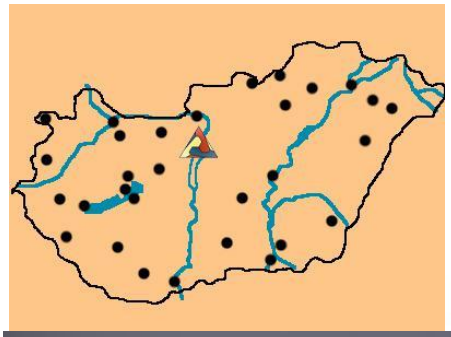
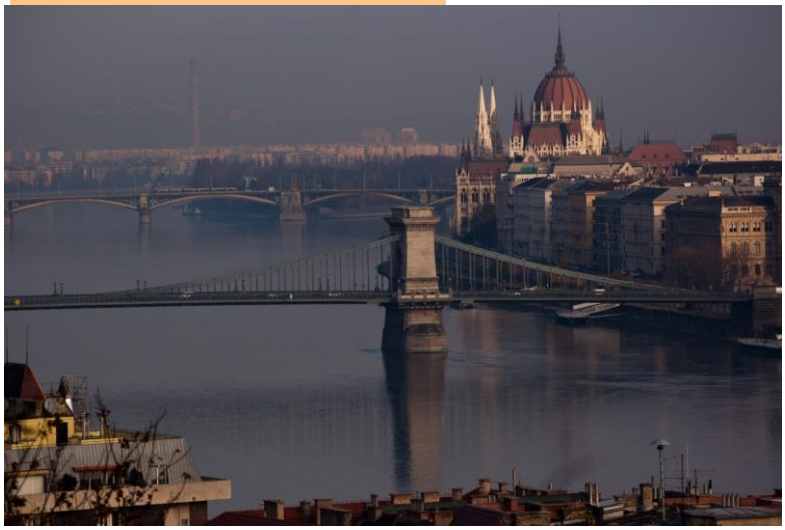
BUDAPEST BIOSTRUCT COURSE ON BASICS IN PROTEIN CRYSTALLIZATION AND CRYSTALLOGRAPHY 2013



BUDAPEST·BioStructX 2013



BioStructX

within the framework of the Biostruct-X project

Dates: August 30 – September 3, 2013

Venue: Budapest University of Technology and Economics Biostruct Laboratory

How to find us

From Hotel Mediterran:

traveling by tram, 61 villamos, to Móricz Zsigmond körtér

getting on: Budaörsi út / Villányi út

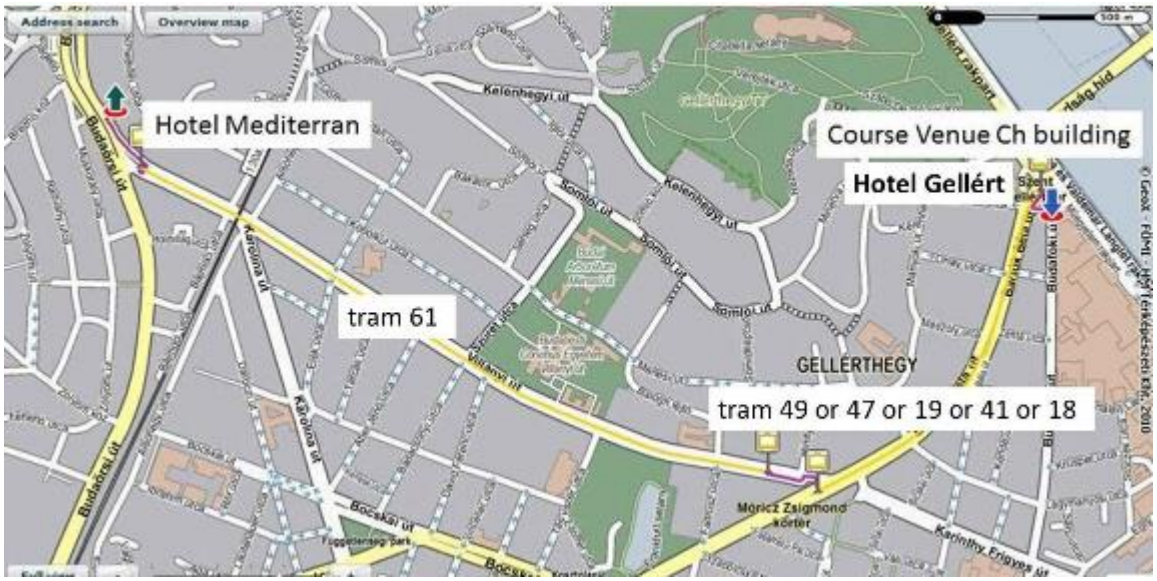
getting off: Móricz Zsigmond körtér

traveling by tram, 49 or 47 or 19 or 41 or 18 villamos, to Szent Gellért tér

getting on: Móricz Zsigmond körtér

getting off: Szent Gellért tér

Using public transportation (you need one change) approx time to get from the hotel to the course is 35 mins



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PROGRAMME

Day 1, August 30 Friday

Arrival-registration

Speakers' Dinner

Day 2, August 31, Saturday

9:15-9:30 **Welcome and opening remarks** (Beáta Vértessy, Budapest Univ. Technology and Hung. Acad. Sci, Budapest, Hungary)

09:30-10.30 **Lecture 1: Structural biology with synchrotron radiation and free electron lasers** (Matthias Wilmanns, EMBL Hamburg Outstation, Hamburg, Germany)

10:30-10:50 **Coffee break**

10:50-12:30 **Students presentations** 10 mins (5+5)

10:50-11:00: Gergely Bánóczi: Investigation and engineering of MIO prosthetic group containing aromatic amino acid ammonia-lyases and 2,3-aminomutases

11:00-11:10: Péter Burkovics: D-loop formation by the HLTF

11:10-11:20: Alicia Contet : *Plasmodium falciparum* cytidylytransferases: key enzymes for membranes biosynthesis of the malaria parasite

11:20-11:30: Péter Ecsédi : Structure determination of S100A4 complexes using X-ray crystallography

11:30-11:40: Éva Gráczer : Atomic level description of the domain closure in a dimeric enzyme: thermus thermophilus 3-isopropylmalate dehydrogenase (IPMDH)

11:40-11:50: Iuliia Iermak : Crystallization of the members of multistep signaling system from *Arabidopsis thaliana*

11:50-12:00: Kajal Kanchan : The structural basis for the regulation of human transglutaminase 2 by calcium ions

12:00-12:10: Aiste Kasiliauskaite : Design of sulfonamides as inhibitors of human carbonic anhydrases

12:10-12:20: Rute Matos: Unravelling the mechanism of action of the RNB family of exoribonucleases

12:20-12:30: Goran Mikleušević: Bacterial purine nucleoside phosphorylase family of proteins

12:30-14:00 **Lunch**

14:00-16:00 **Students presentations** 10 mins (5+5)

14:00-14:10: Botond Nagy: The interaction of various benzofuran-2-yl and phenylthiophen-2-yl-alanines with PAL isolated from different sourses

14:10-14:20: Ana Zuleima Obando: Towards the molecular mechanism of the *E.coli* exchanger AdiC

14:20-14:30: Katja Ota : Bicomponent MACPF pore-forming proteins: a new subgroup in the MACPF superfamily?

