Student abstracts

P1

CHARACTERIZATION OF NOVEL DOPAMINERGIC LIGANDS BY COMPETITIVE RADIOLIGAND BINDING ASSAY

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Dopaminergic receptors are G-protein-coupled receptors (GPCRs), which are involved in a wide variety of physiological processes. Abnormalities in GPCR mediated signal transduction are associated with many different diseases. Therefore, dopamine receptors are targets for variety of drugs involved in diseases like schizophrenia, Parkinson's disease, depression and many others. Drugs, which are selective towards one target, have less side effects. However, dopamine receptor subtypes often have very similar pharmacological profile, which makes finding subtype selective drugs difficult.

In current work HEK293 cell lines with stable expression of individual recombinant D_1, D_{2L} and D_3 receptors were used to assess binding activity and selectivity of different apomorphine derivates. The main purpose of this work was to discover novel, subtype-selective, high-affinity dopaminergic ligands. As a result, several compounds with nanomolar affinities for D_2 receptor were described. Also, some compounds with subnanomolar affinity and notable selectivity for D_3 receptor were identified. In summary, some promising functional groups, which improved binding properties of apomorphine derivates, were discovered. This is an additional step towards novel, subtype-selective dopaminergic drugs.

Work with dopaminergic receptors continues. The goal is to characterize binding properties of novel ligands towards D_4 and D_5 receptors. In addition, the aim is to implement different fluorescence methods for dopamine receptors. This would help to understand and characterize receptor-ligand interactions in further details.

P2

CHALLENGES IN THE VIROLOGICAL ASPECTS OF SPR BIOSENSING AND APPROACH TO THEIR SOLUTION

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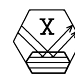
There are a lot of methods for the investigation of virus specific proteins and their interactions, but only few of them can detect intact virus particles. Surface plasmon resonance (SPR) technique is widely used for the exploration of the virus specific macromolecules, however the physical mechanism of this method causes some limitations when applying for direct detection of intact virus. Namely, because the depth of the evanished wave is about 200 nm, some problems appear concerning adequate interpretation of the results of measurements for subjects whose characteristic length is over several tens of nanometers.

For the SPR technique the classical approach is the immobilization of the receptor at the sensor surface, while the analyte remains in solution. For this case the angle shift depends on the effective thickness of the layer of analyte specifically bound to the immobilized receptor layer, the densities of both layers being constant. In other words that angle shift change is due to variation of parameters of the molecular ensemble of interacting molecules in the vertical plane. The shift of the angle depends not only on the layer thickness but on the refractive index variation within the layer. So the molecular layer compactness also affects the response due to refractive index variation. If, at the same time, the thickness of the surface structure could be fixed (due to specificity of the interaction process and constant form of the interacting components), then the shift of the angle will be a one-valued function of the compactness of biomolecular ensemble. For the multi component system containing not only virus and specific antibody but also additional factors affecting the binding capacity of reagents, which is typical for living systems, the use of such approach seems to be reasonable.



P3

STRUCTURAL DIFFERENCES BETWEEN SMALL PEPTIDES WITH AND WITHOUT FLUORESCENT LABEL

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Antimicrobial peptides occur in humans, animals and plants and act as „natural antibiotics". These, mainly positively charged, small peptides function among other things against pathogens, like the *Staphylococcus aureus*, responsible for a number of slight, but also lethal infections [1].

The frequent use of different antibiotics has lead to a variety of multi-resistant *S. aureus* strains appearing especially in hospitals as a huge problem, therefore the development of new drugs concentrates on prevention of stress resistance creation [2].

In this work an artificial dodecapeptide with antibacterial activity and its twelve alanine mutants, all with FITC label (fluoresceinisothiocyanate) [3], were examined with respect to their preformed secondary structure elements in the unbound state. Via the alanine-screen amino acid positions "hot-spots" with crucial influence on the secondary structure were determined and correlated to binding data.

From NMR-parameters, as well as comparison with database structures and algorithmic methods, predictions about the structure of the free peptides could be made.

Additional investigations using the dodecapeptide without FITC label reveal that the fluorescence tag causes significant non-local changes in secondary structure. Therefore for small peptides in general the influence of tagging groups should not be assumed to be negligible.

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P4

PROTEIN-HEPARIN MIMETIC PEPTIDE INTERACTIONS

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Heparin as well as act as inhibitors of blood coagulation, it is the macromolecule with the highest electric charge density in nature also. But after post-translational modifications, it is exposed to many diverse including sulfate distribution. Because of this heterogeneous structure of heparin, the interactions of structure-function can not understood clearly and the structural heterogeneity of heparin how effect the bind it to proteins is not known.

Many heparin-binding proteins have been identified before. The heparin-binding property has been used in the purification of these proteins by heparin affinity chromatography and has suggested further experiments that many define the effects of added heparin on the activities of the protein question. Heparin also has a number of interesting effects on the growth and biological activities of cells in culture.

The aim of this study to investigate the effect of heparin load distribution to protein affinity of heparin on protein-heparin interactions. In this study, colorimetry, light scattering and isothermal titration calorimetry will be used primarily and datum will be considered statistically.

Keywords: heparin, heparin-mimetic peptide, proteins.

