Visegrad Fund

C4Sys

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

2 nd SUMMER SCHOOL IN MOLECULAR BIOPHYSICS AND SYSTEMS BIOLOGY

7TH JULY – 27TH JULY 2014

NOVE HRADY
CZECH REPUBLIC

2nd SUMMER SCHOOL IN MOLECULAR BIOPHYSICS AND SYSTEMS BIOLOGY

The scientific and research Summer school in molecular biophysics and systems biology supported from International Visegrad Fund, jointly organized in the Academy and University Center Nove Hrady, by the Faculty of Sciences of the University of South Bohemia in Ceske Budejovice, the Institute of Nanobiology and Structural Biology of the Academy of Sciences of the Czech Republic, the University of Szeged in Hungary, Jagiellonian University in Krakow and Warsaw University, Poland and Comenius University in Bratislava, Slovakia, gives the chance to students from the Visegrad region as well as foreign university students to work with specialists – tutors in real scientific research, listen to exceptional lectures, and get a broader perspective on current methodologies and research.

Students selected by the committee of tutors-leaders of the research teams according to the strict criteria work in excellent fully-equipped laboratories on topics related to systems biology and molecular biophysics. Invited excellent lecturers present a wide range of methods and techniques used in the study of systems biology. At the end of summer school, the research teams present the results of their work in the summer school conference and competition. Their presentation are evaluated by the scientific committee and the best three presentations are awarded. Each participant is given a certificate of participation.

10 chosen students are sponsored by Visegrad Fund and 7 students by the Institute of Nanobiology and Structural Biology GCRC, Academy of Sciences of the Czech Republic.

PARTNERS

Visegrad Fund

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Universytet Warszawski (University of Warsaw)
Universytet Jagiellonski (Jagiellonian University)
Szegedi Tudomanyegyetem (University of Szeged)
Univerzita Komenského v Bratislave (Comenius University in Bratislava)

PROJECT EVENTS

April 28th and June 6 th, 2014 MEETING OF SUMMER SCHOOL ORGANIZING TEAM

07th – 27th July 2014 SUMMER SCHOOL IN MOLECULAR BIOPHYSICS AND SYSTEMS BIOLOGY

http://auc.cz/summerschool2014

Ladies and gentlemen, dear friends, dear participants,

a warm welcome to Nové Hrady and the 2st Visegrad Summer School in Molecular Biophysics and Systems Biology organized jointly by the Jagiellonian University in Crakow and Warsaw University, Poland, the University of Szeged, Hungary, Comenius University in Bratislava, Slovakia, the Institute of Nanobiology and Structural Biology of the Academy of Sciences here in Nove Hrady, and the University of South Bohemia in Ceske Budejovice, Czech Republic.

The Visegrad Summer School builds on two traditions, the first is the close collaboration of the above mentioned institutions in the field of computational simulations and spectroscopy of biologically relevant systems, following the tradition of the well established Visegrad Symposia 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 Romania and especially from the Belarusian State University in Minsk, which was made possible by a recently signed memorandum of understanding between our institutions, and 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 is sponsored 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 an unique opportunity to get in touch with "real" cutting-edge science and experience how science works. The lecture series held not only by our scienctists but also by various internationally well-recognized speakers complemets 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 summer!

Professor RNDr. Rüdiger H. Ettrich, Ph.D.

Professor of Biophysics
Faculty of Sciences of the University of South Bohemia
and
Academy of Sciences of the Czech Republic

SUPERVISORS

Professor RNDr. RÜDIGER H. ETTRICH, Ph.D.
University of South Bohemia in Ceske Budejovice

Dr. rer. nat. et Doc. JOST LUDWIG
UNIVERSITY OF BONN

JOSEF LAZAR, Ph.D.
ACADEMY OF SCIENCES OF THE CZECH REPUBLIC, INSB GCRC

PROJECT LEADERS

PROF. RNDR. RÜDIGER H. ETTRICH, Ph.D.
JOSEF LAZAR, Ph.D.
RNDr. BABAK MINOFAR, Ph.D.
MGR. DAVID ŘEHA, Ph.D.
MGR. KATSIARYNA SHAMAYEVA
EKATERINA TUTUBALINA, MSc
IULIIA IERMAK, MSc
ALEXEY BONDAR, Ph.D.
ALINA KEVORKOVA, MSc
DR. RER. NAT. HABIL. JOST LUDWIG

Summer school - preparation

SPEAKERS

Prof. RNDr. Rudiger Ettrich, Ph.D.
Institute of Nanobiology and Structural Biology, Academy of Sciences of the Czech Republic, and University of South Bohemia in České Budějovice
Lecture - Was binding of free aminoacids an early innovation in the evolution of allostery?

