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STRUCTURE AND GENOME RELEASE MECHANISM OF HUMAN CARDIOVIRUS SAFFOLD VIRUS-3 (SAFV-3)

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Saffold virus (SAFV) is the human *Cardiovirus* closely related to the Theiler murine encephalomyelitis virus (TMEV), of the family *picornaviridae* (1). It was reported that, SAFV might cause respiratory, gastrointestinal, and central nervous system infections (1,2). To date 11 genotypes of SAFV have been identified (1, 3). In the present study, the three-dimensional structure of SAFV-3 has been determined at 2.5 Å resolution. Although the architecture of the major capsid proteins VP1, VP2 and VP3 of SAFV-3 is similar to other cardioviruses, there are some differences on the surface loops. The presence of disulphide bond on the surface of VP3, surprisingly diminish the stability and infectivity of SAFV-3. Several capsid-binding and replication inhibitors of other picornaviruses fail to have any effect on SAFV-3. It was also shown that SAFV-3 dissociates in to pentameric subunits upon the genome release.

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Thursday, March 19, Session III

L10

STRUCTURE OF LLT1, A LIGAND FOR HUMAN NKR-P1, AND ITS VARIABILITY UNDER VARIOUS CONDITIONS

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Natural killer cells (NK cells) are large granular lymphocytes – a type of white blood cells. They are able to kill virally infected, stressed or tumor cells. Unlike T-cells, the activity of NK cells is innate, they do not need to have previous experience with a tumor – they are natural killers.

NKR-P1 (CD161) is a receptor on a surface of human NK cells. LLT1 is a ligand for NKR-P1 receptor, expressed primarily on activated lymphocytes and antigen presenting cells. The interaction of the ligand with the receptor inhibits NK cell cytotoxicity; however, it may have also activation effects in some cases. Extracellular domains of both binding partners, NKR-P1 and LLT1, have C-type lectin like (CTL) fold.

Using X-ray diffraction, we determined four structures of LLT1 [1] from protein produced in HEK293S GnTI- cells. The protein with $GlcNAc_2Man_5$ glycosylation packs into hexamers (consisting of three dimers) in crystals. The protein deglycosylated after the first N-acetylglucosamine was found in our crystal structures in forms of dimers (in pH 7.0) and monomers (in pH 3.5).

The LLT1 structures (Figure 1) show that LLT1 follows the "classical" mode of dimerization known from other structures with the same fold (CD69 [2], Clr-g [3]). The series of the LLT1 structures bring insight into variability of the dimerization interface, flexibility of the outer long loop of the CTL domain and influence of glycosylation on the structure.

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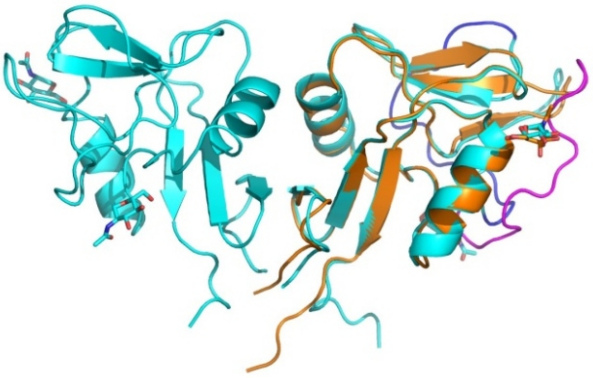

Figure 1. Structure of LLT1 in dimeric (cyan) and monomeric form (orange). The loop which position differs in dimer and monomer is on the right side (dimer - dark blue, monomer - magenta).

L11

A REFINED ATOMIC SCALE MODEL OF THE *SACCHAROMYCES CEREVISIAE* K^+-TRANSLOCATION PROTEIN TRK1P COMBINED WITH EXPERIMENTAL EVIDENCE CONFIRMS THE ROLE OF SELECTIVITY FILTER GLYCINES AND OTHER KEY RESIDUES

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Potassium ion (K^+) uptake in yeast is mediated mainly by the Trk1/2 proteins that enable cells to survive on external K^+ concentration as low as a few μM. Fungal Trks are related to prokaryotic TRK and Ktr and plant HKT K^+ transport systems (the SKT protein family). Overall sequence similarity is very low, thus requiring experimental verification of homology models. Here a refined structural model of the *Saccharomyces cerevisiae* Trk1 is presented that was obtained by combining homology modeling, molecular dynamics simulation and experimental verification through functional analysis of mutants. Structural models and experimental results showed that glycines within the selectivity filter, conserved amongst the K-channel/transporter family, are not only important for protein function, but are also required for correct folding/ membrane targeting.