14:30-14:40: Jana Pisackova : How Pre-Crystallization Analysis Can Save Your Project

14:40-14:50: Vid Puz : Structural characterization of the Z-disc protein complexes centred on myotilin

14:50-15:00: Zsófia Simon-Vécsei : An attempt to investigate the celiac epitope(s) of transglutaminase 2 (TG2) by crystallization of the TG2 - celiac minibody complex

15:00-15:10: Tamás Szimler : Site-directed mutagenesis of 3-isopropylmalate dehydrogenase (IPMDH)

15:10-15:20: Diána Henrietta Szűcs: Homology modeling of multidrug resistant membrane protein in the *Brucella melitansis*

15:20-15:30: Róbert Tóth: The role of the RAD5 Hiran domain in DNA repair

15:30-15:40: Andrea Varga: PAL-PAM tandem for the biocatalytic synthesis of both (S)- and (R)-β- arylalanines

15:40-15:50: Sandra Viegas: Characterization of Dis3L2 exoribonuclease in *S. pombe*

15:50-16:00: Janusz Wiśniewski: Solving of mammalian muscle phosphoglycerate mutase crystal structure

16:00-16:30 **Coffee break**

16:30 **Tour of the Biostruct Laboratory**

Walking tours , ruin pub (romkocsma)

Day 3 September 1, Sunday

9:00-10:30 Lectures in crystallization 1: **From protein solution to protein crystals** (Bernhard Rupp, k. k. Hofkristallamt, USA; visiting Prof. at Medical University Innsbruck, Austria)

10:30-11:00 Coffee break

11:00-12:30 Lectures in crystallization 2 : **Growing, optimizing and handling of protein crystals** (Bernhard Rupp, k. k. Hofkristallamt, USA; visiting Prof. at Medical University Innsbruck, Austria)

12:30-14:00 **Lunch**

14:00-15:30 **Practical 1/2/3:**

Setup of drops

Crystal observation

Seeding and crystal handling

(Ibolya Leveles, Veronika Harmat, Beáta Vértessy, Kinga Nyíri, Klaudia Kovács, László Radnai, Gergely Nagy, Krisztian Fodor, Ábris Bendes, Imre Zagyva)

15:30-16:00 **Coffee break**

16:00-17:30 **Practical 1/2/3 :**

Setup of drops

Crystal observation

Seeding and crystal handling

(Ibolya Leveles, Veronika Harmat, Beáta Vértessy, Kinga Nyíri, Klaudia Kovács, László Radnai, Gergely Nagy, Krisztian Fodor, Ábris Bendes, Imre Zagyva)

17:30-18:00 **Q&A** (box)

Free evening

Day 4 September 2, Monday

9:30-11:00 **Visit to the Protein Crystallography Laboratory of Eotvos Lorand University** (Budapest, Pázmány Péter sétány 1/A)

http://prot.chem.elte.hu/en/laboratories/krisztallografiai-laboratorium

11:00-11:30 **Coffee break**

11:30-13:00 **Practical 1/2/3 :**

Setup of drops

Crystal observation

Seeding and crystal handling

(Ibolya Leveles, Veronika Harmat, Beáta Vértessy, Kinga Nyíri, Klaudia Kovács, László Radnai, Gergely Nagy, Krisztian Fodor, Ábris Bendes, Imre Zagyva)

13:00-14:30 **Lunch**

14:30-16:00 **Basic lectures in crystallography** (Veronika Harmat, Eotvos Lorant University (ELTE) , Budapest, Hungary)

16:00-16:30 **Coffee break**

16:30-18:00 **Data collection** (Manfred Weiss, Helmholtz-Zentrum Berlin für Materialien und Energie (HZB), Berlin, Germany)

Free evening

Day 5, September 3, Tuesday

9:00-10:00 **Data processing** (Veronika Harmat, Eotvos Lorand University (ELTE) , Budapest, Hungary)

10:00-11:30 **Solving the phase problem (phase determination)** (Manfred Weiss, Helmholtz-Zentrum Berlin für Materialien und Energie (HZB), Berlin, Germany)

11:30-12:00 **Coffee break**

12:00-13:30 **Model building, refinement and validation** (Veronika Harmat and Krisztián Fodor, Eotvos Lorand University (ELTE) , Budapest, Hungary)

13:30-14:30 **Lunch**

14:30 -16:00 **Practical 4/5:**

Crystal mounting and data collection

Data processing and model building

(Zoltán Gál, Ibolya Leveles, Veronika Harmat, Kinga Nyíri, Gergely Nagy)

16:00-16:30 **Coffee break**

16:30-18:00 **Practical 4/5:**

Crystal mounting and data collection

Data processing and model building

(Zoltán Gál, Ibolya Leveles, Veronika Harmat, Kinga Nyíri, Gergely Nagy)

18:00-18:30 **How to publish crystallographic results?** (Manfred Weiss, Helmholtz-Zentrum Berlin für Materialien und Energie (HZB), Berlin, Germany)

Followed by discussions on individual cases, how to apply for a synchrotron trip, Q and A (Manfred Weiss, Veronika Harmat, Zoltán Gál, Krisztián Fodor, all lecturers and tutors)

19:00 **Closing remarks** (Beáta Vértessy, Budapest Univ. Technology and Hung. Acad. Sci, Budapest, Hungary)

19:30 **Dinner**

Lecture abstracts

Structural Biology with Synchrotron Radiation and Lasers

Matthias Wilmanns

European Molecular Biiology Laboratory, Hamburg, Germany

During recent decades structural biology has been revolutionized in terms of scope of applications, complexity and size of structures from biological macromolecules, and opportunities to visualize dynamic processes. Synchrotron radiation has become a very powerful source to measure X-ray diffraction data with unprecedented accuracy from even very small crystals. New emerging Free Electron X-ray Laser facilities provide a completely new dimension in terms of radiation source parameters, potentially allowing to solve structures of biological macromolecules from very small assemblies in the near future. During the lecture, these developments will be introduced, reviewed and complemented with a few highlights.

Lectures in crystallization 1: From protein solution to protein crystals

Bernhard Rupp

k.k. Hofkristallamt, Vista, CA 92084, USA and Medical University Innbruck, A 6020, Austria

Pert one of the lectures focusses on the fundamental properties of proteins and their crystals. Part two covers aspects of the actual how-to of crystal screening and harvesting, including post-mortem analysis in case things do not turn out well.
Protein crystallization is the self-assembly of protein molecules into an ordered, periodic structure, the protein crystal. Protein molecules however are large, complex, and flexible molecules and most proteins are therefore difficult to crystallize. To understand how to find conditions that allow crystal formation, we need to understand the physico-chemical nature of proteins and how to modify their solubility and local surface property distribution. Once we understand what conditions must be fulfilled for crystallization to occur, the question is how to (a) obtain a protein that actually can crystallize, and (b) how to efficiently sample the magnitude of possible reagent combinations that might provide the right conditions. The initial screening or sampling then informs us how to proceed further and how to optimize crystal growth, and often also indicates that further examination and modification of the protein itself may be necessary to achieve successful crystallization.

Lectures in crystallization 2: Growing, optimizing and handling of protein crystals

Bernhard Rupp

k.k. Hofkristallamt, Vista, CA 92084, USA and Medical University Innbruck, A 6020, Austria

The second lecture covers aspects of the actual how-to of crystal screening and harvesting, including post-mortem analysis in case things do not turn out well. Practical aspects of protein crystallization include the use of robotics and of prior information aiming to extract the most information from the least amount of precious material, or in other words, to maximize the efficency of the process. We discuss various screening setup techniques, some sampling theory and data mining results, as well as analysis and optimization of crystals. The crystals also need to be harvested and often cryo-protected before they can be exposed to X-rays. Finally, we introduce a few methods to rationalize reasons why no or no well diffracting crystals could be grown, with emphasis is on assessment of conformational purity of the proteins.

