Parallel Session IXb, June 1, Thursday

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TOOLS FOR RNA AND DNA STRUCTURAL DESCRIPTION AT DNATCO.DATMOS.ORG

Bohdan Schneider, Michal Malý, Lada Biedermannová, Jiří Černý

Institute of Biotechnology of the Czech Academy of Sciences, 252 50 Vestec, Czech Republic
bohdan.schneider@ibt.cas.cz

I will introduce our web application *dnatco.datmos.org*, which enables an in-depth analysis and validation of nucleic acid structures, primarily based on the assignment of dinucleotide conformers. The web service was introduced for the first time in 2016 [1] but has been quite significantly redesigned and its functionality extended. We developed several new tools for annotation and validation of nucleic acids that are all based on the concept of the *dinucleotide conformational classes*, NtC [2]. One of the new validation tools are scattergrams correlating the geometries of dinucleotides and their fit to the experimental electron density, RSCC-rmsd plots. We also validate quality of the covalent geometry of analyzed nucleic acids by comparing values of their bond lengths and angles to values expected in a curated set of PDB-deposited structures. The web service dnatco.datmos.org is based on the C++ backend programs for the NtC assignment and runs most user demanded calculations on the user computer; it uses customized Molstar viewer [3].

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This research was funded by Czech Academy of Sciences, grant RVO 86652036 and by grant LM2023055 to ELIXIR CZ from MEYS Czech Republic.

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CLUSTERING OF IMMUNE RECEPTORS ON THE SURFACE OF NK CELLS. STRUCTURE OF THE HUMAN NKR-P1:LLT1 COMPLEX

T. Skálová[1], J. Bláha[2], B. Kalousková[2], D. Cmunt[2], J. Stránský[1], J. Dohnálek[1], O. Vaněk[2]

[1]Institute of Biotechnology, Czech Academy of Sciences, BIOCEV, Průmyslová 595, 252 50 Vestec, Czech Republic
[2]Department of Biochemistry, Faculty of Science, Charles University Prague, Hlavova 8, 128 40 Praha, Czech Republic
t.skalova@gmail.com

Natural killer cells are white blood cells able to kill tumour, virus-infected or stressed cells. NKR-P1 is an immune receptor on the surface of human natural killer cells and LLT1 protein is its binding partner on the surface of another cell interacting with the NK cell. Both NKR-P1 and LLT1 have extracellular domains of the same C-type lectin-like (CTL) fold. LLT1 is expressed on activated monocytes, B cells and cancer cells. Therefore, structural knowledge of the interaction between NKR-P1 and LLT1 is important for understanding physiological and pathogenic processes in the immune system.

Crystal structure of human NKR-P1 in complex with LLT1 was determined using diffraction data collected at the Diamond Light Source (Harwell, UK) at beamline I03 with resolution 1.9 Å [1].

Besides this structure, we solved also three structures of LLT1 (monomeric, dimeric and dimeric fully glycosylated, [2]) and two structures of NKR-P1 (glycosylated and deglycosylated dimer, [1]). These structures confirm that NKR-P1 and LLT1 systematically differ in the way of their

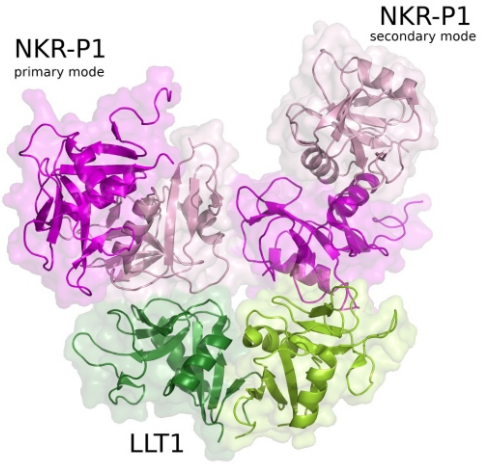

Figure 1. The crystal structure (PDB code 5MGT) revealed two binding modes of immune receptor NKR-P1 with its protein ligand LLT1, here denoted as primary and secondary binding mode.

dimerization. The structure of the NKR-P1:LLT1 complex showed that different mode of dimerization of the binding partners is important for their geometrical arrangement. Moreover, the structure of the complex revealed two different binding modes between the proteins (Fig. 1, primary and secondary binding mode) and virtually "infinite" chains of both proteins existing in the crystal. Biological relevance of both binding modes was confirmed by mutational analysis and clustering of the receptors with ligands was observed also on cell surface using super-resolution microscopy. The proposed interaction model is shown in Figure 2.

This study was supported by the Czech Science Foundation grant 18-10687S, the Ministry of Education, Youth and Sports of the Czech Republic grant LTC17065 (in the frame of the COST Action CA15126 MOBIEU), and the European Regional Development Fund (CZ.02.1.01/0.0/0.0/15_003/0000447). Computational resources were supplied by the project Infrastruktura CZ (e-INFRA LM2018140) provided within the program Projects of Large Research, Development, and Innovations Infrastructures. We acknowledge using CF Biophysical methods of CMS, CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127).

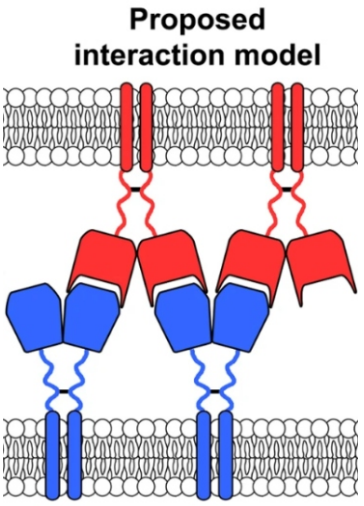

Figure 2. NKR-P1:LLT1, the proposed model of the clustering on the cell surface. Taken from [1].