Interpretation of thermodynamic ligand-binding data through the lens of molecular dynamics has led to a structural and energetic description of the molecular mechanism of allostery for the hexameric E. coli arginine repressor, the master feedback regulator of transcription in L-arginine metabolism, which displays strong negative cooperativity of L-arginine binding. A controversial prediction of the famous allostery model of Monod, Wyman, and Changeux is that constraints imposed on protein subunits by multimerization are relaxed by ligand binding, but with conservation of symmetry in partially-liganded states. Molecular dynamics simulations reveal that conserved Arg and Asp sidechains in each L-arginine binding pocket promote rotational oscillation of apoArgR trimers by engagement and release of salt bridges. Binding of exogenous L-arginine displaces resident Arg residues and arrests oscillation, shifting the equilibrium quaternary ensemble and promoting subunit motions that generate an entropic driving force while maintaining symmetry in partially-liganded states. Computational simulations supported by experimental data indicate that partially-liganded states can be structurally symmetric despite their conceptual asymmetry. The symmetric relaxed state is visualized as a multimer with all subunits anchored near the center, and with motions transferred to the periphery like a bouquet of balloons in strong wind. Thus, even during sequential filling of binding sites, symmetry can be maintained by exploiting the dynamics of the assembly and the distributed nature of its cohesive energy. The mechanism suggests the possibility that binding of free amino acids was an early innovation in the evolution of allostery.

Prof. RNDr. Tomáš Polívka, Ph.D.
University of South Bohemia in České Budějovice
Lecture – History and Present of Nobel Prize

Bohdan Schneider, Ph.D.
BIOCEV - Biotechnology and Biomedicine Center of the Academy of Sciences and Charles University in Vestec
Lecture - Habilitation and presentation in the field of Biophysics - Structure and Dynamics of Nucleic Acids

The lecture discusses nucleic acid chemical composition and spatial architecture, compares local DNA and RNA structures, and shows their simiarities and differences, especially foding of RNA in 3D. After introducing nucleic acids, the talk concentrates on original research results of the author. Summarized are mainly studies of RNA and DNA local structure ("dinucleotide conformers"). Briefly is described original method

of "Fourier Averaging", technique which the author developed. The dinucleotide conformers were used to analyze protein/DNA interaction interfaces; the results of the analysis are briefly discussed.

RNDr. Martina Roeselová, Ph.D.
Institute of Organic Chemistry and Biochemistry ASCR, v.v.i.
Lecture – Water we (sometimes) don't see: Molecular simulations of water on surfaces

Water in the environment exists in three forms: as liquid, solid (ice), and vapor. Water vapor molecules, present in the air, get in contact with various objects (houses, roads, plants, etc.) and can adsorb at the surface of these objects. This water, "bound" to the surface, may dramatically change the surface properties, both physical, such as its optical reflectivity, as well as chemical. For example, some chemical reactions, occurring at surfaces, would not proceed without the "help" of the water molecules present there. The amount of surface-adsorbed water depends on the humidity of air and on the "water-binding" properties of the surface itself. This lecture will show how molecular dynamics computer simulations can be used to investigate – at the level of individual molecules – water adsorption on surfaces of different chemical composition, hence, of different hydrophilic/hydrophobic character. Together, we will try to answer questions such as the following: How much of water is there on the surfaces around us? What is the structure of surface-adsorbed water? Does it look (and behave) the same as "normal" liquid water? Finally, we will discuss few examples of environmental processes in which surface-adsorbed water plays an important role.