Basic lectures in crystallography

Veronika Harmat

Eotvos Lorand University (ELTE) , Budapest, Hungary

Protein crystallography is the leading experimental method of determining 3D structures of proteins, nucleic acids and their complexes. In the last few decades experimental and computational techniques of protein crystallography underwent great improvement, resulting new techniques, user-friendly software and partial or full automation of all steps of the structure determination pipeline (crystallization, data collection, data reduction, solving the phase problem, model building, refinement and validation). The set of macromolecules available for protein crystallography is growing. The aim of the lecture is to introduce basic concepts and definitions of single crystal diffraction: crystal symmetry and crystal lattice; scattering vector, reciprocal lattice; structure factors, phases, and electron density; phase problem; symmetry of the diffraction pattern.

Data Collection Strategies – or how to avoid collecting suboptimal X-ray diffraction data

Manfred Weiss

Helmholtz-Zentrum Berlin für Materialien und Energie (HZB), Berlin, Germany

Once crystals of a biological macromolecule have been obtained, the collection of X-ray diffraction data is the next step towards the determination of the three-dimensional structure of the macromolecule. It is important to realize that the data collection step is the last experimental step in the course of a structure determination. Every mistake one makes here will make the following computational steps more difficult and will eventually results in a structure which is of lower information content than it could be. In the presentation, all aspects of diffraction data collection will be reviewed and potential sources of error identified. At the end, a list of points will be presented, which every experimenter should go through before actually starting the data collection experiment.

Data processing

Veronika Harmat

Eotvos Lorand University (ELTE) , Budapest, Hungary

Data processing, the conversion of detected diffraction patterns to a list of reflection intensities and estimates of their errors is in most cases an automated process using today's data processing software. The basic steps of data processing will be explained: finding and refining the unit cell dimensions and orientation (indexing and cell refinement), integration of diffraction spot intensities, determining the crystal symmetry, scaling and merging the dataset. Data quality indicators show information content of the data (resolution; significance of the anomalous signal), but they are also important tools to sort out if something went wrong during data collection or data processing and help to indentify difficult cases.

Phase Determination Using Synchrotron Radiation

Manfred Weiss

Helmholtz-Zentrum Berlin für Materialien und Energie (HZB), Berlin, Germany

Once diffraction data have been collected from a crystal of a biological macromolecule, phases for each of the X-ray reflections have to be determined in order to be able to calculate the electron density distribution within the unit cell of the crystal. Over the years, several methods have been outlined to achieve this. These methods are mostly based on the identification of a much smaller and much less complex substructure, which can then be used as a reference structure for phase calculations. In isomorphous replacement based methods, the substructure is the heavy atom structure introduced into the macromolecule crystal by co-crystallization or soaking. In the methods based on anomalous scattering (SAD, MAD), the substructure is either part of the protein (Se in case of Se-Met protein) or again introduced into the crystal and identified by utilizing the energy-dependent scattering properties. The only phasing method which does not need a substructure is molecular replacement. However, here a homologous structure, which is similar to the target structure, needs to be available. In this case, the search structure can be oriented and placed in the unit cell so that it can serve as starting point for phase calculations. In the talk, an overview of the various phase determination methods will be given.

Model building, refinement and validation

Veronika Harmat and Krisztián Fodor

Eotvos Lorand University (ELTE) , Budapest, Hungary

A macromolecular structure determined with X-ray crystallography is an interpretation of the experimental data. During the process of structure determination, the first model is a poor representation of the data, and therefore this model needs to be further improved. This is performed by and iterative procedure, by which the model is fitted to the experimental data through consecutive cycles of model building and refinement.

Data quality and resolution are the main factors that determine how much and how detailed information can be gained from the final structure. These, and the availability of experimental phases determine model building and structure refinement strategy to be applied (e.g. possible automation, involvement of information on molecule geometry, refinement algorithms). A high resolution and good quality structure allows the investigation of not only the conformation of the macromolecule, but also its hydrogen bonding system including the water molecules, orientation and conformation of bound ligands, and even small geometric distortions.

As such, reliable high-resolution 3D protein structures are of great importance for both fundamental research activities and the biopharma industry. Increasing popularity of automated data processing methods also require robust structure validation tools.

A good model is consistent with the available experimental data and the known physico-chemical properties of the macromolecule. Structures of macromolecules are widely used by professionals with various backgrounds, such as clinicians, teachers, bioinformaticians or structure biologists. Their interest may vary but they all need good quality structural models. Ensuring that the final model of any structure biology experiment is the best possible solution is therefore of great importance.

Nowadays there are several possible validation tools available, either as part of a standalone-software (like COOT), or as a web-service (e. g. MolProbity). The lecture will give a brief introduction about the basics of model building, refinement and validation.

Students’ abstracts

Investigation and engineering of MIO prosthetic group containing aromatic amino acid ammonia-lyases and 2,3-aminomutases

Gergely Bánóczi [1], Zsófia Bata[1], Klaudia Kovács[1,2], Gábor Hornyánszky[1], Beáta G. Vértessy[2] , Csaba Paizs[3], László Poppe[1]

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Our research group has been working on the preparation and application of aromatic amino acid ammonia-lyases and 2,3-aminomutases for a long time introducing both practical and theoretical methods. Tyrosine, phenylalanine and histidine ammonia-lyases (TAL, PAL and HAL, respectively) catalyze the nonoxidative ammonia elimination from their corresponding substrates L-tyrosine, L-phenylalanine and L-histidine. Tyrosine, phenylalanine 2,3-aminomutases (TAM, PAM, respectively) catalyze the direct conversion of proteinogenic L-α-amino acids to L- or D-β-amino acids. The X-ray structure of HAL revealed first that the enzyme contains 3,5-dihydro-5-methylidene-4H-imidazol-4-one, MIO, as the electrophilic prosthetic group (**figure 1.**).

Our aim is to develop biotransformational processes from the start (protein engineering, protein expression) until the end (immobilization, flow reactor tests). Regarding protein engineering we use molecular modeling to get insights of the structural properties of the enzyme and enzyme-substrate interactions. Homology models were created for various enzymes lacking the proper conformation or the entire 3D structure. Docking/systematic conformational analysis was performed on prepared, adequate active sites to predict substrate activity. More sophisticated QM/MM calculations were performed to discover the reaction mechanisms of different PAMs and PAL. Molecular dynamics and even QM/MM dynamics studies are in preparation to investigate the structural properties of several enzymes. Based on the gained knowledge we want to create a model with strong predicting ability to rationalize enzyme design. For all these methods active protein structures are needed that can be achieved with X-RAY protein crystallography.

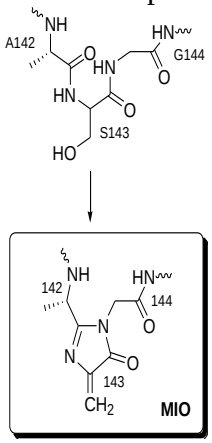

Figure 1. Formation of MIO

D-loop formation by the HLTF

Péter Burkovics

Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Stalling of replication forks at unrepaired DNA lesions can result in discontinuities opposite the damage in the newly synthesized DNA strand. Translesion synthesis or facilitating the copy from the newly synthesized strand of the sister duplex by template switching can overcome such discontinuities. During template switch a new primer–template junction has to be formed and two mechanisms, including replication fork reversal and D-loop formation, have been suggested. Genetic evidence indicates a major role for yeast Rad5 in template switch and that both Rad5 and its human orthologue, HLTF, a potential tumour suppressor, can facilitate replication fork reversal. This study demonstrates the ability of HLTF to form a D-loop without requiring ATP binding and/or hydrolysis. We also show that this ability is independent of RAD51 in vitro, and is not interchangeable on another member of the SWI/SNF family, RAD54. In addition, the 3′ end of the invading strand in the D-loop can serve as a primer and is extended by DNA polymerase. Our data indicate that HLTF is involved in a RAD51-independent D-loop branch of template-switch pathway that can promote repair of gaps formed during replication of damaged DNA.