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PHOSPHORYLATION OF LON PROTEASE – THE EFFECT ON FUNCTION AND STRUCTURE

G. Ondrovičová[1*], N. Kunová[1*], S. Kereúche[2*], J.A. Bauer[1*], V. Kotrasová[1], B. Stojkovičová[1], H. Havalová[1], L. Martináková[1], V. Lukáčová[4], L. Kohútová[3], P. Baráth[3,4], M. Pinkas[5], J. Nováček[5], V. Pevala[1], E. Kutejová[1]

[1]Department of Biochemistry and Protein Structure, Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, 845 51 Bratislava, Slovakia

[2]Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University and General University Hospital in Prague, Albertov 4, 128 01 Prague, Czech Republic

[3]Chemical Institute, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia

[4]Medirex Group Academy, Nitra, Slovakia[5] Masaryk University, Cryo-Electron Microscopy and Tomography Core Facility, Kamenice 753/5, 625 00 Brno, Masaryk University

** authors with same contribution*

Eva.Kutejova@savba.sk, Nina.Kunova@savba.sk

Lon protease is a unique ATP-dependent protease found in all kingdoms of life [1]. In mitochondria, Lon represents a crucial protein responsible for maintaining the mitochondrial homeostasis and is important for degrading of misfolded, disassembled and oxidatively damaged proteins. Lon was also shown to control the levels of several mitochondrial proteins including the subunits of mitochondrial processing peptidase MPP, StAR protein, helicase Twinkle and ribosomal subunit MrpL32 in human cells, and mtDNA-packaging protein Abf2 and mtDNA-maintenance factor Mgm101 in *S. cerevisiae* [1-3]. To better understand the function of Lon, we determined the first 3D cryo-EM structure of a complete human mitochondrial Lon protease [4] and described the rearrangement of its structure when Lon is moving between the ATP-bound and ADP-bound states. Since both, over-expression and reduced expression of Lon, were found in cancer cells [5], it is clear that a more complex understanding of the mechanisms regulating its functions in mitochondria is needed. This regulation could occur on the level of its transcription, but recent studies have suggested that post-translational modifications might also have a large impact here. Phosphorylation represents an effective post-translational modification in cells. To characterize the effect of such modification on function and structure of human Lon protease, we prepared several point mutations in Lon's N-terminal domain, where Tyr was replaced by Glu or, using an orthogonal translation system in modified *E. coli* strain,

para-carboxymethyl-L-phenylalanine (pCMF), a non-hydrolysable analogue of phosphotyrosine, was introduced into the Tyr sites of interest. Our results indicated that phosphorylation of some N-terminal Tyr substantially reduced Lon protease activities without any significant change of its structure. Such regulation might facilitate quick dynamic changes to mitochondrial crucial components, nucleoids and ribosomes, which are inevitable for conducting mitochondrial functions and maintaining mitochondrial homeostasis *in vivo* and could also have significant implications for human medicine.

The authors have received a support from the Slovak Research and Development Agency APVV-15-0375, APVV-19-0298), the Slovak Grant Agency (VEGA 2/0069/23), the Interreg V-A Slovakia-Austria program for the project StruBioMol, ITMS: 305011X666, co-financed by the European Regional Development Fund and Ministry of Education and CF CF CryoEM of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042)) and European Regional Development Fund-Project „UP CIISB" (No. CZ.02.1.01/0.0/0.0/18_046/0015974).

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LOCAL MOTIFS OF DISORDERED PROTEINS: CRYSTAL STRUCTURE, SIMULATIONS, COMPUTATIONS

Rostislav Skrabana[1], Katarina Martonova[1], Ondrej Cehlar[1], Michal Hricovini[2], Milos Hricovini[2], Klaudia Meskova[1]

[1]Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, Bratislava, Slovakia
[2]Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, Bratislava, Slovakia
rostislav.skrabana@savba.sk

In water solution, intrinsically disordered proteins (IDPs) contain no stable three-dimensional structure. IDP's polypeptide chain is fully hydrated and its conformations are composing a conformational ensemble (CE). Individual conformational states of CE are separated by a small energy barrier and freely interconvert [1].

The structure of globular proteins contains large number of intramolecular hydrogen bonds involving main-chain atoms and resulting in large-scale structural elements of beta sheets and alpha helices. In contrast, IDPs satisfy hydrogen-bonding potential of their main chain almost exclusively by hydrogen bonding to the solvent molecules.

We found that there may be exemptions to this general pattern of H-bonding in IDPs. Starting from the observation of crystal structures and molecular simulations, we proved the existence of intramolecular H-bonds in IDPs. These particular bonds are confined locally and create small 9-15 membered ring structures. Results of simulations indicate that these rings may be stable only for a short period of time and frequently renew their existence. Quantum mechanics calculations revealed the proportion of rings in the local CE.

For these structures we coined the name *SPuRs* – side chain propelled rings. It is hypothesized that these small ring structures may influence the composition of IDP CE and regulate corresponding biological activity. We have observed several *SPURS* in crystals of IDP tau with monoclonal antibodies [2]. In the future work we aim to examine the *SPURS* also in other IDPs.

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This work was supported by the grant number APVV-21-0479.