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P5

CALORIMETRY STUDIES OF AN CELLOBIOHYDROLASE I FROM *TRICHODERMA REESEI*

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Cellulases produced by filamentous fungi are widely used in biotechnological applications, including biomass depolymerization and second generation bioethanol production [1]. To make possible the use of biomass it is necessary to degrade cellulose, a constituent of the cell wall, to fermentable sugars [2]. One form of degradation is the enzymatic hydrolysis. The complete enzymatic cellulose hydrolysis involves synergistic actions of endoglucanases (EC 3.2.1.4), exoglucanases/cellobiohydrolases (EC 3.2.1.91) and -glucosidases (EC 3.2.1.21) [3]. Cellobiohydrolase I (CBHI) is the major cellulolytic enzyme produced by *Trichoderma* sp. and is currently one of the most investigated cellulases for biofuel applications. CBHI hydrolyzes crystalline cellulose to soluble cellobiose units, which turns it into a key enzyme for producing fermentable sugars from biomass. CBHI seems to be confined to action at the chain ends by the shape of its active site which is located in a long tunnel formed by several well-ordered loops on the enzyme surface. The structural knowledge of the enzymes as well as the interaction of these with the substrate during the hydrolysis is extremely important. These study aims at understanding the molecular basis of the functioning of enzymes for application in the production of bioethanol from sugarcane bagasse. Thus, our studies might provide insights into understanding, of the interplay between structure and activity of *Tr*CBHI at different cellooligomers and temperature conditions, which can be useful for possible biotechnological applications of the enzyme as bioethanol production.

Keywords: Cellobiohydrolase_I, bioethanol, calorimetry

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P6

STUDY OF THE INTERACTION BETWEEN THE MTGA AND INHIBITORY COMPOUNDS

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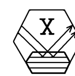
The emergence of multidrug-resistant bacteria causes major public health problems, particularly in medical facilities where the lack of efficient antibiotics could jeopardize the advances made in the treatment of many diseases. New efficient antibiotics against multidrug-resistant strains are urgently needed to counter this worrying situation.

Bacteria have a unique feature called the peptidoglycan (PG), an essential net-like macromolecule that surrounds bacteria, gives them their shape, and protects them against their own high osmotic pressure. PG synthesis inhibition leads to bacterial cell lysis, making it an important target for many antibiotics. The final two reactions in PG synthesis are performed by the membrane inserted penicillin-binding proteins (PBPs). Their glycosyltransferase (GT) activity uses the lipid II precursor to synthesize glycan chains and their transpeptidase (TP) activity catalyzes the cross-linking of two glycan chains via the peptide side chains. Inhibition of either of these two reactions leads to bacterial cell death. -Lactam antibiotics target the transpeptidation reaction while antibiotic therapy based on inhibition of the GTs remains to be developed.

Our objective is to develop a HTS (High Throughput Screening) binding assay based on the fluorescence properties using the monofuctional GT (MtgA) from *S. aureus* as model enzyme.

The structure of the GT domain can be divided into a soluble head subdomain with similarities to the phage lysozyme and a hydrophobic jaw subdomain, specific to the GT51 family, partly embedded in the cytoplasmic membrane. As a model GT enzyme, we choose the MtgA



from *Staphylococcus aureus* because it is a monofunctional GT (without any TP domain) active without its transmembrane segment and its structure is known.

In this work we provide a proof of concept of a fluorescence quenching binding assay that could be applied for HTS of screening for inhibitory compounds that might become leads for antibiotics development. To validate this assay, a HTS need to be performed with a library of small molecules or natural products. We have further characterized the mode of action of substrate analogue inhibitors. Our first result suggests that they bind to both acceptor and donor sites, and that the binding of compounds to acceptor site induce a cooperative binding of moenomycin to the donor site.

P7

CHARACTERIZATION AND SCREENING OF ACETYLCHOLINESTERASE INHIBITORS

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Given that 47% of marketed small-molecule drugs act on enzymes, this group of proteins appears to be one of the most pharmacologically important class of biomolecules. The regulation of enzymes activity as a therapeutic strategy has attracted the attention of researchers for decades. Nevertheless, the development of new rapid and effective screening techniques for the selection of new drug candidates acting as enzyme modulators is still an important issue.

Here we present an application of isothermal titration calorymetry nad as well as a monolithic micro-immobilized-enzyme reactor chromatographic system for the thermodynamic characterization and potency evaluation of acetylcholinesterase (AChE) inhibitors. AChE plays an important role in the termination of the impulse transmission at cholinergic neurons which are involved in learning and memory. Furthermore, the impairment of the cholinergic pathway is involved in the development of neurodegenerative diseases such as Alzheimer's disease. Nowadays, four out of five FDA approved drugs for the symptomatic treatment of Alzheimer's disease are AChE inhibitors. Moreover, modulation of cholinergic function by AChE inhibitors is used as a pharmacological strategy for the treatment of other disorders such as *Myasthenia Gravis* or glaucoma.

In the present work human recombinant AChE was immobilized on a chromatographic support, i.e. ethylenediamine monolithic convective interaction media (CIM) disk. The obtained micro-immobilized-enzyme reactor (IMER) was connected to a HPLC system and showed to be suitable for the characterization of cholinesterase inhibitors. The optimized analysis protocols allowed for the rapid on-line screening of known and new inhibitors, the determination of their activity,their mechanism of action and kinetics of the binding. At the same time the influence of nonspecific interactions between the screened compounds and the chromatographic support was reduced. Moreover, immobilized enzyme showed increased stability compared to the in-solution form. Consequently assay costs were reduced and data reproducibility was increased. The described instrumental set-up can be further automated and the throughput increased. However, due to lack of literature data the orthogonal technique (e.g. SPR) should be used in future to validate the chromatographically derived kinetic parameters of the AChE-inhibitor interaction (k_{on}, k_{off}).

