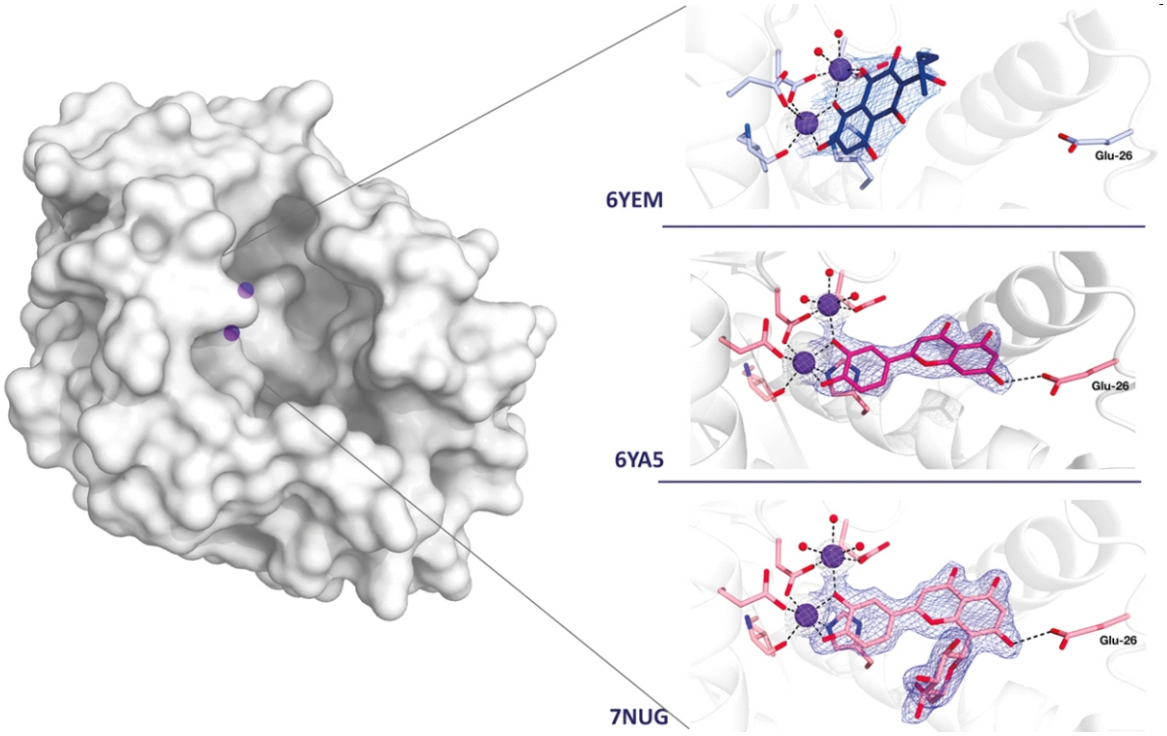

Figure C.

drugging undruggable targets (ChemBioDrug)" (No. CZ.02.1.01/0.0/0.0/16_019/0000729) and the European Union - Next Generation EU, The project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103).

Thursday, March 21, Session II

L3

X-RAY STRUCTURES OF (*S*)-ENANTIOSELECTIVE HALOALKANE DEHALOGENASE DmmarA FROM *MYCOBACTERIUM MARINUM* REVEAL A NEW MODE OF HOMODIMERIZATION THAT IS ATYPICAL FOR THIS ENZYME FAMILY

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Haloalkane dehalogenases (HLDs) are a family of -hydrolase fold enzymes that employ S_N2 nucleophilic substitution to cleave the carbon-halogen bond in diverse chemical structures, the biological role of which is still poorly understood. Their most important biotechnological applications include (i) biodegradation of pollutants such as 1,2-dichloroethane or 1,2,3-trichloropropane, (ii) decontamination of the warfare agent yperite, (iii) pollutant biosensing and (iv) HaloTag cell imaging [1, 2]. Atomic-level knowledge of both the inner organisation and supramolecular complexation of HLDs is thus crucial to understand their catalytic and non-catalytic functions.

Recently, database mining searches identified a new haloalkane dehalogenase, DmmarA, encoded in the genome of a waterborne pathogenic bacterium *Mycobacterium marinum* M. In our work, crystallographic structures of this (*S*)-enantioselective enzyme were determined at 1.6 and 1.85 Å resolution. The structures show a canonical -sandwich HLD fold with several unusual structural features. Mechanistically, the atypical composition of the proton-relay catalytic triad (aspartate-histidine-aspartate) and uncommon active-site pocket reveal the molecular specificities of catalytic apparatus that exhibits a rare (*S*)-enantiopreference of this enzyme family. Additionally, the structures reveal a previously unobserved mode of homodimerization, which is predominantly mediated through unique L5-to-L5 loop interactions. This homodimeric association in solution is confirmed experimentally by data obtained from small-angle X-ray scattering.

Utilizing the newly determined structures of DmmarA, molecular modelling techniques were employed to elucidate the underlying mechanism behind its atypical enantioselectivity. The (*S*)-preference can be attributed to the presence of a distinct binding pocket and variance in the activation barrier for nucleophilic substitution. Our findings thus highlight key molecular features distinguishing the DmmarA enzyme from other HLD family members.

