Posters

P1

ZINC-DEPENDENT S1-P1-LIKE NUCLEASES FROM OPPORTUNISTIC HUMAN PATHOGENS

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The family of S1-P1 nucleases contains zinc-dependent 3'-nucleases/nucleotidases cleaving phosphodiester bond of nucleic acids. Gene coding an S1-P1 type nuclease can be found in many species. In plants and fungi (i.e. TBN1 nuclease from *Solanum lycopersicum*, S1 nuclease from *Aspergillus oryzae*), these nucleases were already functionally and structurally well characterized [1, 2], but the potential role of their homologs from bacteria or protozoan parasites is not fully understood and their crystal structure is yet to be determined. As they occur in some opportunistic human pathogens (i.e. *Legionella pneumophila, Leishmania major*), they became an interesting target for biomedical and biotechnology studies [3].

Here we present crystal structures of recombinant class I nuclease from *Stenotrophomonas maltophilia* (SmNuc1), structure of the native enzyme at 1.4 Å resolution and two crystal structures with 5'-mononucleotides present in the active site. *Stenotrophomonas maltophilia* is Gram-negative aerobic bacterium from *Gammaproteobacteria.* It is a human opportunistic pathogen which causes several nosocomial diseases and has high multidrug resistance. SmNuc1 nuclease is a 28 kDa protein, composed mostly of -helices with two disulfide bridges. The active site composition and protein fold are similar to its eukaryotic homologs, but there are some differences in the surface properties and substrate binding site.

Based on these new structures, we are able to suggest mutations of some specific sites. These mutations could help us fully understand the substrate-binding mechanism, which could lead to specific inhibitors for S1-P1 type nucleases from bacterial pathogens.

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1. Kovaľ T. *et al*., *PLoS ONE*, 2016, 11(12): e0168832.

2. Kovaľ T. *et al., Acta Crystallographica D,* 2013, 69: 213-226.

3. Kovaľ T, Dohnálek J, *Biotechnology Advances*, 2018, 36(3): 603-612.

P2

DESIGN OF CARDIAC GLYCOSIDE CONJUGATES FOR IMPROVED CANCER CELL SELECTIVITY

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Cardiac glycosides, well established therapeutics for treatment of cardiac diseases, have been nowadays subjected to drug repositioning due to their anticancer potential. Their main mode of action is inhibition of Na^+/K^+-ATPase (NKA) which leads to cell apoptosis, a major cause of their cytotoxic properties. However, NKA is present in both cancer and healthy cells, therefore, its inhibition can lead to severe side effects. One of such potential inhibitors is a cardiac glycoside hyrcanoside (Hyr). Hyr contains a carbonyl group C-19, which is probably crucial for NKA binding. Thus a chemical modification of this group by compounds containing an oxime bond could lead to suppression of Hyr ability to bind NKA due to steric hindrance. An oxime bond is unstable at lower pH values, therefore, it is used for conjugations of compounds, for which release at such pH is desired. Based on this, the aim of this work was to design

pH-sensitive Hyr derivatives with an oxime bond and to perform *in silico* simulations of their complexes with NKA. Using molecular docking, our results indicate that modification of C-19 carbonyl leads to loss of Hyr ability to bind to NKA. In the next part of this study, our knowledge of Hyr-NKA complexes will be extended by performing simulations in real-time using molecular dynamics. We believe that a modification of Hyr C-19 carbonyl containing an oxime bond will enhance ability of Hyr to selectively kill cancer cells and that the Hyr conjugates could have a potential to be used in a cancer therapy.

P3

THE FIRST HYDRATION LEVEL AROUND BIOMOLECULES IS SITE-SPECIFIC

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Proteins and nucleic acids evolved in the aqueous environment, and water is therefore deeply interrelated with both biomolecular structure and function. The first layer of water molecules around the biomolecular surface - the hydration shell - has properties different from the bulk water [1]. The dynamics of these water molecules is significantly reduced, and the shell mostly consists of ordered (localized) water molecules. However, the first shell water molecules do not have an ice-like structural properties. These ordered water molecules play significant role in recognition and binding of ligands.

In our work, we utilize crystallographic data to compile the average hydration patterns around biomolecules. Firstly, we investigated hydration of DNA building blocks [2, 3], and later hydration of amino acids in proteins as a function of their rotameric state and the secondary structure [4, 5]. Recently, we analyzed hydration of DNA dinucleotides as a function of their conformation and sequence [6]. Here, we present the overview of these results as well as the methodology we used to obtain the data and the potential application of the results.

1. Biedermannová L. & Schneider B.: Hydration of proteins and nucleic acids: Advances in experiment and theory. A review. *Biochimica et Biophysica Acta* - General Subjects 1860: 1821-1835 (2016).
2. Schneider B. & Berman H.M.: Hydration of the DNA Bases Is Local. *Biophysical J.* **69**: 2661-2669 (1995).
3. Schneider B., Patel K. & Berman H.M.: Hydration of the Phosphate Group in Double-Helical DNA. *Biophysical J.* **75**: 2422-2434 (1998).
4. Biedermannová L. & Schneider B.: Structure of the ordered hydration of amino acids in proteins: analysis of crystal structures. *Acta Cryst.* **D71**: 2192-2202 (2015).
5. Černý J., Schneider B. & Biedermannová L.: WatAA: Atlas of Protein Hydration. Exploring synergies between data mining and ab initio calculations. *Phys. Chem. Chem. Phys.* **19**, 17094 (2017).
6. Biedermannová L. et al., to be published (2020).

P4

EXAMINATION OF INTERACTION BETWEEN REGULATORY DOMAIN OF TYROSINE HYDROXYLASE AND 14-3-3 PROTEIN

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Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine biosynthesis belonging to aromatic amino acid hydroxylases (AAAHs) family [1]. Enzymatic reaction results in hydroxylation of L-tyrosine into L-DOPA, a precursor of crucial neurotransmitters in human body: dopamine, norepinephrine and epinephrine which deficiencies lead to neurological disorders like Alzheimer disease, dystonia and Segawa syndrome [2].

TH has a multi-domain structure with unstructured amino-terminal regulatory domain (RD) followed by catalytic domain and coiled-coil domain responsible for oligomerization at the carboxyl terminus. Enzyme activation is mostly held by phosphorylation of both Ser-19 and Ser-40 residues [3]. These two modifications enable to form a complex with 14-3-3 protein with high affinity and provides TH stabilization and proteolytic protection of the regulatory domain N-terminus [3]. Although TH was first reported enzyme interacting with 14-3-3 protein, the exact mode of its regulation still needs further investigation to provide the molecular targets in drug development [4].

1. Kaufman, S. (1985). Regulatory properties of phenylalanine, tyrosine and tryptophan hydroxylases. *Biochemical Society Transactions,* **13**(2), 433–436.

2. Willemsen, M. A., Verbeek, M. M., Kamsteeg, E.-J., de Rijk-van Andel, J. F., Aeby, A., Blau, N., Wevers, R. A. (2010). Tyrosine hydroxylase deficiency: a treatable disorder of brain catecholamine biosynthesis. *Brain*, **133**(6), 1810–1822.

3 Daubner, S. C., Le, T., & Wang, S. (2011). Tyrosine hydroxylase and regulation of dopamine synthesis. *Archives of Biochemistry and Biophysics*, **508**(1), 1–12.

4. Ichimura, T., Isobe, T., Okuyama, T., Yamauchi, T., & Fujisawa, H. (1987). Brain 14-3-3 protein is an activator protein that activates tryptophan 5-monooxygenase and tyrosine 3-monooxygenase in the presence of Ca^{2+},calmodulin-dependent protein kinase II. *FEBS Letters*, 219(1), 79–82.

P5

EVOLUTIONARY UPGRADE OF STEFINS FOR SECRETION IN PARASITES

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Fasciolosis caused by the liver fluke *Fasciola hepatica* is a worldwide spread parasitic disease of ruminant and an emerging human disease. Cystatin superfamily of cysteine protease inhibitors is composed of intracellular type 1 cystatins (stefins), secreted type 2 cystatins (cystatins), and multidomain type 2 cystatins. Helminth parasites secrete type 2 cystatins to modulate host immune responses for successful parasitism, except for *F. hepatica* that lacks type 2 cystatin genes.

This work is focused on *F. hepatica* type 1 cystatin FhCY2. FhCY2 was localized to gastroderm and tegument - tissues responsible for protein secretion, and was surprisingly detected in the excretory/secretory products. We demonstrated that recombinant FhCY2 is a broad-selective inhibitor of host cysteine cathepsins as well as cysteine cathepsins of *F. hepatica*, suggesting its dual role in the regulation of exogenous and endogenous proteolytic systems. Furthermore, we solved the crystal structure of FhCY2 at 1.6 Å. The structural and phylogenetic analyses revealed that FhCY2 has the sequence and fold of type 1 cystatins but also the signal peptide and disulfides typical for type 2 cystatins, combining all hallmarks in an unprecedented way.

We propose that FhCY2 is an evolutionary upgrade of type 1 cystatins for secretion that occurred in *F. hepatica* in the absence of type 2 cystatins. Furthermore, we found that absence of type 2 cystatins is archetypal for the order of Plagiorchiida and that those species are endowed with type 1 cystatins with various grade of upgrade.

P6

BIOPHYSICAL RESEARCH FACILITIES 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 member of Instruct-ERIC and Czech Infrastructure for Integrative Structural Biology, provides shared resources of instruments and technologies for the determination of size, structure and stability of biomolecules, study of conformational changes and thermodynamics of temperature transitions and characterization of biomolecular interactions.

Following instruments and technologies are currently available: circular dichroism spectroscopy (Chirascan Plus CD spectrometer), spectrophotometry (Specord 50 Plus UV/Vis spectrophotometer), Fourier-transform infrared spectrometry (Vertex 70v spectrometer), fluorescence spectrometry (photoluminescence spectrometer FLS1000), differential scanning fluorescence (Prometheus NT.48), multiangle dynamic light scattering (Zetasizer Ultra) and differential scanning calorimetry (Microcal VP-DSC), isothermal titration calorimetry (Microcal iTC200), microscale thermophoresis (Monolith NT.115 and NT.Label Free) and surface plasmon resonance (ProteOn XPR36).

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P7

PHOTOINDUCED DYNAMICS OF EL222 MONITORED BY MULTI-SITE-SPECIFIC INFRARED SPECTROSCOPY

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EL222 is a blue-light sensitive DNA binding protein from the bacteria *Erythrobacter litoralis.* The dark-state crystal structure of the protein reveals a flavin binding light-oxygen-voltage (LOV) domain and a DNA binding helix-turn-helix (HTH) domain tightly packed against each other and thereby blocking the recognition of DNA. Upon blue light excitation by the flavin moiety, EL222 undergoes conformational changes that ultimately lead to protein dimerization and association with DNA [1]. However, our knowledge of the light-adapted state and the molecular mechanism by which it is formed remains incomplete. Our goal is to elucidate the structure of light-state EL222 and the conformational changes that occur when EL222 is photoactivated.

Fourier transform infrared (FTIR) spectroscopy is a well known method to characterize protein structure and dynamics. However, the spectral congestion makes it challenging to assign the observed bands to particular bonds. This problem can be solved by the introduction of IR-sensitive probe groups in target residues [2]. Here we make use of the cyano group as a site-specific infrared reporter to track the conformational dynamics of photoactivated EL222. EL222 variants containing the non-canonical amino acid p-cyanophenylalanine (CNF) in different locations across the protein were prepared by amber suppression technology. Screening of a large set of labelled positions was done by recording the steady-state difference FTIR spectra between light and dark states spectra. Some mutants showed a clear shift in the position of the CN stretching vibration, suggesting a change in the local environment around the probe upon illumination. Selected mutants will then be further investigated by time resolved IR spectroscopy to detect the site-specific propagation of conformational changes in EL222 from a few femtoseconds (photon absorption) to several seconds (interaction with DNA).

1. Nash AI, McNulty R, Shillito ME, Swartz TE, Bogomolni RA, Luecke H, et al. Structural basis of photosensitivity in a bacterial light-oxygen-voltage/helix-turn-helix (LOV-HTH) DNA-binding protein. *Proceedings of the National Academy of Sciences*. **2011**;108(23):9449-54.
2. Ramos S, Thielges MC. Site-Specific 1D and 2D IR Spectroscopy to Characterize the Conformations and Dynamics of Protein Molecular Recognition. *The Journal of Physical Chemistry B*. 2019;123(17):3551-66.

P8

CATHEPSIN D PROTEASE OF PARASITIC BLOOD FLUKE *SCHISTOSOMA MANSONI* AS A TARGET FOR INHIBITORY DRUGS

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Schistosomiasis, a parasitic disease caused by blood flukes of the genus *Schistosoma*, is a global health problem with over 240 million people infected. Treatment relies on just one drug, and new therapies are needed. Adult schistosomes live in the cardiovascular system, and host blood proteins are a primary source of nutrients. In the schistosome gut, a network of proteases performs the digestion of host proteins and represents a potential intervention target. *Schistosoma mansoni* cathepsin D (SmCD) is a pepsin-family aspartic protease that initiates host hemoglobin digestion in schistosomes.

