biomembranes'16

MIPT, DOLGOPRUDNY, RUSSIA

MECHANISMS OF AGING AND AGE-RELATED DISEASES

MIPT

Moscow Institute of Physics and Technology
(State University)

BIOMEMBRANES 2016:
Mechanisms of Aging and Age-Related Diseases
International Conference

26 – 30 September 2016

Book of Abstracts

Dolgoprudny
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Contents

Posters

Conference Program

26 September 2016 - Monday	
15:00-17:30	Registration
17:30-18:30	Satellite event: Open lecture by Marat Yusupov "Ribosome a Largest Molecular Machinery of the Cell"
18:35-19:35	Satellite event: Open lecture by Valery Fokin "Catalytic Methods for Discovery of Biological Function"

27 September 2016 - Tuesday	
08:00-08:55	Registration
09:00-09:30	Conference Opening. Welcome address from MIPT rectorate, Board of the Research Center for Molecular Mechanisms of Aging and Age-Related Diseases, Conference Committees.
09:30-10:15	Keynote lecture: Werner Kühlbrandt "Cryo Electron Microscopy with Mitochondrial Membrane Complexes"
10:20-11:05	Keynote lecture: Antti Niemi "Folding Proteins at the Speed of Life"
11:05-11:35	Coffee Break and Poster Session
11:35-12:20	Keynote lecture: Vladimir Anisimov "Longevity: Free Choice of Destiny"
12:25-12:55	Christoph Fahlke "Glutamate Transporter Dysfunction in Human Diseases"
13:00-13:25	Alexander Molochkov "Modelling of the structure and phase diagram of protein conformational states caused by local symmetry"
13:25-14:50	Lunch
14:50-15:20	Max Maletta "Cryo Electron Microscopy and the FEI Volta Phase Plate as Tools for Studying Biomembranes"
15:25-15:55	Michiru D. Sugawa "Shaping Strategies in Healthy Longevity Science"
16:00-16:45	Keynote lecture: Isao Shimokawa "Anti-Ageing Effect of Dietary Restriction"

16:50-17:20	Andrei Gilep "Steroidogenic Cytochrome P450s: Protein-Membrane and Protein-Protein Interactions"
17:25-17:30	Andrey Rogachev, Information from the Organizing Committee
17:30-18:00	Coffee Break and Poster Session
18:00-19:00	Satellite event: Open lecture by Antti Niemi "What is Life - a Physics Point of View"
19:05-20:05	Satellite event: Open lecture by Isao Shimokawa "Experimental interventions for longevity"

28 September 2016 - Wednesday	
09:00-09:10	Information from Organizing and Program Committees
09:10-09:40	Sergei Grudinin "Using Machine-Learning, Artificial Intelligence and Integrative Approaches for Protein Structure Predictions"
09:45-10:15	Alexander Myasnikov "High Resolution Electron Microscopy in Protein Synthesis Investigation"
10:20-10:50	Francisco Rodriguez-Valera "Metagenomics of Aquatic Microbes, a Window into the Diversity of Rhodopsins"
10:50-11:20	Coffee Break and Poster Session
11:20-11:50	Boris Gorshunov "Dielectric Spectroscopy of Biological Materials"
11:55-12:40	Keynote lecture: Raymond Stevens "GPCRs and Diseases"
12:45-13:15	Raul Gainetdinov "Emerging Pharmacology of Trace-Amine Associated Receptors (TAARs)"
13:15-14:40	Lunch
14:40-15:10	Vernon Smith "Pushing Back Frontiers: In-house GPCR Crystallography in Structure-based Drug Design"

15:15-15:45	Martin Engelhard "Two-Component Signaling System"
15:50-16:35	Keynote lecture: Norbert Dencher "Mitochondrial Membranes as the Site of Ageing and of Neurodegenerative Diseases"
16:40-17:10	Vadim Cherezov "G-Protein Coupled Receptors and Ageing"
17:10-17:50	Coffee Break and Workshop from Helicon Company & Thermo Fisher Scientific: "Multiparameter Cell Analysis: from Individual Cells to Phenotypic Profiling".
17:50-18:20	Elena Vorontsova "Rapid Analysis of Fluorescence Microscopy Images"
18:25-19:10	Keynote lecture: Marat Yusupov "Structural Studies of the Mechanism of Translation"
19:15-19:40	Eduard Bocharov "«Hot Spots» for Pathogenic Gain-of-Function Mutations in RTK Transmembrane Domains"
19:45-20:10	Olga Bocharova "Structural Insights into Initial Steps of Alzheimer Disease Development via Conformational Variability of APP Transmembrane Domain"

