MS04 P14

Preparation and crystallization of rat natural killer cell receptor NKR-P1B Ondrej Vanek[a], Petr Kolenko[b], Vladimir Kopecky, Jr.[c], Jan Dohnalek[b], Karel Bezouska[a,d] [a]*Department of Biochemistry, Faculty of Science, Charles University in Prague, Hlavova 2030,CZ-12840 Prague, Czech Republic.* [b]*Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovskeho namesti 2, CZ-16206 Prague, Czech Republic.* [c]*Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 5, CZ-12116 Prague, Czech Republic.* [d]*Institute of Microbiology, Academy of Sciences of the Czech Republic, Videnska 1083, CZ-14220 Prague, Czech Republic.* E-mail: kenav3@seznam.cz

Keywords: lectins, preparation and characterization, crystallization

Natural killer cells, the youngest members of lymphocyte family, are specified by being able to directly kill tumours or virally infected cells without previous antigen sensitization, [1]. Apart from cytokine and FcR-dependent activation, this proces is mediated by interaction of various protein receptor molecules displayed on their surface with epitope structures on target cells, such as MHC class I complexes. Natural killer cell receptors, both activation and inhibitory, are divided in two structural groups: immunoglobuline and C-type lectin. From the latter group, first identified signalling molecule was named NKR-P1 ("natural killer receptor-protein 1" also known as CD161 or KLRB1, [2]). Several members of this receptor gene family are known now from mouse and rat genome, but only single one was found in human. Our work focuses on rat NKR-P1B, an inhibitory receptor which despite its high sequence identity with activation rat NKR-P1A shows opposite function, [3]. It is also noteworthy that from all rodent isoforms of NKR-P1 receptor, rat NKR-P1B is most closely related tu human NKR-P1 receptor. As there is no crystal structure of any of NKR-P1 receptor molecules yet, hope is we can shed light onto these closely relative but functionally divergent proteins. We start with C-type lectin-like domain of rat NKR-P1B in full length, comprising amino acids from Ala90 to Lys215. The gene sequence was cloned into pRSETB vector and recombinantly expressed in E. coli as inclusion bodies which were then subjected to in vitro refolding protocol followed by extensive HPLC purification. Optimalization of all of these steps was needed to obtain reasonable amount of pure protein. To test the homogeneity and stability of prepared protein, dynamic light scattering (DLS) and diferential scanning calorimetry (DSC) experiments were performed. Raman spectroscopy analysis of secondary structure elements distribution was done both in solution and in semi-solid state as drop coating deposition Raman (DCDR, [4]) method. Finally, crystallization trials were set up in hanging drop arrangement and first screens yielded promising microcrystals presently. This work was funded by the European Commission as SPINE2-COMPLEXES, Contract No. LSHG-CT-2006-031220.

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MS04 P15

Highly amphiphilic interface in the complex DPPIV (CD26) and adenosine deaminase Wolfram Saenger[a], Wilhelm A. Weihofen[a], Jiango Liu[b], Werner Reutter[b], Hua Fan[b] [a]*Institute for Chemistry and Biochemistry/ Crystallogra- phy, Freie Universitaet Berlin, Takustrasse 6, D-14195 Berlin, Germany.* [b]*Institut for Molecular Biology and Biochemistry, Charité-Universitaetsmedizin Berlin, Arnimallee 22, D-14195 Berlin, Germany*
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Keywords: protein-protein interaction, receptor structure, protease

Dipeptidyl-peptidase IV (DPPIV or CD26) is a homodimeric type II membrane glycoprotein in which the two monomers are subdivided into a propeller domain and an α/β-hydrolase domain.
As dipeptidase, DPPIV modulates the activity of various biologically important peptides and, in addition, DPPIV acts as a receptor for adenosine deaminase (ADA), thereby mediating co-stimulatory signals in T-lymphocytes.
The 3.0-Å resolution crystal structure of the complex formed between human DPPIV and bovine ADA presented here shows that each β-propeller domain of the DPPIV dimer binds one ADA [1]. At the binding interface, two hydrophobic loops protruding from the β-propeller domain of DPPIV interact with two hydrophilic and heavily charged α-helices of ADA, giving rise to the highest percentage of charged residues involved in a protein-protein contact reported thus far. Additionally, four glycosides linked to Asn229 of DPPIV bind to ADA. In the crystal structure of porcine DPPIV, the observed tetramer formation was suggested to mediate epithelial and lymphocyte cell-cell adhesion [2]. ADA binding to DPPIV could regulate this adhesion, as it would abolish tetramerization.

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MS04 P16

CPD Damage Recognition by Transcribing RNA Polymerase II. Florian Brueckner[a,b], Ulrich Hennecke[a], Thomas Carell[a], Patrick Cramer[a,b]. [a]*Department of Chemistry and Biochemistry,* [b]*Gene Center Munich, Ludwig-Maximilians-Universitaet Muenchen, Feodor-Lynen-Str. 25, 81377 Munich, Germany.*
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Cells use transcription-coupled repair (TCR) to efficiently eliminate DNA lesions such as UV-induced cyclobutane pyrimidine dimers (CPDs). Here we present the structure-based mechanism for the first step in eukaryotic TCR, CPD-induced stalling of RNA polymerase (Pol) II [1], [2]. A CPD in the transcribed strand slowly passes a translocation barrier, and enters the polymerase active site. The CPD 5'-thymine then directs uridine misincorporation into mRNA, which blocks translocation. Artificial

replacement of the uridine by adenosine enables CPD bypass. Thus Pol II stalling requires CPD-directed misincorporation. In the stalled complex, the lesion is inaccessible, and the polymerase conformation is unchanged. This is consistent with non-allosteric recruitment of repair factors and removal of a lesion-containing DNA fragment together with Pol II.

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