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MOLECULAR ARCHITECTURE AND LIFE CYCLE OF FILAMENTOUS VIRUSES

Polak Martin[1], Novacek Jiri[1], Plchova Helena[2], Novakova Slavomira[3], Podobnik Marjetka[4], Sink Andreja[4]

[1]Core Facility Cryo-electron Microscopy and Tomography CEITEC, Masaryk University, ČR
[2]Institute of Experimental Botany, AS ČR
[3]Department of Plant Virology, AS SR
[4]Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Slovenia

Potato virus X (PVX) is a member of the family *Alphaflexiviridae* in the genus Potexvirus. It infects several solanaceous crops including potato (it can cause yield losses of up to 15% in some varieties), tomato and tobacco. Symptoms produced by PVX are variable, depending on the strain and host plant. In general, plants often do not exhibit symptoms, but the virus can cause symptoms of mild mottling, chlorosis, mosaic or decreased leaf size. It can be mechanically transmitted.

PVX has a monopartite plus-sense single stranded RNA ((+)ssRNA) of approximately 6.4 kb (Skryabin et al., 1988). PVX virions are flexible rods 515 nm long and 13.5 nm in diameter, consisting of approximately 1350 helically folded identical coat protein (CP) subunits and viral RNA packed between its turns (Tollin and Wilson, 1988).

So far, only low-resolution structure of PVX virion has been determine providing limited information about CP-CP and CP-RNA interactions. The aim of this project is (1) to determine the near-atomic structure of Potato virus X and CP-CP and CP-RNA interacting amino acids and (2) to define the surface exposed regions/loops and test them for possible tolerance of inserted peptides. PVX structure and interacting amino acids was analyzed using cryo-electron microscopy.

Friday, March 23, Session VI

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STRUCTURE OF TICK-BORNE ENCEPHALITIS VIRUS AND ITS NEUTRALIZATION BY A MONOCLONAL ANTIBODY

Tibor Füzik[1], Petra Formanová[2], Daniel Růžek[2,3], Kentaro Yoshii[4], Matthias Niedrig[5], Pavel Plevka[1]

[1]Structural Virology, Central European Institute of Technology, Masaryk University, Kamenice 753/5, 62500 Brno, Czech Republic
[2]Department of Virology, Veterinary Research Institute, Hudcova 70, 62100 Brno, Czech Republic
[3]Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branisovska 31, 37005 Ceske Budejovice, Czech Republic
[4]Laboratory of Public Health, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan
[5]Centre for Biological Threats and Special Pathogens, Robert Koch Institute, Nordufer 20, 13353 Berlin, Germany

Tick-borne encephalitis virus (TBEV) causes 13,000 cases of human meningitis and encephalitis annually. However, the structure of the TBEV virion and its interactions with antibodies are unknown. Here, we present cryo-EM structures of the native TBEV virion and its complex with Fab fragments of neutralizing antibody 19/1786. Flavivirus genome delivery depends on membrane fusion that is triggered at low pH. The virion structure indicates that the repulsive interactions of histidine side chains, which become protonated at low pH, may contribute to the disruption of heterotetramers of the TBEV envelope and membrane proteins and induce detachment of the envelope protein ectodomains from the virus membrane. The Fab fragments bind to 120 out of the 180 envelope glycoproteins of the TBEV virion. Unlike most of the previously studied flavivirus-neutralizing antibodies, the Fab fragments do not lock the E-proteins in the native-like arrangement, but interfere with the process of virus-induced membrane fusion.

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LOOKING FOR INHIBITOR: STRUCTURAL AND FUNCTIONAL ANALYSIS OF NOVEL BANGLE LECTIN PHL FROM *PHOTORHABDUS ASYMBIOTICA*

Josef Houser[1,2], Gita Jančaříková[1,2], Pavel Dobeš[3], Eva Fujdiarová[2], Pavel Hyršl[3], Magdolna Csávás[4], Jitka Moravcová[5], Michaela Wimmerová[1,2]

[1]CEITEC-Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
[2]National Center for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic,
[3]Department of Animal Physiology and Immunology, Institute of Experimental Biology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
[4]Department of Pharmaceutical Chemistry, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary
[5]Department of Chemistry of Natural Compounds, University of Chemistry and Technology, Prague, Technickaě 5, 166 28 Prague, Czech Republic

Photorhabdus asymbiotica is a gram-negative bioluminescent bacterium living in a symbiosis with *Heterorhabditis* nematodes forming a highly entomopathogenic complex. Unlike other *Photorhabdus* species, *P. asymbiotica* can act as an emerging human pathogen as well. In its genome, we identified a gene for a putative lectin, and examined the corresponding recombinant protein PHL from functional and structural point of view. It exhibited high affinity for fucosylated carbohydrates and lower affinity to several other mono- and oligosaccharides including saccharides from bacterial cell wall or human blood epitopes. It was further shown to interact with all types of red blood cells and insect haemocytes, inhibit the production of reactive oxygen species in human blood and inhibit antimicrobial activity both in human blood, serum and insect haemolymph [1].