29 September 2016 - Thursday	
09:00-09:10	Information from Organizing and Program Committees
09:10-09:40	Alexei Nikulin "Regulatory RNA in Bacteria and Archaea: Their Interaction with Sm-like (LSm) Proteins"
09:45-10:30	Keynote lecture: Dietmar Manstein "New Concepts for the Treatment of Myopathies"
10:35-11:05	James Liu "Structural and Functional Study of Human GPCRs"
11:10-11:40	Oxana Galzitskaya "The Mechanism Underlying Amyloid Polymorphism is Opened for Alzheimer Aβ Peptide"
11:40-12:10	Coffee Break and Poster Session

12:10-12:40	Sergei Kozin "Aged Isoform of β-amyloid as Biomarker and Drug Target of Alzheimer's Disease"
12:45-13:10	Plamena Stroh "Mechanism of Neurodegeneration in Parkinson's Disease"
13:15-13:45	Thomas Gensch "Determination of Intracellular Ion Concentrations by Fluorescence Life-Time Imaging"
13:50-14:15	Konstantin Mineev "Role of Membrane Domains and Juxtamembrane Regions in p75 Neurotrophin Signaling"
14:20-14:50	Ravinder Anand-Ivell "Relaxin Family Members and Their GPCRs as Modulators of Health and Aging"
14:50-15:50	Lunch
17:15	Cultural Program in Moscow

30 September 2016 - Friday	
09:00-09:10	Information from Organizing and Program Committees
09:10-09:40	Judith Haendeler "Molecules Relevant for Vascular Aging – Specific Role in Mitochondrial p27"
09:45-10:15	Joachim Altschmied "Role of Mitochondrial Telomerase Reverse Transcriptase in the Cardiovascular System"
10:20-11:05	Keynote lecture: Ernst Bamberg "Optogenetics: Basics, Applications and New Developments"
11:05-11:35	Coffee Break and Poster Session
11:35-12:20	Keynote lecture: Georg Büldt "Retinal proteins inspired biophysical studies"
12:25-12:55	Igor Chizhov "Kinetics and Energetics of Ion Transport in Retinal Proteins
13:00-13:30	Roman Efremov "Biomembranes: Results of Computer Studies"

13:30-14:00	Coffee Break and Poster Session
14:00-14:25	Michael Proskurin/Alla Karpova "FlinChR: a New Optogenetic Tool and Its Application for Neural Circuit Dissection"
14:30-15:00	Olga Sokolova "Conformational States Underlying Autoregulation of F-BAR Domain Membrane Remodelling"
15:05-15:30	Anton Chugunov "Unique Structural Organization of Prokaryotic Membranes Probed by High-Performance Computing"
15:35-15:50	Conference Closing

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Preface

Cardiovascular, neurodegenerative diseases and cancer are main causes of death in developed countries. Their probability dramatically increases with age. Even healthy aging leads to significant deterioration in the quality of life. Being in the focus of modern life science, biomembranes and membrane proteins play an important role in aging. A view of aging through the understanding of molecular mechanisms of biomembrane functioning can be very productive.

At the Conference we see world leading scientists from various fields of modern integrative structural biology and aging research. We believe that a complementary use of methods of both fields may help to uncover basic mechanisms of aging and age-related diseases. The participants of the Conference will learn a lot from lectures overviewing their cutting-edge research. We are especially happy to see students and young scientists among the participants and hope that they will enjoy fascinating scientific discoveries highlighted in the lectures.

The Conference was organized by the Laboratory for Advanced Studies of Membrane Proteins, the Laboratory for Structural Biology of GPCRs, the Laboratory for Chemistry and Physics of Lipids and the Laboratory for Structural Analysis and Engineering of Membrane Systems which are parts of the Research Center for Molecular Mechanisms of Aging and Age-Related Diseases at MIPT.

The Conference is greatly supported by the MIPT rector board, the Research Center Jülich and the Joint Institute for Nuclear Research, Dubna and by our sponsors.

We especially acknowledge the 5-100 program of the Ministry for Education and Science of the Russian Federation, the Russian Foundation for Basic Research. This Conference would never be possible without them.