Plasmodium falciparum cytidylytransferases: key enzymes for membranes biosynthesis of the malaria parasite

Alicia Contet[1], Emilie Pihan[2], Marina Lavigne[1], Gergely Nagy[3], Beata Vertessy[3], Henri Vial[1], Dominique Douguet[2] and Rachel Cerdan[1]

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[2]Institut de Pharmacologie Moléculaire et Cellulaire, 660 Route de Lucioles, 06560 Sophia-Antipolis, France.
[3]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, 29 Karolina street 1113 Budapest, Hungary.

Malaria caused an estimated 660 000 deaths in 2010, mostly children under the age of five years old according to the World Health Organization. In the absence of an effective vaccine and because of the emergence of resistance towards current chemotherapies, it is crucial to develop new therapeutic strategies. The intra-erythrocytic development of the most deadly malaria parasite *Plasmodium falciparum* requires massive biosynthesis of membranes which contain phospholipids and lack cholesterol unlike the host membranes. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) together represent approximately 80% of the total membrane lipids and inhibition of their biosynthesis leads to parasite death. In the absence of metabolic activity within the erythrocyte, PC and PE are synthesized by the parasite's machinery, mainly through the *de novo* CDP-choline and CDP-ethanolamine (Kennedy) pathways using choline and ethanolamine as precursors. CTP:phosphocholine and CTP:phosphoethanolamine cytidylytransferases (CCT and ECT respectively) catalyze the rate limiting step of these two pathways and are essential for parasite survival, what make them putative drug targets for the development of new antimalarial drugs. In *Plasmodium* species, CCT contain two catalytic cores each with a cytidylyltransferase (CT) domain associated to a regulatory region. Interestingly, this feature is found only in three different organisms, all from the Apicomplexa phylum: *Babesia*, *Theileria* and *Plasmodium.* We established the kinetic properties of both the recombinant *Pf*CCT and the endogenous enzyme within the parasite. Analysis of the individual CT domains showed that both are active and behave similarly in contrast to what occurs for *Pf*ECT (Maheswhari et al. 2013, Biochem J.). Potential inhibitors were identified by virtual screenings of drug libraries using docking tools against the 3D model of *Pf*CCT obtained by homology modeling using the X-ray structure of the catalytic rat CCT domain as template. Two compounds showed inhibition of the recombinant *Pf*CCT with an IC50 of less than 1 mM. For one compound, the mechanism of inhibition was confirmed by inhibition of the endogenous enzyme, competitive inhibition with the substrate phosphocholine, ITC experiments and inhibition of the parasite growth.

Structure determination of S100A4 complexes using X-ray crystallography

Ecsédi Péter

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S100A4 is a member of the S100 family of small (~10 kDa), dimeric, EF-hand Ca^{2+}-binding proteins. S100 proteins are present exclusively in vertebrates. Elevated expression of S100A4/metastasin is often observed in aggressive and metastatic tumors causing higher death rate. Moreover, S100A4 can activate metalloproteinases in the synovial fluid causing the degradation of the cartilage and other tissues inducing rheumatoid arthritis. Although S100A4 is a promising molecular target for cancer and RA therapy, the structural details of its interaction with its protein partners are poorly understood.

Numerous complex structures were earlier determined in the S100 family, but they do not show a uniform binding mode, as the orientation of bound peptides are considerably different. Nevertheless, all the structural models show that one S100 dimer binds symmetrically to two peptides (PDB: 2JTT, 1BT6, 1DT7). Last year our research group published the 3D structure of the non-muscle myosin IIA (NMIIA) − S100A4 complex revealing a novel asymmetric mode of peptide binding, where one myosin chain binds to a dimeric S100A4 (PDB: 3ZWH). The results of binding studies with other known S100A4 partners strongly suggest that this type of interaction may not be confined to the S100A4 - NMIIA complex. To prove this hypothesis we plan to crystallize S100A4 in complex with p53, MDM2 and annexin A2 proteins (and perhaps other partners). In addition we want to test complexes of NMIIA with other S100 family members that are known or predicted to bind to two different isoforms of this important motor protein.

So far our crystallization attempts using the automatic liquid handler Mosquito (TTPLabTech) in standard sparse-matrix screens did not provided suitable crystals for diffraction data collection. Crystal growth is monitored by Rock Imager (Formulatrix).

Atomic level description of the domain closure in a dimeric enzyme: thermus thermophilus 3-isopropylmalate dehydrogenase (IPMDH)

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A so-called „hinge-bending" motion, i.e. opening and closing of two respective domains, has been observed in a number of proteins, both monomeric and oligomeric. Isopropylmalate dehydrogenase molecule consists of two identical subunits and each of the subunits can be divided into two structural domains. Comparison of various known crystal structures of IPMDHs indicates that the relative position of the two domains may vary between open and closed. This suggests that domain closure plays an essential role during the catalytic cycle, similar to the mechanisms proposed also for other multidomain enzymes.
Different substrate-IPMDH complexes were determined by X-ray crystallography. Molecular graphical analysis was used to describe a possible molecular mechanism of substrate-induced conformational transitions. The role of the side-chains in the domain motions and the structural background of the catalysis by IPMDH were investigated by site-directed mutagenesis and different biochemical methods, for example SAXS and FRET experiments.
Mapping of all contact of the conserved side-chains and their changes upon substrate binding have led us to identify two notable hinges: hinge 1 in the turn connecting α-helix d and β-strand F as well as hinge 2 in the loop between the α-helix h and the following β-strand E. The analysis has also revealed that the substrate MnIPM complex has an essential role in operation of these hinges. NADH also influences the domain closure, but in a much smaller extent. In addition, the subunit-subunit contacts formed in the cavity between the two domains were also found to contribute to the domain motions. The site-directed mutants of IPMDH suggest the role of the conserved side-chains in the active centre of IPMDH not only in the substrate binding and catalysis, but also in the domain closure.

Crystallization of the members of multistep signaling system from *Arabidopsis thaliana*

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In higher plants multistep signalling system (MSS) play a major role in cytokinin reception and contribute to ethylene signal transduction. Some components of MSS take part in osmosensing, megagametogenesis and flowering promotion in plants. Therefore it is important to investigate mechanisms of functioning of MSS for deeper knowledge about higher plants vital activities. Multistep signalling systems are composed of hybrid histidine kinases (HKs) that perceive a signal, histidine-containing phosphotransfer proteins (HPs) that mediate the phosphate signal downstream to their corresponding response regulators (RRs), which usually play a role of transcription factors. One of HKs, CKI1, was suggested to participate in female gametophyte development in *Arabidopsis thaliana*. CKI1 has specificity in interactions with HPs, in particular it has affinity in binding to AHP2 and AHP3 [1].

The main aim of this project is structural characterization of the interactions among *Arabidopsis* MSS members what will allow us to get inside into details of its functioning.

For this purpose crystallization of protein complexes with further X-ray diffraction analysis are used.