We succeeded in determining structure of PHL in complex with several monosaccharides revealing its unusual properties. It was shown that fucose and galactose occupy different group of well-defined sites, making PHL the first confirmed case of barrel-shape lectin with two sets of sites displaying different specificity and arranged in two layers. As it further forms dimer, the maximal number of potential binding sites per biological unit is 28. This arrangement lead to forming a new type of lectin called bangle lectin [1].

As being promising target for treatment of *P. asymbiotica* related infections, we tested a wide range of various mono-, di- and oligovalent carbohydrate-based molecules. Their ability to bind PHL and subsequently inhibit its interactions with natural ligands was studied using heamagglutination, isothermal titration calorimetry and surface plasmon resonance [2]. For several of them, we also succeeded in preparing crystals of corresponding PHL complexes and analyzing their structures (Fig. 1). Data gained so far show the way for design and synthesis of potential therapeutics.

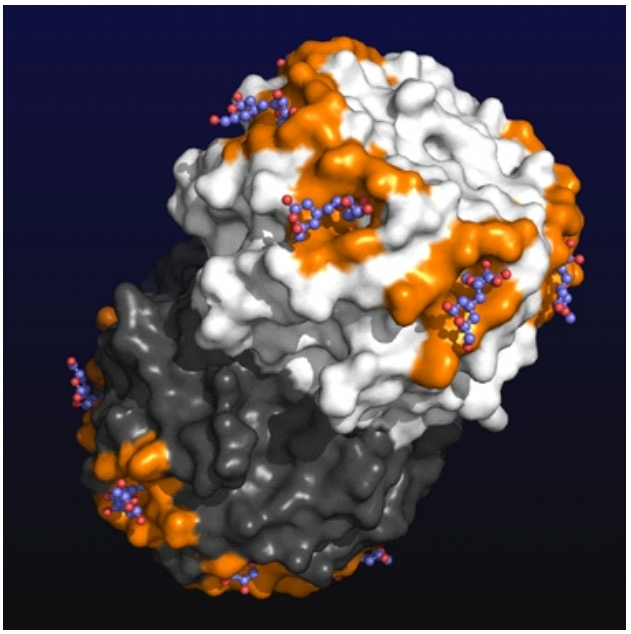

Figure 1. PHL dimer in complex with one of the studied inhibitors (shown as ball and sticks). Individual monomers shown in white and black, respectively, and recognized binding sites highlighted in orange.

This work was supported by the Czech Science Foundation (project 18-18964S) and from European Regional Development Fund-Project „CIISB4HEALTH“ (No. CZ.02.1.01/0.0/0.0/16_013/0001776). CIISB research infrastructure project LM2015043 funded by MEYS CR is also gratefully acknowledged for the financial support of the measurements at the CF Biomolecular Interactions and Crystallization, CF X-ray Diffraction and Bio-SAXS and CF Proteomics at CEITEC (Brno, Czech Republic). We wish to thank the BESSY II (Berlin-Adlershof, Germany) and PETRA III (Hamburg, Germany) for access to their synchrotron data collection facilities and allocation of synchrotron radiation beam time.

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CRYSTAL STRUCTURE OF GLOBIN DOMAIN OF *Af*GcHK HISTIDINE KINASE

T. Skálová[1], P. Kolenko[1,2], M. Stráňava[3], J. Bláha[3], J. Dohnálek[1], M. Martínková[3]

[1]*Institute of Biotechnology CAS, v.v.i., Biocev, 252 50 Vestec, Czech Republic*
[2]*Department of Solid State Engineering, Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University in Prague, Brehova 7, 115 19 Praha 1, Czech Republic*
[3]*Department of Biochemistry, Faculty of Science, Charles University, Hlavova (Albertov) 2030/8, Prague 2, 128 43, Czech Republic*
t.skalova@gmail.com

Histidine kinase from *Anaeromyxobacter* sp. Fw109-5, denoted as *Af*GcHK, is a homodimer, each chain containing two distinct domains. The N-terminal domain is a heme-binding globin domain and the C-terminal domain is the active domain of the histidine kinase. The globin domain regulates the activity of the histidine kinase as an oxygen sensor: oxygen binding to the globin domain heme has structural and dynamic effect on both domains of the histidine kinase.

Here we present the first crystal structures of the isolated globin domain of *Af*GcHK – the structure with cyanide bound to the heme (PDB code 5OHE) and the structure of the partially reduced globin (PDB code 5OHF) [1]. The protein shows relatively large structural changes upon its reduction.

The protein was crystallized and red wedge-shaped crystals with dimensions up to 80 x 80 x 300 μm were obtained. The unit cell has a rather large size (unit cell parameters 78 Å, 78 Å, 441 Å, space group $P4_12_12$) and the asymmetric unit contains eight chains A-H (four dimers AB, CD, EF and GH) of the globin domain. This is not advantageous during structure solution; however, it is convenient for stability of the crystal during reduction as only dimer GH of the four dimers in the asymmetric unit shows changes when a reduction agent is added. Ca. 30 % of the protein chain G was found in two alternative positions connected with the change of the heme position and with the loss of its ligand. Neighboring parts of chain H are changed too. The other three globin dimers are intact and form a scaffold in the crystal so that this observation in chain G was possible without destroying the crystal.