We greatly acknowledge scientists who are giving talks and sharing the results of their beautiful research with the participants of the Conference. Certainly, we are very grateful to all the participants for their active role. This conference is for you and we are happy if you enjoy it. For the organizers this will be a major acknowledgement of their work.

The Organizing Committee, the Program Committee

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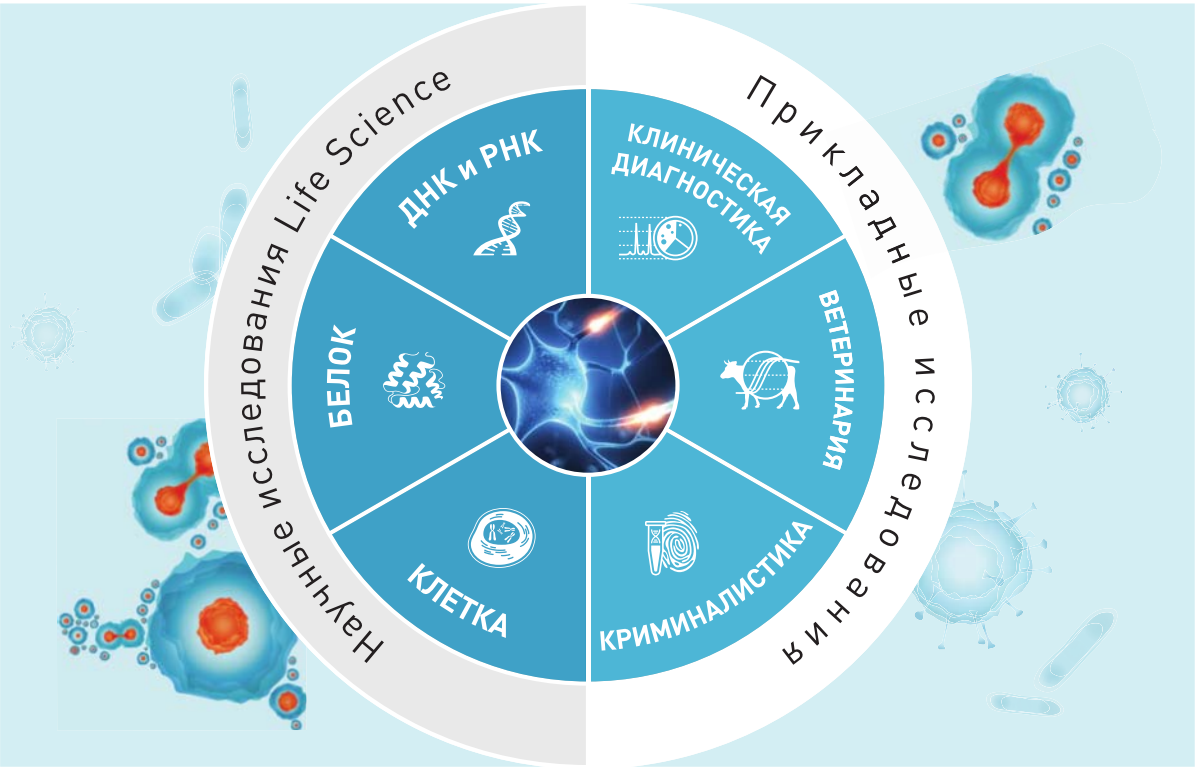

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Мощь синергии

Объединение групп по монокристальной дифракции корпорации Rigaku и компании Oxford Diffraction привело к созданию организации, одна из целей которой состоит в том, чтобы предоставлять структурным биологам наилучшую комбинацию технологий

Серия XtaLAB

Платформа The XtaLAB включает в себя гониометр ¼ χ и выбранные генератор рентгеновского излучения, оптику и детектор, которые в максимальной степени отвечают потребностям вашей лаборатории. В качестве источников рентгеновского излучения мы предлагаем микрофокусные отпаянные трубки (MicroMax-003), не требующие большого объема технического обслуживания, и источники с вращающимся анодом (генераторы MicroMax-007 HF и FR-X), доказавшие свою надежность.

