Visegrad Fund

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Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

Annual SUMMER SCHOOL IN MOLECULAR BIOPHYSICS AND SYSTEMS BIOLOGY

6th JULY – 26th JULY 2015

NOVE HRADY
CZECH REPUBLIC

Annual SUMMER SCHOOL IN MOLECULAR BIOPHYSICS AND SYSTEMS BIOLOGY

The scientific and research Summer school in molecular biophysics and systems biology is supported from International Visegrad Fund, jointly organized by the Institute of Nanobiology and Structural Biology GCRC, Academy of Sciences of the Czech Republic and the University of South Bohemia. In partnership with University of Szeged, Hungary, University of Warsaw, Poland and Comenius University in Bratislava, Slovakia, summer school gave the chance to Czech and foreign university students to work with experienced scientists and tutors. Overall the students took a part in excellent lectures and got a new view of cutting-edge methodologies and research.

The students were selected in the selection procedure by the committee of scientific leaders and worked in excellent fully-equipped laboratories on topics related to systems biology and molecular biophysics. At the end of summer school, the research teams presented the results of their work and competed with others. Participants were evaluated by scientific committee and were awarded with prizes and certificates.

Summer school 2015 was attended by 21 distinguished students from Poland, Czech Republic, Hungary, Slovakia, Ukraine, Belarus, Croatia, Turkey, Iran and Russia. 10 students from Visegrad countries were sponsored by Visegrad Fund and students from other countries were co-funded by the Institute of Nanobiology and Structural Biology GCRC, Academy of Sciences of the Czech Republic and the University of South Bohemia.

SPONSORS

Visegrad Fund

Jihočeská univerzita
v Českých Budějovicích
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University partners:
Univerzita Komenského v Bratislavě (Comenius University in Bratislava)
Szegedi Tudomanyegyetem (University of Szeged)
Universytet Warszawski (University of Warsaw)

PROJECT held:
06th – 26th July 2015 SUMMER SCHOOL IN MOLECULAR BIOPHYSICS AND SYSTEMS BIOLOGY

http://auc.cz/summerschool2015

Ladies and gentlemen, dear friends, dear participants,

A warm welcome to Nové Hrady and the Annual Visegrad Summer School in Molecular Biophysics and Systems Biology organized jointly by the South Bohemian University, the Warsaw University, Poland, the University of Szeged, Hungary, Comenius University in Bratislava, Slovakia, and the Institute of Nanobiology and Structural Biology of the Academy of Sciences in Nove Hrady, Czech Republic.

The Visegrad Summer School builds on two traditions, the first is the close collaboration of the five institutions in the field of computational simulations and spectroscopy of biologically relevant systems, following the tradition of the well established Visegrad Symposium on Structural Systems Biology, which were initiated as a scientific meeting by Dr. Babak Minofar back in 2009 and are co-organized by the four Visegrad countries annually in one of the Visegrad countries. The second tradition is the long history of summer schools in the Academy and University Center in Nove Hrady, dating back to the first "Schola Ludus" in Biophysiscs organized by Prof. Ladislav Nedbal in 2002. It is a great pleasure for us not only to welcome this year's students from the four Visegrad countries but also from Croatia, Turkey, Iran, Ukraine, Russia and especially from the Biological Faculty of the Belarusian State University in Minsk, which was made possible by a recently signed memorandum of understanding between our institutions. We are hoping that this newly established link will be mutually fruitful and become an established part of the summer school in the future. This year's summer school was sponsored again by the Visegrad fund, a fact which we greatly appreciate.

We believe that the wide range of topics, ranging from various computational methods used in the study of biologically relevant macromolecules up to microscopic methods in living cells offer the students a unique opportunity to get in touch with "real" cutting-edge science and experience how science works. The lecture series held not only by our scientists but also by various internationally well-recognized speakers complements the scientific work and shall give a broader overview about molecular biophysics and systems biology. We believe that the unique setting in the chateau makes social contacts easier and helps to further emphasize the collaborative atmosphere of the summer school.

On behalf of the organizing team, I wish you an enjoyable and inspiring year!

Professor Rüdiger H. Ettrich, Ph.D.
Director of the Institute of Nanobiology and Structural Biology GCRC AS CR

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JIRI HELLER, MSc

TOMAS FESSL, Ph.D.

LUKASZ BUJAK, Ph.D.

Summer school preparation and meetings

SPEAKERS

David Doležel, Ph.D.

Institute of Entomology, Laboratory of Molecular Chronobiology, Biology Centre AS CR in České Budějovice

Lecture – Chronobiology -- daily rhythms and seasonality in animals

Most of organism from unicellular prokaryotes to elephant synchronizes their metabolism, physiology and behavior with periodic alternations in environment (light, temperature, predators). These natural cycles include tide, periodic changes of day and night, lunar cycles and alternations of seasons. It turned out, that many organisms not only adapt to specific conditions affected by these events, but also anticipate these changes before they actually happen. Hence we find circadian, circatidal, circalunar and seasonal clocks. Our understanding of mechanisms and architecture behind these clocks differs remarkably. Circadian clock is well characterized both at the anatomical and molecular level in several model organism. On the other hand circatidal and seasonal clocks are only poorly characterized. The lecture will cover some basic introduction to chronobiology including brief historical perspective, followed by more detail description of research methods, current "hot topics" and possible future research directions.

Prof. Marek Jindra, Ph.D.