Prof. Maik Kschischo, Ph.D.
University of Applied Sciences, Koblenz, RheinAhrCampus Remagen
Lecture - Modelling with uncertainty: Examples from Cancer & Microbial Systems Biology

Mathematical models are useful tools for analysing and predicting the emergent properties of living systems. However, mechanistic as well as statistical modelling of complex biological systems is often hindered by insufficient or uncertain knowledge. Tools and methods for handling these uncertainties are urgently needed. For mechanistic (ordinary differential equation) models we recently proposed the Reverse Tracking Algorithm (RTA) for the inference of dynamical modelling errors. The utility of this algorithm is presented for a recent model of potassium homeostasis in yeast cells, where essential and previously unknown regulatory inputs were predicted and subsequently verified by experiments.

Another source of uncertainty is intrinsic heterogeneity between individual cells. In solid tumours, cell to cell variability in genetic copy numbers is frequently observed and associated with prognosis and drug sensitivity. An important aspect of this tumour heterogeneity is chromosomal instability and aneuploidy and we will review recent

results regarding the origins and core regulatory mechanisms involved. Based on the analysis of many data sets, we will discuss the hypothesis that chromosomal instability is a design principle of cancer evolution involved in proliferation and progression and that a deeper understanding of tumour heterogeneity is essential for personalised treatment strategies.

RNDr. Babak Minofar, Ph.D.
Institute of Nanobiology and Structural Biology, Academy of Sciences of the Czech Republic
Lecture - Structure and dynamics of biomolecules in aqueous and non-aqueous solutions

Biomolecules can be solvated in both aqueous and non-aqueous media such as organic solvents and ionic liquids, leading either to conformational changes in their structure, or changes in their enzymatic activity. Using enzymes in aqueous solution of organic solvents and ionic liquids bring benefits forindustries as majority of substrates are insoluble in water and organic media can improve solubility. Although organic solvents and ionic liquids can improve the solubility of such non-soluble compounds, solutions containing organic solvents can not be used at all concentrations for enzymatic reactions thus limiting the usage of organic solvents in industrial applications. Non-aqueous solutions are not only changing the native structure of enzymes or their catalytic activity, but they may lead even to denaturation of biomolecules.

Mrg. David Řeha, Ph.D.
Institute of Nanobiology and Structural Biology, Academy of Sciences of the Czech Republic
Lecture - Biological Applications of QM/MM calculations

During the lecture the two most common approaches to calculate energy of molecules used for computational biology will be introduced; quantum mechanics (QM) and Molecular mechanics (MM). The advantages and disadvantages of both approaches will be compared and demonstrated on the examples of typical applications used for computational biology. The most common MM applications where the advantages of MM (speed and efficiency, possibility to model system with more than 100000 atoms) can be applied are MD and MC simulations and molecular docking. Disadvantages of MM approach (need for parameterization, no explicit polarization and impossibility to model chemical reactions) can be tackled by QM methods.

PROJECTS

1. Crystallization of proteins with Ionic Liquids

Project`s aim:

During the Summer School 2014 student get a basic information about protein, its parameters, properties and structure and how do they form crystals. Also the intern will know how to crystallize protein samples using several standard methods and modern crystallographic techniques; find optimal condition due to obtain "nice' crystals of proteins trying to use all crystallization methods and broad spectrum of precipitant solutions.

After getting basic knowledge, selected enzymes (model proteins such as lysozyme, thaumatin, glucose isomerase) will be crystallized with ionic liquids as additives in various crystallization plates (Hampton Research – CA, USA, MDL – UK, Emerald BioStructures, WA, USA) for hanging or sitting drops and in capillary tubes (Triana Sci & Tech, Spain) at room temperature and at 4 deg. Conditions that yield crystals of any kind will be repeated once again but without ILs to compare influence of ILs on crystallization process (quality of crystals, their shape and size, etc)..

Project leader: Ekaterina Tutubalina, MSc, tutubalinakate@mail.ru

2012 – current PhD student at the Faculty of Science, University of South Bohemia in Ceske Budejovice

2010 Biology Faculty of the Belarusian State University in Minsk

Student: Mihail Shapira, Belarusian State University, Belarus

Abstract:

Using Ionic Liquids in Protein Crystallization

The fact that many enzymatic catalytic reactions take place in aqueous solutions, some of them can also take place in non-aqueous solutions of organic solvents or ionic liquids (ILs), can open a new opportunity for scientists to work with enzymes, which are unstable in aqueous solutions. Ionic liquids are organic salts composed of separate cations and anions, which are liquid at room temperature. They often have negligible vapor pressure, high thermal stability, but their key feature is their 'tunable' nature. ILs has been reported to influence on thermal stability of proteins, to stabilize molecular structure and enzyme's activity, to increase crystallization rates and crystal size, to enhance the kinetics of crystallization process.