Модели детекторов и источников рентгеновского излучения

Источник	Тип источника	Мощность	Возможность использования двух длин волны
MicroMax™-003	Мирокофокусная отпаянная трубка	30 Вт	Нет
MicroMax-007 HF	Микрофокусный вращающийся анод	1,2 Квт	Есть
FR-X	Микрофокусный вращающийся анод	2,970 кВт	Есть

Название детектора	Активная зона	Время считывания
PILATUS3 R 200K	83.8 мм x 70 мм	7 мс
PILATUS3 R 300K	83.8 мм x 106,5 мм	7 мс
EIGER R 1M	77,2 мм x 79,9 мм	Непрерывное считывание, время нечувствительности 4 пс
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Lectures

Role of mitochondrial of Telomerase Reverse Transcriptase in the cardiovascular system

Altschmied J.[1]

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e-mail: Joachim.Altschmied@uni-duesseldorf.de

Age-associated cardiovascular disorders are associated with mitochondrial dysfunction, which causes reduced respiratory chain activity, decreased mitochondrial ATP production, an increase in reactive oxygen species (ROS) and cell death. We could show that the catalytic subunit of Telomerase, Telomerase Reverse Transcriptase (TERT), has a beneficial effect on heart and endothelial function. Furthermore, we recently demonstrated that TERT is also localized within the mitochondria and as such belongs to the group of dual-targeted proteins with additional functions outside the nucleus. Therefore, we aimed to characterize the functions of mitochondrial TERT in cardiovascular aging and diseases.

To analyze specifically the role of TERT in the mitochondria we expressed TERT fused to a strong mitochondrial targeting sequence and showed that mitochondrial TERT potently inhibits hydrogen peroxide induced endothelial cell apoptosis and specifically reduces mitochondrial ROS production. Moreover mitochondrial TERT seems to be required for cardiac myofibroblast differentiation, a critical process after myocardial infarction.

Furthermore, we created mice containing TERT exclusively in the mitochondria (mitoTERT mice). Oxygraphy showed a significantly lower state 3 respiration in heart mitochondria from TERT-deficient mice compared to mitochondria from hearts of wildtype littermates, whereas the mitochondria isolated from hearts of mitoTERT mice had a significant higher state 3 respiration. Furthermore, we could demonstrate that this effect is not dependent on the Telomerase RNA subunit TERC. Moreover, mitoTERT mice showed elevated ATP levels in heart mitochondria when compared to TERT-deficient animals

With these studies we could for the first time demonstrate compartment-specific roles of TERT in the cardiovascular system *ex vivo* and *in vivo*.

Optogenetics: basics, applications and new developments

Bamberg E.[1], Mager T.[1], Wood P.[1]

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Optogenetics is the use of genetically encoded light activated proteins for manipulation of cells in an almost noninvasive way by light. The most prominent example is Channelrhodopsin2(ChR2), which allows the activation of electrical excitable cells via the light dependent depolarization. The combination of ChR2 with hyperpolarizing light driven ion pumps as the Cl^- pump halorhodopsin (NphR) enables the multimodal remote control of neural cells in culture, tissue and living animals. It is obvious that this method offers completely new possibilities in neuroscience and cell biology. Also the chance for a gene therapy for some neurodegenerative diseases. For this optogenetic tools have to be optimized with respect to speed and absorption maxima. In this line promising tools and their applications will be presented. The basics of optogenetics and some applications are presented. Possible biomedical applications with the focus on blindness and deafness are discussed.

“Hot spots” for pathogenic gain-of-function mutations in RTK transmembrane domains

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Signal transduction by receptor tyrosine kinases (RTKs) has been in the spotlight of scientific interest owing to the central role of these single-spanning membrane receptors in the regulation of development, cell motility, proliferation, differentiation, and apoptosis. Nowadays, the elucidation of high-resolution structure of full-size RTK having flexible multiple-domain composition is still a challenge. During signal transduction across plasma membrane, RTKs are activated by proper ligand-induced homo- and hetero-dimerization or by reorientation of monomers in preformed receptor dimers upon ligand binding. Specific helix-helix interactions of transmembrane domains (TMD) are believed to be important for RTK lateral dimerization and signal transduction. Either destroying or enhancing such helix-helix interactions can result in many human diseases: developmental, oncogenic, neurodegenerative, immune, cardiovascular etc. Observed the RTK TMD helix-helix packing diversity appears in favor of the recently proposed the lipid-mediated rotation-coupled activation mechanism [1], which implies that the sequence of structural rearrangements of RTK domains is associated with perturbations of the lipid bilayer in the course of ligand-induced receptor activation, considering the receptor together with its lipid environment as a self-consistent signal transduction system.