In the research major attention is embedded to the complexes of receiver domain of histidine kinase ($CKI1_{RD}$) with *Arabidopsis* histidine-containing phosphotransfer proteins AHP2 and AHP3.

Screening of crystallization conditions was performed by means of different crystallization screens. Some hits were found for complexes of $CKI1_{RD}$ and AHP2 (in different concentrations) in Morpheus screen. All promising conditions contain MPD, PEG 1000 and PEG 3350, but different buffers and ligands. Obtained crystals are 3D, quite large, but too fragile. X-ray diffraction analysis for testing of crystals will follow.

Through X-ray diffraction analysis structures of complexes CKI1 with AHPs are expected to be solved.

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The structural basis for the regulation of human transglutaminase 2 by calcium ions

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Transglutaminase 2 (TG2) is the member of a large family of enzymes that catalyze protein crosslinking in presence of calcium. Besides its crosslinking activity, TG2 also has several other biochemical activities such as isopeptidase, deamidase and amine incorporation into the proteins and calcium is essential for catalyzing all of these activities. It is clear from the biochemical experiments that TG2 binds six calcium ions which lead to large conformational change in the protein structure. Crystal structure of TG2 has been solved in presence of GTP/GDP and an active site inhibitor. Calcium and GDP/GTP inversely regulates transamidation activity of TG2. When TG2 is bound to GTP it is in a closed conformation which corresponds to inactive form of the enzyme while when it is bound to calcium, it gets activated and adopts an open conformation. Crystal structure of TG2 bound to inhibitor also adopts an open conformation and it is speculated that it is the active conformation of TG2. Until now, no TG2 structure is available which is bound to calcium hence it is difficult to predict whether inhibitor bound structure is the real active form of the enzyme. Therefore, the main aim of our project is to crystallize TG2 in presence of calcium.

TG2 besides crosslinking other proteins, also gets crosslinked it self in presence of calcium hence it has always been difficult to purify TG2 and crystallize it. We use wild type TG2 as well as active site mutant (C277S) for crystallization trials. The active site mutant lacks transamidation activity. Both the constructs were purified using cobalt affinity column and then gel filtrated to remove the aggregated and crosslinked proteins. Before loading the protein on the gel filtration column, it was incubated with 0.5 mM $CaCl_2$ for an hour. Gel filtration buffer also contained 0.5 mM $CaCl_2$. TG2 was concentrated to 20 mg/ml and crystallization screens were performed in 96-well-plates using PACT premier, JCSG+ and Structure1&2 screens available from Molecular Dimensions. There was good precipitation and phase separation but until now no crystal were seen. We are in process of optimizing the conditions further as well as cloning TG2 with a shorter N' terminal tag.

Design of sulfonamides as inhibitors of human carbonic anhydrases

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Carbonic anhydrases (CA) are a class of zinc metalloenzymes present in both prokaryotes and eukaryotes. They contain a metal ion core, which is coordinated by three histidine imidazole groups and a single hydroxide ion, and form a distorted tetrahedral shape. There are 15 different isozymes of human carbonic anhydrases, with different tissue distributions, subcellular locations, and expression levels. Many CA isozymes constitute valid targets for the design and development of selective CA inhibitors for clinical applications [1].
A series of sulfonamide inhibitors were designed and synthesized in the Department of Biothermodynamics and Drug Design. X-ray crystallographic data reveals a network of interactions between carbonic anhydrases and ligands. This information was correlated with the thermodynamic parameters obtained from the Isothermal Titration Calorimetry (ITC) and the Thermal Shift Assays (TSA) [2, 3]. X-ray crystallographic cocrystal structures and computational docking studies provided structural details of inhibitor binding to CA. Our aim is to learn how to predict binding constants from the compound chemical structure, and to use these predictions to design novel compounds with desired properties.
In my work, I cloned, determined the expression conditions, grew bacterial culture, and purified carbonic anhydrase isozyme VB using metal chelation and affinity chromatographies. The protein was characterized by determining its activity and inhibitor binding by ITC and TSA. Unfortunately, this isozyme did not crystallize. One of the goals is to cocrystallize CA VB with lead inhibitors and determine the structural features of inhibitor selectivity towards this isoform.

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Unravelling the mechanism of action of the RNB family of exoribonucleases

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Enzymes from the RNase II-family are ubiquitous and play a crucial role in RNA metabolism. They are involved in virulence, growth and viability, mitotic control and chloroplast biogenesis. *E. coli* RNase II is the prototype of this family. An important breakthrough in the understanding of the mechanism of action of this protein was the resolution of its structure. The 3D structure showed that this protein is formed by four domains, two N-terminal CSD and a C-terminal S1 domains involved in RNA binding, and a central RNB domain, which is responsible for catalysis (Frazão et al, 2006, Nature). Moreover, the co-crystallization of this protein with a RNA molecule allowed seeing which residues are crucial for catalysis; the role of these amino acids was then biochemically confirmed (Barbas et al, 2008; 2009, JBC). RNase R, the other member of this family in *E. coli*, shares 60% of sequence homology with RNase II, however, these proteins behave differently. While RNase II activity is blocked by the presence of secondary structures, RNase R is capable of degrading highly structured RNA. Another difference is that the final degradation product of RNase II is a 4 nt fragment, whereas the end product of RNase R is a 2 nt fragment. We performed a biochemical analysis and constructed several mutants in order to understand the mechanism of action of RNase R. We discovered that the RNB domain from RNase R is sufficient for the degradation of structured substrates (Matos et al, 2009, Biochem J). At the C-terminal, this protein has a lysine-rich tail, which probably helps to unwind the substrate before it enters into the catalytic cavity (Matos et al, 2011, Proteins). A definitive model for RNA degradation by RNase R is still open, and only the resolution of its structure will answer the many questions about its remarkable mode of action.

Bacterial purine nucleoside phosphorylase family of proteins

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Purine nucleoside phosphorylases (PNPs) are the key enzymes of purine salvage. They catalyse the reversible phosphorolytic cleavage of the glycosidic bond of purine nucleosides and some analogues: beta-purine nucleoside + orthophosphate ↔ purine base + alpha-D-pentose-1-phosphate (1). Our scientific research is focused on studying complex catalytic mechanism of two PNPs from bacterial origin: *Escherichia coli* and very recently, from human pathogen *Helicobacter pylori* (HpPNP) whose purine methabolism is solely dependant on purine salvage (2). In contrast to human homologue, *E. coli* PNP has a broad substrate specificity which makes it a good candidate in gene therapy against solid tumours (3). Based on protein sequence analysis, HpPNP and *E. coli* PNP share 70 % similarity indicating their common catalytic mechanism. Hence, specific inhibitors of *E. coli* PNP may also inhibit HpPNP and in turn help in *H. pylori* eradication without affecting human host. Therefore, understanding the catalytic mechanism of these enzymes is of utmost importance. So far, we have conducted detailed kinetic studies and determined numerous 3D-structures of *E. coli* PNP binary and ternary complexes which enabled us to predict the main steps in its catalytic mechanism (4).

Up to date we were able to grow crystals for X-ray studies only from conditions containing sulphate or phosphate ions. Since sulphate is an inhibitor and phosphate is a substrate of this enzyme, and, on the other hand, it is very difficult to distinguish these two ions in the electron density maps, new crystallisation conditions with no phosphate and/or sulphate are needed for further studies. For example, the crystals of ternary complex of *E. coli* PNP with phosphate and formycin A (structural analogue of adenosine) grown from highly concentrated ammonium phosphate (1.2 M) showed unusual phosphate binding mode (5) which could be an artefact and may not have biological relevance. For HpPNP only preliminary crystallisation experiments has been acomplished and its crystal structure is yet to be determined.

