Short Lectures - D, Tuesday, June 12

SL - D1

COMPARISON OF DIFFERENT PROTEINS CRYSTALLIZATION CAPABILITY

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The crystallization and crystallographic studies of selected soluble and membrane proteins and protein complexes are the main aims of the scientific research at the Laboratory of crystallogenesis and biomolecular crystallography of Institute of Nanobiology and Structural Biology GCRC AS CR joined with Laboratory of molecular structure and dynamics of School of complex systems of FFPW University of South Bohemia in Ceske Budejovice. During last 10 years more than 20 different proteins, protein complexes and mutant variants (e.g. HsdR subunit of restriction enzyme of EcoR124I from *E.coli* plus its 3 mutant forms, PsbP protein from *Spinacia oleracea*, WrbA apo and holo forms of *E.coli*, Fe-regulated protein D (FrpD) of *Neisseria meningitides*, haloalkan dehalogenases DhaAwt, DhaA04, DhaA12, DhaA13, DhaA14, DhaA15, DhaA31 from *Rhodococcus rhodochrous* NCIMB 13064 and their complexes with substrates isopropanol (IPA) and 1, 2, 3 – trichloropropane (TCP), DbeA and DbeA1 of *Bradyrhizobium elkani* USDA94, two new haloalkan dehalogenases DpcA from *Psychrobacter cryohalolentis* K5 and DmxA from *Marynobacter* sp ELB 17, etc.) were crystallized, tested and crystals were measured at the synchrotron radiation sources. Diffraction data were used to solve and refine protein structures that have been deposited in PDB under specific codes. Enzymes were crystallized using standard, advanced and alternative crystallization techniques. The cross influence procedure was directly developed and tested in our laboratory.

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Short Lectures - D, Wednesday, June 13

SL - D2

BORON CLUSTER DERIVATIVES AS INHIBITORS OF IMPORTANT THERAPEUTICAL TARGETS: HIV-1 PROTEASE AND CARBONIC ANHYDRASE IX

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Boron cluster derivatives have been previously identified and characterized as a new class of HIV protease inhibitors [1,2]. Structural studies of metallacarboranes in complex with HIV protease as well as novel type of boron cluster derivatives designed on basis of previous structural studies [3,4] ENREF3 and their specific inhibitory effect on human carbonic anhydrase IX (CA IX) will be presented.

CA IX is overexpressed in several hypoxic cancer tissues. The structure and enzyme inhibition effects of boron cluster based inhibitors will be presented. This novel types of selective CA IX inhibitors could play important role in cancer diagnostics and therapy.

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inorganic polyhedral metallacarboranes. *Journal of Medicinal Chemistry* 52, 7132-41, 2009.

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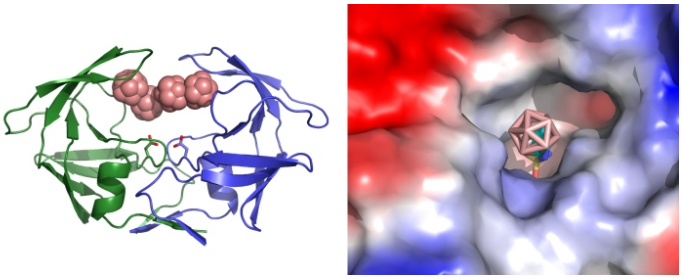

Figure 1. Boron cluster derivatives as novel types of inhibitors of HIV protease (left) and carborane-sulfamide molecule binding to the active site of hCA II (right).