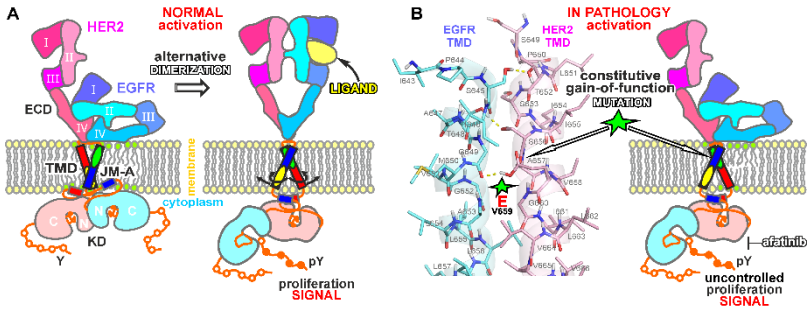

Fig. 1. Normal and pathological activation of EGFR/HER2 hetero-dimer as a model RTK. (A) Presumable structural rearrangements of EGFR/HER2 hetero-dimer in the course of ligand-induced receptor activation according to the lipid-mediated rotation-coupled mechanism proposed in [1]. (B) Additional inter-monomeric hydrogen bonding caused by HER transmembrane polar mutations (e.g. V659E-HER2) can stabilize the N-terminal dimerization mode of TMD allowing release of cytoplasmic juxtamembrane regions (JM-A) from the membrane leaflet and activation of kinase domains (KD) independently of ligand binding and regardless of the states of extracellular domains (ECD) and surrounding lipids.

The human epidermal growth factor receptors (HER) and the fibroblast growth factor receptors (FGFR) families serves as excellent model RTK to illustrate how ligand-induced conformational rearrangements and specific dimerization of extracellular domains lead to the allosteric activation of the cytoplasmic kinase domains, resulting in signal propagation across the membrane (Fig. 1A). Besides, HER and FGFR relatives are known oncogenic drivers in many cancers, and inhibitors of these receptors have been among the most successful examples of targeted cancer therapies to date. Pathogenic transmembrane mutations found for the HER and FGFR relatives are located as a rule in narrow regions within the specific TMD

helix-helix interfaces (Fig. 1B) assuming that the intermolecular interactions inside membrane are important for the RTK cell signaling dysfunction in human organism [1, 2]. Such regions can be characterized as a “hot spot” for gain-of-function mutations associated with different human pathologies. This finding justifies a prediction that similar gain-of-function mutations, e.g. enhancing the TMD dimerization in certain conformation and thus activating the receptor independently of ligand binding, can be found for other RTK representatives and suggests searching for them is a promising idea for future clinical studies. It can also have potential therapeutic implications, broadening the spectrum of targets for pharmaceuticals by inclusion of plasma membranes and their constituents.

The work is supported by the Russian Foundation for Basic Research (project #15-04-07983-a).

[1] E.V. Bocharov, D.M. Lesovoy, K.V. Pavlov, Y.E. Pustovalova, O.V. Bocharova, A.S. Arseniev, Alternative packing of EGFR transmembrane domain suggests that protein-lipid interactions underlie signal conduction across membrane, Biochim. Biophys. Acta, 1858 (2016) 1254–1261.

[2] E.V. Bocharov, D.M. Lesovoy, S.A. Goncharuk, M.V. Goncharuk, K. Hristova, A.S. Arseniev, Structure of FGFR3 transmembrane domain dimer: implications for signaling and human pathologies, Structure, 21 (2013) 2087–2093.

Structural insights into initial steps of Alzheimer disease development *via* conformational variability of APP transmembrane domain

Bocharova O.V.[1], Urban A.S.[1, 2], Nadezhdin K.D.[1, 2], Kuzmichev P.K. [1, 2], Volynsky P.E.[1], Bocharov E.V.[1], Arseniev A.S.[1, 2]

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Despite some progress in study of the molecular mechanisms of Alzheimer's disease (AD) development the initial steps of the pathogenesis are still puzzling. Amyloid Aβ-peptides forming plaques in brain during AD are the products of sequential cleavage of a single-span membrane amyloid precursor protein (APP). More than half of mutations associated with familial forms of AD were found in the APP transmembrane (TM) domain. The pathogenic mutations presumably affect structural-dynamic properties of the APP TM domain, e.g. changing its conformational stability, lateral dimerization and intermolecular interactions, which can result in enhanced and alternative cleavage by γ-secretase in membrane.