Institute of Entomology, Laboratory of Developmental Genetics, Biology Centre AS CR in České Budějovice

Lecture – Hormonal and genetic regulation of development and metamorphosis of insects and genesis

Babak Minofar, Ph.D.

Academy of sciences of the Czech Republic, INSB GCRC AS CR

Lecture - How much do we know about water?

Guido Grimm, Ph.D.

Department of Paleontology, University of Vienna

Lecture - The two towers: What do genes and fossils tell us about evolutionary history (of plants)

The molecular revolution and the advent of "big data" have fundamentally influenced our perception of phylogeny and evolutionary history. Since Darwin's Origin of Species, common ancestry was defined by overall similarity or the sharing of a few unique, derived traits, termed 'synapomorphies' in 1950 by Hennig (e.g. the double integument in flowering plants, the angiosperm clade). Already in 1963 Cavalli and Sforza produced the first statistically obtained phylogenetic tree, based on a genetic similarity matrix. Today researches can rely on thousands to ten-thousands of nucleotides or other

molecular characters and highly sophisticated mathematical models to infer their evolutionary histories. This led not a few to believe that all other data sets have come out of date. But there is one place; no molecular data will ever have access to. The evolutionary past. We can obtain fragments of DNA from material that is some thousands and even 10,000 years old, but evolutionary history covers millions of years. Hence, reconstructing the past using genes relies on models but not factual evidence. The only hard evidence for the existence of an organism at a given point in space and time is a fossil, something dug out. Fossils can be (partly) organic but often they are just mineral impressions of a once living and evolving thing. Accordingly, the identification of fossils rely mostly on form; and palaeontology has long ignored the molecular revolution that affected so deeply their neontological sister sciences, systematic biology and biogeography. Using just form, not rarely delinked from function (form-genera and form-species), can be tricky when it comes to phylogenetic relationships was the first lesson they learned from the molecular data.

Thus, identifying the "dark spots" of molecular and fossil data is the first step towards a holistic reconstruction of evolutionary history. I will show a number of examples that demonstrate the inadequacy of molecular and morphological data sets when taken alone, and how methodological advances and a bit of thinking-out-of-the box can assist in bridging the still widening gaps between the two towers of evolutionary history: the genes and the fossils.

Thomas Stockner, Ph.D.
Medical University of Vienna
Lecture - Principles of molecular dynamics simulations

Christa Fittschen, Ph.D
French National Centre for Scientific Research
Lecture - Atmospheric chemistry and pollution phenomena
The lecture will introduce the composition and structure of the atmosphere and will present basic principles of atmospheric chemistry. The origins of the different pollution phenomena such as photochemical smog, London smog, acid rain, ozone depletion, climate change and urban air pollution will be briefly presented. Few more details on the role of major key species in atmospheric chemistry (OH radicals, reactive nitrogen species (NO+NO2), hydrocarbons and ozone) will be presented as well.

Jeroen R. Mesters, Ph.D
Institute of Biochemistry, University of Lübeck
Lecture I. - Snapshots of a journey through time in Biocrystallography
Lecture II. - High-thoughput, structure- and fragment-based techniques in lead compound identification

Wojciech Mrozik, Ph.D
School of Civil Engineering and Geoscience, Newcastle University
Lecture - Micropollutants in the environment

Micropollutants consist of various chemical groups among them most important are: Pharmaceutical and Personal Care Products (PPCPs) and Endocrine Disrupting Compounds (EDCs).These compounds are becoming of the major concerns in natural waters as the results of their continuous release into the environment. They are widely used and consumed in modern societies or for agricultural purposes. Moreover large number of them is poorly or not treated at all in conventional waste water treatment facilities. Eventually they end up in natural matrices like rivers, sediments or ground waters. Although many of PPCPs do not exhibit bioactivity in the environment; but it has been well documented that aquatic fauna are at major risk of exposure from some of these compounds *i.e.* hormonal disruption in wild fish caused by estrogenic hormones. It must be underlined major of the data and studies has been performed in the developed industrialized countries like USA or in Western Europe. Countries with rapid economic, urban and industrial growth (*i.e.* India), with little regulation of chemicals or treatment of wastewaters still lack of valuable information about the extent of micropollutants in natural environment.

An example on of studies on identification and determination of selected micropollutants in two major rivers in India; the River Ganges and the River Yamuna will be presented. Those rivers are very important from cultural and economic aspects, both of which are also the subject of important governmental plans to improve water quality.

PROJECTS

1. Expression, purification and crystallization of photosynthetic protein PsbR using different vectors and crystallization techniques.

Project`s aim:
During the project the student will be introduced into the field of molecular biology methods such as protein overexpression, purification using AKTA and others like: CD, fluorescence and DLS measuring prior to prepare protein sample suitable for crystallization. Basic crystallization techniques will follow and the student will learn to distinguish different structures which will occur in the drop.

Project leader: **Mgr. Jiří Heller, hellej01@prf.jcu.cz**

2013 - Current	PhD student of molecular biology and genetics at the University of South Bohemia
2011 - 2013	Master student of molecular and evolutionary biology in the University of South Bohemia

Students: Artem Dubovetskyi - National Aviation University, Ukraine
Valeria Kopats - Researcher Training Institute of the National Academy of Science, Belarus

Abstract:
Expression and purification of photosynthetic protein PsbR for crystallization

The PsbR protein is a nuclear-encoded photosystem (PS) II subunit specifically found in higher plants, in particular this 10 kDa protein is a component of oxygen evolving complex (OEC) of PSII. The role of PsbR has not been clarified yet. It is reported that PsbR might have auxiliary role for the PsbP binding to PSII or cooperate with manganese ions having complex, where the O_2 is formed[1]. Also previous studies showed this hydrophobic protein is located in luminal side of the thylakoid membrane but the exact position, region of the contact with OEC and function of the PsbR remains unknown[2]. PsbR diffracting crystals from higher plants were not

obtained in order to discover their precise 3D structure, for this reason the aim of this work was to obtain and isolate the PsbR protein for crystallization.