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The interaction of various benzofuran-2-yl and phenylthiophen-2-yl-alanines with PAL isolated from different sourses

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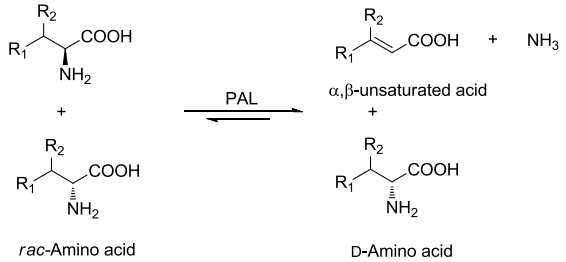
Phenylalanine ammonia-lyase (PAL) is an important plant enzyme, as its product, (E)-cinnamic acid is the precursor of lignines, coumarins, and flavonoids. Its mechanism of action has been the subject of great interest[1]. PAL contains the electrophilic prosthetic group 5-methylene-3,5-dihydroimidazol-4-one (MIO), which is able to attack the phenyl group of the substrate. Thereby, the β-protons of the amino acid are activated allowing their extraction by an enzymatic base, a step which is followed by elimination of ammonia.

Another interesting property of PAL is its ability to react with a large number of arylalanines, which makes it a useful enantiospecific biocatalyst[2]. In this work we investigate the interaction of phenylalanine ammonia-lyase (PAL) with various types of heteroaryl – alanines. Several substituted benzofuranyl-2-yl and phenylthiophenyl-2-yl-alanines and their acrylic acid counterparts were synthesized starting from commercially available aldehydes or anilines. The kinetic measurements are based on the UV-spectrophotometrical determination of the concentration of acrylates formed. First, their extinction coefficients (ε) were determined in Tris-buffer (0.1 M, pH 8.8) at wavelengths (λ) for which the corresponding amino acids do not show absorbtion. The enzymatic assays were carried out in 1mL cuvettes in the above mentioned buffer at 30°C in the case of benzofuranyl-derivatives, and at various temperatures for phenylthiophenyl-alanines, respectively, in presence of 25 μg PAL with the substrate concentration varying between 0.5-2.5 mM.

Figure 1. Reactions catalyzed by PAL



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Towards the molecular mechanism of the *E.coli* exchanger AdiC

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AdiC is an exchanger used by enteric bacteria, like *E. coli,* to survive to extreme acidic conditions in the host stomach to further establish several types of gastrointestinal infections [1]. AdiC forms a homodimer in the plasma membrane exchanging extracellular Arg^{1+} by intracellular Agm^{2+}, in this process one proton is consumed increasing the internal pH of bacteria to 4.7 [2, 3]. Additionally, AdiC shares about 18% of identity with the light subunits of the eukaryotic L-Amino acid Transporters (LATs). LATs form with a heavy subunit (rBAT or 4F2hc) the Heteromeric Amino acid Transporters (HATs), which are involved in human pathologies like inherited aminoacidurias, tumor growth and invasion, viral infection and cocaine addiction [4]. AdiC and LATs belongs to the amino acid/polyamine/organocation (APC) superfamily of transporters, and structure-function studies suggest that AdiC and LATs share the LeuT-fold, characteristic of several transporter families [5, 6]. In these transporters, the internal pseudo two-fold symmetry dictates the conformational changes during the transport cycle [7, 8]. AdiC structure has been solved facing outwards: without substrate [9, 10], with substrate bound (mutant N101A) [11], and with substrate occluded (mutant N22A) [12], bringing insights about the substrate-induced fitting mechanism of AdiC. However the complete mechanism of transport (e.g., translocation) remains unknown because crystal structures of the inward-facing states are missing. In this regard, it is fundamental to solve inward-facing structures of AdiC. Thus the objective of this project is to solve the crystal structure of AdiC in inward-facing conformation. Guide by the outward-facing structural data and the inward-facing models of AdiC, we have been using point mutation as a strategy to stabilize the transporter facing inward. I have been crystalizing AdiC mutants designed to try to weaken the interaction of the substrate in the outward binding site or to strengthen the interaction in the inward binding site.

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Bicomponent MACPF pore-forming proteins: a new subgroup in the MACPF superfamily?

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Recent advances in the resolution of MACPF (membrane attack complex/perforin) protein structures have caused changes in structural definition and classification of the MACPF domain-containing pore-forming toxins (PFTs). A study by (Gilbert et al., 2012) has disproved the original concept of a four-domain structure of cholesterol-dependent cytolysins (CDCs) as they have shown that domains 1 and 3 should be seen as a single functional MACPF domain. These results additionally supported the joint assignment of CDC and MACPF protein families to the MACPF/CDC superfamily.
Through our re-investigation of a fungal aegerolysin, ostreolysin A (OlyA) we have found it to be a crucial component of a bicomponent PFT, acting in concert with a MACPF protein, pleurotolysin B (PlyB). Sequence analysis, 3D structural modeling, proteins' membrane binding, oligomerization, and permeabilization characteristics and EM membrane pore visualization have enabled us to propose a new model of bicomponent MACPF protein pore formation.
In contrast to other metazoan cytolytic proteins with the MACPF domain, the PlyB C-terminal domain seems to be designed to interact specifically with membrane-bound OlyA. This interaction is prerequisite for PlyB attachment and structural rearrangement of the MACPF domain to create a transmembrane □-barrel pore. We have confirmed that the all-beta structured OlyA exhibits the traits of the C-terminus of other MACPF proteins and targets PlyB to the membrane.
We propose that, based on the bicomponent nature of fungal MACPF proteins the C-terminal domain, analogous to the domain 4 in other MACPF/CDC pore-forming protein, should be supplemented with a separate aegereolysin superfamily protein. This renders the bicomponent fungal MACPF proteins to be a distinct protein family within a MACPF superfamily of PFTs. However, the structural information supporting our claims is still lacking as crystal structures of the monomeric and/or pore structure of both proteins have not yet been solved.

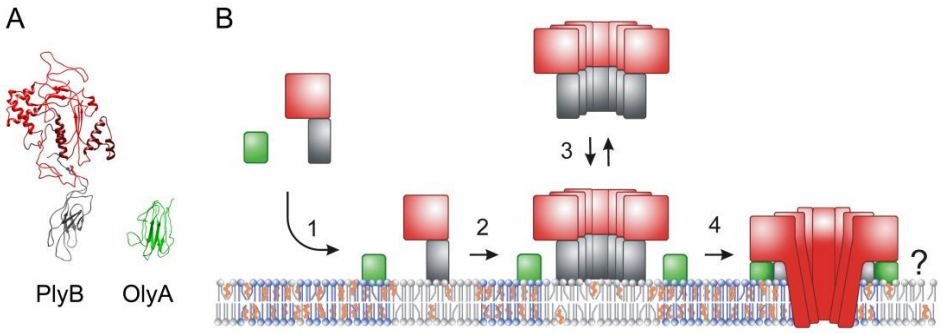

Figure: A. 3D models of PlyB and OlyA. **B.** Hypothetical model of pore formation by PlyB and OlyA: Step 1**.** Monomeric OlyA (green) and monomeric PlyB, depicted with the MACPF domain (red) and C-terminal region (grey) bind to the membrane. Step 2. PlyB oligomerizes to form the presumed pre-pore complex. Step 3. The oligomerised PlyB complex can dissociate from the surface of the bilayer or associate with OlyA in step 4, to form the transmembrane pore-complex. The location and number of OlyA monomers in the final complex is not certain.