In addition isothermal titration calorimetry was applied to obtain thermodynamic characteristics of inhibitor-AChE interaction. Based on the protocol proposed by Todd and Gomez (2001) not only the affinity but also activity of tested compounds was determined.

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P8

Zn^{2+} LIGAND BINDING BY HUMAN TRAIL CYTOKINE AND ITS DERIVATIVES

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Human TRAIL cytokine can induce apoptosis selectively in cancer cells[1] and therefore is a potential candidate for a novel anti-cancer therapy. In its active form, TRAIL is a homotrimer and binds a zinc ion[2]. The zinc-binding site locates on the interface between the subunits.

While it is known that the presence of the zinc ion affects TRAIL's activity[2,3] and hepatotoxicity[4], little is still known about the structural aspects of the ligand binding. We have observed that TRAIL stability changes upon Zn^{2+} binding and so do its fluorescence properties, suggesting that some structural alterations accompany the ligand binding. We aim to characterize them thermodynamically and kinetically.

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P9

ROLE OF DNA IN POSTULATED INTRAMOLECULAR INTERACTION OF YIN YANG 1 TRANSCRIPTIONAL FACTOR BETWEEN N-TERMINAL INTRINSICALLY DISORDERED ACTIVATORY DOMAIN AND C-TERMINAL ZINC FINGER REPRESSION DOMAIN

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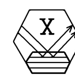
The Yin Yang 1 protein is transcription factor involved in the regulation of diverse fundamental cellular processes through DNA-protein and protein-protein interactions. YY1 exerts itsspecific effects on gene expression depending on the context in which it binds to DNA. In some promoters YY1 plays the role of constitutive repressor and upon binding of coactivator, i.e. AAV E1A protein, to YY1-DNA complex, the activation domain is exposed or released, thereby converting repression to an activator. What is more this effect can be artificially induced by deletion of last zinc finger of YY1. This phenomenon is called "an activation domain masking effect" and an unknown structural interaction is responsible for it. This type of mechanism could be confirmed by investigation of the YY1 structure and its intramolecular interaction.

Our preliminary results (SPR, EMSA, fluorescence anisotropy, CD) show no direct binding of N–terminal domain to C–terminal domain alone or its complex with DNA. Deletion of last zinc finger significantly decreases the YY1 binding constant to DNA. With FRET technique we would like to exclude or confirm existence of week electrostatic interaction between both oppositely charged ends of YY1 in complex with DNA.



P10

C-TERMINAL HELICAL DOMAIN OF THE MOTOR SUBUNIT HSDR FROM THE TYPE I RESTRICTION-MODIFICATION SYSTEM ECOR124I: STRUCTURE AND FUNCTIONAL IMPLICATIONS

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The published structure of the HsdR subunit of EcoR124I[1] suggested the motor subunit to be a planar array of four functionally integrated domains, with the fourth, C-terminal domain being all helical and implied to play a role in complex assembly and/or DNA binding. However, the last 150 amino acids of this domain are unresolved in the crystal structure.

A single point mutation lead to a new crystal structure that allowed to trace the backbone of the unresolved C-terminal residues, and homology and energetic modeling was applied to generate an all-atom 3-D model of wild-type HsdR and complemented by in vivo and in vitro studies to establish the function of the helical domain.

In vitro DNA cleavage assays, gel mobility shift assays and in vivo restriction tests were performed on WT and mutant HsdRs with selectively deleted parts of the helical domain.

Our results strongly support the suggested role in subunit interaction and demonstrate the importance of the C-terminus in complex assembly.

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P11

CYSTEINYL LEUKOTRIENES

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Cysteinyl leukotrienes are a family of potent inflammatory lipid mediators synthesized in human organism from arachidonic acid via lipoxigenase pathway. These molecules perform their physiological functions by interacting with receptors belonging to the protein-coupled receptors (GPCR) superfamily: cysteinyl leukotriene receptor 1 (CysLTR1), cysteinyl leukotriene receptor 2 (CysLTR2) and GPR17.

CysLT1 and CysLT2 receptors are proved to play an important role in pathogenesis of asthma, cancer, allergic response, cardiovascular and other diseases. While CysLTR1 has a number of antagonists available as medicaments, there is no specific treatment having CysLT2 receptor as a target. Obtaining a high resolution structure of CysLTR2 will provide an opportunity to create ligands being specific drugs.

Method of X-ray diffraction on protein crystals gives the best resolution of protein structure but it demands a high purity of protein together with high stability and monodispersity. Thus, several genetic constructions of target protein CysLTR2 with fusion partner protein BRIL were created to get required quality of protein and improve possible crystal contacts. It is also possible to stabilize receptor by interacting with some ligands that shoud be experimentaly selected. Constructions with target protein CysLTR2 were expressed using baculovirus expression system in Sf9 insect cells and are studied using western-blotting, size-exclusion chromatography and thermal-shift assay methods in presence of ligands and without them.