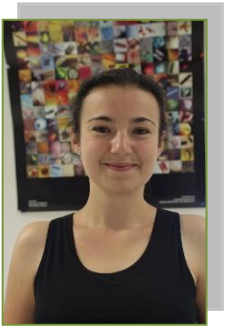
PsbR has been expressed in *Escherichia coli* BL21. A plasmid vector pET41a(+) allowed expression of the PsbR as a glutathione-S-transferase (GST) fusion protein[3], after that it was cleaved from the carrier protein with thrombin (1 NIH/mg protein) and purified on glutathione sepharose high performance column using FPLC AKTApurifier system. We adopted the GST expression system and modified it for production of electrophoretically pure PsbR protein in sufficient quantities (~mg) to be used in crystallography experiments and others structural studies. In this work we performed measurements of circular dichroism (CD) of the PsbR that confirmed that PsbR contains α-helix.

PROJECTS

2. Crystallization of glyceraldehyde dehydrogenase or how to overcome the main bottleneck of macromolecular crystallography

Project`s aim:
During the project students will go through all steps of protein structure determination from obtaining a good-quality crystal to the structure refinement of glyceraldehyde dehydrogenase from *Thermoplasma acidophilum* (*Ta*AlDH). Crystallization part will include optimization of obtained crystallization conditions by variation of protein and precipitant concentrations and pH and using microseeding procedure. In the second part students will study how to work with software for diffraction data processing and structure refinement.

Project leader: Iuliia Iermak, MSc, julia.ermak90@gmail.com



2013 - current	PhD student at the Faculty of Science, University of South Bohemia in C. Budejovice
2011 – 2012	M.Sc. at the Department of Biological and MedicaL Physics, V.N.Karazin Kharkov National University
2007 – 2011	B.Sc. at the Department of Biological and Medical Physics, V.N.Karazin Kharkov National University

Current research of Iuliia Iermak concerns structural characterization of various bacterial and plant proteins such as haloalkane dehalogenase LinB mutants, glyceraldehyde dehydrogenase TaAlDH and members of multistep phosphorelay from *Arabidopsis thaliana*.

Students:

Bianka Kőhegyi - Budapest University of Technology and Economics, Hungaria
David Novak - Palacky University, Czech Republic

Abstract:

Crystallization of glyceraldehyde dehydrogenase or how to overcome the main bottleneck of macromolecular crystallography

The glyceraldehyde dehydrogenase from *Thermoplasma acidophilum* is a part of cell-free system for production of isobutanol and ethanol from glucose. It participates in oxidation of D-glyceraldehyde to D-glycerate in this synthetic pathway. Wild type of *Ta*AlDH has high substrate selectivity and product tolerance but leaves place for optimization. In order to improve enzyme properties various mutants of *Ta*AlDH were constructed using random approach. Still, for further enhancement of the enzyme knowledge of its three-dimensional structure will be useful[1]. Nowadays one of the main techniques that are widely used for structural characterization of macromolecules is X-ray diffraction analysis of macromolecular crystals. Crystals of *Ta*AlDH wild type and two mutants were already obtained; however, optimization of crystals quality is required.

Optimization of *Ta*AlDH F34M+Y399C+S405N crystals was carried out by alteration of precipitant cocktail parameters, such as protein concentration, concentration of different components of precipitant solution and pH. Best crystals with the size of 130 µm were found in concentration of protein 10 mg/ml in 0,1 M Bicine pH 9,0 and 10% PEG 20 000 as precipitant. To avoid active site cysteine oxidation two different reduction agents (reduced glutathione and DTT) in 1mM concentration were added to protein solution prior to crystallization. It was found that in presence of DTT crystals had flower-like shape, and in presence of glutathione crystals had cubic shape.

For *Ta*AlDH F34M+S405N X-ray diffraction data set was already collected and structure was solved by molecular replacement method using *Ta*AlDH wild type structure as a model. Structure refinement and validation using programs *Refmac5* of the *CCP4* package[2] and *Coot*[3] is now in progress.

PROJECTS

3. Modeling interactions in biomolecules using methods of quantum and molecular mechanics

Project`s aim:
The study of interactions between proteins and several ligands (drugs) and other related bimolecular processes by means of various computational methods, particularly quantum mechanics (QM), hybrid QM/MM methods, molecular dynamics (MD) simulations and molecular docking.

Project leader: David Řeha, Ph.D., reha@nh.cas.cz

2011 - current	Research group leader, Dpt. of Computational Biology, INSB GCRC AS CR
2009 - 2011	Post-doc fellowship, Deparment of Biological Sciences, University of Essex, Colchester, UK
2007 - 2009	Post-doc fellowship, School of Physics and Astronomy, University of Leeds, UK
2005	PhD. in physical chemistry, Faculty of Sciences, Charles University, Prague
2000	Master of sciences (Mgr.) in physical and macromolecular chemistry, Faculty of Sciences, Charles University, Prague

The research of David Řeha is focused theoretical study (ab initio calculations, MD simulations) of bimolecular systems (DNA, RNA, proteins, ...) analysis of inter- and intramolecular interactions (i.e. biomolecules-drug interactions); molecular docking; calculation of spectroscopic constants (IR, NMR, UV) of biomolecules as well as their model systems; QM/MM calculations; performance of existing methodologies; application and development of new methods for computational study of the biomolecules.