A conserved aspartic acid in the P_A helix (D79) and a lysine in the $M2_D$ helix (K1147) were proposed earlier to interact. Our results suggested individual roles of these residues in folding, structural integrity and function. While mutations of D79 completely abolished protein folding, mutations at position 1147 were tolerated to some extent. Intriguingly, a secondary interaction of D79 with R76 could enhance folding/stability of Trk1 and enable a fraction of Trk1[K1147A] to fold.

The part of the ion permeation path containing the selectivity filter is shaped similar to that of ion channels. However below the selectivity filter it is obstructed or regulated by a proline containing loop. The presented model could provide the structural basis for addressing the long standing question if Trk1 is a passive or active ion-translocation system.

L12

THE EUROPEAN XFEL AND ITS POTENTIALS IN STRUCTURAL BIOLOGY

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Uppsala University and The European XFEL

At the beginning of 2017, the European X-ray Free-Electron-Laser (XFEL) in Hamburg will begin user operations. Free-electron lasers are the most brilliant sources of X-rays to date, exceeding the peak brilliance of conventional synchrotrons by a factor of 10 billion, and improving. In the duration of a single flash, the beam focused to a micron-sized spot has the same power density as all the sunlight hitting the Earth, focused to a millimetre square. The interaction of an intense X-ray pulse with matter is profoundly different from that of an optical pulse. A necessary goal of research with these machines is to explore photon-material interactions in strong X-ray fields. The aim in biology is to step beyond conventional damage limits and develop the science and technology required to enable high-resolution imaging of both crystalline and non-crystalline biological objects at high resolution. Eligible targets include single virus particles, organelles, cells, nanocrystals, engineered nanoclusters and isolated macromolecules. The talk will summarise developments at the European XFEL and provide an overview of some of the biological results from the Linac Coherent Light Source (LCLS), the first hard X-ray free-electron laser. One of the aims of the talk us explore possibilities for interested Czech scientists to participate in revolutionary new experiments at the European XFEL.

Friday, March 20, Session IV

L13

STRUCTURAL BIOINFORMATICS - A BRIDGE BETWEEN STRUCTURAL BIOLOGY AND BIOINFORMATICS

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Structural biology of today is a well-defined field of science. It is not so for bioinformatics, which is understood from very narrow classical view (informatics of the genome) on the one hand, to very wide concept of informatics of any biology related information. In all cases, bioinformatics becomes an important field of science as the amount of bio-related information, especially from Next Generation Sequencing (NGS), is increasing dramatically, and, for the time being, there is no software tool available that would be able to extract all the biological information hidden in the data.

In contrary, structural bioinformatics is relatively well defined part of bioinformatics (see, for example [1-2]), which is related to the analysis and prediction of the three-dimensional structure of biological macromolecules.The term *structural* has the same meaning as in *structural* biology, and structural bioinformatics can be seen as a part of *computational structural* biology. Even if the grow of 3D structural data is much lower compared to NGS, also here the increase is exponential and calls for new approaches to extract structurally and/or biologically relevant information.

In our group, we have developed several software tools that are able to help in solving such a task. These are MotiveQuery [3] for quick finding and extraction of biomacromolecular fragments, SiteBinder [4] for fast and accurate comparison of these fragments, MotiveValidator [5] and ValidatorDB [6] for validation of ligands and non-standard residues, and AtomicChargeCalculator [7] for calculation of partial atomic charges. Last but not least, we have developed also MOLE [8], a software tool for detection and characterization of channels and pores in biomacromolecules. All the software tools are accessible from the link http://ncbr.muni.cz/WebChemistry.

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