P12

PHOTOSYSTEM II PSBO PROTEIN FROM HIGHER PLANTS

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Photosystem II (PSII) is a huge complex of proteins in thylakoid membranes of algae, higher plants, and cyanobacteria that conducts light-driven water oxidation and produces molecular oxygen, electrons and protons. The splitting of water and release of oxygen appear in the catalytic centre of PSII – the oxygen-evolving centre (OEC) that contains manganese-calcium cluster (4:1 Mn:Ca) situated near to the luminal surface of the transmembrane domain and hemmed by intrinsic and extrinsic components in thylakoid membranes. PsbO (33kDa), PsbP (23kDa), PsbQ (17 kDa), PsbR (10 kDa) are extrinsic proteins attached to the luminal side of PSII in higher plants, which keep stability of water oxidation site and right ionic environment during oxidation of water. PsbO might be implicated in the OEC associated Ca^{2+} binding site creation by enhancing the balance between free and fixed Ca^{2+} that prefers the bound metal (Barber, 2004). The aim of our project is to receive recombinant PsbO proteins from *Spinacia oleracea* and *Pisum sativum* and use them in interaction experiments with other extrinsic proteins and structural studies. The isolation of mRNA from leaves and convertion to cDNA were our first attempts to obtain the *psbO* gene. The conditions of polymerase chain reaction (PCR) were adjusted and DNA fragments encoding the *psbO* gene were acquired. Vector pBluescript II SK(+) was utilized as a cloning vector and pET-28b(+) vector was employed for overexpression of recombinant PsbO protein. PsbO protein was overexpressed in insoluble inclusion bodies and purification protocol had to be optimized. Isolation of inclusion bodies from *E. coli* and their solubilisation with urea was done, refolding of protein by more steps dialysis was provided and finally ion-exchange chromatography was employed to purify the PsbO protein. Crystallization screenings are under construction.

P13

BINDING OF LOW MOLECULAR WEIGHT LIGANDS TO NLP PROTEINS

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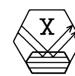
Nep-1 like proteins (NLPs) are widely distributed among non-related microorganisms like fungi, bacteria and oomycetes. These microorganisms are widespread and infect different important crops like potato, tomato, soya and tobacco. NLPs cause necrosis in plants and enormous economic loss worldwide.

The NLPs are structurally similar to actinoporins, the cytolytic proteins which are known to bind the specific lipid in membrane and make pores. The action of NLPs in plants is still unknown, but it is presumed that one kind of glycerosfingolipid is the binding target. We are using different methods with the emphasis on surface plasmon resonance to investigate more this protein-membrane interaction.

The second area of our research is screening the ligands that would bind to the NLPs and prevent their necrotic activity in plants. For this purpose we have employed the ligand-based virtual screening to obtain compounds that would bind to speculated binding cavity of the protein. With surface plasmon resonance, isothermal titration calorimetry and microscale thermophoresis we are determining the affinity data for the interactions of ligands with three different proteins from NLP family. For some ligands strong affinity was shown and these compounds were also proved to have inhibitory effect when infiltrated together with NLPs into plants.



P14

CHANGING NATURE OF THE CTD STRUCTURE

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RNA polymerase II associates with a large number of enzymes and protein/RNAbinding factors through its C-terminal domain (CTD). The CTD consists of multiple tandem repeats of the heptapeptide consensus Y1S2P3T4S5P6S7 which is specifically phosphorylated depending on the transcription cycle event. The CTD does not possess any rigid structure, it is represented by an ensemble of multiple conformations. Equilibrium between different conformations can be shifted by reversible post-translational modifications and the subsequent binding with protein factors recognizing modification.

Flexibility and repetitive nature of the CTD makes structural studies a challenging task. Current structural information is limited to proteins bound to a few CTD repeats but the full-length CTD structure and the requirement for the repetitiveness of the CTD are poorly understood.

In order to describe overall structure of the CTD and transitions between differently bound states, we have created model system mimicking the full-length CTD with specific phosphorylation pattern. By combining this system with advanced nuclear magnetic resonance (NMR) methods (PRE, spin relaxation, NOEs) and small angle X-ray scattering (SAXS) we follow changes in structural behavior of both the CTD and respective binding factor at atomic level resolution. Using this approach we visualized for the first time how the structure of full-length CTD is modulated upon binding with multiple copies of CTD-interacting domain (CID) of Rtt103, a subunit of yeast Rat1 exonuclease complex. Our study shows that one CID is accommodated on 2 repeats of the CTD heptadepeptide and the strength of CID-CTD interaction is affected by the CTD sequence adjacent to its minimal binding site. Resulted ensemble of structures indicates that CTD retains its highly flexible character upon binding, no fixed contact between CIDs is formed.

P15

RECOMBINANT PREPARATION AND INITIAL NMR SPECTROSCOPIC INVESTIGATION OF EXTRINSIC PSBO FROM THE OXYGEN-EVOLVING COMPLEX OF HIGHER PLANTS

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Photosystem II (PSII) is a protein complex of photosynthetic apparatus located in thylakoid membrane which is present in higher plants, algae, and cyanobacteria. Water, as the ultimate source of electrons in oxygenic photosynthesis, is oxidized within a remarkable molecular device called oxygen-evolving complex (OEC) which is the part of the PSII. The OEC contains a metal cluster consisting of four manganese ions, a calcium ion, and five oxo ligands (Mn_4CaO_5 cluster). Water molecules are split in the cluster by light driven oxidation into protons, electrons, and molecular oxygen. PsbO (33kDa) is one of the extrinsic proteins attached to the PSII and is present in all oxygenic organism. The extrinsic proteins optimize oxygen evolution at physiological conditions and protect the Mn_4CaO_5 cluster from exogenous reductants. PSII complex lacking the PbsO retains the cluster but the ability of oxygen evolution is very low. Additional low affinity calcium-binding sites are present in PsbO but their function is also not fully understood.