Recombinant SmCD was produced in the *Leishmania tarantolae* protozoan expression system. Screening of a library of more than 30 macrocyclic statin-based peptidomimetics against SmCD selected potent inhibitors with low nanomolar activity. These inhibitors displayed anti-schistosomal properties and thus represent a new lead scaffold for developing potential drugs for schistosomiasis treatment. Furthermore, we solved the crystal structure of the SmCD zymogen, and currently we are working on the structural analysis of SmCD complexes with macrocyclic inhibitors.

P9

INTERACTION OF DYNAMIC SPORULATION PROTEIN SPOIIE WITH NOVEL CELL CYCLE REGULATOR PROTEIN GPSB

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Spore formation is an ultimate response of Bacillus subtilis to hostile conditions. The process starts with an asymmetric cell division, at the onset of which dynamic sporulation protein SpoIIE has a key role in effecting the switch from normal mid-cell division to asymmetric division [1]. Asymmetric cell division requires the same set of division proteins as vegetative division. Both types of division require peptidoglycan synthesis to take place at the site of septation. Proteins involved in peptidoglycan synthesis are however also needed for the lateral cell growth, thus their trafficking between a division site and lateral cell wall must take place, depending on the stage of a cell cycle. One of the recently identified proteins, GpsB, operates in such shuttle system, transporting peptidoglycan synthesis protein PBP1 from a mature cell pole, which is a former division site, to the lateral cell wall. EzrA, on the other hand serves to return PBP1/GpsB complex back to the septum for division [2]. Very recently, GpsB was assigned with a role of general adaptor for peptidoglycan synthesizing enzymes PBPs, directing them to large protein complexes responsible for cell wall synthesis during cell elongation and cell division, facilitating thus these crucial interactions [3].

We demonstrated that GpsB interacts directly with SpoIIE and co-localizes with SpoIIE in the asymmetric septum, indicating that GpsB keeps its PBP-docking role also during asymmetric cell division.

We suggest that a multi-protein complex which includes sporulation specific SpoIIE and proteins involved in peptidoglycan synthesis (GpsB, EzrA, PBPs, RodZ) [4] may represent a direct link between asymmetric division and peptidoglycan biosynthesis.

1. S. Ben-Yehuda & R. Losick. *Cell* **109** (2) (2002), 257–266.
2. D. Claessen, R. Emmins, L.W. Hamoen, R.A Daniel, J. Errington, D.H. Edwards. *Molecular Microbiology* **68** (4) (2008), 1029–1046.
3. R. M. Cleverley, Z. J. Rutter, J. Rismondo, F. Corona, H. T. Tsui, F. A. Alatawi, R. A. Daniel, S. Halbedel, O. Massidda, M. E. Winkler, R. J. Lewis. *Nature Communications* **10** (1) (2019), 261.
4. K. Muchová, Z. Chromiková, N. Bradshaw, A.J. Wilkinson, I. Barák.*Plos One* **11** (2016), 1-21.

P10

MOLECULAR MECHANISM OF PHASE SEPARATION OF RNA POLYMERASE II

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In cells, alongside organelles with lipid membrane, exist organelles without membrane. These dynamic membranelles organelles are formed by liquid-liquid phase separation (LLPS) mechanism; they serve as microreactors, increasing a local concentration of components and accelerate the reaction. In eukaryotic cells, the complex responsible for transcribing of protein-coding is RNA polymerase II (RNAPII). This enzyme clusters into droplets at transcribed genes via its C-terminal, disordered domain. The molecular mechanism of RNAPII clustering remains unknown.

Here, we present a unique technique of co-expression and purification of the complex of human RNAPII from Hi5 cells. Three subunits of RNAPII were fused with specific affinity tags, which allows us to purify protein using affinity chromatography. Because preparation of RNAPII in suitable concentrations from Hi5 cells seems to be problematic, we simultaneously work on a protocol to purify RNAPII from HEK293 cells.

The purified RNAPII will allow us to study the process of phase separation of human RNAPII into droplets *in vitro* by a combination of structural and functional studies.

P11

STRUCTURAL STUDIES OF PURINE NUCLEOSIDE PHOSPHORYLASE INHIBITORS

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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.

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 thus has been established as prospective target for drug design. Several hPNP inhibitors recently entered human clinical trials.

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.

We are using X-ray crystallography in structure-based drug design of novel acyclic nucleotide analogues. Our goal is to design inhibitors with high affinity towards hPNP, PfPNP and MtPNP, 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, PfPNP and MtPNP were identified through wide screening and optimization. Selected inhibitors were successfully co-crystallized with MtPNP 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 in the enzyme will further help with the designing of specific PNP inhibitors.

P12

PREDICTING ION BINDING SITES IN PROTEINS

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From oxygen delivery, to protein phosphorylation and pH maintenance, ions are heavily involved in a wide range of biological processes and they have also been a target of pharmacological studies. About one third of known proteins include at least one metal ion and many metalloproteins are found in humans.

A number of experimental techniques are being used to identify metal ion binding sites in proteins; however, they are often tedious and time consuming, raising challenges in their wide application. UniProtKB contains over 500,000 annotated protein sequences while another 180 million sequences are pending annotation. Additionally, there is an abundance of protein structures solved for structural genomics projects that have no functional annotation. There is a dire need for computational methods that could process the bulk of accumulating data and produce meaningful annotations.

Several methods have been developed, typically training machine–learning algorithms in order to reveal patterns within protein–ion interactions and allow the prediction of ion binding sites within a given protein. Despite the remarkable progress in the field, and the high accuracy achieved by the top-of-the-line predictors, most of them seem to suffer from low sensitivity and low MCC values. Furthermore, the entirety of current predictors focusses on specific subsets of metal ions and even specific protein residues as possible binding candidates. Moreover, there are non-metal ions that are well-represented in the PDB but are not being considered in the ion binding prediction scheme. These are indications that the predictive capacity can be extended and improved upon.

Here, we are planning to cover all well-known metal ions, but also include non–metals and other previously neglected ions. In addition to the established prediction features that are found within the broader context of ligand

binding site prediction, we want to explore properties that are distinctive within the protein–ion binding scheme such as electrostatics and coordination geometry.

We are employing state-of-the-art machine learning algorithms P2Rank and PrankWeb, which have emerged through our collaboration with David Hoksza's group, and are adopted by PDBe-KB, and we are updating and optimizing them, for the purpose of ion binding site prediction in proteins. We assemble a robust dataset of 102,700 ion-binding protein structures, including 42,294 unique, non–redundant, ion–binding sites within those structures, and we create separate ion subsets on which machine-learning will be performed.

1. Burnett, G. & Kennedy, E. P. J. Biol. Chem. 211, 969–980 (1954).
2. Hsia, C. C. N. Engl. J. Med. 338, 239–247 (1998).
3. Bonar, P. T. & Casey, J. R. Channels (Austin) 2, 337–345 (2008).
4. Ndagi, U., Mhlongo, N. & Soliman, M. E. Drug Des Devel Ther 11, 599–616 (2017).
5. Degtyarenko, K. Bioinformatics 16, 851–864 (2000).
6. Jernigan, R., Raghunathan, G. & Bahar, I. Current Opinion in Structural Biology 4, 256–263 (1994).
7. Jendele, L., Krivak, R., Skoda, P., Novotny, M. & Hoksza, D. Nucleic Acids Res 47, W345–W349 (2019).
8. Krivák, R. & Hoksza, D. J Cheminform 10, (2018).
9. PDBe-KB consortium. Nucleic Acids Res. (2019).

P13

PLL3, A NOVEL MONOMERIC MEMBER OF THE SEVEN-BLADED BETA-PROPELLER LECTIN FAMILY

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

Our research is focused on studying lectins from bacterium *Photorhabdus*, which is known for its complicated life cycle, including mutualism with microscopic nematode and pathogenicity towards insects [2]. Moreover, some species of *Photorhabdus* are also able to infect humans. In this bacterial genus, two lectins with a seven-bladed beta-propeller fold and a high level of homology were described previously [3, 4]. This contribution is focused on the PLL3 lectin, a novel member of the seven-bladed beta-propeller lectin family. PLL3 exhibited the highest affinity toward L-fucose and its derivatives but was also able to interact with *O*-methylated glycans and other ligands. Unlike the other members of this family, PLL3 was discovered to be a monomer, which might correspond to a weaker avidity effect compared to homologous lectins.

1. Lis, H. and Sharon, N. Chem Rev. 1998, 98, 637-74.
2. Waterfield NR, Ciche T, Clarke D. Annu Rev Microbiol. 2009,63: 557–574.
3. Kumar, A., Sýkorová, P., Demo G., Dobeš, P., Hyršl, P., Wimmerová, M., JBC, 2016, 291(48), 25032 - 25049.
4. Jančaříková G, Houser J, Dobeš P, Demo G, Hyršl P, Wimmerová M. PLoS Pathog. 2017;13: e1006564.

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P14

^{19}F LABELLING OF 14-3-3 RECOMBINANT PROTEIN FOR ^{19}F NMR SPECTROSCOPY

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

Here, we present our progress in the preparation of protein samples for ^{19}F NMR measurements, labelled with ^{19}F modified aromatic amino acids (AAs): 5-^{19}F-Trp, 4-^{19}F-Phe, and 3-^{19}F-Tyr. Even though we used identical protocols, different AAs had different incorporation efficiency rates. ^{19}F tryptophan was readily incorporated with 100% efficiency. However, the extent of incorporation of ^{19}F phenylalanine and tyrosine ranged only between 30-50%, presumably due to the similar biosynthetic pathways or non-optimal culture conditions. On the other hand, the amount and purity of samples was sufficient for pilot titration experiments, as demonstrated by well-resolved 1D ^{19}F NMR spectra.

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

1. C. Frieden, S. D. Hoeltzli, J. G. Bann. *Methods Enzymol*. **380**, 2004, pp. 400-415.
2. J. C. Jackson, J. T. Hammill, R. A. Mehl, *J. Am. Chem. Soc.* **129,** 2007, pp. 1160-1166.
3. E. N. G. Marsh, Y. Suzuki, *ACS Chem. Biol*. **9**, 2014, pp. 1242-1250.
4. J. G. Bann, J. Pinkner, S. J. Hultgren, C. Frieden. *PNAS* **99(2)**, 2002, pp. 709-714.
5. C. Li, E. A. Lutz, K. M. Slade, R. A. S. Ruf, G-F. Wang, G. J. Pielak. *Biochemistry* **48**, 2009, pp. 8578-8584.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic within programme INTER-ACTION (project no. LTAUSA18168). CIISB research infrastructure project LM2018127 funded by MEYS CR is gratefully acknowledged for the financial support of the measurements at the CF Josef Dadok National NMR Centre.

P15

HERMES – A SOFTWARE TOOL FOR PREDICTION AND ANALYSIS OF MAGNETIC FIELD-INDUCED RESIDUAL DIPOLAR COUPLINGS IN NUCLEIC ACIDS

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HERMES is a web application for prediction of fiRDC and analysis of magnetic field induced RDC. fiRDC prediction is based on input 3D model structure(s) of nucleic acid (NA) fragment(s) [1] and built-in library of nucleic acid base specific magnetic susceptibility tensors and reference geometries [2]. When 3D model of NA is provided alongside experimental fiRDC, the program allows validation of the structure against calculated fiRDC data. When multiple models are provided, it allows identification of NA model(s) consistent with experimental fiRDC and/or quick assessment of nucleic acid fragment oligomeric state [3]. Additionally, the program built-in routine for rigid body modeling allows assessment of relative orientation of two domains in the nucleic acid structure. The program is written in MATLAB language and is executed on an Apache server interfaced with HTML, JavaScript and PHP. The web application and the source code in MATLAB are publically accessible at hermes.ceitec.muni.cz.

1. van Buuren BN, Schleucher J, Wittmann V, Griesinger C, Schwalbe H, Wijmenga SS (2004) NMR spectroscopic determination of the solution structure of a branched nucleic acid from residual dipolar couplings by using isotopically labeled nucleotides. *Angew Chem Int Ed Engl,* **43**(2):187-92.

2. Vavřinská A, Zelinka J, Šebera J, Sychrovský V, Fiala R, Boelens R, Sklenář V, and Trantírek L (2016) Impact of nucleic acid self-alignment in a strong magnetic field on the interpretation of indirect spin–spin interactions. *J Biomol NMR*, **64**: 53–62.

3. Al-Hashimi H, Tolman J, Majumdar A, Gorin A, Patel D (2001) Determining Stoichiometry in Homomultimeric Nucleic Acid Complexes Using Magnetic Field Induced Residual Dipolar Couplings. *J Am Chem Soc*, **123**, 5806-5807.

