Saturday, March 23, Session VI

L24

CENTRE OF MOLECULAR STRUCTURE IN BIOCEV – INFORMATION AND UPDATES

J. Pavlíček

Centre of Molecular Structure, Institute of Biotechnology AS CR, v. v. i., BIOCEV, Průmyslová 595, 252 50 Vestec, Czech Republic, jiri.pavlicek@ibt.cas.cz

The Centre of Molecular Structure (CMS) operates in BIOCEV as a part of the Institute of Biotechnology, AS CR. It represents a complex of core facilities specialized in the solution of structure and interactions of biomolecules. CMS includes facilities for crystallization and diffraction, physicochemical characterization of biomolecules, and mass spectrometry analysis. As a part of CIISB and Instruct infrastructures, CMS provides a comprehensive service and consultation for researchers from BIOCEV, from other Czech locations and even for users from abroad. During last year, the number of techniques provided to researchers in CMS had grown again.

CIISB research infrastructure project LM2015043 funded by MEYS CR is gratefully acknowledge for the financial support of the measurements at the CMS.

L25

STRUCTURE OF *LEISHMANIA* RNA VIRUS 1

M. Procházková[1], T. Füzik[1], D. Grybtchuk[1], L. Podešvová[2], F. Falginella[1], R. Vácha[1], V. Yurchenko[2], P. Plevka[1]

[1]*CEITEC-MU, Kamenice 753/5, 625 00 Brno, CZ*
[2]*LSRC, Faculty of Science, University of Ostrava, Chitussiho 10, 710 00 Ostrava, CZ*
michaela.veselikova@ceitec.muni.cz

Leishmania RNA virus 1 (LRV1, Totiviridae) infects human protozoan parasite *Leishmania*. LRV1-carrying parasites are more virulent and cause more serious mucocutaneous form of leishmaniasis characteristic by massive inflammation and facial tissue damage [1, 2] . Despite its role in pathogenesis of leishmaniasis, the LRV1 was not structurally characterized up to date. Here we present a structure of LRV1 capsid and demonstrate that it binds the host RNA.

To circumvent the issue of low virus titer in *Leishmania* cells, we took advantage of the recombinant capsid protein tendency to form stable virus-like particles (VLPs). The LRV1 VLP is an empty particle 42 nm in diameter with an icosahedral symmetry (Fig 1, b) and triangulation number T = *2 typical for totiviruses. The asymmetric unit consists of two copies of the capsid protein (Fig 1, a). We determined the structure of LRV1 VLP to the resolution of 3.65 Å using cryo electron microscopy. The LRV1 capsid protein model was constructed *ab initio* and refined against experimental data in Phenix suite.

Capsid proteins of related L-A virus of yeast [3] possess the ability to de-cap cellular mRNAs to overload the RNA degradation machinery of the host cell and thus protect viral mRNAs [4, 5]. The residues responsible for m^{7}GTP binding and hydrolysis (His154, Tyr150, Asp152, and Tyr452) are not conserved between L-A virus and LRV1. Instead, the molecular simulations point at the cap4 preference for positively charged amino acids on the bottom of a trench formed by three antiparallel alpha-helices in subunit B at the interface with subunit A (Fig 1, c).

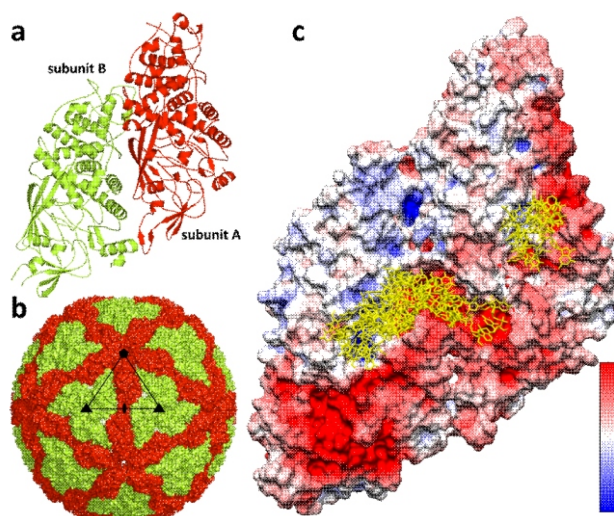
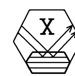


Figure 1. (a) Structure of the LRV1 capsid protein asymmetric unit (subunit A in red, subunit B in green). (b) Capsid of LRV1 with icosahedral symmetry axes marked – threefold (triangle), twofold (oval), fivefold (pentagon) and subunit A in red, subunit B in green. (c) Electrostatic surface coloring of LRV1asymmetric unit (red-blue) with top 10 docked cap4 structures in yellow.