The investigation of using ionic liquids as additives for advanced crystallization was held. Seven ILs (Ionic Liquid Screen kit, Hampton) were incorporated in the experiment for their effect on the lysozyme, glucose-6-phosphate isomerase and trypsin crystallization. Experiments were set up with 50% w/v ILs added to the crystallization solutions in ionic liquid/reservoir ratios 1:5, 1:9, 1:14 and into the drops in ionic liquid/drop ratios 1:5, 1:9 and 1:14. Crystallization droplets were set up at protein/precipitant ratio 1:1. Crystals were obtained using Sitting Drop Vapor Diffusion under non-optimal conditions for each protein. During this work the molecular dynamics simulation was held using GROMACS packet. In this simulation interactions between protein molecule and molecules of ILs were studied. Crystallization experiments showed that addition of ILs could led to less crystal polymorphism and larger the protein crystals or ILs could cause the opposite effect. The influence of ILs on crystallization process depends on concentration of ILs and its chemical properties.

PROJECTS

2. Crystallization of haloalkane dehalogenase LinB mutants

Project`s aim:

Haloalkane dehalogenase LinB isolated from a bacterium Sphingobium japonicum UT26 has relatively broad substrate specificity and can be potentially used for biosensing and biodegradation of environmental pollutants. During the project the student will study various techniques of proteins crystallization such as sitting drop vapour diffusion, hanging drop vapour diffusion and microseeding. These techniques will be then applied for crystallization of LinB mutants.

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

2013 - current PhD student at the Faculty of Science, University of South Bohemia in Ceske 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*.

Student:

Alzbeta Roeselova, Gymnázium Jana Keplera Prague

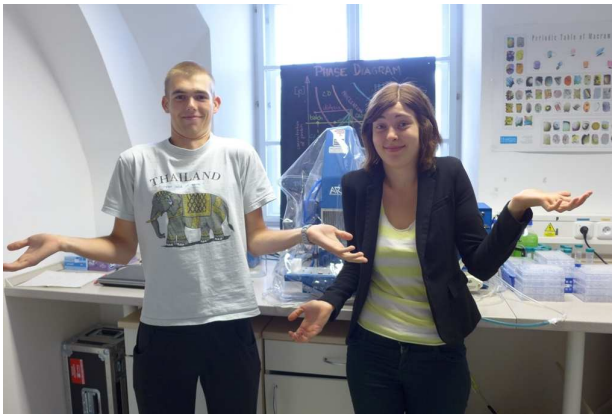
Mykyta Markevych, Kharkiv National Medical University, Ukraine

Abstract:

Crystallization of haloalkane dehalogenase LinB mutants

LinB is a microbial enzyme of the haloalkane dehalogenase family that catalyze the cleavage of the carbon-halogen bond in halogenated aliphatic pollutants, resulting in the formation of a corresponding alcohol, a halide ion and a proton. Haloalkane dehalogenase LinB isolated from a bacterium Sphingobium japonicum UT26 has relatively broad substrate specificity and can be potentially used for biosensing and biodegradation of environmental pollutants [1]. Different variants of haloalkane dehalogenase LinB were constructed with a goal to study the effect of mutations on enzyme functions [2]. In LinB73 (D147C+L177C) variant mutations lead to blocking of the main tunnel by formation of disulfide bridge. In LinB81 (W140A+F143L+D147C+L177C+I211L) main tunnel is also closed by disulphide bridge and alternative way is opened for connection of the deeply buried active site with the surrounding solvent.

For crystallization of LinB mutants various techniques were applied such as vapour diffusion (hanging and sitting drop), microbatch under oil and free interface diffusion in capillary. Crystals of LinB mutants were obtained in crystallization conditions from different commercial screens: PEGs Suite (QIAGEN, Netherlands), PGA Screen (Molecular Dimensions Ltd., UK) and Structure Screen 1&2 (Molecular Dimensions Ltd., UK).



Optimization of crystals and co-crystallization with enzyme's ligands following by further X-ray diffraction analysis will follow.