P16

ROLE OF HEXAMERIN IN REGULATION OF PUPA DEVELOPMENT

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The most abundant proteins in haemolymph of larva and pupa of holometabolous insects are hexamerins. Hexamerins function as a source of amino acids for development during non-feeding stages of life cycle. Furthermore, there has been an evidence that hexamerins act as juvenile hormone binding proteins. However, the details of this interaction remain unknown. Here we present the crystal structure of native hexamerin 70b, isolated from honeybee (*Apis mellifera*) pupae, determined to 2.0Å resolution. Hexamerin is a homohexameric complex with D3 symmetry and each subunit possesses an enclosed hydrophobic cavity occupied by a molecule of juvenile hormone. Upon proteolysis of hexamerin the hormone is released into haemolymph and affects the development of pupa. We propose that this novel mechanism of hexamerin-based regulation might be conserved among holometabolous insects.

P17

CRYSTALLOGRAPHIC STUDIES OF TBEV NS5 RDRP DOMAIN

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Tick-borne encephalitis virus (TBEV) is a major human pathogen, transmitted by ticks from *Ixodidae* family [1, 2]. TBEV is an enveloped virus with a ~ 11 kb positive-sense single-stranded 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 viral replication machinery, forming replication complex [5]. Despite many functional studies of various TBEV NS proteins, there is actually no crystal structure of any TBEV NS proteins deposited in the Protein Data Bank.

Non-structural protein NS5 is a large bi-functional protein comprising of two domains connected by highly flexible 10aa linker. 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 3D structure determination of TBEV RdRp domain. Two constructs of TBEV RdRp domain with modification of the flexible linker were designed. Both constructs were cloned into pET28-SUMO expression vector and the heterologous production of recombinant enzymes was optimized using *E. coli* BL21-CodonPlus competent cells (Agilent). SUMO-RdRp fusion proteins were purified using IMAC. SUMO fusion partner was cleaved off and the recombinant protein was further purified via reversed IMAC. Circular dichroism analysis was used to verify the correct fold of the protein and purified RdRp was then used for initial crystallization screening applying various commercially available crystal -lization screens (Molecular Dimensions, Hampton Research).

1. Bogovic, P. & Strle, F. (2015). *Word J. Clin. Cases.* **3**, 430.

2. Taba, P., Schmutzhard, E., Forsberg, P., Lutsar, I., Ljostad, U., Mygland, A., Levchenko, I., Strle, F. & Steiner, I.(2017). *Eur. J. Neurol.* 24, 1214-1261.

3. Lindenbach, B. D., Murray, C. L., Thiel, H. J. & Rice, C. M. (2013). In *Fields Virology.* Lippincott Williams & Wilkins, PA, USA.

4. Fuzik, T., Formanova, P., Ruzek, D., Yoshii, K., Niedrig, M. & Plevka, P. (2018). *Nat. Commun.* **9**, 436.

5. Mackenzie, J. (2005). *Traffic.* **6**, 967-977.

6. Bollati, M. *et al.* (2009). *Antiviral Res.* **87**, 125-148.

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P18

PREPARATION OF A SET OF ANTI-TAU MONOCLONAL ANTIBODIES IN CHO CELL LINE USING A ROBUST SYSTEM FOR EUKARYOTIC EXPRESSION

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Alzheimer's disease (AD) is characterized by dementia, which usually begins with subtle changes and memory loss and slowly increases in severity until it affects the patient's daily life and eventually ends in death of the patient. AD is pathologically defined by deposits of aggregated -amyloid (A) and hyperphosphorylated tau protein. In pathological conditions, tau polymerizes to neurofibrillary tangles composed of tau filaments, which overlap with regions of neuronal loss in AD and related tauopathies. Monoclonal antibodies are in many instances sole tools able to reveal subtle conformation changes on tau leading to neurodegeneration. A method permitting reproducible preparation of monoclonal antibodies is highly needed for structural, biophysical and functional studies on tau, with the consequences for the development of diagnostics and therapy of AD.

We constructed a eukaryotic expression vector pCMV_3'UTR derived from the pCMV-Script backbone. The new vector contains CMV promoter, signal peptide driving expression to culture media, multiple endonuclease cleavage sites for flexible cloning and 3′ untranslated region of EF1 alpha gene, which starts immediately after the sequence of antibody chain (Fig.1). The presence of 3'UTR domain enhances the level of expression in CHO cell line.

We have analysed the production of recombinant form of Fab monoclonal antibodies DC25, MN423, DC11 and DC39N1 cloned in the pCMV_3'UTR vector. The antibodies were purified by affinity chromatography on Protein G. We compared three different protocols for CHO cell cultivation after transfection. In the final optimized conditions, we were able to prepare more than 10 mg of purified antibody from 40 ml culture in three weeks. Prepared Fabs were successfully used for crystallization of tau protein complexes and biophysical studies.

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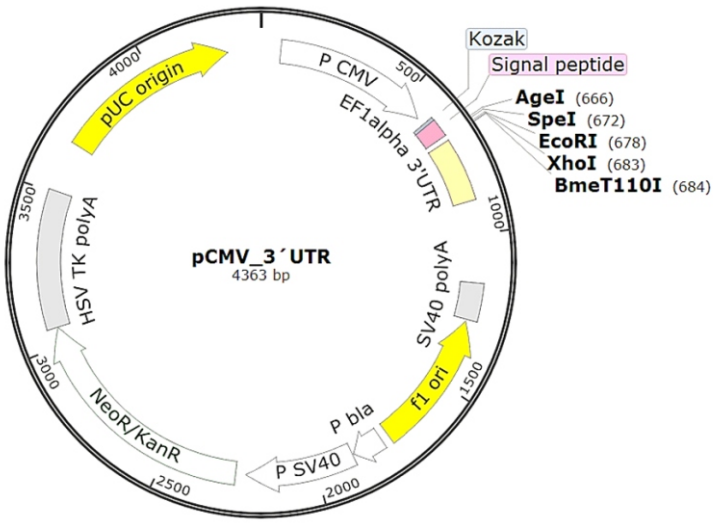
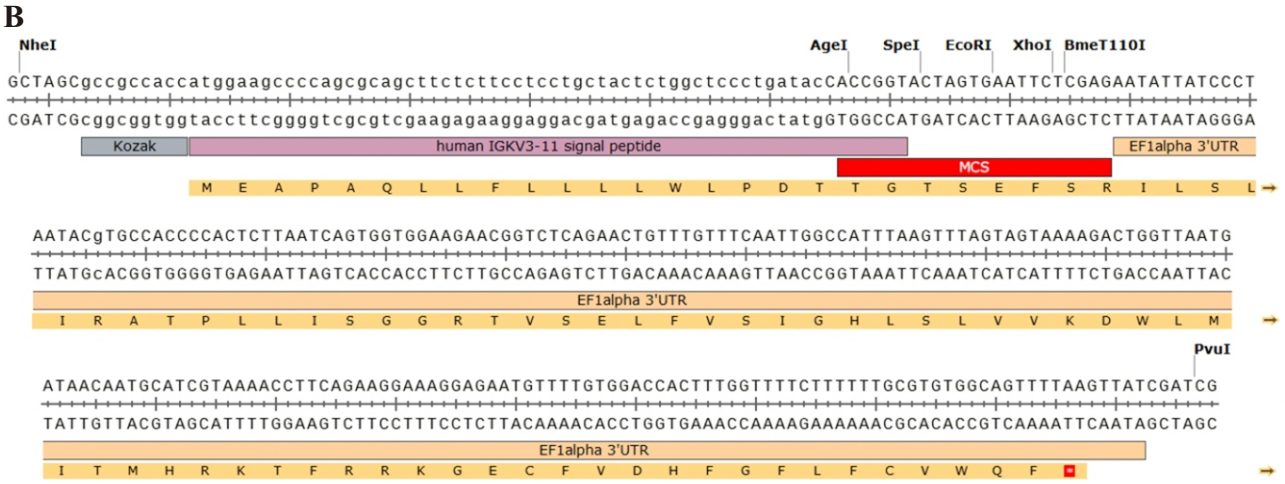


Figure 1. **Overview of new vector.** A - Map of pCMV_3'UTR vector with highlighted sequence features (P- promoter). AgeI and XhoI were chosen as cloning sites for monoclonal antibodies used in this study. B – Detailed view of pCMV_3'UTR vector with highlighted endonuclease sites, human IGKV3-11 signal peptide and elongating factor 1 alpha 3´UTR.



P19

STRUCTURAL STUDY OF KIX DOMAIN BY NMR

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KIX domain is part of transcriptional coactivator CREB binding protein (CBP) which is an important part of the gene regulation. KIX domain is a structured globular protein that consists of three alpha-helices and provides two allosteric binding sites named after its characteristic binding partners – the MLL site and the c-Myb site [1-2]. KIX domain binding partners are often unstructured and become folded only after interacting with the KIX domain. Despite its relatively small size (10 kDa), KIX domain serves as a great model protein for understanding molecular allostery, coupled folding and binding mechanisms of transactivation domains of intrinsically disordered proteins [3]. Recently established Nine amino acid Transactivation Domain (9aaTAD) motif unifies a prevalent number of transactivation domains of transcription factors [4]. This 9aaTAD pattern can be predicted using the prediction tool and can be further explored using the KIX domain and mutants of its typical binding partners.

This phenomenon was investigated by NMR spectroscopy 2D HN-HSQC experiment using ^{15}N isotopically labelled KIX domain titrated with MLL WT and its mutants to observe structural changes and dynamics.

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1. Korkmaz, E., Nussinov, R., Halilođlu, T., Plos Comput Biol, 2012, 8(3), e1002420.
2. Thakur, J.K., Yadav, A., Yadav, G., Nucleic Acids Res., 2014, 42(4), 2112-2125.
3. Brüschveller, S., Konrat, R., Tollinger, M., ACS Chem. Biol., 2013, 8(7), 1600-1610.
4. Piskacek, M., Vasku A., Hajek, R., Knight A., Mol. BioSyst., 2015, 11, 844-851.a.

P20

BIOPHYSICAL CHARACTERIZATION OF THE N-TERMINAL PART OF ASK1 AND ITS INTERACTION WITH TRX

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Apoptosis signal-regulating kinase 1 (ASK1), a member of mitogen-activated protein kinase kinase kinase (MAPKKK) family, is a serine/threonine kinase, which is a fundamental component of the MAP kinase signal transduction pathway [1]. Its dysregulation is associated with the pathogenesis of neurodegenerative, tumor, cardiovascular and other diseases [2, 3, 4]. The balance between up- and down-regulation is maintained through oligomerization and protein-protein interactions, however the molecular mechanism is largely unclear [5]. The main aim of this study is to describe the oligomeric behavior of three different N-terminal constructs of ASK1 and its interaction with thioredoxin (TRX). We performed SV-AUC, SAXS, XL-MS, rigid-body modelling and protein-protein docking for either ASK1 variants alone or in complex with TRX. Altogether, experiments performed up to now have shown, that all N-terminal constructs of ASK1 are at least partially capable of forming a dimer in solution. The concentration-dependent dimerization is driven by the kinase domain, however according to SV-AUC and XL-MS it appears that the central regulatory region might be also involved. The role of TRX in the process of ASK1 dimerization is still unclear, neither AUC nor SAXS analysis of ASK1:TRX complexes were able to provide satisfying results, therefore further experiments have been planned to determine its role.

1. Ichijo, H. *et al*., *Science* **275**, 90-94 (1997).
2. Okazaki, T., *Adv. Biol. Regul*. **66**, 37-45 (2017).
3. Liu, T. *et al*., *Adv. Biol. Regul*. **66**, 54-62 (2017).
4. Guo, X. *et al*., *Adv. Biol. Regul*. **66**, 63-71 (2017).
5. Rusnak, L. & Fu, H., *Adv. Biol. Regul*. **66**, 23-30 (2017).

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P21

THE REACTIVITY OF THE C-TERMINAL ANTIBODY SHOWS DIFFERENCES POSSIBLY DUE TO THE CHANGES IN THE GLOBAL FOLDS OF TAU ISOFORMS

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Alzheimer's disease (AD) is the most common cause of dementia and thus a disease that radically reduces the quality of life not only for patient but also for the patient's family. An important role in the development and progression of this disease is played by the accumulation of two proteins, namely the tau protein and -amyloid, caused by changes in their structures.

The structural insights into the pathological and physiological tau protein conformations may help to answer the key questions of the pathogenesis of AD and other tauopathies. All isoforms of tau protein consist of four main domains and differ according to number of N-terminal inserts (0N, 1N, 2N) and microtubule binding repeat regions - MTBRs (3R, 4R). An important regulatory role is played by the C-terminal domain that has shown to have an inhibitory effect on tau pathological aggregation [1].

We have been studying the kinetics of the interaction between the antibody DC39C, which epitope lies inside the last 12 C-terminal amino acids of tau, and various tau proteins by surface plasmon resonance. According to recent insights into the structure of tau protein obtained by

cross-linking mass spectrometry guided discrete molecular dynamics, where tau molecule was modelled as a rather globular and compact [2], the epitope of DC39C antibody should be hidden in between the beta strands of the first N-terminal insert and the N-terminus of tau protein. Global conformation that was previously observed by FRET method supports the model that places the C-terminal domain between the MTBRs and the N-terminal domain [3]. Our results have shown that isoforms without the N-terminal inserts bind to DC39C antibody better than isoforms containing them; same results were obtained when tau with various N-terminal domain truncation were used.