SL - D3

STRUCTURE-BASED DRUG DESIGN OF SELECTIVE 5'-NUCLEOTIDASES INHIBITORS

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The monophosphate 5'-nucleotidases, including 5'(3')-deoxyribonucleotidase, belong to a family of enzymes that catalyze the dephosphorylation of nucleoside monophosphates. The ribonucleotides and deoxyribonucleotides could be synthesized *de novo* from low-molecular-weight precursors or by a salvage pathway from nucleosides or nucleobases originating from catabolism of nucleic acids. In this salvage pathway, ribonucleotides and deoxyribonucleotides are phosphorylated by nucleoside and nucleotide kinases to maintain sufficient pools of dNTP's and NTP's for nucleic acid synthesis. The phosphorylation by cellular nucleoside kinases is opposed by tha activity of 5'-nucleotidases that dephosphorylate ribo- and deoxyribonucleoside monophosphates. Besides their role in the regulation of physiological dNTP pools, substrate cycles between ribonucleotidases and kinases may affect the therapeutic action of pyrimidine nucleoside analogs used as anticancer and antiviral agents. Such compounds require the nucleoside kinases activity for phosphorylation to their active forms. Results of clinical and in vitro studies propose that an increase in nucleotidase activity can interfere with nucleoside analogue activation resulting in drug resistance.

The main goal of this project is structure-assisted design of a potent and selective inhibitors of mammalian 5'-nucleotidases based on nucleoside phosphonic acid derivative scaffold. In general, compounds of a strong and selective inhibitory potency are of high medicinal interest as antimetabolites for anticancer and antiviral therapy.

We have used the structure of human mitochondrial 5'-nucleotidase [6] and performed *in silico* screening of a virtual library containing 29 thousand compounds. A set of compounds with highest scores was synthesized and screened for their inhibitory effect toward two isoforms of human 5'-nucleotidase: cytosolic (cdN) and mitochondrial (mdN).

Using this approach we have identified compounds specifically inhibiting mdN and cdN with inhibitory potency being 100 and 50 times better compared to the reported mdN and cdN inhibitors, respectively. Structural details of interactions of newly identified compounds with mdN were investigated through determination of high-resolution crystal structures.

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SL - D4

SIGNIFICANT CHANGES BETWEEN THE X-RAY STRUCTURE AND NMR STRUCTURE OF -SUBUNIT OF RNA-POLYMERASE

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RNA polymerase (RNAP) is an essential enzyme that is responsible for transcription of DNA into RNA. It is a multi-subunit enzyme and its composition is well conserved throughout all bacterial species. Gram-positive bacteria in comparison to gram-negative bacteria contain two additional subunits that associate with RNAP: [1] which is the subject of this work, and $_1$ [2]. The recombinant form of -subunit from *Bacillus subtilis* is a 173 aa long protein with the acidic pI of 3.6. It was shown to consist of two domains: the N-terminal domain displaying an ordered structure as determined by CD spectroscopy, and the C-terminal domain, which appeared flexible and unstructured. The N-terminal domain was shown to interact with RNAP [3]. The structure of N-terminal domain was determined by NMR. It consists mainly of three -helixes and two short -sheets, yet the N-terminal part remains unstructured. The cause of this flexibility is probably the His_6-Tag attached at the N-terminus [4].

The N-terminal domain of -subunit was conquested to high-throughput screening (sitting drop), where several crystallization conditions were found. Further optimization showed two conditions that are more favourable for the crystal growth and it´s quality. Vapour diffusion hanging drop and under oil crystallization techniques were used for the crystallization in the optimizing steps. Higher quality crystals were obtained after 7-9 days. An iodine compound was used as a co-crystalline substance to have a possibility to provide a Single Anomalous Dispersion (SAD) experiments. Diffraction data were collected at BESSY II Berlin, beamline MX-14.2. The collection of SAD and native data of -subunit was successful. Both data were collected on the same crystal with average size 500 μm x 350 μm. The resolution of the SAD data was 1.66 Å and the native data 1.8 Å. The data were processed by MOSFLM and the structure was solved using SAD data by determination of the positions of heavy atoms, in this case iodines from the iodine substance used in optimizing step of the crystallization procedure. The space group was determined as C222(1), which means orthorhombic centro-symmetric space group. For the native data, the structure determination was done using molecular replacement with the structure solved from SAD data. The -helical part of the protein is in a good agreement with the NMR structure. However,the X-ray structure showed completely different behaviour (folding) inthe region corresponding to -sheets present in the NMR structure The problematic N-terminal part with His_6-Tag on the end is not observable due to high flexibility. In the X-ray structure Ni^{2+} ions were observable, which can be explained by usage of Ni-NTA column for purification of the N-terminal domain of -subunit. Further investigations were concerned on the Ni^{2+} ions as a possible main factor of a different folding of the protein in the crystal structure. This is a strong proof that some of the proteins can be differently structured in the solution as in the crystalline phase.