PROJECTS

3. Expression, purification, restriction and ATPase activities in mutated subunit HsdR from EcoR124I restriction-modification complex

Project`s aim:

During the project, students are able to get practical experience in production, purification and analysis of biochemical properties of proteins.

Project leader: Mgr. Katsiaryna Shamayeva, shamayeva@nh.cas.cz

2008-2013 PhD student at the Faculty of Science,
University of South Bohemia in Ceske Budejovice
2011-2012 Schola Ludus, project leader
2008 Schola Ludus, participant
2003-2008 Belarusian State University,
Biological Faculty, Belarus, Minsk

The research of Katsiaryna Shamayeva is focused on restriction-modification enzyme EcoR124I, mechanism of restriction and translocation.

Student: Gabriela Fiser, Biological Research Centre of the Hungarian Academy of Sciences

Abstract:
Expression, purification, restriction and ATPase activities in mutated subunit HsdR from EcoR124I restriction-modification complex

EcoR124I is restriction-modification complex which protect bacteria against foreign DNA. It consist three different subunits: HsdS which responsible for binding DNA, two HsdM subunits which responsible for DNA methylation and two HsdR subunits which responsible for the endonuclease DNA cleavage and it is also an ATP-dependent DNA translocase. HsdR subunit is biggest part of whole complex and consists of four different domains: endonuclease domain with catalytic site, two rec-A-like helicase domain and helical domain. In the work presented here, we selected one residue D881 from helical domain. D881 was replased by Alanine with neutral charge. The aim of the work is preparation of expression systems for purification of mutant HsdR. Obtained mutant HsdR subunit will be after in vitro reconstitution wit methyltransferase (MTase) tested for ATPase and restriction activity.

PROJECTS

4. 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 leaders: Mgr. David Řeha, Ph.D., reha@nh.cas.cz

Research group leader, Dpt. of Computational Biology, INSB GCRC AS CR
PhD. in physical chemistry, Faculty of Sciences,
Charles University, Prague, 2005.
Master of sciences (Mgr.) in physical and macromolecular chemistry, Faculty of Sciences, Charles University, Prague, 2000.

Students:

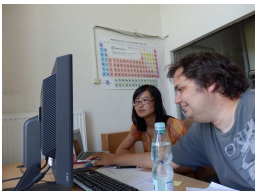
Yani Zhao, University of Szczecin, Poland
Stanislav Šimko, Comenius University in Bratislava, Slovakia
Marta Strumillo, University of Warsaw, Poland

Abstract:

The study of the stability of E. coli WrbA dimer by Molecular Dynamics simulations

Author: Yani Zhao

The WrbA protein from Escherichia coli is a multimeric flavodoxin-like protein, which was crystallized in the presence of FAD or the native cofactor FMN. The WrbA protein forms tetramer under standard conditions. The four subunits bind together over small and large interfaces. It has been found experimentally that, WrbA in solution participates in a dimer-tetramer equilibrium at low temperature T=278K, while for T=300K, the only tetrameric structure of WrbA dominate. It still remains unknown, whether the structure of dimers is formed over the small or large interface. To find out which



kind of structure is favorable for dimers, we have performed the molecular dynamics simulations for dimers formed over both the small interface and large interface. In both cases, the apoprotein and holoprotein are considered. Our calculations have shown that at higher temperature T=300K, the dimers formed over small interface are more likely than the dimers formed over large interface. We have also studied the stability of E. coli WrbA at low temperature T=278K, the calculated interaction energies of dimers suggested that there is no special preference for dimers formed over small or large interface.

QM/MM study of 3-hydroxyaspartates as inhibitors of human serine racemase (hSR)

Author: Stanislav Šimko

D-serine is naturally occurring amino acid that acts as coagonist in glycine site of N-methyl- D-aspartate (NMDA) signalling receptors in mammals brains. These receptors are important especially at beginning of the brain development, for learning abilities and for memory. Their over-activation or under-activation can lead for example to Alzheimer's disease or schizophrenia. Therefore, by regulating the D-serine concentration one could improve health of patients with these diagnoses. This regulation can be driven by PLP dependent protein serine racemase (SR) that catalyzes direct conversion (racemization) of L-serine to D-serine. It has been shown that mouse SR also converts L-serine and L-serine-O-sulfate into pyruvate by β-elimination, that probably occurs at the same active site [1] .