Students: Joanna Macnar – University of Warsaw, Poland
Monika Litvinukova – University of South Bohemia, České Budějovice
Johannes Kepler University, Linz, Austria
Sara Matić - University of Zagreb, Croatia

Abstract by Joanna Macnar:
Theoretical analysis of organic solvents effects on Chorismate Claisen Rearrangement using QM/MM calculations

Chorismic acid is an important biochemical intermediate in plants and microorganisms. Its Claisen - reaarranged form - prephenate - can subsequently be converted to aromatic products such as tyrosine or phenylalanine. Chorismate mutase (E.C. 5.4.99.5) is an homotrimer enzyme that catalyzes the rearrangement of chorismate[1]. The enzymatic and nonenzymatic reactions are concerted, asynchronous reactions, which proceed via a transition state with chair-like geometry[2, 3]. Chorismate mutase (CM) has become a popular model system for studies of enzyme catalysis[2].

We performed Molecular Dynamic simulations for explicit solvents rearrangement and hybrid Quantum Mechanics/Molecular Mechanics calculations to analyze influence of the organic solvents on activation energy of Claisen rearangement.

Abstract by Monika Litvinukova:
Examination of the point mutation H134A in the Calcium release-activated Calcium channel Orai 1

The Ca^{2+} release-activated Ca^{2+} (CRAC) channel presents the major calcium transport pathway in many cell types, thereby controlling different vital cell functions. The channel complex is composed of the endoplasmic reticulum Ca^{2+} sensor STIM1 and the plasma membrane Ca^{2+} channel Orai1. Depletion of endoplasmic Ca^{2+} induces STIM1/Orai1 channel aggregates and activation of a highly Ca^{2+} selective influx.

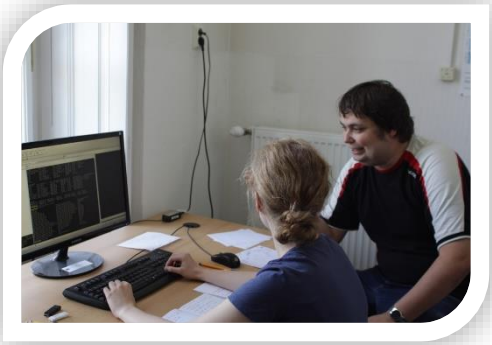
Recent studies in the human patients point out the connection between the defects in the CRAC channel function and the pathophysiological processes as immunity, allergy and cancer. Constitutively active STIM1/Orai1 mutants bypass the endoplasmatic reticulum stop depletion and result in thrombocytopenia, bleeding diathesis, miosis and myopathy.[1,2] The altered expression of STIM1/Orai1 was also linked to the different stages of cancer progression as proliferation, migration and apoptosis.[3]

In this project the point mutation H134A was examined using the molecular dynamics approach. The focus of the experiments was aimed on the pore size of the channel and the mobility of the single chains in comparison to the wild type.

Abstract by Sara Matić:
Molecular dynamics study of LinB haloalkane dehalogenase behavior in different organic solvents

LinB is a haloalkane dehalogenase isolated from *Sphingomonas paucimobilis*. This type of enzymes can catalyze hydrolitic dehalogenation of synthetic haloalkanes, which are hazardous soil contaminants. Haloalkane dehalogenases were originally isolated from soil bacteria and were found to exhibit different specificity for various haloalkane substrates. The substrate specificities of haloalkane dehalogenases mostly depend on differences in the geometry and the aminoacid composition of the active site as well as the entrance tunnel connecting the active site to the protein surface. The reaction mechanism involves an activated water molecule acting as the catalytic base.

However, the haloalkane solubility in water is critical criteria for their availability to the enzyme, in order to enhance their degradation; a different type of co-solvent must be introduced. On the other hand, organic solvents can impact the protein structure and activity. Therefore it is necessary to analyze the protein stability in different solvents as well as the ability of these solvents to enter the active site of the enzyme. Penetration of solvents into the active site, however, must not necessarily lead to the disruption of the enzyme activity, but it is also possible that it can contribute to the solvation of the halide produced in the reaction. Elucidating the behavior of LinB with three different types of co-solvents; acetone, choline cloride and mixture of choline cloride and ethylene glycol, in a reasonable range of concentrations, gives us valuable insight into the mechanisms that can explain their effect on LinB's catalytic activity with co-solvents observed experimentally.



PROJECTS

4. Theoretical investigation of the interactions of hydrated ionic liquids with membranes for bio-applications and drug delivery

Project`s aim:
The objective of this project is to study theoretically the interaction of aqueous solutions of ionic liquids with biologically related compounds in order to understand their roles in possible bio-applications such as drug delivery, protein folding and protein crystallization.