Subsequently, we have measured the kinetics of the heparin-induced filament formation by ThT fluorescence to observe the effect of the C-terminal domain removal on tau aggregation. We have compared the longest full length 3R tau isoform with two truncated tau proteins: tau151-391/3R which causes Alzheimer´s like pathology in the rat model [4] and the full length 3R tau isoform without the C-terminus (1-391/3R). The double truncated tau construct (151-391/3R) indicated very rapid filament formation; slightly slower filament formation was observed for tau protein without C-terminal, and the slowest filament formation was given by the isoform tau39 (2N3R). These findings also support the previously proposed hypothesis of C-terminal domain inhibition of tau aggregation.

1. Abraha, A., Ghoshal, N., Gamblin, T. C., Cryns, V., Berry, R. W., Kuret, J. & Binder, L. I., *J Cell Sci* **113**, (2000), 3737-3745.
2. Popov, K. I., Makepeace, K. A. T., Petrotchenko, E. V., Dokholyan, N. V. & Borchers, C. H., *Structure* **27**, (2019), 1710-1715 e1714.
3. Jeganathan, S., von Bergen, M., Brutlach, H., Steinhoff, H. J. & Mandelkow, E., *Biochemistry-Us* **45**, (2006), 2283-2293.
4. Filipcik, P., Zilka, N., Bugos, O., Kucerak, J., Koson, P., Novak, P. & Novak, M., *Neurobiol Aging* **33**, (2012) 1448-1456.

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P22

ROLE OF 14-3-3 PROTEIN IN THE REGULATION OF DEATH-ASSOCIATED PROTEIN KINASE 2 (DAPK2)

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Apoptosis and autophagy are tightly regulated biological processes, acting in synchro to secure proper development or survival of cells and multicellular organisms [1]. Death-associated protein kinase (DAPK) family of proteins plays an important role in commitment stage of apoptosis and progression of autophagy. Human DAPK family consists of five serine/threonine kinases with high homology in their catalytic domains located on N-terminus but vary in remaining regions [2]. 370 amino acid long DAPK2 is the shortest member of the DAPK family that consists of three domains: catalytic domain (23-285), autoregulatory domain (288-330) and dimerization domain (331-370). DAPK2 is activated by Ca^{2+}/Calmodulin (Ca^{2+}/CaM) binding to the autoregulatory domain or by phosphorylation at S299 and inhibited by the phosphorylation at S318 and T369 [3]. Phosphorylation at S318 triggers conformational change that prevents both: Ca^{2+}/Calmodulin from binding and substrate from entering the active site, as visible from the crystal structure of DAPK2 [4]. Phosphorylation at T369 is recognized by the 14-3-3 protein which disrupts dimerization and decreases the activity of DAPK2 [5, 6]. Primary focus of this study is to characterize the 14-3-3/DAPK2 complex and propose a molecular mechanism of 14-3-3 role in negative regulation of DAPK2. Based on our low-resolution structural data combined with biophysical characterisation of the complex and functional studies, we propose a mechanism where 14-3-3 acts as a scaffold for autoinhibitory conformation of DAPK2 and prevents dephosphorylation of regulatory phospho-sites. In this conformation, the 14-3-3 protein not only interacts with C-terminal regulatory domains of DAPK2 but also with the kinase domain, hence explaining the observed inhibitory effect of 14-3-3 on DAPK2 kinase activity. Additionally, the 14-3-3/DAPK2 complex can be stabilized by small molecule compound Fusicoccin. Thus, this study not only provides mechanistic insight into DAPK2 regulation but also suggests an alternative way how to inhibit DAPK2 during cancer treatment.

1. Gump, J. M. & Thorburn, A., *Trends Cell Biol.* **21**, 387–392 (2011).
2. Gozuacik, D. & Kimchi, A., *Autophagy* **2**, 74–79 (2006).
3. Geering, B., *Int. J. Biochem. Cell Biol.* **65**, 151–154 (2015).
4. Patel, A. K. *et al.*, *J. Mol. Biol.* **409**, 369–383 (2011).
5. Yuasa, K. *et al*., *Biochem. Biophys. Res. Commun.* **464**, 70–75 (2015).
6. Gilad, Y. *et al.*, *Cell Rep.* **8**, 909–921 (2014).

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P23

CRYO-EM STRUCTURE OF HUMAN RHINOVIRUS 14 IN COMPLEX WITH ITS RECEPTOR ICAM-1

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Human rhinovirus 14 (HRV14) belongs to the major group of human rhinoviruses, which utilize intercellular adhesion molecule 1 (ICAM-1) as their receptor. It was shown before that the binding site for ICAM-1 is located at so called 'canyon' region of the HRV14 in a vicinity of the five-fold symmetry axis. However, precise binding interface between HRV14 and ICAM-1 is not known. Here we present cryo-EM structure of HRV14-ICAM-1 complex at 2.8 Å resolution. We show that the interaction causes conformational changes in BC and FG loops of D1 domain of ICAM-1 and loop 151-161 in VP1 of HRV14. We describe extensive ionic and hydrogen bonds between the virus and the receptor. This information will provide a guide for development of small molecule inhibitors interfering with the cell entry of HRV14. Blocking of attachment of virus to its receptor is in scope of many drug development trials since it disrupts the viral life cycle in its very beginning.

P24

STRUCTURAL AND BIOPHYSICAL STUDY OF THE FIRST N-TERMINAL INSERT OF TAU PROTEIN IN THE COMPLEX WITH MONONCLONAL ANTIBODY

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Alzheimer's disease (AD) is neurodegenerative disorder characterized by deposits of -amyloid plaques and aggregated tau protein. Tau is associated with microtubules and stabilizes them maintaining their highly dynamic state. Under disease conditions tau undergoes aberrant posttranslational modifications, *e.g.* truncation and hyperphosphorylation [1]. Pathological form of tau protein does not bind microtubules and is prone to aggregation creating dimers and oligomers, which next are forming straight filaments and paired helical filaments leading to typical neurofibrillary tangles.

Tau protein is an intrinsically disordered protein (IDP) without any stable secondary or tertiary structure [2]. The conformational regulation and structural changes of tau are of great interest because they can be targeted in the treatment of AD. For monitoring the structure, dynamic biophysical methods are employed. Very useful can be studies involving interaction partners of tau, particularly monoclonal antibodies recognizing specific (conformational) epitopes on tau. Biophysical characterization of tau-antibody complexes may elucidate kinetic and thermodynamic regulation of individual tau epitopes, whereas crystallography of complexes may confer molecular details of tau structural propensity.

Monoclonal antibody DC39N1 used in this work was prepared after mice immunization with short peptide corresponding to the first alternatively spliced N-terminal insert of tau. It was used for detailed analysis of the structure of N-terminal domain of tau protein alone and in cooperation with other tau protein domains. Previously, DC39N1 epitope was refined to nine amino acids from 58 to 67 residues in 2N4R isoform [3, 4].

Fab of DC39N1 was cloned in eukaryotic expression vector and produced in ExpiCHO cells. After cultivation, the antibody was purified by affinity chromatography, characterised by dynamic light scattering and SDS-PAGE and prepared for isothermal titration calorimetry (ITC), surface plasmon resonance (SPR) and crystallisation experiments. ITC and SPR measurements of complex formation with a large panel of tau proteins revealed subtle allosteric regulation of flexible tau protein by alternatively spliced parts of molecule. In crystallization experiments we were able to obtain crystals of antibody alone and of putative complexes with four tau protein variants of sufficient quality (Fig. 1).

1 M. Novak, R. Jakes, P. C. Edwards, C. Milstein, C. M. Wischik, *Proc. Natl. Acad. Sci. U.S.A.* **88**, (1991), 5837 – 5841.

2. D. W. Cleveland, S. Y. Hwo, M. W. Kirschner, J. Mol. Biol. **116**, (1977), 207 – 225.

3. K. Soltys, G. Rolkova, L. Vechterova, P. Filipcik, N. Zilka, E. Kontsekova, M. Novak, *NeuroReport*, **16,** (2005), 1677 – 1681.

4. N. Zemanova, Magister thesis, (2016).

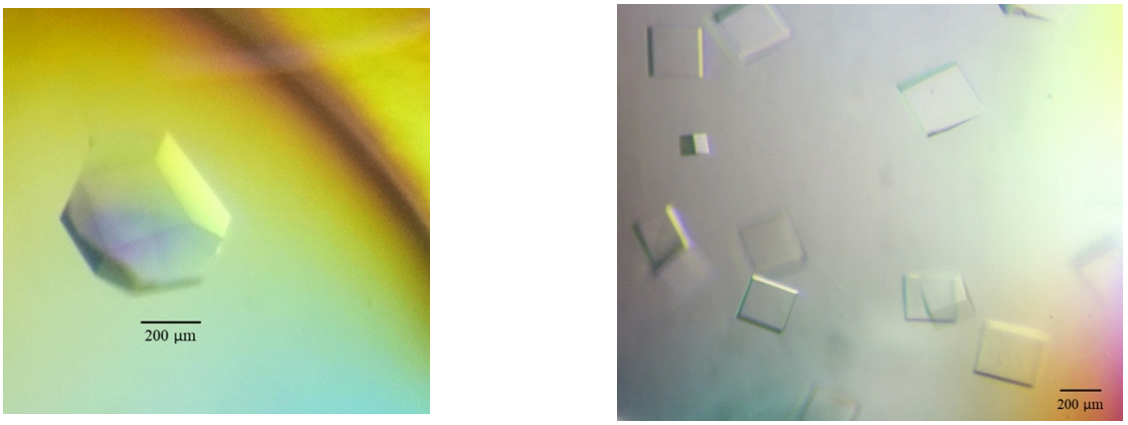

Figure 1. Microphotography of crystals created by Fab monoclonal antibody DC39N1 (left) and the DC39N1 Fab in complex with tauN98 (right). Crystal of Fab monoclonal antibody DC39N1 grown in 0.1M Sodium HEPES pH7.5 10%w/v PEG 6000 5%v/v MPD and crystal of the DC39N1 Fab in complex with tauN98 grown in 0.2 M Ammonium sulfate, 0.1 M BIS-TRIS pH 6.5, 25% w/v PEG 3350.

P25

STRUCTURAL AND BIOPHYSICAL PRINCIPLES OF NATURAL PROTECTION OF ORGANISM MEDIATED BY LACTOFERRIN – A MILK GLYCOPROTEIN

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Lactoferrin (LF) is an iron-binding glycoprotein belonging to the transferrin family. LF is abundant in milk and is present also in other exocrine fluids (*e.g.* saliva, tears) and in the secondary granules of neutrophils. LF has a bilobal structure and a high isoelectric point. A cluster of positively-charged amino acids is located at its N-terminus, wherefrom the bioactive peptide lactoferricin (LFcin) is derived. Basic character of LF and its ability to bind iron mediate many beneficial biological properties, which have been attributed to LF – antimicrobial, antiviral and immunomodulatory activities.

Recently, LF has been shown to block activation of plasminogen (Plg), a zymogenic form of serine protease plasmin (Pm) [1]. Plg-Pm system plays an important role in many physiological processes, *e.g.* fibrinolysis. However, aberrant activation of Plg may aid invasion of tumour cells and certain bacteria [1, 2]. Therefore, inhibition of pathological Plg activation by LF can have a therapeutic potential. It has been shown by surface plasmon resonance (SPR) that holo-LF can bind directly to Plg with a high affinity; however, the interaction does not obey a simple 1:1 mechanism and needs a pre-activation of LF by acids [1]. For an effective exploitation of LF inhibitory activity on Plg activation it will be of major importance to elucidate the molecular structure of LF-Plg complex as well as the pathways of complex formation. These are the aims of our work.

As the apo-LF needs to be exposed to acidic pH before being able to bind Plg, and this treatment can alter its structure and induce oligomerization, we studied in details the conformation of LF in lowering pH by dynamic light scattering. We observed a reversible acidic expansion of LF with no direct implication on its dimerization. Further, we compared reactivity of various forms of LF with Plg by SPR.

The X-ray structures of LF and Plg are known, however, LF was until now crystallized only in conditions employing high concentration of propanol, which can influence the structural features. We have performed large crystallization screening of LF and have found crystallization conditions without alcohol. Finally, because lactoferricin peptide is suspected to mediate the interaction of LF and Plg [1], we have optimized preparation of LFcin by pepsine digestion of LF and purification by a two-step chromatography procedure, and tested the inhibitory activity of purified peptides on Plg activation.