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L4

SOLVING X-RAY STRUCTURE AT LOW RESOLUTION: NATURAL KILLER CELL RECEPTOR NKP30 WITH ITS TUMOR LIGAND B7-H6

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NKp30 is an activating receptor on the surface of human natural killer (NK) cells. B7-H6 is an activating ligand expressed by various tumor cells. The crystal structure of the complex, with NKp30 protein from bacterial production and B7-H6 protein from baculovirus-infected insect cells, was published by Li *et al*. [1], PDB code 3PV6.

We have solved a new crystal structure of NKp30: B7-H6, in which both components come from eukaryotic cell lines (HEK293S GnTI$^-$ cells). NKp30 was used with complete glycosylation, while B7-H6 was deglycosylated after the first GlcNAc for better crystallization. The structure has been deposited in the Protein Data Bank under code 6YJP and published [2]. The structure confirmed the NKp30:B7-H6 interaction interface, as observed by Li *et al*. However, we observed dimers of NKp30, which are likely biologically relevant and different from dimers described previously in [3], PDB code 3NOI. The newly observed dimer of NKp30 is placed among two B7-H6 molecules in the crystal structure. This arrangement may indicate the possibility of binding of the NKp30 dimer between two B7-H6 ligands even during the contact of the NK cell and the tumor cell.

In this talk, I will focus on issues connected with the solution and refinement of the crystal structure. The data were processed to resolution 3.1 Å; however, they were anisotropic and had resolution only 4.4 Å in the weakest direction. The crystals lost diffraction during data collection, and data from several crystals were necessary to merge. Both proteins have the same immunoglobulin-like fold, so it was uneasy to place the molecules into the unit cell correctly. Refinement was performed using LORESTR (REFMAC pipeline for low-resolution structures). Electron density indicated presence of an additional very flexible protein chain; this chain was not included in the final deposited coordinates.

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L5

STRUCTURAL IMMUNOLOGY OF (SUPER)NATURAL KILLER CELL RECEPTOR-LIGAND RECOGNITION

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Natural killer (NK) cells, a subset of effector lymphocytes, are an essential component of non-specific immunity, whose primary function is to recognise and spontaneously destroy damaged, infected, or malignant cells. The cytotoxicity of NK cells is regulated by their surface receptors through which they examine ligands on target cells. Some receptors enhance cytotoxicity (activating receptors), while others suppress it (inhibitory receptors). Which of the signals prevails then determines the action of the NK cell. NK cell cytotoxicity is further modulated by various other stimuli, such as cytokines, and it is executed upon direct cell-to-cell contact with the target cell by a deathly cocktail of enzymes released from lytic granules into the immune synapse. Thus, NK cells promise great therapeutic potential, which is currently being explored using various protein immunotherapeutics and genetically engineered CAR NK cells [1].

Over the last fifteen years, we have contributed to the structural description of various activating and inhibitory NK cell receptors of mouse, rat, and man, as well as of their cognate protein ligands and their mutual complexes. Using a combination of protein X-ray crystallography, small-angle X-ray scattering, and in-solution biophysical methods utilizing individual soluble recombinant proteins, with single-cell localization microscopy techniques observing the proteins expressed on the cell surface, we have become unravelling the almost supernatural nature of NK cell ligand recognition where the usually rather low affinity of single receptor-ligand interaction is overcome by their oligomerization and/or cross-linking/clustering within the immune synapse, thereby deploying avidity instead of affinity [2-4].

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L6

VIRAL RNA-METHYLTRANSFERASES: FUNCTION, STRUCTURE AND INHIBITION

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Methyltransferases (MTases) associated with coronaviruses, such as nsp10/16 and nsp14, are responsible for the final stages of RNA-cap formation within the cytoplasm. This cap is crucial for maintaining the stability of viral RNA and plays a pivotal role in evading the innate immune response. Uncapped RNA is swiftly detected by the innate immune system, triggering degradation and activating antiviral defenses. Consequently, the coronaviral MTases have become focal points of extensive scientific investigation. Recently, through the use of X-ray and cryo-electron microscopy techniques, the structures of both enzymes have been elucidated, even in complex with other components of the viral replication machinery. The application of high-throughput screening methods and the design of inhibitors guided by structural insights have resulted in the identification of potent inhibitors targeting these MTases. This talk critically examines the remarkable progress made in the field of coronaviral MTases since the onset of the COVID-19 pandemic. Additionally, it is worth noting that similar investigations into poxviruses' MTases have also contributed significantly to our understanding of viral replication and immune evasion strategies.

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L7

MODERN LEICA OPTICAL MICROSCOPY – PRODUCT AND APPLICATION VIEW

M. Kopecký

Specion, s.r.o., Prague

The presentation explores the transformative role of Leica's optical microscopy in various fields, from Live Cell Imaging to Clinical and Anatomic Pathology. Delve into the applications of Leica products in Neuroscience, Organoids, 3D Cell Culture, Virology, Cancer Research, and beyond. Highlighting Correlative Light & Electron Microscopy, along with Advanced Techniques like Cryo-Electron Tomography, FLIM, Photomanipulation, Super-Resolution, and STED, this session showcases how Leica is shaping the future of optical microscopy for enhanced scientific discovery and diagnostic precision.