Preparation of PsbO for NMR spectroscopy, investigation of its dynamics and characterization of the structure by this technique are the goals of our project. Recombinant PsbO from spinach was expressed in *Escherichia coli*. The overexpression of PsbO in various temperatures and different composition of minimal medium was tested. Glycerol was used for proper refolding of the expressed protein. Due to the high viscosity, glycerol needs to be replaced by mannitol or sorbitol in the NMR measurements. Proper folding of PsbO in different buffers was monitored by CD spectroscopy. The NMR spectra of ^{15}N labelled protein were recorded during the initial stages of the assignment.

P16

PROTEIN SPECIFICITY OF CHARGED SEQUENCES IN POLYANIONS AND HEPARINS

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Long-range electrostatic interactions are generally assigned a subordinate role in the high-affinity binding of proteins by glycosaminoglycans, the most highly charged biopolyelectrolytes. The discovery of high and low sulfation domains in heparan sulfates, however, suggests selectivity via complementarity of their linear sulfation patterns with protein charge patterns. We examined how charge sequences in anionic/nonionic copolymers affect their binding to a protein with prominent charge anisotropy. Experiments and united-atom Monte Carlo simulations, together with Delphi electrostatic modeling for the protein, confirm strongest binding when polyanion sequences allow for optimization of repulsive and attractive electrostatics. Simulations also importantly identified retention of considerable polyion conformational freedom, even for strong binding. The selective affinity for heparins of high and low charge density found for this protein is consistent with nonspecific binding to distinctly different protein charge domains. These findings suggest a more nuanced view of specificity than previously proposed for heparinoid-binding proteins.

P17

INTERACTION OF PLANT SERYL-TRNA SYNTHETASE AND BEN1, PROTEIN INVOLVED IN BRASSINOSTEROID METABOLISM

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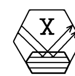
Aminoacyl-tRNA synthetases (AARS) are essential cellular enzymes that play significant role in protein synthesis by charging amino acids to their cognate tRNAs. In recent years more evidence appeared showing involvement of these enzymes in diverse cellular functions beyond translation. Characterization of these non-canonical functions broadens our knowledge in functional proteomics. The studies of aaRS assemblies in plants are scarce. Our main research goals were to determine potential protein interacting partners of seryl-tRNA synthetase (SerRS) in plant cell, its potential nuclear localization and kinetic characterization. In search for SerRS interacting protein partners in plant *Arabidopsis thaliana, L.* we conducted yeast-two hybrid (Y2H) screen on cDNA libraries and tandem affinity purification (TAP) in plant. Potential interacting partners were identified either by DNA sequencing (Y2H) or mass spectrometry (TAP). BEN1, protein involved in metabolism of brassinosteroid hormones, was identified as the most promising interacting partner. Interaction of BEN1 and SerRS was analyzed *in vitro* using isothermal calorimetry titration (ITC), pull-down and surface plasmon resonance method (SPR). Probably due to the nature of interaction we were not able to retrieve positive results using pull-down assay and ITC, but SPR gave us positive confirmation and information about dissociation constant. To determine regions responsible for protein-protein interaction we planned to prepare shortened variants of both SerRS and BEN1 proteins and use them in biophysical analysis. So far we managed to construct, successfully express and purify BEN1 variant lacking 37 amino acids long polar region at the N-terminus and SerRS variant lacking hydrophilic region at C-terminus. Although we successfully cloned other truncated variants of SerRS protein, thus far their expression yielded inclusion bodies. Biophysical determination of possible interactions between SerRS and BEN1 variants will give us insight in additional cell functions and physiology of both SerRS and BEN1 proteins.



P18

ROLE OF EF-HAND MOTIF IN THE ACTIVATION OF NEUTRAL TREHALASE

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Trehalases hydrolyze the non-reducing disaccharide trehalose amassed by cells as a universal protectant and storage carbohydrate. Recently, it has been shown that the activity of neutral trehalase (Nth1, EC 3.2.1.28) from *Saccharomyces cerevisiae* is mediated by PKA-phosphorylation, Ca^{2+} and the yeast 14-3-3 protein (Bmh1) binding [1, 2]. Bmh1 modulates the structure of both the catalytic domain of Nth1 and the region containing the EF-hand like motif which is conserved among many Ca^{2+} binding proteins and which role in the activation of Nth1 is unclear [3]. In this work, the structure of the Nth1:14-3-3 complex and the importance of the EF-hand like motif were investigated using site-directed mutagenesis, hydrogen/deuterium exchange coupled to mass spectrometry, chemical cross-linking and small angle X-ray scattering (SAXS) [4]. The low resolution structural views of Nth1 alone and the Nth1:14-3-3 complex show that the 14-3-3 protein binding induces a significant structural rearrangement of the whole Nth1 molecule. The EF-hand like motif-containing region forms a separate domain that interacts with both the 14-3-3 protein and the catalytic trehalase domain. The structural integrity of the EF-hand like motif is essential for the 14-3-3 protein-mediated activation of Nth1 and Ca^{2+} binding, although not required for the activation, facilitates this process by affecting its structure. Our data suggest that the EF-hand like motif-containing domain functions as the intermediary through which the 14-3-3 protein modulates the function of the catalytic domain of Nth1.

This work was supported by the Czech Science Foundation (Project P207/11/0455) and by Grant Agency of Charles University (Grant 644313).