1. A. Zwirzitz, M. Reiter, R. Skrabana, A. Ohradanova-Repic, O. Majdic, M. Gutekova, O. Cehlar, E. Petrovcikova, E. Kutejova, G. Stanek, H. Stockinger, V. Leksa, *J. Biol. Chem.* **293**, (2018), 8600–8613.
2. K. Dano, N. Behrendt, G. Hoyer-Hansen, M. Johnsen, L. R. Lund, M. Ploug, J. Romer, *Thromb Haemost* **93**, (2005), 676–681.

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STUDY OF PROTEIN-PROTEIN INTERACTIONS OF HUMAN E3 UBIQUITIN LIGASE NEDD4L USING FLUORESCENCE SPECTROSCOPY

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Post-translational modifications by ubiquitination are important for conserved roles in the regulation of membrane proteins. Nedd4-2, an ubiquitin ligase (E3) of the HECT family, targets membrane proteins such as ion channels and transporters for ubiquitination [1,2]. 14-3-3 proteins, a family of the conserved regulatory molecule, negatively regulates Nedd4-2 through phosphorylation by PKA. This regulation is performed by providing scaffolding for Nedd4-2, thereby preventing the interaction with Nedd4-2 and other membrane proteins. Though this is known, the molecular mechanism of this regulation largely remains unknown and is under scientific scrutiny [3, 4].

We aim to understand the structural and functional basis of 14-3-3 mediated regulation of Nedd4-2. Nedd4-2 consists typically of three domains: C2 domain, WW domain and HECT domain. Possible mechanism of the 14-3-3 mediated inhibition of pNedd4-2 includes stabilization of inactive conformation of Nedd4-2 in which, HECT and C2 domains are involved in the intramolecular interaction and steric masking of WW domains surfaces. To test this hypothesis, we plan to perform the time resolved fluorescence spectroscopy measurements using phosphorylated Nedd4-2 variants labelled by extrinsic fluorophore and monitor their interaction with 14-3-3 protein. Fluorescence spectroscopy will provide basic information on the dynamics of the interaction between Nedd4-2 ligase and 14-3-3 protein. Therefore, we prepared seven Nedd4-2 protein variants with the single amino acid cysteine, which will be fluorescently labelled, monitoring different WW domains of Nedd4-2. The position selected for cysteine variants in Nedd4-$2_{190\text{-}581}$; WW1: C209, C218; WW2: C389, C414: WW3: C508, C522; WW4: C571. Measuring of rotational correlation time and determination of the mean lifetime values of excited fluorophore in Nedd4-2 alone and in the complex with 14-3-3 protein (containing no Cys residues) will allow us to trace the microenvironment of one particular cysteine amino acid, which is located at different positions within Nedd4-2 construct. Expression and purification of selected cysteine mutants has been successfully performed using a bacterial expression system.

We also crystallized the complex of 14-3-3 C with the peptide containing phosphorylated Ser^{342}, solved its structure using molecular replacement and refined it at 1.61Å resolution.

1. J. A. Manning and S. Kumar, *Trends Biochem. Sci.* **43**, (2018), 635–647.
2. P. Goel, J. A. Manning, and S. Kumar, Gene, 557, (2015), 1–10.
3. Nagaki K, Yamamura H, Shimada S, Saito T, Hisanaga S, Taoka M, Isobe T, Ichimura T, Biochemistry, 45, (2006), 6733-40.
4. Ichimura T, Isobe T, J Biol Chem., 280, (2005), 13187-94.

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P27

STRUCTURE COMPARISON OF SALIVARY SERPINS FROM *IXODES RICINUS*

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The serpins are a large superfamily of structurally conserved proteins that are widely distributed [1]. Our structural study is focused on serpins, serine protease inhibitors, found in tick saliva. This group of proteins has primarily immunological and haemostatic functions, but their functions can vary. The tick serpins act as modulators of immune responses by using their anti-coagulation, anti-complementary functions and play role in immunosuppression [2].

The serpins rearrange their structural conformation required for inhibitory activity. The secondary structure typically consists of 3 -barrels, 9 -helices and exposed, flexible reactive center loop (RCL) that contains proteinase recognition site. There are different types of conformation and each of these structural rearrangements is important in the inhibitory pathway. The process of serine proteinase inhibition required irreversible suicide substrate mechanism [3].

Structural and functional studies of inhibitory serpins help understand the serpinopathies, diseases such as cirrhosis, hypertension or familial dementia. Here, we present the crystal structures of two serpins found in tick saliva of *Ixodes ricinus* (iripin3 and IRS-4) compared to the structure of *Ixodes ricinus* serpin 2 (IRS-2) describe previously [4].

1. J. Potempa, E. Korzus & J. Travis (1994) *J. Biol. Chem*. **269**, 15957-15960.
2. Ooi, C. P., Haines, L. R., Southern, D. M., Lehane, M. J., & Acosta-Serrano, A. (2015). PLoS Negl. Trop. Dis. 9:e3448. doi: 10.1371/journal.pntd.0003448
3. P. G. W. Gettins, P. A. Patston & S. T. Olson(eds) (1996) Serpins: Structure, Function and Biology, *Molecular biology Intelligence Unit*, R. G. Landes Co., and Chapman & Hall, Austin, TX.
4. J. Chmelar, C. J. Oliveira, P. Rezacova, I. M. B. Francischetti, Z. Kovarova, G. Pejler, P. Kopacek, J.M. C. Ribeiro, M. Mares, J. Kopecky & M. Kotsyfakis; *Blood* 2011; **117** (2): 736–744. doi: https://doi.org/10.1182/blood-2010-06-293241

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

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Transcription is considered one of the major threats for genome stability; as the conflicts of the transcription machinery with the replication fork, or another barrier, can lead to double-stranded DNA breaks. Although maintaining genome integrity is crucial for the cell viability, the mechanisms responsible for avoiding these conflicts are poorly characterized. Therefore, my research project focuses on structural characterization of the interaction between RNA polymerase II (RNAPII) and BRCA1-BARD1 complex, one of possible players involved in maintaining the genome stability. Recently, we have confirmed the interaction between the C-terminal domain of RNAPII and the BRCT domains of BRCA1 and BARD1, respectively, and we are reconstituting the full-length complex for cryo-electron microscopy studies. Structural characterization of the complex, as well as description of the conditions under which it is formed will help us to analyse its function in preventing transcription-borne DNA damage. This, in turn, will help us to understand how cells coordinate transcription and other competing processes on DNA, such as replication or DNA repair.

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X-RAY STRUCTURE OF PDZ DOMAIN OF DISHEVELLED-3 PROTEIN

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Dishevelled (DVL) is a key component of the Wnt signalling pathway, which governs a wide range of biological processes (cell proliferation, migration and differentiation, stem cell renewal, cell polarity). It is involved in both canonical (-catenin-dependent) and non-canonical (-catenin-independent) cell signalling pathways [1]. Dishevelled protein was first discovered in Drosophila mutants with disordered hair and bristle polarity [2], and it was proved that it plays an important role in embryogenesis. Dishevelled proteins are also connected with the process of cancerogenesis in humans, and mutations/dysregulation of Wnt pathway components are associated with certain carcinomas [3].

Dishevelled proteins consist of three conserved domains: N-terminal DIX domain, central PDZ domain, and C-terminal DEP domain, that are linked by an unstructured basic region (DIX-PDZ) and proline-rich region (PDZ-DEP) [4]. As a scaffolding protein, DVL uses its domains for interaction with a wide range of human proteins [1]. Its activity is affected by post-translational modifications (phosphorylation, ubiquitination and acetylation) [3, 5], however, the exact way how Dishevelled proteins integrate and relay the complex signals to perform such a broad spectrum of biological activities remains unknown.

This work is focused on the crystallization and structure determination of the PDZ domain of human Dishevelled-3 (DVL3) protein. A 1.4 Å diffraction data were collected and the structure of PDZ was solved by the molecular replacement method.

1. C. Gao, Y. G. Chen, *Cell Signal* **22(5)** (2009), 717-27.
2. O. G. Fahmy, M. J. Fahmy, *Genetics* **44(6)** (1959), 1149-71.
3. M. Sharma, I. Castro-Piedras, G. E. Simmons, K. Pruitt, *Cell Signal* **47** (2018), 52-64.
4. M. Boutros, M. Mlodzik. *Mech Dev* **83(1-2)** (1999), 27-37.
5. M. Sharma, D. Molehin, I. Castro-Piedras, E. G. Martinez, K. Pruitt. *Sci Rep* **9(1)** (2019) 16257.

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STUDY ON ALDEHYDE DEHYDROGENASES FROM LOW AND HIGH PLANTS USING X-RAY CRYSTALLOGRAPHY, MICROSCALE THERMOPHORESIS AND SITE-DIRECTED MUTAGENESIS

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Aldehyde dehydrogenases (ALDHs) comprise a protein superfamily of $NAD(P)^+$-dependent enzymes (EC 1.2.1.-) and catalyze irreversible oxidation of aldehydes to carboxylic acids. ALDHs play a crucial role in detoxifying aldehydes produced by various metabolic pathways and during various stress conditions such as salinity, heat, cold and drought. At least 13 distinct families have been found in plants. Genomes of primitive plants such as algae comprise often a single *ALDH* gene per family while those of higher plants contain one to several *ALDH* gene copies per family. Gene duplication is a predominant evolutionary force behind existence of new genes, which undergo either partitioning of gene function or development of a new function. As plant organisms moved to land, there was a need for gene expansion linked to adaptation to new terrestrial stressors as well as to vascularization, tissue distribution and differentiation or regulation of hormone responses. As many studies on plants revealed that up-reg-

ulation of *ALDH* genes is a common target of stress response pathways, there is an economical interest in utilizing *ALDH* expression to improve crop growth and quality.

Here, we focused on ALDH10 and ALDH12 family members from moss *P. patens*, *A. thaliana* and maize (*Z. mays*) as well as ALDH22 family from maize and barley (*H. vulgare*) and ALDH23 from moss. Members of plant ALDH10 family are known to oxidize various aminoaldehydes arising from polyamine oxidation while ALDH12 oxidize semialdehyde dehydrogenase arising upon proline oxidation. So far, metabolites linked to ALDH22 and ALDH23 are unknown. The optimal buffer composition was analyzed using a nano differential scanning fluorimetry to achieve the highest stability of particular ALDH isoform. Substrate specificity was further evaluated spectrophotometrically, the best substrates were identified and kinetic constants were determined. Affinities towards the best aldehyde substrates and coenzyme NAD^+ were further determined using microscale thermophoresis. The crystal structure of ZmALDH12 was solved at 2.2 Å resolution and subsequent site-directed mutagenesis was performed to identify key residues affecting the substrate and coenzyme preferences.

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LEDGF/P75 INTERACTION NETWORK IN THE NUCLEOSOMAL CONTEXT

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Lens Epithelium Derived Growth Factor/p75 (LEDGF/p75 or PSIP1) is a transcriptional co-activator responsible for tethering other factors to the regions of actively transcribed genes using its PWWP domain that specifically binds di/trimethylated lysine 36 on histone 3 (H3K36me2/3). Cellular partners bound to its C-terminal integrase-binding domain (IBD) are thus drawn near the active chromatin. Through this interaction, LEDGF/p75 is associated with two distinct diseases, HIV infection and mixed-lineage (MLL) leukemia, and therefore becomes an attractive therapeutic target. The aim of our work is to understand the biological roles of LEDGF/p75 by tracking its interaction network in the nucleosomal context. The binding affinity for PWWP domain is six orders of magnitude higher when interacting with the whole nucleosome than H3K36me3 peptides or DNA alone because of its ability to bind both gyres and modified histone tail simultaneously. This would have an impact on the IBD domain-bound cellular partners that are readily available in the proximity of transcribed genes. To further elucidate the whole process, we examine the PWWP domain-nucleosome interactions using full-length LEDGF/p75, as well as LEDGF/p75 in complex with its known cellular partners. Our data present obtained cryo-EM structure of H3K36me2 nucleosome bound to the LEDGF/p75-POGZ(1117-1410) complex. These results will have an impact on current MLL leukemia drug discovery efforts by finding specific inhibitors of LEDGF/p75-MLL1 interaction and will contribute to a better understanding of transcriptional regulation in eukaryotic cells.

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STUDY OF DNA-PROTEIN INTERACTIONS IN LIVING CELLS BY IN-CELL NMR SPECTROSCOPY

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In each organism, there are macromolecules - elementary components of living machinery responsible for the proper function of their cells. There are number of established structural and chemical biology approaches to investigate relationships between macromolecule structure and function under in vitro conditions. However, conventional structural and chemical biology methods for analysis of macromolecules are not conducted in their native context. Consequently, information on important structural and functional features of macromolecules are lost as macromolecules inside the living cells are influenced by many

environmental factors such as temperature, molecular crowding, pH, viscosity, or metabolites.

In-cell NMR spectroscopy can provide invaluable biological, structural, and functional information about behavior of proteins and nucleic acids (DNA/RNA) in the complex physiologically relevant environment of living cells at close-to-atomic resolution[1]. Here, we report on development of the in-cell NMR based approach for investigation of DNA-protein interactions in living human cells (Fig.1). The procedure is based on inducible overexpression of the isotopically labeled protein of interest encoded by corresponding DNA sequence stably incorporated in human genome of cells that are subsequently transfected with desired DNA/RNA target. Our ambition is to reconstitute protein-NA binding interface susceptible to in-cell NMR investigation inside intracellular space of living cells.