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This work was supported by Grant Agency of the Czech Republic Grant 15-19883S, Ministry of Education, Youth, and Sports of the Czech Republic Grant LM2015043 CIISB, Biocev -Crystallization, Diffraction and LTC17065 in frame of COST Action CA15126 MOBIEU. This work was also partly funded by Instruct, part of the European Strategy Forum on Research Infrastructures (ESFRI) and supported by national member subscriptions.

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CRYSTAL STRUCTURE OF NOVEL ARYL-ALCOHOL OXIDASE FROM THERMOPHILIC FUNGUS *CHAETOMIUM THERMOPHILUM*

L. Švecová[1, 2], T. Skálová[1], T. Kovaľ[1], L. H. Østergaard[3], J. Dohnálek[1]

[1]*Institute of Biotechnology of the Czech Academy of Sciences, v.v.i., Biocev center, Průmyslová 595, Vestec, 252 50, Czech Republic*
[2]*Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University in Prague, Břehová 7,115 19, Praha 1, Czech Republic*
[3]*Novozymes A/S, Brudelysvej 26, DK-2880 Bagsvćrd, Denmark*
leona.svecova@ibt.cas.cz

Cheatomium thermophilum var. thermophilum (*Ct*) is a cellulose–degrading thermophilic fungus living in soil, dung or compost heaps, which supply appropriate conditions (air access, heat and humidity) for its growth. The interest in *Ct* has increased in recent years, as a source of new thermostable proteins for industrial and biotechnological purposes, where the high temperatures are needed [1].

Here we present a novel aryl-alcohol oxidase (EC 1.1.3.7) from *Chaetomium thermophilum* (*Ct*AAO), a monomeric extracellular oxidoreductase catalyzing FAD-dependent two-electron oxidation of aromatic alcohols to aldehydes during reductive half-reaction, accompanied by two-electron reduction of O_2 to H_2O_2 during oxidative half-reaction. H_2O_2 is further utilized by peroxidases during lignin degradation process [2]. *Ct*AAO is a member of the glucose-methanol-choline oxidoreductase (GMC) family, whose members contain a highly conserved residue – the active-site histidine – in the C-terminal part. This residue plays the crucial role of a catalytic base activating electron-donating substrate for hydrogen transfer to the FAD

isoalloxazine ring during the reductive half-reaction [3]. We determined the crystal structure of native *Ct*AAO at 2 Å resolution. Compared to other members of the GMC family, the active site of *Ct*AAO including the catalytic base His has a different arrangement of residues on the re-side of the isoalloxazine ring than has been observed previously.

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This work is supported by the project BIOCEV (CZ.1.05/ 1.1.00/02.0109) and the project Structural dynamics of biomolecular systems (CZ.02.1.01/0.0/0.0/ 15_003/ 0000447) from the ERDF, institutional support of IBT CAS, v. v. i. RVO: 86652036) and by the Grant Agency of the Czech Technical University in Prague, grant No. SGS16/246/OHK4/3T/14.

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IDENTIFICATION AND CHARACTERIZATION OF MICROTUBULE-BINDING DOMAIN OF HDAC6

Kseniya Ustinova, Zora Nováková, Zsofia Kutil, Zdenek Lansky and Cyril Bařinka

Institute of Biotechnology CAS, v. v. i., AS CR, Průmyslová 595, 252 50 Vestec, Czech Republic

In the cell, tubulin is post-translationally modified to create functionally distinct microtubules (MTs) endowed with specialized functions. Acetylation of Lys-40 of -tubulin (K40) is one of major post-translational modifications that provides long-lived MTs with mechanical resilience. The K40 level is controlled by histone deacetylase 6 (HDAC6), a multidomain cytosolic protein that acts as a major tubulin deacetylase. While the tubulin deacetylase activity of HDAC6 has been unequivocally assigned to the second of the duplicated catalytic domains, there is virtually no information on contribution of other domains on HDAC6/tubulin interactions and deacetylation efficacy.

Using deletion mutagenesis, we identified the N-terminal part of human HDAC6 as a microtubule-binding (MTB) domain and functionally characterized it up to the single molecule level. We show that the MTB domain is solely responsible for 100 nM HDAC6 affinity for stabilized MTs and the MT-binding motif spans a positively charged patches comprising amino acids 32 – 37 and 51 – 56. HDAC6/MT interactions are fully independent of the presence catalytic domains and are mediated by ionic interactions with C-terminal unstructured tubulin tails. At the same time, the interplay between the MTB and deacetylase domains is critical for recognition and efficient deacetylation of tubulin, but not small peptidic substrates. Overall, our data reveal that recognition of natural substrates by HDAC6 is more complex than previously appreciated and domains outside the tandem catalytic core are essential for proficient substrate deacetylation *in vivo*.