In this work we have studied interactions of human SR (hSR) with its ligand L-erythro-3- hydroxyaspartate and its enantiomers in neutral and ionic form and calculated their interaction energies by means of QM/MM methods. For QM region we chose ligand with PLP cofactor of hSR and MM region was rest of the protein. We docked all of the enantiomers with hSR and chose the best conformations of dimers according to GLIDE docking score for which we calculated interaction energies. Minimization of energies of these dimers was performed on * DFT-B3LYP level theory with 6-31G basis set of QM region and OPLS_2005 force field for ** MM region. Energies of final structures were calculated by the same methods with 6-31G++ basis set. All MM calculations were performed utilizing Schrödinger programs package [2]. Most stable ionic dimer that we observed was that with D-erythro-3-hydroxyaspartate followed by L-erythro-3-hydroxyaspartate enantiomer which was previously specified as best competitive inhibitor of the hSR of the studied molecules [1].

Marta unexpectedly had to depart earlier from the project, therefore there is not abstract of her work.

Marta Strumillo

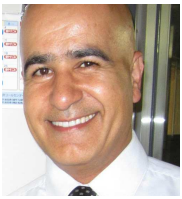
PROJECTS

5. 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: RNDr. Babak Minofar, Ph.D., minofar@nh.cas.cz



Junior research group 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.

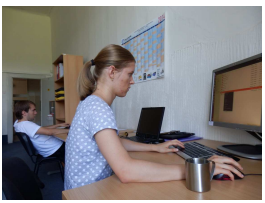
Students:

Katarína Stančiaková, Comenius University in Bratislava, Slovakia

Pavel Kviatko, Belorusian State University, Belarus

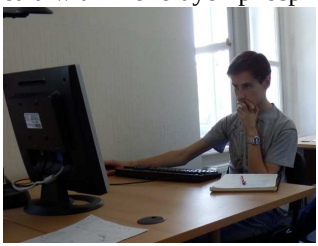
Abstract:

Theoretical investigation of the interactions of chemical pollutants with phospholipids



In recent time various chemicals and materials were developed for a range of various applications and now are replacing their old and inefficient analogs. Though, their eco- and cell toxicity are still not well-studied. The aim of our study was the investigation of interactions of such compounds as room- temperature ionic liquids (RTILs), carbon nanostructures (CNS), humic acids and trichloroacetic acid (TCAA)

with phospholipids, as there is some evidence that such interactions underlie their cell toxicity. During the study we utilized methods of MD simulations to evaluate the effect of structure of both phospholipids and the above-mentioned chemicals as well as their concentrations and combinations on the resulting interactions. Phospholipids are the major compound of natural surfactants that are responsible for reducing the surface tension for breathing. Because the main pathway of infiltration of the above-mentioned pollutants is inhalation into lungs (or gills for aquatic organisms), in our simulations we dealt with monolayer phospholipid structures, that are present in surfactants. In our



simulations we observed differences in interactions with phospholidids according to different content of hydrophilic and hydrophobic groups in the studied compounds. The analysis of the density profiles of the resulting configurations of our systems as well as the surface tension of their components was made. In most cases we observed just small quantitative effects on these parameters, while in some cases the changes were dramatic (formation of water pores with TCAA or penetration of monolayer by fulvic acid ions). The dependency of the effect from the concetration was mostly linear, though in some cases the were obvious critical points. Generally, DPPC monolayer was more sensitive to the pollutants, than the POPC monolayer. As we can see, the obtained results undreline the need for the evaluation of toxicity of the above- mentioned compounds due to their possible toxic effect on organisms.

PROJECTS

6. 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 leaders: 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

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:
Katsiaryna Maskalenka, Biology faculty at Belarusian State University
Kajetan Sawa, Jagiellonian University in Kraków

Abstract:

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

G proteins and G protein-coupled receptors are key players of cell signaling and intercellular communication. They detect and transduce signals from a multitude of physical and chemical stimuli, including hormones, neurotransmitters, odorants, light, flavours etc. We were interested in molecular mechanisms of signal transduction through various G-proteins. cholesterol-enriched membrane compartments, such as lipid rafts or caveolae. Cholesterol of these compartments is thought to affect the G protein conformation and regulate the G protein functional activity.