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How Pre-Crystallization Analysis Can Save Your Project

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X-ray crystallography is the principal method of determination of bio-molecular structure with protein crystallization being a bottleneck in the process of structure determination. Protein crystallization is influenced by numerous factors. From these factors, protein sample properties are the most important variable. Pre-crystallization analysis of protein sample by the combination of biochemical and biophysical methods such as size-exclusion chromatography, dynamic light scattering, and thermofluor assay [1] can thus be used for the optimization of protein crystallizability. This approach is demonstrated on the crystallization of deoxyribonucleoside regulator protein (DeoR) from *Bacillus subtilis* [2]. DeoR negatively regulates the expression of enzymes required for deoxyribonucleosides and deoxyribose utilization [3], deoxyribose-5-phosphate is the preferred ligand of DeoR and induces expression of the catabolic enzymes [4].

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Structural characterization of the Z-disc protein complexes centred on myotilin

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Although all types of muscle cells use actin and myosin for contraction, only in skeletal and cardiac muscle these proteins are organized into sarcomeric units. Sarcomere is defined between two neighbouring Z-discs, and plays a central role as the site organizing thick filaments and titin into the molecular machinery that is required for muscle contraction. A striking feature of muscle and Z-disc is the diversity of multiple protein-protein interactions that form a complex network. These include its major component α–actinin–2 and other Z-disc proteins such as F-actin, filamin C, Z-repeats of titin, ZASP, FATZ, telethonin, myopalladin, and myotilin to mention a few.

The focus of our research is myotilin, 57 kDa homodimeric protein, consisting of two immunoglobulin-like domains flanked by a unique serine-rich N-terminus and a short C-terminal tail. It is involved in the dynamic scaffolding during myofibrillogenesis and important for the Z-disc integrity during contraction. Literature data report that it binds five other Z-disc proteins: α–actinin–2, filamin C, ZASP, FATZ, G- and F-actin.

The goal of this research is structural elucidation of the various myotilin constructs in complex with selected binding partners. After screening of conditions for complex formation *in vitro*, viable complexes will be upscaled for crystallization screening. The main structural technique employed will be X-ray crystallography, supplemented with other complementary structural biology techiques, in particular small angle X-ray scattering and circular dichroism.

An attempt to investigate the celiac epitope(s) of transglutaminase 2 (TG2) by crystallization of the TG2 - celiac minibody complex

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Transglutaminase 2 (TG2) is a Ca2+-dependent, ubiquitous enzyme with various enzymatic functions such as protein cross linking, deamidation, amine incorporation and isopeptidase activity. The enzyme has four structural domains: N-terminal β-sandwich, catalytic core and two C-terminal β-barrels. Two crystal structures of TG2 exist: the GDP-bound "closed" form and an active-site inhibitor-bound "open" form, when the two β-barrels are displaced as much as 120 Å. The crystal structure of the Ca2+-bound form of the enzyme has not been solved yet. The enzyme was identified as the major autoantigen of celiac disease (CD), which disease is a gluten-dependent autoimmune disorder featured with the presence of anti-TG2 autoantibodies. During our earlier work we identified the main celiac epitope of TG2 by site directed mutagenesis and now our aim is to verify this binding site by crystallization of the TG2-celiac antibody complex. To this end we selected and cloned TG2-specific miniantibodies from an antibody library of a celiac patient. We expressed one of the minibodies in 293T cells and purified it by protein A column, while His-tagged TG2 is derived from a bacterial expression system and it was purified by Ni-affinity chromatography. We examined the binding properties of the celiac minibody to TG2 by ELISA and by surface plasmon resonance (SPR) method (K_d (M) = $2{,}87*10^{-8}$). We also try to clarify the role of Ca2+ in the antibody-binding (in the presence of Ca2+ the binding affinity is higher) and solve the Ca2+-bound structure of the enzyme, which may can be stabilized by the minibody binding.

Site-directed mutagenesis of 3-isopropylmalate dehydrogenase (IPMDH)

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3-Isopropylmalate dehydrogenase (IPMDH) catalyzes the oxidative decarboxylation of 3-isopropylmalate (IPM). It is an essential enzyme of the leucine biosynthesis pathway, and is found exclusively in prokaryotes, plants and fungi. Since the enzyme is absent in humans, it may be an appropriate target of antibiotics. In order to develop an effective inhibitor of IPMDH, structural basis of its catalytic mechanism must be understood. The aim of my work was to identify the catalytic side-chains of *Thermus thermophilus (Tt*) IPMDH by the aid of site-directed mutagenesis and detailed physico-chemical and enzymatic characterization of the mutants. The major results are the following:

1.) The alanine mutants of the active site side-chains K185 and Y139 of *Tt* IPMDH were produced in homogeneous form by site-directed mutagenesis.
2.) Native gelelectrophoresis have shown that both mutants possess dimeric structure with a somewhat increased hydrodynamic radius compared to the wild type enzyme.
3.) The far UV CD spectra of the mutants have shown no detectable changes in their global secondary structure. The near UV CD and the fluorescence emission spectra indicate small extent of local perturbations of the tertiary structure in the surroundings of aromatic side-chains.
4.) DSC experiments showed no substantial change in the heat-stability of either mutants.
5.) Enzyme kinetic experiments have revealed that K185A and Y139A exhibit 0.06% and 3%, respectively, of the k_{cat} value of the wild-type enzyme, demonstrating the critical catalytic role of both side-chains. Both mutants show increased K_m values of IPM and Mn^{2+}, confirming the essential role of both K185 and Y139 side chains in substrate binding.
6.) While IPM-binding to the wild-type enzyme is characterised by a hyperbolic curve, the binding curves of both K185A and Y139A exhibit sigmoidicity, indicating operation of cooperative interactions between the mutant subunits.
7.) The pH-dependence of the wild-type enzyme activity (pK=7,4) indicates the existence of an ionizable chemical group that should be deprotonated for maximal activity. The different pH-dependence of the activity of K185A mutant (pK=8,6) as well as abolishment of pH-dependence of the activity of Y139A mutant are both in accordance with the assumed role of Lys185 in the acid-base catalysis, as well as with the activating role of Y139 in it.

Homology modeling of multidrug resistant membrane protein in the *Brucella melitansis*

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Introduction

The infections caused by Brucella species, besides the human morbidity, are involved in significant economic damages in agriculture. Brucella melitensis is one of the main source of zoonosis. The long latent period (10-30 days) and the flu-like symptoms of the disease make the identification and the subsequent treatment difficult. Nowadays antibiotic cocktails, such as streptomycin-tetracyclin and rifampicin-tetracyclin, are used in therapy. These cocktails are necessary for the treatment because of the increasing number of resistant strains, however according to previous studies not all of them were found to be effective. Also, the resistance is not properly proven so far.

The protein, encoded by the gene bmeI 1645, shows homology to AcrB of *Escherichia coli*. AcrB is a member of an MDR efflux system a tripartite complex integrated into the inner membrane and pumps a large spectrum of chemically different drugs out from the cell.

Our main aim was to prepare the homogy model of the BME1645 membrane protein based on the X-ray structures of AcrB from *Escherichia coli* to understand the mechanism of substrate binding, the first step in the MDR transport process.

Materials and methods

The X-ray structures of the AcrB protein with and without ligands found in the RCSB Protein Data Bank were used as templates to model *Brucella melitensis* AcrB homologue transporter by the MODELLER 9.11 program package. The structural overlap between the modelled target BMEI 1645 and the templates was used to qualify the model. The structure obtained by multi template homology modelling was considered the best approach for the drug-MDR interactions of this *Brucella* MDR transporter. VMD 1.9.1 was used for visualization.