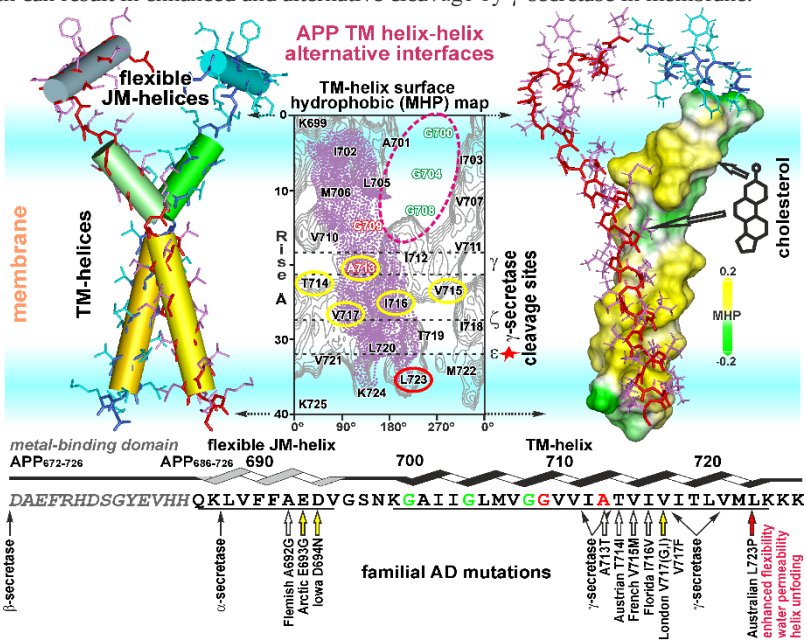

Fig. 1. Schematic representation of the dimeric APP TM domain [1] embedded into the membrane mimicking micelles, hydrophobic/hydrophilic properties of the TM-helix surface, possible alternative TM helix-helix interfaces and cholesterol binding sites. Residues, which mutations are associated with familial AD forms, are indicated by small ovals.

We designed high-performance systems of bacterial and cell-free expression and purification for biochemical and biophysical studies of the APP TM fragments (the substrates of γ-secretase complex), containing metal-binding, juxtamembrane (JM) and TM regions of different length, as well as the fragments with some familial AD mutations situated in the JM and TM regions [2] (Fig. 1). The systems allow obtaining milligram quantities of the APP TM fragments with isotope labeling more than 95%. The $^{2}H/^{13}C/^{15}N$-isotope labeled samples were

produced for NMR studies, which subsequently make it possible to characterize in detail spatial structure, dynamics, and kinetic parameters of specific dimerization of the APP TM fragments. The fragments were solubilized in detergent micelles and lipid bicelles, 40-60 kDa supramolecular membrane-mimicking complexes, which allows acquiring proper NMR spectra despite low sample stability and aggregation. MD relaxation of obtained high-resolution NMR structures in hydrated explicit lipid bilayers provided a detailed atomistic picture of the intra- and intermolecular interactions. As a result, the dimerization mechanism of the TM domain APP and cholesterol-binding sites were described that can be important for the understanding of the APP functioning and AD development.

"Australian" mutation, L723P, is identified to be associated for developing autosomal-dominant, early onset Alzheimer's disease (*red* arrow in Fig. 1). To investigate mechanism of L723P mutation influence on Aβ-peptide formation we carried out high-resolution NMR studies of structural-dynamical properties of mutant fragment $APP_{686-726}$ corresponding to the initial step of sequential proteolysis of APP by γ-secretase in membrane. We detected enhanced flexibility and partial unfolding of the C-terminal region of the TM-helix of L723P mutant compared to wild-type peptide, which can facilitate the APP proteolysis in the ε-site. We also found that unlike wild-type fragment the L723P "Australian" mutant gradually converts from α-helical to β-conformation and this process accompanied by high molecular weight aggregates formation. Thereby the mutant APP TM fragments are shown to be promising objects for determination of molecular mechanisms of amyloidogenesis and for identifying the structural and functional determinants of APP, which is necessary for understanding of AD pathogenesis.