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SL - D5

THE STRUCTURAL AND BIOCHEMICAL DATA OF ENZYME CAPABLE OF ORGANOPHOSPHATES DEGRADATION

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Extremophiles are organisms living in extreme conditions on Earth (for illustration: temperatures of -50 °C or 113 °C, hydrostatic pressures of 120 MPa or pH values of 0.5 or 12.0). Psychrotrophs are a group of extremophilic microorganisms with the minimal temperature of growth around 0 °C and with the optimal temperature of growth around 20 °C. Halophiles are a group of extremophilic microorganisms requiring at least 0.2 M concentrations of salt for their growth.

The examined enzyme organophosphorus acid anhydrolase (OPAA) is able to catalyze hydrolysis of proline dipeptides (Xaa-Pro), and of several types of organophosphate compounds commonly used as pesticides or as nerve agents. The enzyme, with the pH optimum around 8, offers a large potential for biotechnological application as a tool for bio-degradation of these dangerous compounds. Two different types of the OPAA enzyme from different bacteria – psychrothropic and slightly halophilic *Pseudoalteromonas haloplanktis* and slightly halophilic *Alteromonas macleodii* – were studied and compared with the sequence related human prolidase.

Three molecular structures of the OPAA enzyme from *Alteromonas macleodii* have been determined. The structure data were collected at the beam line PX 14.1 of the source of synchrotron radiation Bessy II (Helmholtz-Zentrum, Berlin). Native amOPAA crystallized in the space group $C2$ with unit cell parameters a = 134.3 Å,, b = 49.1 Å, c = 97.2 Å and = 125.0°. The crystals were measured native and soaked with ligand Pro-Gly, too. Data were collected up to resolutions 1.8 Å and 1.9 Å, respectively. The third crystal structure determined is the native amOPAA crystallized in the space group $P2_12_12_1$ (unit cell parameters a = 75.6 Å, b = 111.2 Å, c = 138.1 Å). Data were collected to resolution 2.2 Å in this case. Refinement of all these structures performed well with the average B factor around 20 Å^2 and the final R factors in the range 15.3 – 16.6 %. The structure of was deposited in the PDB under the accession code 3RVA.

To modulate the enzymatic activity profiles and to explain the enzymatic function of OPAA, we made site-directed mutagenesis of OPAA from *Pseudoalteromonas haloplanktis* near in its substrate binding site (**Tyr212**, **His226**, Asp244, Asp255, **His335**, His339, Arg370, Glu384, Arg421, Glu423, Val345, His346) found in our structure with the dipeptide Pro-Gly complex. Only two of seven tested single-mutations (**Y212F,** Y212S - H226N,

Figure 1. The cartoon representation of 3D structure of the OPAA from *Alteromonas macleodii.* The binuclear metal center is highlighted by two spheres.

H226K - H334N, H334K, H334Q) retained the enzymatic activity.