In the same time we would like to expression and purification our target protein for the crystallisation, that so we can our computational modell with experimental results with help of the X-ray crystallography.

Results

The spatial arrangement of both the side-chains and the residues of backbone atoms accommodating the ligands changes when the model is built in the presence of ligands. The resulting BMEI 1645 structural model obtained by this manner is considered more realistic and suitable to perform further docking studies to produce AcrB homologue-ligand complexes explaining its MDR activity.

The role of the *RAD5* Hiran domain in DNA repair

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The sequence of DNA is an important information for the life of cells. Any damage of DNA leads to inaccurate function of the cell, or occasionally to its death. Therefore proteins of DNA repair have a critical role in preserving the initial state of DNA.

At DNA damage the replication polymerase stalls and the complex of Rad6 and Rad18 proteins ubiqutinates the Proliferating Cell Nucleare Antigene (PCNA) at its lysine 164 residue. PCNA is the processivity factor of replication polymerases. Subsequently, this PCNA residue is polyubiquitinated by the protein complex of Rad5, Mms2 and Ubc13.

The Rad5 has three domains: RING, Helicase/ATPase and Hiran domain. The RING domain has E3 ubiqutine ligase activity. The Helicase/ATPase domain has a replication fork reversal activity, and it forms a chicken-foot DNA structure. The function of Hiran domain is unknown, it is predicted to a DNA binding domain.

We examined the role of Hiran domain.

Mutations were generated in the conserved regions of the domain. Five from the twelve mutants showed sensitivity to DNA damaging agents. Two mutants from the five showed the same growth curve like wild type on mutagenic treatment if over-expression of proteins were induced. It means the low expression level of this two mutant proteins caused their sensitivity in our previous experiments. The other three mutant proteins were purified and tested *in vitro* in biochemical assays. The LI265,266RR had the same activity like wild type protein. GA177,178RR and G183R showed no activity neither in helicase nor in ubiquitine ligase assay.

The GA177,178 and G183 parts of the domain are likely to have a basic role in both of the two functions of Rad5. Nevertheless it is possible that these mutations modify the whole structure of the protein and it loses all of its activities. To answer this question more structural studies are needed with both wild type and mutant proteins.

PAL-PAM tandem for the biocatalytic synthesis of both (*S*)- and (*R*)-β-arylalanines

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The synthesis of optically pure α- and β-amino acids constitutes a challenge for the preparative chemistry. These molecules occur as components of numerous bioactive compounds. Essentially, the reactions mediated by ammonia lyases can be used for the stereoselective synthesis of amino acids via two alternative pathways. One way implies the use of the stereoconstructive, reversible reaction of ammonia-lyases; thereby, the addition of ammonia to unsaturated, achiral precursors is used for obtaining L-amino acids. On the other hand, the stereodestructive nature of the ammonia-lyases mediated reactions can be exploited in order to obtain the D-enantiomer through the enantiomer selective destruction of the L-enantiomer from the corresponding racemic mixture.[1] Aminomutases carry out the chemically challenging exchange of a hydrogen atom and an amine substituent present on neighboring carbon atoms.

PAL from parsley accepts a wide range of L-arylalanines as substrates, while PAM from *Pantoea agglomerans* can transform a wide range of (*S*)-α-arylalanines into the corresponding (*S*)-β-amino acids.[2]

We analyzed sevaral factors that can influence the velocities of the transformation of both reaction counterparts into the other one since in presence of PAM the equilibrium concentration of (*S*)-α-arylalanines and (*S*)-β-amino acids are arround 50-50%. Moreover using both PAL and PAM a preparative scale procedure was set up for the synthesis of both (*R*)- and (*S*)-β-amino acids. (**Fig.1.**).

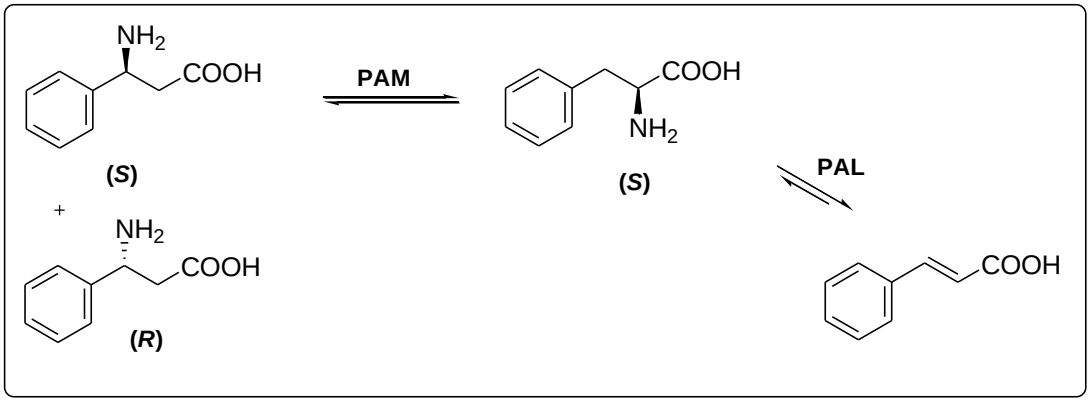

Fig.1. Biotransformations mediated by PAM and PAL

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Characterization of Dis3L2 exoribonuclease in *S. pombe*

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The final step of cytoplasmic mRNA degradation proceeds in either a 5'-3' direction catalyzed by Xrn1 exoribonuclease or in a 3'-5' direction by the protein complex exosome. The Dis3/Rrp44, an RNase II family protein, is the active subunit of the exosome. In *Saccharomyces cerevisiae* there is only one copy of DIS3 in the genome, but for example in humans there are three isoforms of this enzyme- DIS3, DIS3L and DIS3L2. Recently, different publications have linked some of these proteins with important human diseases, namely cancer. The product of the *S. pombe* Dis3L2 gene encodes an active 3-5' RNA exonuclease that is localized in the cytoplasm and in cytoplasmic foci that are docked to P-bodies. Our data proves that this protein does not interact with the exosome complex. Although single deletion of this gene does not have an obvious phenotype, it is synthetically lethal with Xrn1 deletion and the double deletion with Lsm1 protein has a considerable negative impact on cell growth. DIS3L2 has a preference towards uridylated substrates. In *S. pombe* and possibly in most other eukaryotes, DIS3L2 is an important factor in mRNA degradation. This novel 3'-5' RNA decay pathway represents an alternative to degradation by Xrn1 and exosome.

Solving of mammalian muscle phosphoglycerate mutase crystal structure.

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The main goal of my project is to solve the crystal structure of rabbit muscle phosphoglycerate mutase (PGAM2). PGAM is a glycolytic enzyme that catalyzes the convertion of 3-phosphoglycerate to 2-phosphoglycerate. Overexpression of PGAM is linked to the increased glycolitic activity in cancer cells, whereas its deficiency is related to the glycogen storage disease type X.
The crystal structure of human brain isoenzyme of PGAM has been recently resolved providing some information on the mechanism of the enzyme regulation. However, there are several differences in amino acid composion between the muscle and the brain PGAM isoenzymes, including the regulatory phosphorylation residue. Despite clinical significance, the structure of mammalian muscle isoenzyme of PGAM remains unknown.
Rabbit muscle has been chosen as the source of PGAM because of significant identity of rabbit and human PGAM2 (~96%).

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