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.

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STRUCTURE OF LEISHMANIA RNA VIRUS 1

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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 $T = \ast 2$ 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 mGTP 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 LRV1 asymmetric unit (red-blue) with top 10 docked cap4 structures in yellow.

LABORATORY EVOLUTION REVEALS STRUCTURAL ELEMENTS DRIVING ENZYME EVOLVABILITY

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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 position of the cap-domain-forming α4 helix. Moreover, electron density maps for the α4 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 α4 helix distortion. Complementary protein simulations supported that the gained conformational flexibility of the cap-domain-forming elements favors 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.

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

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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-kinases (PI4Ks), namely phosphatidylinositol 4-kinase IIIβ. 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.