Enzyme assay studies showed a loss of activity as a consequence of site-directed mutagenesis in five cases. Two mutations highlighted in bold retained the enzymatic activity according to the results of biochemical characterization (DLS, SDS-PAGE). The pH profiles of the OPAA mutants Y212F and H226K preserving activity remain

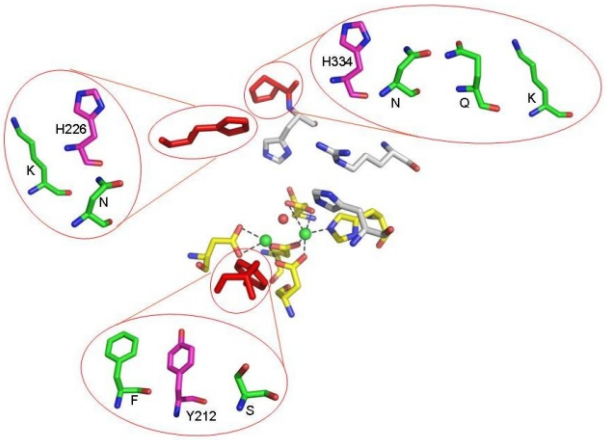

Figure 2. Tested mutations around the active site of OPAA. The red residus are in positions found in the native OPAA. The magenta residues are residues which were mutated in phOPAA; the green residues show the particular mutations. The manganese ions are shown as green spheres.

similar to the wild type, but in the case of mutants are shifted slightly to more basic pH.

The comparative study shows that the OPAA enzyme is very similar to human prolidase. High similarities in structure and also in substrate specificities of OPAAs and prolidases have brought up some interesting questions regarding the historical classification of this group of enzymes with related but not identical activity profiles.

The structures discussed here together with other three structures of protein-ligand complexes of the low temperature active -galactosidase are part of the PhD thesis [3].

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ADVANCED COURSES OF PROTEIN STRUCTURE ANALYSIS 2012 - 2015

Series of seminars, courses and discussion meeting on analysis of protein structure, dynamics and function supported under the program OPVK MŠMT will be organized during the period 2012-2015.

The actions are determined for scientific workers, teachers, for PhD students and students, etc. **All participants receive preliminary materials for study** before visiting the course to ensure better perception of problematics and possible active participation in the discussion meetings.

The participation is free of charge for the participants employed in the research or educational institutions all over the Czech Republic with exception of the city Prague from the OPVK program.

The participants who are not able to prove affiliation to the region outside of Prague city should cover the current fee. The fee will be announced derived from real costs of the individual action in will be announced in the invitation to the particular seminar. It depends on the length of the seminar and whether the accommodation and meals will be required, etc.

Detailed information and the final programs of particular courses and seminars will be announced continuously to all registered using the attached form, mostly about 3 months in advance.

The courses will be registered in the educational program of the Charles University and if the student decides to take part in examinations, he can register his participation in his curriculum of graduate or postgraduate study.

THE PLANNED TOPICS

1. Structure-function correlations – practical use of the Protein Structure Databank, the Cambridge Structure Database of Organic and Organometalic structures, the Polymer Structure Database, and the related software.
2. Experimental tool for structure determination (X-ray diffractometers, synchrotron facilities, free-electron lasers, NMR spectrometers, ELI).
3. *Ab initio* structure determination of macromolecular structure (X-ray diffraction, neutron diffraction, electron dif fraction, NMR, transmission electron microscopy, optical spectroscopy). The structure refinement, constraints and restrains, template libraries (X-ray, NMR)
4. Accuracy and reliability of protein structure determination (X-ray, NMR, TEM)
5. Function principles of organized molecular systems
6. Expression, purification, deuterization, advanced crystallization methods. Genomic analysis, structure alignments, correlations, etc.
7. Molecular modelling, molecular dynamics, the energy estimates, model and reality
8. Protein structure and function in different environment. Using methods for checking a status of protein – small angle scattering, optical spectroscopy, diffuse light scattering, etc.
9. Principles of molecular recognition, protein-protein and protein-ligand interactions, drug design

The courses are mutually independent, i.e. the participation in any of these courses is not bound to participation in any other course of this series.

For more specific information or proposals of missing topics or lectures, please, send e-mail to hasek@imc.cas.cz

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