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P19

INTERACTION OF TROPANE DERIVATIVES WITH DOPAMINE TRANSPORTERS

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The dopamine transporter (DAT) is a transmembrane protein belonging to the ion-coupled secondary transporter superfamily [1,2]. This protein is located in presynaptic membranes and mediates the reuptake of dopamine that is released into the synaptic cleft, and through this mechanism regulates the concentration of this neurotransmitter [3].

Tropane derivatives are bicyclic alkaloids, some found naturally, especially in plants from *Solanaceae* family [4], and some of synthetic origin. Numerous tropane derivatives have been developed for PET as selective DAT imaging compounds. DAT is quite sensitive to the configuration of stereo centers and to the functional groups [5].

It has been reported that the DAT inhibitors can bind to DAT in two different mechanisms: (i) the fast ligand binding to protein taking place under equilibrium conditions; (ii) after initial fast binding step the ligand induces protein conformation change that can be identified by kinetic experiments [6]. This extra stadium consists of a slow conformational change and enhances the apparent binding effectiveness of the ligand. Some tropane derivatives (e.g. PE2I) exhibit this behavior, while in other cases [e.g. cocaine] this phenomenon is absent [7]. We suggest that the property of a ligand to induce isomerization of the ligand-protein complex should dependent on the structure of the ligand, but we do not have information about the ligand structural factors which are responsible for switching on the isomerization process. This information can be obtained by kinetic study of the ligand binding, using a radioligand, which is able to induce the isomerization step.

Among several radioligands, which have been used for *in vitro* binding experiments with DAT (e.g. [^{3}H]WIN35,428; [^{3}H]GBR-12935 and [^{3}H] -CIT), a specific radioligand [^{3}H]PE2I is known to induce protein isomerization with DAT, and therefore can be used for experimental study of protein-ligand complex isomerization step [6]. Although radioligand binding is a very sensitive and reliable procedure, it has some drawbacks, which may be less significant in the case of alternative methods as SPR and fluorescence spectroscopy. Therefore testing of these methods may be very challenging for investigation of ligand-DAT interaction mechanism.

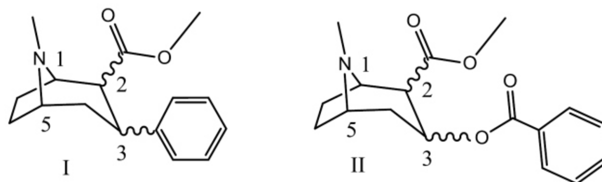

Figure 1. The stereo centers of phenyltropane (I) and cocaine (II).

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P20

PHYTOESTROGEN INTERACTIONS WITH OESTROGEN RECEPTOR AND BREAST CANCER PROGRESSION

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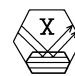
This study aims to understand the interaction between endogenous oestrogens, selective oestrogen receptor modulating compounds and dietary foodstuffs with oestrogen-like properties (phytoestrogen, PE), in order to determine hypothetical influences on a population of breast cancer patient prognosis. There is evidence that consumption of such phytoestrogenic compounds plays a role in the regulation of the disease, as those interactions influence the action of both synthetic and endogenous estrogen in cancer cells, in turn modulating cellular proliferation. In particular, genetics variants (e.g. SNPs) may have a major role in breast cancer progression.

Computer and in vitro models will include SNPs analysis of the ER alpha and beta genes from the DietCompLyf study. Through the SNPs analysis an understanding will be provided about their role in breast cancer progression. Subsequently, the ERά, ERâ isoforms, and different ligands (E1, E2, E3, PE, tamoxifen, ICI 182,780) will be modeled and assessed using in vitro laboratory models of both isoforms. The in silico and in vitro studies will lead to further understanding of the molecular structure and function in the pathophysiology of BC.

Data gathered from the study will allow for a better understanding of the interactions between ER isoforms and endogenous/exogenous oestrogens in breast cancer as well as with other oestrogen-like agents and of his role as a transcription factor in breast cancer proliferation. The bioinformatics approach and the results of laboratory tests will elucidate the extent of the role of diet on BC prognosis.



P21

INVESTIGATING THE MECHANISM OF ACTION OF PFCCT, A KEY LIPID BIOSYNTHETIC ENZYME FROM THE MALARIA PARASITE

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At the dawn of the 21st century, malaria still presents as the most serious tropical infectious disease whith more than half million death cases annually (1). Due to the emerging antimalarial drug resistence of causing agent Plasmodium parasites, there is an inevitable need for the development antimalarials targeting novel biosynthetic pathways. D*e novo* phosphatidylcholine biosynthesis of the rapidly proliferating parasites has recently been validated as novel antimalarial target as choline-mimicking drug candidates acting on this pathway selectively and efficiently eradicate parasitaemia(2,3) Within this pathway CTP:choline-phosphate cytidylyltransferase (CCT) was shown to catalyze a rate-limiting step in Plasmodia (4), converting CTP and choline-phosphate (ChoP) to generate the high energy intermediate CDP-choline metabolite.

As a first step towards characterization of key elements of *Plasmodium falciparum* CCT (*Pf*CCT) mechanism of action, we optimized the expression of an engineered construct encompassing its catalytic domain in *E. coli.* We then performed steady kinetic characterization as well as equilibrium substrate and product binding followed by tryptophan fluorescence and isothermal titration calorimetry (5). Results indicate that in the presence of the Mg^{2+} cofactor, the binding of the CTP substrate is attenuated by a factor of 5. The weaker binding of CTP:Mg^{2+}, when paralleled to earlier observations regarding aminoacyl tRNA synthetases, suggests that Mg^{2+} is necessary for catalysis but not for ligand binding of *Pf*CCT. We hypothesize that in lack of Mg^{2+}, positively charged side chain(s) of CCT may contribute to CTP accommodation.