1. Luchinat E, Banci L. A Unique Tool for Cellular Structural Biology: In-cell NMR. J Biol Chem. 2016 Feb 19;291(8):3776–84.

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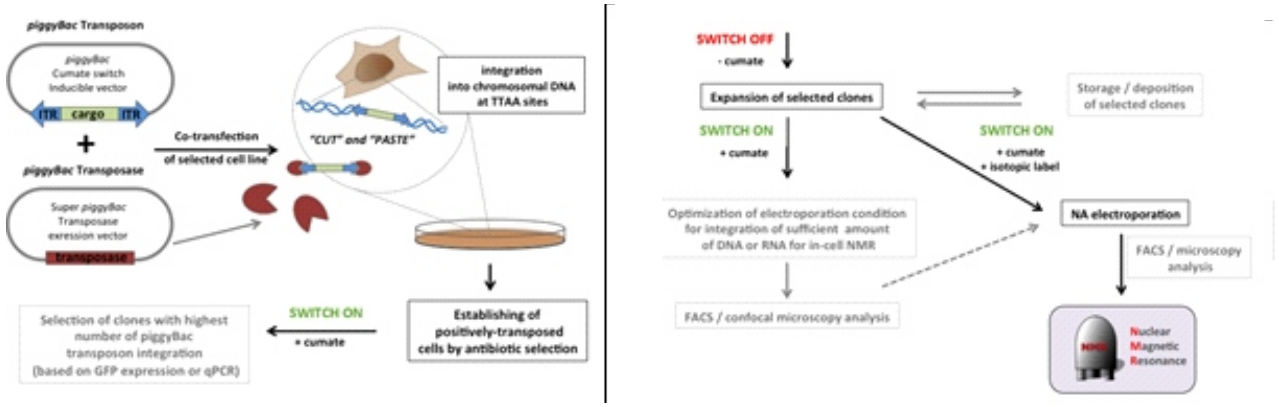

Figure 1. Scheme of DNA-protein experiment.

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RECOGNITION OF RNA POLYMERASE II C-TERMINAL DOMAIN BY RPRD2

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

In this work, we show that human transcription factor, RPRD2, recognizes specifically pSer2 or pThr4 phosphorylated forms of CTD via its CTD-interacting domain (CID) in a similar way to its yeast homologue, Rtt103. The interaction of RPRD2 CID with pSer2 phosphorylated CTD is further enhanced by additional phosphorylation on pSer7. To provide mechanistic details of the interaction between RPRD2 CID and pSer2,7 CTD, the solution structure was obtained using NMR spectroscopy. pSer2 and pThr4 phosphomarks occur mainly during the late elongation and termination. RPRD2s preference for these two phosphomarks suggests possible involvement of RPRD2 in transcription termination.

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LIFE PRIOR TO AROMATIC RESIDUES: REVERSE ENGINEERING OF A DEPHOSPHOCOENZYME A KINASE

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It is well-known that the large diversity of protein functions and structures derives from the broad spectrum of physicochemical properties of the 20 canonical amino acids that make up modern proteins. According to the generally accepted hypothesis [1], evolution of protein structures and functions was continuously associated with enrichment of the genetic code, with aromatic amino acids being considered the latest addition to the genetic code to increase structural stability of proteins and enhance clarification of its catalytic functions [2].

The main objective of this study was to test whether enzymatic catalysis can spare the structural stability provided by aromatics by determining the effect of amino acid alphabet reduction on structure and function of dephosphoCoA kinase (DPCK). Two mutant variants of a putative DPCK from Aquifex aeolicus (PDB code 2IF2) without aromatic amino acids were designed, substitution by Leu residues (DPCK-LH) and by non-aromatic residues based on the best predicted preservation of thermodynamic stability (DPCK-M).

Structural characterization of DPCK variants (using CD and 1D-NMR) suggest that there is less tertiary structure and partial loss of secondary structure content upon loss of the aromatic residues. Intriguingly, DPCK-LH seems to be structurally more similar to DPCK-WT than the DPCK-M variant. The results of structural studies reflect on catalytic activities of protein variants with the gradual decrease of protein folding echoing in the steady loss of substrate specificity in a row DPCK-WT – DPCK-LH – DPCK-M. Whereas DPCK-WT shows phosphotransferase activity, DPCK-LH and DPCK-M rather demonstrate ATPase activity and 150 and 500 times lower catalytic efficiency in ATP conversion, respectively, mainly as a result of a decreased turnover number. The HPLC-MS analysis detected significant CoA formation only in the reaction catalysed by DPCK-WT but 10x and 100x less formation was detected also in the reactions catalysed by DPCK-LH and DPCK-M, respectively.

The obtained data provide evidence to the hypothesis that proteins in the early stages of life could support at least some enzymatic activities, probably with lower efficiencies as a result of lack of firm hydrophobic core.

1. J. Tze-Fei Wong, *Bioessays*, **27 (4)** (2005), pp.416-25.
2. E. D. Mauro, A. K. Dunker, E. Trifonov, in *Cellular Origin, Life in Extreme Habitats, and Astrobiology*, edited by J. Seckbach (Springer, Dordrecht), **22** (2012), pp. 415-435.

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P35

MOLECULAR INSIGHT INTO THE INTERACTION BETWEEN FORKHEAD BOX O4 (FOXO4) AND P53 TRANSCRIPTION FACTORS

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The forkhead box O (FOXO) transcription factor belongs to the forakhead (FOX) protein family and controls diverse signaling pathways to regulate cell cycle, metabolism, differentiation, stress response, and apoptosis [1,2]. The FOXO subfamily has four members (FOXO1, FOXO3, FOXO4 and FOXO6) which share approximately 110 amino acid long DNA binding domain (called forkhead domain) throughout evolution [3,4]. They are expressed in a wide range of organisms (yeast to human) and their functional activity highly regulated by post-translational modification and interaction with other proteins including p53 [4,5]. The p53 protein acts as a central hub in the biological network which can modulate the activity of its interacting partners. In the past few years, the growing evidence suggests that both FOXO4 and p53 can interact with each other upon ionizing radiation which leads to cellular senescence [6]. An accumulation of senescent cell accelerates the aging process and other age-related diseases. However, the molecular mechanisms of this interaction remain unknown due to the lack of structural data. So our deep interest is to understand which regions of p53 and FOXO4 are involved in complex formation and what is the structure of the FOXO4-p53 complex. Therefore, pull-down assay, sedimentation velocity analytical ultracentrifugation (SV-AUC), 2D ^{1}H-^{15}N HSQC NMR spectroscopy was used to investigate the interaction between various constructs of FOXO4 and p53. Our preliminary data suggested that both FOXO4 and p53 are interacting with each other with the affinity (K_D) in the micromolar range. HSQC NMR spectroscopy data reveals that the N-terminal segment as well as other regions of the FOXO4 DNA-binding domain is involved in p53 binding.

1. D. Accili, K.C. Arden, Rev. Cell., **117**, (2004), 421–426.
2. D.R. Calnan, A. Brunet, Rev. Oncogene., **27**, (2008), 2276-2288.
3. D. Weigel, H. Jackle, Cell.,**63,** (1990), 455-456.
4. T. Obsil, V. Obsilova, Rev. Oncogene., **27**, (2008), 2263-2275.
5. F. Wang, C.B. Marshall, K. Yamamoto, G-Y Li, M.J. Plevin, H. You, T.W. Mak, M. Ikura, J. Mol. Biol., **384** (2008), 590-603.
6. M.P. Baar, R.M.C. Brandt, D.A. Putavet, ..., J.H.J, Hoeijmakers, J. Campisi, P. L.J.de Keizer, Cell., **169**, (2017), 132–147.

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IN-CELL NMR SPECTROSCOPY AS A TOOL FOR STUDYING THE INFLUENCE OF CHANGING INTRACELLULAR ENVIRONMENT ON NON-B DNA STRUCTURES

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In-cell NMR spectroscopy is useful technique enabling structural characterization of macromolecules such as proteins or DNA inside the complex environment of living cell [1]. This is very important for molecules sensitive to the parameters of their environment such as pH, ionic strength and composition, or molecular crowding.

I-motifs represents one of these structures. DNA i-motif is a tetrameric DNA structure based on the formation of hemi-protonated cytosine-cytosine (C+.C) base pairs [2]. Under in vitro conditions, i-motif formation is favored by acidic pH and low ionic strength [3], [4]. However, the existence of i-motifs in living cells has remained unclear until recently [5], [6].

In eukaryotic genomes, i-motif-prone sequences are enriched in biologically important regions, such as gene promoters where they were proposed to play an active role in regulation of gene expression [7]. Parameters of the intracellular environment vary during different cellular events such as cell cycle, apoptosis or hypoxia [8], which can trigger changes of occurence of i-motifs in vivo, as was

recently demonstrated by [6]. Here, we present possibility to investigate formation of i-motifs in separate phases of the cell cycle using in-cell NMR spectroscopy.

1. Z. Serber, A. T. Keatinge-Clay, R. Ledwidge, A. E. Kelly, S. M. Miller, and V. Dötsch, 'High-Resolution Macromolecular NMR Spectroscopy Inside Living Cells', *J. Am. Chem. Soc.*, vol. 123, no. 10, pp. 2446–2447, Mar. 2001, doi: 10.1021/ja0057528.
2. K. Gehring, J. L. Leroy, and M. Guéron, 'A tetrameric DNA structure with protonated cytosine.cytosine base pairs', *Nature*, vol. 363, no. 6429, pp. 561–565, Jun. 1993, doi: 10.1038/363561a0.
3. H. A. Day, P. Pavlou, and Z. A. E. Waller, 'i-Motif DNA: structure, stability and targeting with ligands', *Bioorg. Med. Chem.*, vol. 22, no. 16, pp. 4407–4418, Aug. 2014, doi: 10.1016/j.bmc.2014.05.047.
4. J.-L. Mergny, L. Lacroix, X. Han, J.-L. Leroy, and C. Helene, 'Intramolecular Folding of Pyrimidine Oligodeoxynucleotides into an i-DNA Motif', *J. Am. Chem. Soc.*, vol. 117, no. 35, pp. 8887–8898, Sep. 1995, doi: 10.1021/ja00140a001.
5 S. Dzatko *et al.*, 'Evaluation of the Stability of DNA i-Motifs in the Nuclei of Living Mammalian Cells', *Angew. Chem. Int. Ed Engl.*, vol. 57, no. 8, pp. 2165–2169, 19 2018, doi: 10.1002/anie.201712284.
6. M. Zeraati *et al.*, 'I-motif DNA structures are formed in the nuclei of human cells', *Nat. Chem.*, vol. 10, no. 6, pp. 631–637, Jun. 2018, doi: 10.1038/s41557-018-0046-3.
7. H. Abou Assi, M. Garavís, C. González, and M. J. Damha, 'i-Motif DNA: structural features and significance to cell biology', *Nucleic Acids Res.*, vol. 46, no. 16, pp. 8038–8056, Sep. 2018, doi: 10.1093/nar/gky735.
8. I. H. Madshus, 'Regulation of intracellular pH in eukaryotic cells.', *Biochem. J.*, vol. 250, no. 1, pp. 1–8, Feb. 1988.

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STRUCTURAL CHARACTERISATION OF PHAGE INFECTION IN BACTERIAL BIOFILM

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Pseudomonas aeruginosa is a multi-drug resistant bacterial pathogen identified by the World Health Organization as a high-priority target for the development of new treatments. *P. aeruginosa* has intrinsic resistance mechanisms such as efflux systems and antibiotic-inactivating enzymes and can also acquire resistance with mutations. As an additional adaptive resistance mechanism, adherent *P. aeruginosa* cells can form a biofilm, shielding the colony from the immune system and from pharmaceutics [1].

Phage therapy is a promising alternative for treating such resistant infections. Bacteriophage LUZ19 is a lytic phage from the *Podoviridae* family that infects a variety of clinical *P. aeruginosa* strains [2]. The viral particle consists of an icosahedral capsid *cca* 60 nm in diameter with 43,5 kbp dsDNA genome packaged inside, and a short tail. The first aim of this project is to characterize the structure of LUZ19 at near-atomic resolution. Phage particles purified on CsCl gradient were used for cryo-electron microscopy data collection; the single-particle analysis of this dataset is in progress. The second aim is to study the interaction of phages with bacterial cells using cryo-electron tomography. Finally, we plan to employ the CRISPR/Cas system to prepare fluorescently labelled phage strains, allowing us to observe the progress of phage infection in bacterial biofilm. Obtained results will further our understanding of LUZ19 infectious cycle in both planktonic and biofilm-forming *P. aeruginosa* populations.