Project leader: Babak Minofar, Ph.D., minofar@nh.cas.cz

2012 - current	Research group leader, Department of molecular liquids, INSB GCRC AS CR
2010 -2012	Post-doc fellowship, Japanese society of science, Kyushu University and Niigata University, Japan,
2007 -2010	Post-doc fellowship INSB GCRC AS CR
2007	PhD. in physical chemistry, Faculty of Sciences, Charles University, Prague
1998	Master of sciences (Msc.) in applied chemistry, Faculty of Sciences, Azad University, Tehran

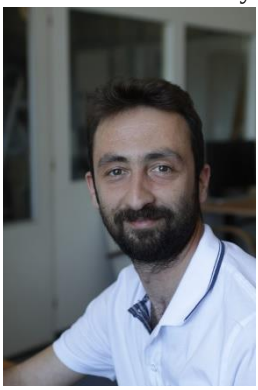
The research of Babak Minofar is focused on structure and dynamics of ions and biomolecules in aqueous and non-aqueous solution in order to understand the dynamics of ions and biomolecules in such media which is not the natural environment for biomolecules which can give valuable information for bio-catalysis in non-aqueous media.

Students:
Kadri GÜLEÇ – Ege University, Izmir, Turkey
Saeid Vakilian – Sharif University of Technology, Tehran, Iran

Abstract:

Computational investigations of haloalkane dehalogenase (DhaA) stability in aqueous solutions of acetone and choline-chloride

A number of halogenated compounds in industrial by-products are environmentally toxic and it has been suggested that haloalkane dehalogenases may be useful catalysts for their biodegradation. Therefore, the optimized functionality of haloalkane dehalogenases in industrial process is limited to find suitable organic solvent system which could stabilize protein and increase the solubility of substrates. Computer simulation was applied as a potent tool to study the effect of aqueous solutions of acetone and choline chloride on the stability of haloalkane dehalogenas DhaA using molecular dynamics simulations. Analysis of root-mean-square-deviations (RMSD) revealed that the presence of a given solvent mainly affects the dynamical behavior of the protein structure. The stability of protein was varied in different concentrations for both acetone and choline chloride. For further investigation, structure flexibility was quantified using root mean square fluctuation (RMSF) which revealed that the presence of a given organic solvent mainly affects the dynamical behavior of the protein. All the residues were more stable in water compared to the both solutions except just 2 residues of protein, which may attribute to the location of these in unfavorable region of the Ramachandran plot. In acetone-water mixture, RMSF shows high fluctuations for loops in the cap domain and those linking the main and the cap domain which can be important for binding and catalysis of different substrates.



PROJECTS

5. Molecular mechanisms of G protein signaling investigated by two-photon polarization microscopy

Project`s aim:
The aim of the project is to determine whether cholesterol in plasma membrane affects conformation and functional activity of heterotrimeric G proteins.

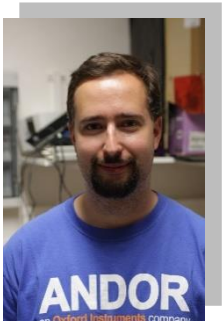
Project leader: Josef Lazar, Ph.D. lazar@nh.cas.cz

2006 – 2007	Columbia Science Fellow, Dept. of Biological Sciences, Columbia University, USA
2002 – 2005	Postdoctoral research fellow, Dept. of Biological Sciences, Columbia University, USA
2001	Ph.D., Medicinal Chemistry, University of Utah, Salt Lake City, USA
1996	M.Sc., Organic Chemistry, Charles University /Institute of Organic Chemistry & Biochemistry AS CR, Prague, Czech Republic

Head, Department of Cell Biology INSB GCRC AS CR

The research of Josef Lazar's group is focused on the development of a voltage sensitive fluorescent protein / Organization of the mammalian odorant and pheromone detection systems

Project leader: Alexey Bondar, Ph.D., bondar@nh.cas.cz



2014	Postdoctoral Research Fellow, Institute of Nanobiology and Structural Biology
2007 – 2014	Doctor of Philosophy (Ph.D.) Biophysics, University of South Bohemia in Czech Budweis
2001 – 2006	Master of Science, Biochemistry, Belarusian State University

Students:
Alina Ralovets - Belarusian State University, Minsk, Belarus
Viktor Navrulin - Kharkiv National University, Kyiv, Ukraine

Abstract:

Regulation of G protein signaling by cell membrane components

G protein signaling cascade is a crucial way of cellular signal transduction. Heterotrimeric G proteins are peripheral membrane proteins which transduce signals from a multitude of extracellular stimuli including hormones, neurotransmitters, light, etc. Malfunction of G protein signaling is directly related to such diseases as stroke, type 2 diabetes and morbid obesity. It has long been speculated that G protein functional activity is regulated by their membrane environment. We have set out to determine the effect of individual cell membrane components on conformation and functional activity of the inhibitory Gi/o proteins.

In order to determine how individual membrane components affect G protein conformation we utilized the technique of two-photon polarization microscopy (2PPM)[1]. 2PPM allows determination of fluorescent protein (FP) orientation with respect to the cell membrane. 2PPM relies on measurements of selective excitation of FP molecules by linearly polarized light (FP linear dichroism) in live cells. It has been previously shown that 2PPM allows sensitive monitoring of protein conformational changes and protein-protein interactions, in living cells, in real time, using a single fluorescent protein tag[2]. We applied 2PPM to determine whether cholesterol and sphingomyelin in the cell membrane affect the conformation of G proteins. We have found that membrane components have distinct effects on G protein conformation. Our results indicate that membrane plays an active role in regulation of the G protein signaling.

PROJECTS

6. Development of fluorescent proteins sensitive to cell membrane voltage

Project`s aim:
To develop a fluorescent protein suitable for observing electrical signals in neurons.