We also aim to integrate the current knowledge of CTP:phosphocholine cytidylyltransferase mechanism of action by exploring the pre-catalytic enzymatic stage for which neither crystallographic nor ligand binding data are published. To investigate the ternary complex of enzyme with two substrates before catalysis, we examine the interaction of native substrates with such an engineered inactive enzyme, whose ligand binding is expected to be only marginally perturbed. We currently plan to characterize Isothermal titration calorimetry Thermodynamics of these enzyme-substrate interactions are to be characterized by isothermal titrational calorimetry. Our further plans include providing structural insights of the ternary enzyme:substrates complex by generation of a three-dimensional protein crystal structure.

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P22

PREVALENCE OF VIRULENCE FACTORS OF *AGGREGATIBACTER ACTINOMYCETEMCOMITANS* IN SLOVENE POPULATION

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Aggregatibacter actinomycetemcomitans (AAC) is a small, fastidious, non-motile, non-encapsulated, slow-growing, capnophilic, Gram-negative cocco-bacillus. It´s habitat is human mouth and can be recovered on culture of oral secretions in up to 20% of healthy people and in the great majority of those with localized juvenile periodontitis (LAP). The most studied toxins of AAC are leukotoxin (LtxA) and cytolethal distending toxin (CDT), they both play a role in immune evasion, but probably differ in their target-cell specificity and pattern of expression during disease.

The aim of the study is to assess the prevalence of mentioned virulence factors of *Aggregatibacter actinomycetemcomitans* isolated from subgingival pocket from 15 patients diagnosed with LAP. By comparing the results of the survey with other published clinical data from abroad, we might better understand the disease and hopefully obtain some new genetic and biochemical insight.

Background:

Localized juvenile periodontitis (LAP) is an oral disease caused by *Aggregatibacter actinomycetemcomitans*. By genetically analysing the virulence factors of the strains

obtained from patients with LAP we hope to obtain better understanding of the disease.

Approach:

Main method for identifying the presence of virulence factors in strains is isolating bacterial DNA and specifically multiplying sequences in the bacterial genome by PCR (polymerase chain reactions).

Key Findings:

Strains of *Aggregatibacter actinomycetemcomitans* isolated from patients diagnosed with LAP contain almost all virulence factors.

Implications & Conclusion:

By knowing which virulence factors are present in a strain of *Aggregatibacter actinomycetemcomitans* obtained from potential patient we can better formulate the treatment.

P23

THERMODYNAMIC ANALYSIS OF LIGAND SPECIFITY FOR 17B-HSD TYPE 1

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17b-Hydroxysteroid dehydrogenases (17b-HSDs) are oxidoreductase enzymes which catalyze (NAD/NADPH-dependent) reduction/oxidation of steroids at C17, regulating the concentration of active biological hormones via inter-conversion of ketone/alcohol forms. In vitro 17b-HSDs appear capable of catalyzing both oxidation and reduction reactions, and the molecular basis of ligand preference remains unclear. Moreover, because levels of steroidal hormones, such as androgens and estrogens, are important in breast cancer, prostate cancer, endometriosis and osteoporosis; designing small molecule regulators of 17b-HSD function may be a useful strategy for the treatment of these diseases. Analysis of the substrate specificity of 17b-HSD and the thermodynamics of ligand binding could thus provide insight into the molecular basis of substrate preference and aid in the design of more effective inhibitors. For this study, recombinant human 17b-HSD1 was expressed and purified from BL21(DE3) E.coli by nickel chromatography. Protein and ligand will be freshly prepared in identical buffer conditions by gel filtration chromatography and dialysis. We propose to measure binding of oxidized and reduced forms of estrogen to 17b-HSD1 using a battery of methods, focusing on isothermal titration calorimetry (ITC). Newly synthesized inhibitors of 17b-HSD1 could also be analyzed. To our knowledge this will be the first thermodynamic study of 17b-HSD ligand binding, and results could be used to guide synthesis of novel 17b-HSD inhibitors. Molecular docking studies will be conducted to complement experimental results. The range of binding studies made possible by this course could result in new molecular models of 17b-HSD substrate binding, and has potential to open new avenues of research in the field.

P24

STRUCTURE AND FUNCTION OF FIMBRIAL POLYADHESINS

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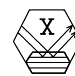
Fimbriae, proteinaceous structures that project from bacterial cell wall, mediate bacterial attachment to the host epithelium. Bacterial pathogens make monovalent or polyvalent attachment to the host epithelium to create niches in which to reside and transfer pathogenic material to elicit infection. Fimbrial adhesins are being recognized and bound by certain type of host receptors (i.e. fibronectin, and galactose and lactose residues) to make this attachment. Crystallographic structure of fimbria-receptor complex depicts the binding site(s) of fimbriae and these sites are biologically important to design antibacterial drug or subunit vaccine. Surface plasmon resonance and isothermal titration calorimetry are state of the art technologies to study the binding affinity and kinetics of those biologically active binding sites and their receptors. We have studied a few Gram-negative bacterial fimbriae and their receptors.