1. Z. Pang, R. Raudonis, B. R. Glick, T-J. Lin, Z. Cheng, (2019) Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies, BiotechAdv.
2. P-J. Ceyssens, *Isolation and Characterization of lytic bacteriophages infecting Pseudomonas aerugionsa*. (2009).

P38

14-3-3 PROTEIN BINDING BLOCKS BOTH THE NUCLEAR LOCALIZATION SEQUENCE AND THE DIMERIZATION INTERFACE OF CASPASE-2

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Among all species, caspase-2 (C2) is the most evolutionarily conserved caspase required for effective initiation of apoptosis following death stimuli. C2 is activated through dimerization and autoproteolytic cleavage and inhibited through phosphorylation at Ser^{139} and Ser^{164}, within the linker between the caspase recruitment and p19 domains of the zymogen, followed by association with the adaptor protein 14-3-3, which maintains C2 in its immature form procaspase (proC2) [1, 2]. Moreover, the caspase-2 region with both 14-3-3-binding motifs also contains the nuclear localization sequence (NLS), thus suggesting that 14-3-3 binding may regulate the subcellular localization of caspase-2. However, the mechanism of 14-3-3-dependent inhibition of C2 activation remains unclear.

We recently reported the structural characterization of the complex between proC2 and 14-3-3 using a combined approach based on small angle X-ray scattering, NMR, chemical cross-linking, fluorescence spectroscopy, hydrogen/deuterium mass spectrometry (HDX-MS) and protein crystallography to determine the molecular basis for 14-3-3-mediated inhibition of C2 activation [3, 4]. Our data revealed that the 14-3-3 dimer interacts with proC2 not only through ligand-binding grooves but also through other regions outside the central channel, thus explaining the isoform-dependent specificity of 14-3-3 protein binding to proC2 and the substantially higher binding affinity of 14-3-3 protein to proC2 than to the doubly phosphorylated peptide. The formation of the complex between 14-3-3 protein and proC2 does not induce any large conformational change in proC2. Furthermore, the 14-3-3 protein interacts with and masks both the nuclear localization sequence (NLS) and the C-terminal region of the p12 domain of proC2 through transient interactions in which both the p19 and p12 domains of proC2 are not firmly docked onto the surface of 14-3-3. Because this masked region of p12 domain is involved in caspase-2 dimerization, 14-3-3 likely inhibits proC2 activation by blocking the dimerization surface of this procaspase.

In conclusion, the structural analysis of the 14-3-3:caspase-2 complex suggested that 14-3-3 protein binding may inhibit caspase-2 activation through interference with caspase-2 oligomerization and/or its nuclear localization by sterically occluding caspase-2 p12 domain as well as NLS, which is bordered by the two phosphorylated 14-3-3-binding motifs of caspase-2. Thus, these results corroborate the hypothesis that 14-3-3 binding is an important regulatory element of caspase-2 activation.

1. L. K. Nutt, M. R. Buchakjian, E. Gan, R. Darbandi, S. Y. Yoon, J. Q. Wu, Y. J. Miyamoto, J. A. Gibbons, J. L. Andersen, C. D. Freel, W. Tang, C. He, M. Kurokawa, Y. Wang, S. S. Margolis, R. A. Fissore, and S. Kornbluth, *Dev Cell* **16**, (2009), 856-866.
2. D. Kalabova, A. Smidova, O. Petrvalska, M. Alblova, D. Kosek, P. Man, T. Obsil, and V. Obsilova, *Biochem Biophys Res Commun.,* **493**, (2017), 940-945.
3. A Smidova, M. Alblova, D. Kalabova, K. Psenakova, M. Rosulek, P. Herman, T. Obsil T, and V. Obsilova, *FEBS J.,* **285**, (2018), 4196-4213.
4. D. Kalabova, F. Filandr, M. Alblova, O. Petrvalska, M. Horvath, P. Man, T. Obsil T, and V. Obsilova, *FEBS J.,* **doi: 10.1111/febs.15215** (2020).

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

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

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

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CENTRE OF MOLECULAR STRUCTURE IN BIOCEV – CURRENT STATUS AND UPDATES

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The core facilities of the Centre of Molecular Structure (CMS) offer a wide range of methods of structural biology. They are located in BIOCEV, as part of the Institute of Biotechnology, AS CR. CMS consists of facilities devoted to crystallization of macromolecules, X-ray diffraction and scattering, biophysical characterization and structural mass spectrometry. The services are provided via the Czech Infrastructure for Integrative Structural Biology (CIISB) and the European infrastructure for structural biology Instruct-ERIC. The latest improvements of our instruments portfolio are the NT8 crystallization dropsetter (Formulatrix) and the upgrades of the diffractometer D8 Venture including new detector, automated robotic goniometer head and the HC-lab device for controlled dehumidification of crystals (Arinax).

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MICROTUBULE ASSOCIATED PROTEIN 2C INTERACTS WITH MULTIPLE PARTNERS AT SPECIFIC SITES

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The Microtubule Associated Proteins (MAPs) regulate the stability and the dynamics of the cytoskeleton. The main structural MAPs, tau and MAP2, are expressed in neurones and are involved in neurodegenerative diseases, such as Alzheimer's disease. Tau and MAP2 are both intrinsically disordered proteins, lacking a fixed three-dimensional structure. However, nuclear magnetic resonance revealed propensities of MAPs to form transient local structures and long-range contacts in the free state [1, 2]. MAP2c, the juvenile MAP2, is expressed during brain development. In addition to its role in the control of dynamics of microtubules and actin filaments, MAP2c is involved in interactions with multiple proteins or small ligands, implied in neuronal development, in the regulation of the cytoskeleton plasticity, or anchoring the cytoskeleton to organelles.

Here, we present the interaction of MAP2c with different proteins, such as 14-3-3 [3], the cytolinker plectin and the RII regulatory subunit of PKA, as well as with neurosteroids. Interestingly, the sites of interaction with the different proteins can be linked to transient structural features observed in free MAP2c [1, 2].

1. K. Melková, V. Zapletal, S. Jansen, E. Nomilner, M. Zachrdla, J. Hritz, J. Nováček, M. Zweckstetter, M. R. Jensen, M. Blackledge, L. Žídek, *J. Biol. Chem.*, **293**, (2018), 13297–13309.
2. K. Melková, V. Zapletal, S. Narasimhan, S. Jansen, J. Hritz, R. Škrabana, M. Zweckstetter, M. Ringkjřbing Jensen, M. Blackledge, L. Žídek, *Biomolecules*, **9**, (2019), 105.
3. S. Jansen, K. Melková, Z. Trošanová, K. Hanáková, M. Zachrdla, J. Nováček, E. Župa, Z. Zdráhal, J. Hritz, L. Žídek, *J. Biol. Chem*., **292**, (2017), 6715–6727

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P42

INITIAL STRUCTURAL STUDIES OF REGULATION OF UBIQUITIN LIGASE NEDD4-2 BY 14-3-3 PROTEINS

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14-3-3 proteins belong to evolutionarily highly conserved family of regulatory proteins which regulate a variety of biological processes by binding to specific phosphorylated motif of their binding partners [1]. One of several hundreds of 14-3-3 protein binding partners is ubiquitin ligase Nedd4-2 (NEDD4L), whose role is ubiquitination of various ion channels and membrane transporters [2]. The best-described example is the regulation of the epithelial sodium channel (ENaC), which with its activity in the distal renal tubule, contributes to maintaining Na^+ homeostasis of the whole organism. Mutations of Nedd4-2 gene are associated with developmental disorders, hypertension and epilepsy. Dysregulation of Nedd4-2 in mice also leads to respiratory, renal, cardiac, and neural disorders and negatively affects the immune system [3–8]. Phosphorylation of specific serine and/or threonine residues allows the binding of 14-3-3 proteins, which results in the inhibition of interaction between Nedd4-2 and its substrate [9]. However, the structural nature of the mechanism of regulation by 14-3-3 protein has not been elucidated yet.

We have expressed and purified stable constructs of Nedd4-2 ligase and we confirmed its interaction with 14-3-3 protein in phosphorylation-dependent manner. We also identified the amino acids involved in the interaction between these molecules and performed initial biophysical characterization using analytical ultracentrifugation and small angle X-ray scattering. Our results show that: Nedd4-2$^{186\text{-}975}$ is monomeric and the stoichiometry of

Nedd4-2$^{186-975}$:14-3-3 complex is 1:2 (with possible shift to 2:2 in high concentrations). The fluorescence polarization assays with short peptides of Nedd4-2 containing single 14-3-3 binding motif, in combination with site-directed mutagenesis and native TBE electrophoresis, show, that the key Nedd4-2 residues important for the interaction with 14-3-3 proteins are phosphorylated Ser^{342} and Ser^{448}. We also crystallized the complex of 14-3-3ăÄC with the peptide containing phosphorylated Ser^{448} and solved its structure using molecular replacement and refined it at 1.73 Å resolution.

1. J. A. Manning and S. Kumar, *Trends Biochem. Sci*., **43**, (2018), 635–647.
2. G. Messaritou, S. Grammenoudi, and E. M. C. Skoulakis, *J. Biol. Chem*., **285**, (2010), 1692–1700.
3. O. B. Kashlan and T. R. Kleyman, *Exp. Cell Res*., **318**, (2012), 1011–1019.
4. C. C. Goulet, K. A. Volk, C. M. Adams, L. S. Prince, J. B. Stokes, and P. M. Snyder, *J. Biol. Chem*., **273**, (1998), 30012–30017.
5. L. Broix et al., *Nat. Genet*., **48**, (2016), 1349–1358.
6. F. Rizzo and O. Staub, *Curr. Opin. Nephrol. Hypertens.*, **24**, (2015), 111-116.
7. E. N. Vanli-Yavuz et al., *Acta Neurol. Belg*., **115**, (2015), 241–245.
8. J. H. Hansson et al., *Nat. Genet*., **11**, (1995), 76–82.
9. P. Goel, J. A. Manning, and S. Kumar, *Gene*, **557**, (2015), 1–10.

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P43

EXCURSION INTO THE *STAPHYLOCOCCUS AUREUS* CELL DURING BACTERIOPHAGE INFECTION

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Global impact of antibiotic-resistant strains of *Staphylococcus aureus* on patients' health is not mitigated by newly developed antibiotics. The phage therapy is an effective approach employing natural enemies of bacteria – bacteriophages – to battle the infection [1]. It is currently of limited availability and should be made accessible to patients with multi-resistant bacterial infections. However, current knowledgebase on *Staphylococcus* phages, which serves authorities to approve wider use of phage therapy, is incomplete [2]. To investigate ultrastructure of phage infection mechanism, we employed a cryo electron tomography on a single cell thinned by focused ion beam milling. Here we present a snapshot of intracellular environment during lytic cycle stages of polyvalent *Myoviridae* phage phi 812 [3, 4] in planktonic *Staphylococcus aureus* cell. We show, that within 15 minutes of infection new phage capsids are apparent on the inner surface of cellular membrane. After 30 minutes infection, the empty, filling, and full phage capsids with or without connected tail are assembled in cytosol. In contrast to a widely accepted assumption on precise timing of a phage life cycle, we show that a considerable amount of newly formed phage particles is incomplete upon lysis.

Increased resistance to treatment in *Staphylococci* caused by a biofilm formation can be addressed by use of a combined phage-antibiotic therapy [5, 6]. Research on the behaviour of phages in biofilm will provide kinetic frameworks for application of a combined therapy to minimize inhibition of a phage component. Here we show, that with super resolution fluorescence microscopy, we can track phage particles during infection of native *S. aureus* biofilm. To track overall phage movement and determine the time frame for application of antibiotic, we will use a light sheet fluorescence microscopy. The native biofilm in a flow-cell setting will be recorded during infection by labelled phages. In order to minimize background autofluorescence of growth media with casein component, we developed transparent defined minimal media with comparable growth capacities. Characterization of phage infection dynamics can facilitate progress in phage therapy approval and availability to patients with urgent need of alternative therapy.

1. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, Russell DA, Ford K, Harris K, et al. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus. *Nat Med.* 2019; **25**(5):730-3. Epub 2019/05/10. doi: 10.1038/s41591-019-0437-z. PubMed PMID: 31068712; PubMed Central PMCID: PMCPMC6557439.
2. Young R, Gill JJ. Phage therapy redux--What is to be done? *Science*. 2015; **350**(6265):1163-4. Epub 2016/01/20. doi: 10.1126/science.aad6791. PubMed PMID: 26785457; PubMed Central PMCID: PMCPMC6005185.
3. Eyer L, Pantucek R, Zdrahal Z, Konecna H, Kasparek P, Ruzickova V, et al. Structural protein analysis of the polyvalent staphylococcal bacteriophage 812. *Proteomics*. 2007; **7**(1):64-72. Epub 2006/12/13. doi: 10.1002/pmic.200600280. PubMed PMID: 17154272.
4. Botka T, Pantucek R, Maslanova I, Benesik M, Petras P, Ruzickova V, et al. Lytic and genomic properties of spontaneous host-range Kayvirus mutants prove their suitability for upgrading phage therapeutics against staphylococci. *Sci Rep.* 2019; **9**(1):5475. Epub 2019/04/04. doi:

10.1038/s41598-019-41868-w. PubMed PMID: 30940900; PubMed Central PMCID: PMCPMC6445280.

