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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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 GlcNAc2-Man5 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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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 P₀ helix (D79) and a lysine in the M2₀ 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.
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 is to explore possibilities for interested Czech scientists to participate in revolutionary new experiments at the European XFEL.