Project leader: Josef Lazar, Ph.D. lazar@nh.cas.cz

2006 – 2007	Columbia Science Fellow, Dept. of Biological Sciences, Columbia University, USA
2002 – 2005	Postdoctoral research fellow, Dept. of Biological Sciences, Columbia University, USA
2001	Ph.D., Medicinal Chemistry, University of Utah, Salt Lake City, USA
1996	M.Sc., Organic Chemistry, Charles University /Institute of Organic Chemistry & Biochemistry AS CR, Prague, Czech Republic

Head, Department of Cell Biology INSB GCRC AS CR

The research of Josef Lazar is focused on the development of a voltage sensitive fluorescent protein / Organization of the mammalian odorant and pheromone detection systems

Students:
Barbora Hoffmannova – Comenius University in Bratislava, Slovakia
Marharyta Semenikhina - Sumy State Pedagogical University, Sumy, Ukraine
Adéla Brzákova, Charles University, Prague, Czech Republic

Abstract:
Development of fluorescent proteins sensitive to cell membrane voltage

Being able to see how brain works has been a dream of scientists for decades. One of the ways to visualize the electrical activity of neurons is using modern genetically encoded fluorescent voltage sensors. Such sensors can be used to visualize the electrical activity of cell population of interest, with high spatial and temporal resolution.

One of the best existing genetically encoded fluorescent voltage sensors is ArcLight. ArcLight's dynamics and large changes in fluorescence intensity have enabled visualizing single action potentials in cultured neurons and even in vivo (Jin, Han et al. 2012; Cao, Platisa et al. 2013). Interestingly, the mechanism of ArcLight's voltage sensitive fluorescent response is not clear. Deciphering the molecular mechanism of ArcLight's response should allow directed improvements of its functional properties. To distinguish between distinct proposed mechanisms of ArcLight's of function is the goal of the present project.

In order to achieve this goal, we have performed combined microscopy/single cell electrophysiogy experiments on ArcLight expressing cells, and analyzed the kinetic profiles of the observed voltage-driven fluorescence responses. Our results got us just one step from revolutionizing neuroscience.

7. Development of optical microscopy into a structural biology technique

Project´s aim:
To develop two-photon polarization microscopy into a novel quantitative technique of structural biology.

Project leader: Josef Lazar, Ph.D. lazar@nh.cas.cz

2006 – 2007	Columbia Science Fellow, Dept. of Biological Sciences, Columbia University, USA
2002 – 2005	Postdoctoral research fellow, Dept. of Biological Sciences, Columbia University, USA
2001	Ph.D., Medicinal Chemistry, University of Utah, Salt Lake City, USA
1996	M.Sc., Organic Chemistry, Charles University /Institute of Organic Chemistry & Biochemistry AS CR, Prague, Czech Republic

Head, Department of Cell Biology INSB GCRC AS CR

The research of Josef Lazar is focused on the development of a voltage sensitive fluorescent protein / Organization of the mammalian odorant and pheromone detection systems

Students:
Olga Rybakova - Saint-Petersburg State Polytechnic University, Russia
Robin Kryštůfek – Charles University, Prague, Czech Republic

Abstract:
Two-photon polarization microscopy[1] (2PPM) is a microscopy technique that allows sensitive observations of molecular processes taking place in living cells and animals. 2PPM should also be able to yield quantitative information on membrane protein structure. Recent work has shown that the combination of 2PPM and single photon polarization microscopy can provide accurate information on orientation of fluorescent

dyes in artificial membranes. It is the goal of the current project to extend the structural biology capabilities of 2PPM, from fluorescent dyes and artificial membranes to fluorescent proteins and living cells.

In order to accomplish this goal we have performed a wide range of experiments involving methods of molecular biology, biochemistry, advanced microscopy, biological image analysis and mathematical modeling. Our experiments have yielded quantitative information on orientation of fluorescent proteins with respect to the membrane in living cells. We have also made progress in obtaining similar information in artificial lipid systems.

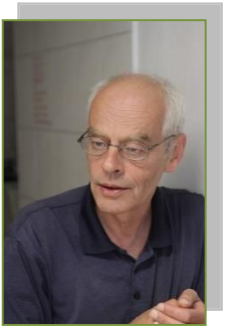
Using our results, we have been able to derive quantitative information on orientation of fluorescent proteins and on the structure of the cytoplasmic membrane in living cells. Our results expand the abilities of 2PPM as a technique of structural biology.

PROJECTS

8. Monitoring intracellular pH changes of yeast cells

Project`s aim:
Analysis of intracellular pH changes of yeast (Saccharomyces cerevisiae) cells upon changes in extracellular pH and external K+ concentration. In the project we'll generate different yeast strains (carrying mutations in K+ translocation system genes) producing the genetically encoded pH sensor pHluorin. These strains will be verified by fluorescence microscopy. Eventually time resolved measurements of intracellular pH will be carried out using a fluorescence microplate reader. Mainly the response of intracellular pH upon changes of external pH and external K+ concentration will be analyzed.

Project leader: PD Dr. Jost Ludwig, jost.ludwig@uni-bonn.de



Leader of Department of membrane physiology and bioenergetics INSB GCRC AS CR
Dr. Ludwig's work and his group is best known for functional analysis of ion channels and transporters, cation transport in yeast (Saccharomyces cerevisiae), cation homeostasis in yeast (more specifically: Localization of cation transport proteins and regulatory proteins, cation flux measurements using ion selective electrodes), and his research in multiple drug resistance with the analysis of promoters involved in expression of MDR relevant genes.