5. Morris J, Kelly N, Elliott L, Grant A, Wilkinson M, Hazratwala K, et al. Evaluation of Bacteriophage Anti-Biofilm Activity for Potential Control of Orthopedic Implant-Related Infections Caused by Staphylococcus aureus. *Surg Infect* (Larchmt). 2019; **20**(1):16-24. Epub 2018/09/13. doi: 10.1089/sur.2018.135. PubMed PMID: 30207891.

6. Abedon ST. Phage-Antibiotic Combination Treatments: Antagonistic Impacts of Antibiotics on the Pharmacodynamics of Phage Therapy? *Antibiotics* (Basel). 2019; **8**(4). Epub 2019/10/17. doi: 10.3390/antibiotics8040182. PubMed PMID: 31614449; PubMed Central PMCID: PMCPMC6963693.

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STRUCTURAL CHARACTERIZATION OF A G-RICH REGION WITHIN EGFR PROMOTER

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G-quadruplexes (G4) are nucleic acid secondary structures formed by planar arrays of four guanines (G-quartets), held together through Hoogsteen hydrogen bonds, stacking one on each other to generate a non-canonical tetra-helix. Many studies reported their involvement in several cellular processes, such as gene expression, DNA replication and telomere maintenance. G-rich sequences are enriched in promoter regions of oncogenes and at these sites G-quadruplex formation has been implicated in modulation of gene transcription, suggesting potential for G4-based anticancer therapies [1].

Whereas it is quite easy to map the distribution of putative G-quadruplex forming sequences (PQS) by in silico analytical tools, the real folding properties of these sequences are currently poorly predictable due to the highly polymorphic behaviour of the G4 arrangements. Indeed, both the nucleic acid sequence and the environment greatly affect the structure, stability and folding kinetics of these elements. Thus, in order to assess any potential biological role for these non-canonical DNA structures, a detailed evaluation of the ability of these PQS to fold into stable G4 is required [2].

In this context, we considered EGFR (Epidermal Growth Factor Receptor) oncogene as a case-study. It encodes a receptor tyrosine kinase, which overexpression or mutations, that make it constitutively active, induce uncontrolled cell proliferation, survival, adhesion, migration and differentiation [3]. A bioinformatic search identified some G-rich regions within the EGFR promoter gene. Here we explore the conformational equilibria of one of them, a 30 residue long sequence located at 272 bp upstream the transcription start site (EGFR-272). By merging spectroscopic and electrophoretic analysis performed on the wild-type sequence as well as on a wide panel of related mutants, we were able to prove that in the presence of K+ ions this sequence folds into two main G-quadruplex structures, presenting parallel and hybrid topology. They show comparable thermal stabilities and affinities for the metal ion and, indeed, they are always co-existing in solution. The folding process is driven by a hairpin occurring in the domain corresponding to the terminal loop which is an important stabilizing element for both the identified G-quadruplexes.

1. D. Rhodes, H. J. Lipps, *Nucleic Acids Res.,* **43**, (2015), 8627–8637.

2. R. Rigo, M. Palumbo, C. Sissi, *Biochim. Biophys. Acta,* **1816**, (2017), 1399-1413.

3. J. R. Goffin, K. Zbuk, *Clin. Ther.,* **35**, (2013), 1282-1303.

P45

A NOVEL INHIBITORY MOTIF AGAINST ASPARTIC PROTEASES IDENTIFIED IN THYROPINS

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Equistatin from sea anemone belongs to the thyropin (thyroglobulin type-1-like) protease inhibitor family. The equistatin domain 2 (Eqd2) is a potent inhibitor of aspartic proteases of cathepsin D type from the pepsin family. We prepared and crystallized a complex of recombinant Eqd2 with a prototype cathepsin D-like protease (CatD). Here we present the crystal structure of the Eqd2-CatD complex solved at 1.7 Å Õolution. This revealed the architecture of the reactive site on Eqd2 with a unique inhibitory motif against aspartic proteases that directly targets catalytic diad residues.

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STRUCTURAL INSIGHTS INTO THE FUNCTION OF ZRANB3 IN REPLICATION STRESS RESPONSE

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DNA replication is essential prerequisite for successful cell division. Its accurate and timely execution is crucial for maintenance of genome stability. At the same time, the integrity of DNA is constantly challenged by endogenous as well as exogenous factors that may impinge on the progression of DNA replication by stalling the replication forks. An intricate network of pathways, collectively known as DNA damage tolerance (DDT), operates to restart the stalled replication forks. The choice of a particular pathway is governed by PCNA and its post-translational modifications, mainly (poly)ubiquitination and to a lesser extent SUMOylation. PCNA is the key replication factor that recruits a diverse array of factors required for both replication and DDT. Polyubiquitination of PCNA is triggered at sites where replication forks stall. ZRANB3 is the only vertebrate factor known to be recruited these sites. ZRANB3 can act in two modes: it may remodel the stalled replication forks thereby promoting bypass of DNA damage, or it may initiate DNA repair by creating a nick on the leading strand ahead of the replication fork. The molecular mechanism of the latter mode is poorly understood. We therefore focused our interest on understanding how ZRANB3 nicks DNA using its nuclease domain of HNH type. We determined the structure of the HNH domain and performed a detailed mutational analysis. Additionally, we studied the role that PCNA has in regulating the nuclease activity both at a functional and structural level. Interestingly, we found that the HNH domain of ZRANB3 contains a unique insertion that is essential for its activity. Moreover, we found out that PCNA stimulates the nuclease activity of ZRANB3 via a direct protein-protein interaction. Taken together, our data indicate that PCNA first recruits ZRANB3 to its site of action, but later plays a distinct role in promoting its nuclease activity. Importantly, we reveal that a subset of cancer-associated mutations in ZRANB3 abolish its nuclease activit.

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STRUCTURAL DATA-BASED IDENTIFICATION OF SUBSTRATE NATURE FOR NOVEL FAD-DEPENDENT OXIDOREDUCTASE FROM *CHAETOMIUM THERMOPHILUM*

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Chaetomium thermophilum is a thermophilic cellulose-degrading fungus living in soil, dung, and compost heaps. It flourishes at higher temperatures (between 45 – 60 °C) and, on that account, it is of wide interest as potential source of thermostable enzymes for high-temperature industrial processes [1]. The subject of our study is a novel thermostable FAD-dependent oxidoreductase from *Chaetomium thermophilum var. thermophilum* (*Ct*FDO), which is an extracellular glycoprotein of molecular mass around 85 kDa.

Here we present a 1.3 Å resolution crystal structure of *Ct*FDO. It belongs to the glucose-methanol-choline (GMC) oxidoreductase family, members of which share the two-domain character, core structural elements, the conserved N-terminal GxGxxG sequence motif characteristic for the Rossmann fold binding the ADP moiety of flavine adenine dinucleotide, and a conserved active-site histidine [2]. A usual feature is also a narrow tunnel or a cleft to the active site pocket containing typically His–His, or His–Asn active-site pair in the re-face of FAD isoalloxazine ring [3]. For the first time, the *Ct*FDO structure reveals a His–Ser active-site pair in the active-site pocket accessible from the exterior via a wide open tunnel. Moreover the active-site pocket is extended by an unusual, mainly hydrophobic, side-cavity.

The GMC family enzymes catalyse the oxidation of primary and secondary alcohols yielding aldehydes or ketones. The measurements of *Ct*FDO catalytic activity with over 1100 compounds did not lead to identification of any strongly reacting substrate. *Ct*FDO appears to be inactive also with common substrates of GMC family enzymes. To get a better insight into the possible substrate moieties and their organization, we performed co-crystallization and crystallographic fragment screening. Five determined structures of complexes with aromatic compounds reveal the potential substrate is of more complex polyaromatic nature.

1. A.-N. Li and D.-C. Li, *J. Appl. Microbiol.*, **106**, (2009), 369-380.
2. W. P. Dijkman, G. de Gonzalo, A. Mattevi, M. Fraaije, *Appl. Microbiol. Biotechnol*., **97**, (2013), 5177-5188.
3. L. Sützl, G. Foley, E. M. J. Gillam, M. Bodén, D. Haltrich, *Biotechnol Biofuels*, **12**, (2019), 1-18.

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STRUCTURE OF *T. CASTANEUM* STORAGE PROTEIN SOLVED BY CRYO-ELECTRON MICROSCOPY

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Tribolium castaneum is a worldwide pest causing damages of food and stored crops. The beetle became resistant to many types of insecticides. Hexamerin is the most abundant protein in the haemolymph of larvae and pupae of holometabolous insect. It serves as an amino acid source during metamorphosis. Hexamerin was isolated from *Tribolium castaneum* pupae and purified by ion exchange and gel chromatography. Hexamerin structure was resolved at resolution of 3.28 Å. Hexamerin particle consists of 6 protomers organized in D3 symmetry. Each subunit is N-glycosylated on Asn^{187}. The glycosylation is located in the cleft between subunits and probably increases the stability of the whole protein. The knowledge of hexamerin structure might shed light on *T. castaneum* lifecycle and potentially might enable the development of substances for the control of this pest.

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STRUCTURAL RELATIONSHIP BETWEEN SUBUNITS OF THE NON-CANONICAL BAF CHROMATIN REMODELING COMPLEX

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The non-canonical (BRG1/BRM-Associated Factor) BAF complex is one of the final assembly forms of the major class of ATP-dependent SWI/SNF chromatin remodeling complexes which are large multi-subunit complexes that play a crucial role in the dynamic regulation of the chromatin architecture and DNA accessibility in eukaryotic cells [1, 2]. Recent studies revealed the high prevalent mutational frequency in genes encoding for SWI/SNF subunits in over 20% of human cancers leading to perturbations in the complex formation and function and providing strong support for their driving role in oncogenesis [3, 4, 5]. The non-canonical BAF complex was identified as a vulnerable target in several BAF-related cancer types as it seems to maintain the oncogenic gene expression at retained mSWI/ SNF sites [6, 7]. In particular, the complex-specific subunits (Bromodomain-containing protein 9) BRD9, GLTSCR1 (glioma tumor suppressor candidate region gene 1) and its paralog GLTSCR1L (GLTSCR1- like) are suggested to intake an essential role in the complex functional assembly [8, 9]. The underlying structural mechanisms and crucial interaction sites within the subunits contributing to the complex organization and function in the cell remain unknown. The aim of the proposed project is to reveal the molecular basis for the interactions between the specific subunits of the non-canonical BAF complex that might shed light on its biological role. First, we will identify the minimal interaction regions within the individual proteins and then perform their detailed structural characterization. Our goal is to construct a detailed structural map of the human non-canonical BAF complex that will reliably predict its behavior under pathological conditions. We will use structural and biophysical methods not only for detailed structural characterization of the interaction network within the complex, but also for validation of results in cells. Clarification of the structural relationships between the individual subunits and their interaction properties under normal and pathological conditions will lead to a better understanding of the regulation and function of the non-canonical BAF complex at the molecular level and will help to design new therapeutic approaches.

1. Kadoch, C. & Crabtree, G. R. Mammalian SWI/SNF chromatin remodeling complexes and cancer: Mechanistic insights gained from human genomics. *Sci. Adv.* **1**, e1500447 (2015).
2. Mashtalir, N. et al. Modular Organization and Assembly of SWI/SNF Family Chromatin Remodeling Complexes. *Cell* **175**, 1272-88.e20 (2018).
3. Kadoch, C. et al. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. *Nat. Genet.* **45**, 592-601 (2013).
4. Roodolph, S. P. & Kadoch, C. Mammalian SWI/SNF Complexes in Cancer: Emerging Therapeutic Opportunities. *Curr. Opin. Genet. & Dev.* **42**, 56-67 (2017).
5. Schick, S. et al. Systematic Characterization of BAF Mutations Provides Insights into Intracomplex Synthetic Lethalities in Human Cancers. *Nat. Genet.* **51**, 1-12 (2019).
6. Michel, B. C. et al. A Non-Canonical SWI/SNF Complex Is a Synthetic Lethal Target in Cancers Driven by BAF Complex Perturbation. *Nat. Cell Biol.* **20**, 1410-20 (2018).
7. Inoue, D. et al. Spliceosomal Disruption of the Non-Canonical BAF Complex in Cancer. *Nat.* 574, 432-36 (2019).
8. Wang, X. et al. BRD9 Defines a SWI/SNF Sub-Complex and Constitutes a Specific Vulnerability in Malignant Rhabdoid Tumors. *Nat. Comm.* **10**, 1881 (2019).
9. Alpsoy, A. & Dykhuizen, E. C. Glioma Tumor Suppressor Candidate Region Gene 1 (GLTSCR1) and Its Paralog GLTSCR1-like Form SWI/SNF Chromatin Remodeling Subcomplexes. *J. Biol. Chem.* **293**, 3892-903 (2018).