We determined the role of cholesterol in G-protein signal transduction by studying fluorescently labelled G-proteins subunits in intact and cholesterol-depleted cells using the technique of two-photon polarization microscopy. It has been proposed that certain types of G-proteins localize to The other aspect we found worth inquiring was whether G-proteins are interacting with receptors even before stimulation. Using wild type constructs and non-dissociating mutants we acquired data suggesting, that there is no precoupling between adrenergic receptor and G-protein. Further enquiry is required in order to confirm our findings. It is worth noting, however, that those findings are in consistency with data of other researchers.

PROJECTS

7. Development of fluorescent proteins sensitive to cell membrane voltage

Project`s aim:

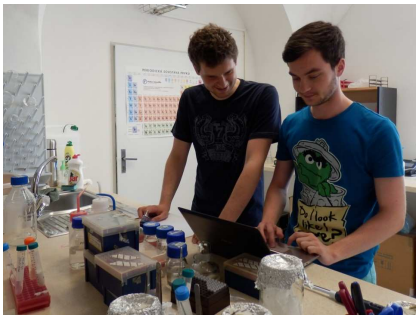
To develop a fluorescent protein suitable for observing electrical signals in neurons.

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

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:
Ivan Kulik, Belarusian State University
Jakub Czuchnowski, Belarusian State University

Abstract:
Two-photon polarisation microscopy (2PPM) is a novel technique that enables monitoring orientational changes in fluorescent molecules associated with lipid membranes through measurements of differences in fluorescence intensity excited by orthogonal linear polarizations of the excitation laser beam. The main aim of this project was to evaluate the ability of polarization microscopy to make quantitative determinations of fluorescent molecule orientations in a simple model system consisting of a dye (DiI) embedded in giant unilamellar vesicles (GUVs). Our experimental data suggest that the dye molecules are oriented close to parallel to the membrane. Quantitative analysis shows an excellent agreement of our experimental data and results of molecular dynamics simulations. We conclude that polarization microscopy can indeed be used for quantitative determinations of molecular orientations in simple model systems and suggest that our approach could be also applicable to complex molecules in biological membranes.



PROJECTS

8. 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
Alina Kevorkova, MSc, kevorkova@nh.cas.cz

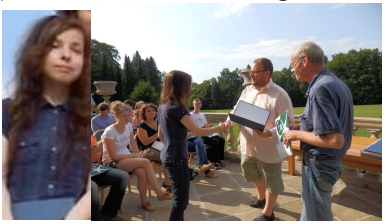
Josef Lazar, Ph.D.
Head, Department of Cell Biology INSB GCRC AS CR
The research of Josef Lazar is focused on the development of a voltage sensitive fluorescent protein / Organization of the mammalian odorant and pheromone detection systems

Alina Kevorkova, MSc
2011 – current PhD student at the Faculty of Science, University of South Bohemia in Ceske Budejovice
2004 – 2010 Master of Science, BSU – Belarusian State University

Students:
Elena Vileishikova, Belarusian State University
Maryia Barashkava, Belarusian State University

Abstract:
Development of optical microscopy into a structural biology technique

Polarization microscopy using single-photon (1P) and two-photon (2P) excitation allows sensitive observations of processes taking place in living cells and animals. Polarization 1 microscopy also allows yielding insights into the structure of the observed proteins. To develop the ability to obtain quantitative data on structure of membrane proteins labeled with fluorescent proteins (FPs) by polarization microscopy, we need to determine the orientation of transition dipole moments (TDMs) in FP molecules. The goal of the present project was to determine the orientation of TDMs in a red fluorescent protein, mCherry. We have overexpressed, purified and crystallized mCherry and carried out 1P and 2P optical measurements on mCherry solutions and crystals. Our results yield information on the 1P and 2P transition moments.



PROJECTS

9. 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 analysed.