Students:
Sabina Chubanova - Belarussian State University, Minsk, Belarus
Katarína Mackova - Pavol Jozef Šafárik University, Košice, Slovakia
Katarina Siroka - Comenius University Bratislava, Bratislava, Slovakia

Abstract:
The role of the *Saccharomyces cerevisiae* K^+ translocation system Trk2

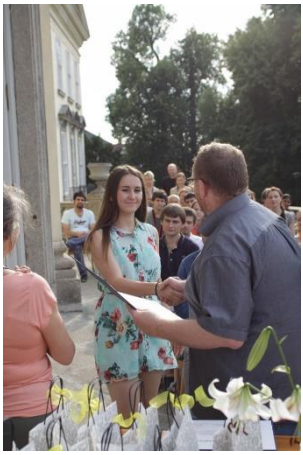
Potassium ions play an important role in the maintenance of living cells homeostasis through the retention osmotic pressure and plasma membrane potential. The intracellular concentration of cations also influences the regulation of enzymatic reactions speed and rate. It is known that yeast cells are able to grow in environments with a wide range of external [K^+] from less than 100 μM to more than 1 M while keeping intracellular [K^+] almost constant. This is achieved by a diverse net of ion translocation systems. In *Saccharomyces cerevisiae*. K^+ translocation system consists mainly of Trk1 and Trk2 for K^+ uptake, and Ena1, Nha1 and Tok1 for efflux. Trk1 is the primary K^+ uptake system [1, 2], whereas Trk2 is less effective,

as for its low expression level. The aim of our study was to analyze whether Trk2 in any way influences or modulates K^+-translocation at all.

In our study, *S. cerevisiae* mutants lacking *TRK2* (*Δtrk2)* or lacking *TRK1*, *TRK2* and TOK1 (*Δtrk1,2*, *tok1)* were compared with wild type cells. In order to analyze the properties of Trk1 and Trk2 independent from their amount, TRK1 and TRK2 genes were re-introduced into the *Δtrk1,2*, *tok1 strain* under control of the (in *S. cerevisiae* constitutively active) *Candida albicans* CDR1 promoter (P_{CDR1}). Ion flux measurements were carried out using FLISE (flux determination using ion-selective electrodes, [1]). Otherwise isogenic strains, transformed with a plasmid from which pHluorin, a pH-dependant GFP derivative was expressed, were used to determine changes in intracellular pH.

Using FLISE we observed a slower onset of K^+ uptake when K^+-starved and de-energized cells lacking Trk2 were exposed to KCl and energized with glucose as compared to wt cells. Consistent with that, internal alkalization of Δ*trk2* cells after KCl addition and energization was also slowed down compared to wt. The kinetics of K^+ uptake in P_{CDR1}-*TRK1* and P_{CDR1}-*TRK2* cells was very similar; we did not observe any significant kinetic or pH changes.

The results of this work indicate that Trk2 might play a role in the rapid sensing of changes of external [K^+]. Our results are also consistent with the existence of a heteromeric K^+-translocation system containing Trk1 and Trk2.



9. Single-molecule investigation of mechanisms behind epigenetic regulation of insulin gene

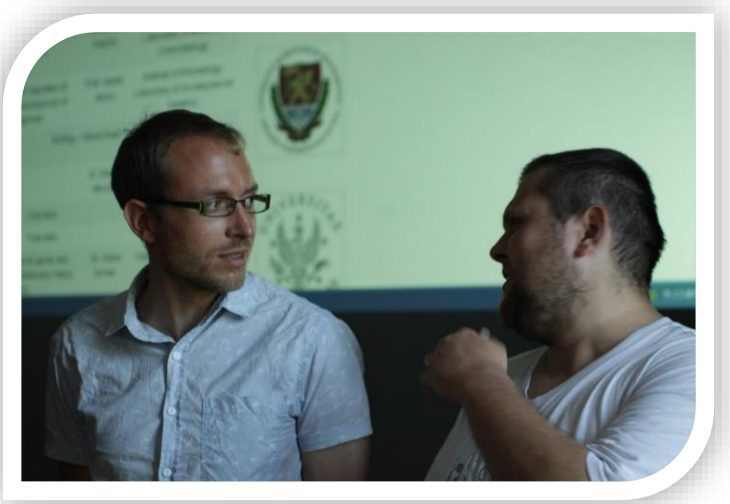
Project's aim:
The goal of this project is to elucidate structural mechanisms behind epigenetic control of insulin gene. In particular we will assess structural impact of methylation on two candidate regulatory sequences. First, G-rich sequence, with propensity to form G-quadruplex with complex folding topology. Second, complementary C-rich sequence, which is known to be in dynamic equilibrium between i-motif and hairpin in near-physiologic region of pH.

Project Leader: Tomáš Fessl, Ph.D., fessl@prf.jcu.cz

2014-now	Postdoc: Single-Molecule Spectroscopy & Nucleic Acid Structure and Dynamics Lab, Faculty of Science, University of South Bohemia, ČR
2012-2014	Postdoc, Nucleic Acid Structure Research Group CRUK, College of Life Sciences, University of Dundee, UK.
2013	Ph.D. (hons) in Biophysics, Faculty of Science, University of South Bohemia, ČR.
2010	FEBS collaborative internship at the Astbury Centre for Structural Molecular Biology, University of Leeds, UK.

Project Leader: Łukasz Bujak, Ph.D., lbujak@prf.jcu.cz

Tomas and Lukasz focus on single-molecule studies of structure and dynamics of nucleic acids, with particular interest in elucidation of mechanism of pigenetic regulation of transcription via chemical modification of DNA.