1. Hartley M-A, Ronet C, Zangger H, Beverley SM, Fasel N. Frontiers in Cellular and Infection Microbiology. 2012;2: 99.



2. Motta L, Ferreira C, Ito MM, Porrozzi R, Cupolillo E, Godoi R De, et al. PLOS Neglected Tropical Diseases. 2015;9.

3. Naitow H, Tang J, Canady M, Wickner RB, Johnson JE. Nature Structural Biology. 2002;9: 725–728.

4. Blanc A, Ribas JC, Wickner RB, Sonenberg N. Molecular and cellular biology. 1994;14: 2664–74.

5. Masison DC, Blanc A, Ribas JC, Carroll K, Sonenberg N, Wickner RB. Molecular and cellular biology. 1995;15: 2763–71.

L26

LABORATORY EVOLUTION REVEALS STRUCTURAL ELEMENTS DRIVING ENZYME EVOLVABILITY

Schenkmayerova Andrea[1,2], Gaspar P. Pinto[1,2], Martin Toul[1,2], Lenka Hernychova[3], Veronika Liskova[2], Klara Markova[2], Stephane Emond[4], Mark Dörr5, Daniel Pluskal[2], Joan Planas[1,2], Radka Chaloupkova[2], David Bednar[1,2], Zbynek Prokop[1,2], Uwe T. Bornscheuer[5], Florian Hollfelder[3], Jiri Damborsky[1,2], Martin Marek[2]

[1]Internat. Clinical Research Center, St. Anne's Univ. Hospital Brno, Pekarska 53, 656 91 Brno, Czech Rep.
[2]Loschmidt Laboratories, Department of Experimental Biology and RECETOX, Faculty of Science, Masaryk University, Kamenice 5, Bld. A13, 625 00 Brno, Czech Republic
[3]Regional Centre for Appl. Molecular Oncology, Masaryk Memorial Cancer Inst., Zluty kopec 7, 656 53 Brno,
[4]Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, UK
[5]Department of Biotechnology & Enzyme Catalysis, Institute of Biochemistry, Felix-Hausdorff-Str. 4, 17487 Greifswald, Germany
andrea.schenkmayerova@fnusa.cz

We have recently resurrected a bifunctional ancestral enzyme that putatively existed prior to the functional diversification into modern-day haloalkane dehalogenases (EC 3.8.1.5) and coelenterazine-converting *Renilla* luciferase (EC 1.13.12.5). This ancestor, which exhibited markedly enhanced thermal stability, was subjected to InDel mutagenesis to uncover molecular determinants important for the evolution of luciferase activity. Generated libraries were screened and the best hits carrying alterations in three hot-spot regions were comprehensively characterized. The most potent hit was crystallized and its 2-Å-resolution structure was solved. There are two monomers (A and B) present in the asymmetric unit, which form a non-crystallographic dimer related by 2-fold axis of symmetry. Although the overall structures of the both monomers are very similar, they markedly differ in the positioning of the cap-domain-forming 4 helix. Moreover, electron density maps for the 4 helix and its flanking loops are not perfectly resolved, some side chains are poorly visible or not seen at all, which illustrates a conformational flexibility in this region. Monomer A is similar to the template structure, although the replacement of a bulky phenylalanine by proline makes the active-site cavity spatially bigger. However, the active-site access tunnel in monomer B is markedly reduced due to the 4 helix distortion. Complementary protein simulations supported that the gained conformational flexibility of the cap-domain-forming elements favours the accommodation of bulkier substrates such as coelenterazine. Collectively, we highlight enzyme molecular determinants required for evolvability of luciferase activity, and propose a new framework to switch enzyme functions by engineering of flexible elements.

L27

WITH OR WITHOUT YOU: STRUCTURE VS. LIGAND-BASED DRUG DESIGN

Radim Nencka

Gilead Research Center at IOCB Prague, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic.

There are two main approaches towards discovery of new therapeutics. We can differ target-based drug design (TBDD), which usually deals with proteins of interest and tries to find various molecules that interact with them, and approach based on phenotype screening, which uses phenotype observation on cells or whole animals. There are also two different approaches towards TBDD - structure-based and ligand-based drug design (SBDD and LBDD, respectively). The lecture will show both the approaches on an example of phosphatidylinositol 4-kineses (PI4Ks), namely phosphatidylinositol 4-kinase III . Our first steps towards the novel inhibitors of this enzyme were purely ligand-based since a structure of the protein was unknown. Lately, our team was able to obtain the crystal structure of this protein, which proved very nice correlation with the structure-activity relationship obtained in our medicinal chemistry efforts and helped us to enhance both activity and selectivity of second-generation inhibitors.