Project leader: Dr. rer. nat. et Doc. JOST LUDWIG, jost.ludwig@uni-bonn.de

Leader of Department of membrane physiology and bioenergetics INSB GCRC AS CR
Ludwig 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: Localisation 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:

Daria Polyanina, Lomonosov Moscow State University

Madalina Oana Popa, Faculty of Biology / ''Alexandru Ioan Cuza'' University (Iasi, Romania)

Abstract:

Investigation of potassium transport in yeast cells by monitoring intracellular pH changes

The intracellular H ion concentration (usually expressed as pH) is an important determinant of the ability of cells to perform their tasks. H homeostasis is strongly connected to K (the most abundant intracellular cation) homeostasis. Being able to adapt to strongly changing environments, yeast cells (Saccharomyces cerevisiae) are a good model for studying eukaryotic cells homeostasis. A series of yeast strains carrying (combinations of) mutations in K translocation system genes(trk1,2 ,tok1, nha1, ena1-5) and expressing the pHluorin [1] gene, a GFP (green fluorescent protein) variant that changes its fluorescence properties depending on pH, has been generated. These strains were used to analyze the response of intracellular pH upon changes of the external K or Na concentration. The measurements of intracellular pH have been carried out using a fluorescence microplate reader. In the wild type the pH changes were dependent on the external K concentration. No

dependence of pH values on external [K] was observed with the strain with all transport system genes deleted. All strains, in which cation-concentration dependence was observed, demonstrated a clear preference for K over Na . Comparisons between strains Δtrk1 and Δtrk2, revealed that both Trk1 and Trk2 play a role in K transport, but TRK1 has a much + + + + + + + + + higher impact. NHA1 and ENA1-5 seemed top be less important under the conditions that were used.

Best presentation award

Jakub Czuchnowski - Ivan Kulik - Yani Zhao

Conference of the 2nd Summer School in Molecular Biohphysics and Systems Biology

2nd Summer School in Molecular Biophysics and Systems Biology, Nove Hrady, 2014		
	Presentation of Results	
	Friday, 25th of July 1014	
	Dipolmas and Awards: 17:00	
9:00	**Welcome**	**9:05**
9:05	**Session 1 (Jost Ludwig)**	**10:40**
	-	
9:05	Maryia Barashkava and Elena Vileishikova: Development of optical microscopy into a structural biology technique	9:35
9:35	Jakub Czuchnowski and Ivan Kulik: Development of optical microscopy into a structural biology technique	10:05
10:05	Katsiaryna Maskalenka and Kajetan Sawa: Molecular mechanisms of G-protein signaling investigated by two-photon polarization microscopy	10:35
10:35	**Coffee (and tea maybe)**	**11:00**
11:00	**Session 2 (Jozef Lazar)**	**12:25**
11:00	Dariya Polyanina and Madalina Oana Popa: Investigation of potassium transport in yeast cells by monitoring intracellular pH changes	11:30
11:30	Gabriella Fiser: Expression, purification, restriction and ATPase activities in mutated subunit HsdR from EcoR124I restriction-modification complex	11:55
11:55	Alžběta Roeselová and Mykyta Markevych: Crystallization of haloalkane dehalogenase LinB mutants	12:25
12:25	**LUNCH**	**13:30**
13:30	**Session 3 (David Reha)**	**14:25**
13:30	Mihail Anatolevich Shapira: Using Ionic Liquids in Protein Crystallization	13:55

13:55	Katarína Stančiaková and Pavel Kviatko: Theoretical investigation of the interactions of chemical pollutants with phospholipids	14:25
14:25	**Coffee (and tea maybe)**	14:50
14:50	**<u>Session 4 (Babak Minofar)</u>**	16:20
14:50	Yani Zhao: The study of the stability of E. coli WrbA dimer by Molecular Dynamics simulations	15:15
15:15	Stanislav Šimko: QM/MM study of 3-hydroxyaspartates as inhibitors of human serine racemase (hSR)	15:40
15:40	Bennett A. Macintosh: Exploring the conformational space of a new domain in EcoR124I's translocation/restriction subunit	*16:10*
16:20	*Babak Minfar, David Reha, Jost Ludwig, Jozef Lazar, Rudi Ettrich,*	*16:50*
	Evaluation of talks, awards	
17:00	**Awards and Diplomas**	**18:00**
18:00	**Dinner and Farewell party**	

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 in the castle, and finally a large park, which is due to its location an ideal for exploring the beautiful surrounding countryside Novohradske Mountains.

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, focussing 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.

The Institute of Nanobiology and Structural Biology, (INSB) was established 1. 1. 2011 by a decision of the board as an independent institute within the public research institution GCRC.