Students:
Katsiaryna Nikitsenka - Belarusian State University, Belarus
Ewa Tratkiewicz - Jagiellonian University, Kraków, Poland

Abstract:

All-optical investigation of mechanisms behind epigenetic regulation of insulin gene

The insulin-linked polymorphic region (ILPR) is upstream of the gene coding for insulin and it is able to form an i-motif. This structure is formed by dimerization of two C–C+ hairpins under acidic conditions and it was widely assumed that i-motives are not stable under physiological pH. However, recent advances have shown that certain i-motives are stable under near-physiological range of pH`s. The stability is also highly dependent on other factors such as sequence, environmental conditions (macromolecular crowding, presence of small cosolutes…) and epigenetic modifications. Stability of an i-motif structure is crucial because its folding and/or unfolding plays important role in regulation of RNA transcription.

In this summer school project, the dependence of above mentioned factors on stability of ILPR i-motif was investigated using circular dichroism (CD) and Förster Resonance Energy Transfer (FRET). CD and FRET analyses showed that methylation of cytosines in three different sites of the ILPR i-motif has different effect on stability of the structure. Extent of this stabilization differed depending on methylation site and presence of macromolecular crowding. In detail, the most stable methylation site was in the center of i-motif core and macromolecular crowding significantly increased the stability.

Single-molecule FRET experiments have further indicated that i-motives are in dynamic equilibrium between unfolded and folded states.

Best presentation award

From left: Jacob Fondriest, Daniel Wood, John Martin, Robin Krystufek, Olga Rybakova, Sabina Chubanova, Katarina Siroka, Katarina Mackova, Sara Matić

Sponsored by Visegrad Fund
Robin Krystufek
Olga Rybakova
Sabina Chubanova
Katarina Siroka
Katarina Mackova
Sara Matić

Sponsored by National Science Foundation
Jacob Fondriest
Daniel Wood
John Martin

Conference of the
Annual Summer School in Molecular Biohphysics and Systems Biology

Nové Hrady, 2015

Presentation of results - Friday July 24, 2015 **Diplomas and Awards**		
Start time	***Topic / title***	***Student***
9:00	*Welcome / Introduction*	
9:10	***Session 1***	
9:10		Artem Dubovetskyi Valeria Kopats
9:30		Bianka Kőhegyi David Novak
9:50		Sabina Chubanova Katarína Mackova Katarina Siroka
10:15	*Coffe / tea*	
10:35	***Session 2***	
10:35		Viktor Navrulin Alina Ralovets
10:55		Barbora Hoffmannova Marharyta Semenikhina Adela Brzakova
11:15		Olga Rybakova Robin Krystufek
11:35		Ewa Tratkiewicz Katsiaryna Nikitsenka
11:55	*Coffea / tea*	
12:10	***Session 3***	
12:10		Joanna Macnar

12:22		Monika Litvinukova
12:34		Sara Matić
12:46		Kadri GÜLEÇ
12:58		Saeid Vakilian
13:10	*Lunch*	
14:20	***Session 4***	
14:20		Virginia Lane
14:35		Nicholas Luedtke
14:50		Brianna L. Hnath
15:05		Jacob Fondriest
15:20	*Coffea / tea*	
15:40	***Session 5***	
15:40		Emma Stavropoulos
15:55		John Martin
16:10		Laura Tociu
16:25		Daniel Wood
18:00	*Ceremonial presentation of participation certificates and best presentation awards.*	Rector prof. Libor Grubhoffer
19:00	*Farewell party*	

More about annual summer school can be found at:

http://auc.cz/summerschool2015

PHOTO GALLERY

Conference Center AV ČR

Château Nové Hrady

Conference Centre ASCR Nove Hrady - New Castle in the historical building of the Empire Château offers modernly equipped conference facilities, comfortable accommodation, gastronomic services, restaurant and finally a large park, which is due to its location an ideal for exploring the beautiful surrounding countryside Novohradske Mountains.

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INSTITUTE OF NANOBIOLOGY AND STRUCTURAL BIOLOGY GCRC AS CR

The Institute of Nanobiology and Structural Biology, (INSB) in Nové Hrady and Ceske Budejovice carries out research in two basic fields:

- systems biology on a molecular, cell, tissue, and organism level. Hence it provides knowledge on the molecular structure of structural system elements, their principal metabolic and control pathways, identifies links between these elements and thus describes the structure of biological systems.

- nanobiotechnology, focusing on research, development and application of new types of biocompatible (nano)composites, and further on interactions of biological structures with nano- and microparticles and chemical and physical factors.

In the application field, INSB carries out highly specialized activities in targeted applications and development. The laboratories and educational center in Nové Hrady were established in 2002. Methods were successfully implemented from bioinformatics, molecular biology, microscopy, molecular modeling and structure determination, mainly X-ray diffraction.

The Campus in Nove Hrady was, from the very beginning, meant to serve not only as a research but also as a scientific training facility, and organizes a reasonable large number of courses, workshops, conferences and symposia. Despite the short time of their existence, the INSB research groups already achieved excellent scientific results published in highly visible journals such as Nature, Nature Structural & Molecular Biology,

PLOS Computational Biology, etc. Thanks to that, scientists from Nove Hrady are regularly invited to speak at international conferences and symposia, get invitations to contribute review papers, book chapters and invited papers. INSB is regularly visited by foreign scientists and participants of international internship programs.