contact with the host cell wall. This interaction triggers a cascade of structural changes in the baseplate, resulting in phage tail contraction and genome ejection [3]. Mechanistic description of the baseplate re-organization, however, remains unknown.

Using cryo-electron microscopy (cryo-EM), we reconstructed the phage baseplate in native and contracted states (Fig. 1). The reconstruction of native baseplate reaches resolution of 4-5 Å and we are in process of building individual protein structures. Also, selected proteins involved in host cell wall attachment and degradation were produced in recombinant form and their structures were solved using X-ray crystallography and cryo-EM single-particle reconstruction. The protein structures will be fitted into reconstruction of the contracted baseplate.

Our results provide first structural characterisation of contractile phage infecting a Gram-positive bacterium. Comparison of the two distinct baseplate states will allow us to describe molecular mechanism of initial stage of phage infection in detail.

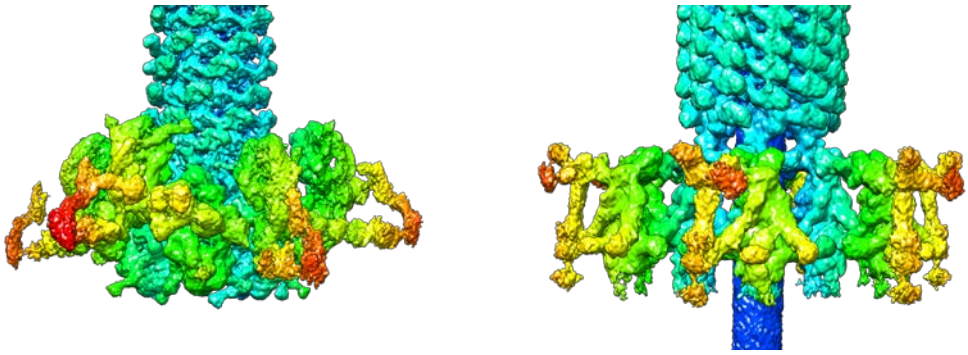

Figure 1. Cryo-EM reconstruction of phi812K1/420 baseplate in native (left) and contracted (right) state. Color-coded according to the distance from the tail axis.

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Virion structure and mechanism of genome delivery of bacteriophage SU10 from the family *Podoviridae*

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Phages from the family *Podoviridae* use short non-contractile tails to deliver their genomes into bacteria. However, there is limited information on how the tails of *Podoviridae* phages penetrate cell walls of Gram-positive bacteria.

Here we present the structures of virion and genome release intermediate of phage SU10. The virion of SU10 is formed by a prolate capsid with a tail decorated by long and short fibers. To infect cells SU10 binds to a cell surface by long tail fibers. Binding of short tail fibers to bacterial surface requires their rotation by 135°, which is connected to reorganization of tail proteins. In the new conformation, the short tail fibers and tail proteins form a 200 Å long nozzle. We employed cryo-electron microscopy to visualize interactions of SU10 with *E. coli* cell wall. Attachment of short tail fibers to the cell surface forces the tail needle, which protrudes from the baseplate, through the outer membrane of the bacterial cell. The tail needle dissociates from the baseplate. Core proteins of SU10 with transglycosylase activity are ejected from the head to degrade cell wall peptidoglycan. Other core proteins form a translocation complex that extends the nozzle across the periplasm and inner membrane.

The extended nozzle together with the attached translocation complex enable delivery of SU10 DNA into bacterial cytoplasm.

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