P25

LPTC-LPS BINDING: A NEW TARGET FOR ANTIBACTERIAL COMPOUNDS

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The development of bacterial resistance to current antibiotics provides considerable interest in the discovery of new targets to kill Gram negative organisms. Gram-negative bacteria are typically surrounded by two membranes, an inner (IM) and an outer (OM) membrane separated by the periplasm [1]. The OM is an asymmetric lipid bilayer with lipopolysaccharide (LPS) in the outer leaflet. LPS is an essential molecule in most Gram negative organisms and a potent activator of the innate immune response in mammalians. Due to its important biological properties, the biogenesis of LPS represents an ideal target for the development of new antibacterial compounds.

The structure and the biosynthetic pathway of LPS have been established, however the precise mechanism of transport and assembly at the cell surface are still poorly understood. The biosynthesis of LPS starts in the cytoplasm and it is completed at the periplasmic face of the IM; then, the export of mature LPS to the cell surface is mediated by a seven protein transporter (LptABCDEFG) spanning IM and OM [2]. LptC plays a crucial role within Lpt complex connecting IM and OM via the periplasmic component LptA and previous studies have demonstrated that LptC binds LPS *in vitro* [3].

In this work we present the study of the *in vitro* interaction of immobilized LptC and fluorescent LPS defining the thermodynamic parameters. Moreover, we found that a fluorescent synthetic glycolipid ligand, originally designed as a TLR4 antagonist, binds LptC in the same site of LPS indicating that it could be a scaffold for the development of a new class of antibacterial molecules.

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P26

INSECT DIGESTIVE ASPARTIC PROTEASE TARGETED BY A PLANT DEFENSE PROTEIN

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LdCD is a digestive cathepsin D-type protease of the Colorado potato beetle, *Leptinotarsa decemlineata*. Recombinant LdCD was produced in *Pichia pastoris* and its crystal structure was determined at 2.0 Ĺ resolution. Using two approaches, FRET-based activity assay and MicroScale Thermophoresis, we demonstrated that LdCD is inhibited by the interaction with PDI (Potato Cathepsin D Inhibitor), a Kunitz-type wound-inducible protein from potato leaves. This suggests that LdCD is a target for PDI acting as an antifeedant in plant defense against insect herbivory. Crystallization of LdCD-PDI complex aimed to determine the inhibition mechanism is currently in progress.

P27

DISASSEMBLY OF THE VITAMIN B_{12} TRANSPORT COMPLEX FROM *E. COLI*

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ATP-binding cassette (ABC) transporters constitute one of the largest membrane protein families, and prevail in all domains of life. These proteins actively transport their substrates across the lipid bilayer, and are directly related to human disease and multidrug resistance.

In E. coli, the inner membrane ABC transporter BtuCD interacts with the periplasmic binding protein BtuF to form the vitamin B12 transport complex, BtuCD–BtuF. The conventional model assumes that BtuF binds the substrate and then associates with BtuCD. Consequently, BtuCD–BtuF complex must dissociate and re-associate in a cyclic manner to resume vitamin B12 transport into the cytoplasm. However, in vitro, BtuCD–BtuF complex is extremely stable ($K_D = 1.16\times10^{-13}$ M). Hence we ask how the complex disassembles.

We aim to first assemble BtuCD–BtuF complex in vitro, and then to dismantle it. As was shown in vitro, either vitamin B12 or ATP reduces the affinity between BtuF and BtuCD. Moreover, when both ligands are present, there is no complex formation. For this reason, the substrate and the nucleotide are the prime candidates for BtuCD–BtuF complex disassembly. Another possibility is that a different molecule of BtuF (pre-loaded with vitamin B_{12}) is necessary for disassembly of the present transport complex and assembly of the next one. Also, we should take other factors – yet to be identified – into consideration.

In any case, we expect to gain insights as to how the vitamin B_{12} transport complex falls apart; and this will further our understanding of complex recycling.

P28

RESONANCE ASSIGNMENT OF PSBP FROM PHOTOSYSTEM II OF *SPINACIA OLERACEA*

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The PsbP is a 23 kDa extrinsic protein from Photosystem II which is located in the so called oxygen evolving center. This is a place where the fundamental reaction of photosynthesis, the water splitting, takes place. PsbP together with other extrinsic proteins regulates the concentration of the necessary cofactors – Ca^{2+} and Cl^-. The PsbP protein also induces structural changes in the thylakoid membrane upon binding which are necessary for a stable oxygen production[1]. However the mechanism of these interactions has not yet been clarified. Recent X-ray crystallographic structure has brought detailed information about the structure of PsbP. Unfortunately the N-terminus and dynamic loops are missing[2]. PsbP is therefore an ideal candidate for the study by NMR.

PsbP was expressed in *E. coli* as 6x His-tagged protein and purified using various chromatographic techniques (affinity, ion - exchange, size - exclusion). In order to study protein by NMR, one needs the complete assignment of its resonances. A set of 2D and 3D NMR experiments was recorded: ^{15}N HSQC, ^{13}C HSQC, HNCACB, CBCA(CO) NH, HNCO, HCCH - COSY, H(CC)(CO)NH, (H)CC(CO)NH, (H)CCH – TOCSY and ^{15}N NOESY – HSQC. Using these spectra, 91% of the backbone and non-aromatic side chain chemical shifts have been assigned up to this date. Another step is to derive 3D solution structure of PsbP and continue with the study of interactions between Psb extrinsic proteins.

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This research was supported by the Austrian Science Funds (project M1404 to KC), and the European Union through the EFRE INTERREG IV ETC-AT-CZ program (project M00146 “RERI-uasb”) and by OEAD through the Aktion program AT-CZ (project nr. 69p26 to NM and JK).