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A new story of old bacteriorhodopsin

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Bacteriorhodopsin is a membrane protein from *Halobacterium salinarum*. The protein harvests energy from light to create an electro- chemical gradient across a cell membrane (for a review, see [1]). bR has been one of the most extensively studied membrane proteins during the past 40 years. The Protein Data Bank currently holds >90 published structures of native and mutated bR in the ground and intermediate states [2]. However, these attempts have failed to propose a complete picture for the molecular mechanism of proton transport [3,4] consistent with infrared spectroscopy, which has significantly contributed to our understanding of proton transport across bR [5,6].

Here we present an overview of the reasons for this situation, disucuss how they can be overcomed and what we learned about bacteriorhodopsin after that.

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Retinal proteins inspired biophysical studies

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X-ray experiments of Blaurock and Stoeckenius [1] demonstrated that the retinal protein bacteriorhodopsin (bR) is organized in high quality lattices the Purple Membranes (PM) in the plasma membrane of *H. salinarum*. These experiments inspired electron microscopy [2], neutron [3] and X-ray studies [4] leading to 7 Å resolution structures showing for the first time conformational changes of intermediate states in an α-helical membrane protein. In meso crystallization of bR [5] and sensory rhodopsin [6,7] allowed to resolve the 3D structures of all the intermediate states to high resolution.

Retinal proteins have a variety of functions. They are proton pumps, ion channels and sensors. These properties were used in optogenetic studies. In a first experiment bR was introduced into the inner mitochondrial membrane of the yeast *S. pombe* in order to support or replace the proton gradient made by the respiratory chain [8]. The proper function of bR in the mitochondrial membrane was demonstrated by the photocycle of bR in isolated mitochondria and by dramatic changes in the uptake of glucose from the medium by transformed yeast cells. These studies stimulated us to investigate the import mechanism of proteins into mitochondria in more detail by fluorescence microscopy. A key question is whether the import is a co- or post-translational process. For these investigations we had to look more closely to the synthesis of proteins by the ribosome [9,10,11]. First experiments by optical tweezers revealed surprising details [12]. In future holographic X-ray studies with femtosecond pulses from free electron lasers will deliver snap shots of events happening in unfrozen wet single cells [13].

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G protein-coupled receptors and ageing

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G protein-coupled receptors (GPCRs) comprise the largest protein superfamily in the human proteome with about 800 members. GPCR-mediated signaling pathways are implicated in numerous human diseases, many of which, such as cardiovascular and neurodegenerative diseases and cancer are associated with ageing. During the last decade, structural biology of GPCRs has experienced exponential growth. By 2016, structures of over 30 different GPCRs have been determined, giving unprecedented details on diversity of ligand-receptor interactions and the mechanism of signal transduction. In this talk I will describe some of the technological advances that enabled the GPCR structural revolution and present the work on structure and function of GPCRs at MIPT.

Kinetics and energetics of ion transport in retinal proteins

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Bacteriorhodopsin was discovered in early 70s as a 7-alpha helical, transmembrane protein. The protein absorbs the green light and converts the energy to the transport of hydrogen ions through a biological membrane against gradient. This process occurs in a multistep manner and is accompanied by changes of absorption spectrum (the so-called photocycle). Over the last 40 years many other retinal-containing proteins have been determined including halide anion pumps, light-gated passive ionic channels, and recently, the alkaline cation pumps. All of them share quite complicated chain of reaction steps upon photoexcitation with detected life-times from femtoseconds to seconds. In last decade the progress in technique of genome screening and synthesis lead to discovery of quite interesting rhodopsins with new photocycle features. Comparative analysis of their properties with the key members of retinal proteins might provide insight into mechanism of the energy transduction and ionic transport. Photocycles of some recently discovered retinal proteins are discussed in context with the bacteriorhodopsin, halorhodopsin, and sensory rhodopsin.

Mitochondrial membranes as the site of ageing and of neurodegenerative diseases

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When in the near future we will understand structure, dynamics, and physiology of all different cellular membranes at the molecular level, we can understand most of the vital cellular processes and can manipulate malfunctioning. This will be illustrated focusing on the involvement of membranes in ageing as well as in two neurodegenerative diseases, Alzheimer's (AD) and Parkinson's dementia (PD). Biological membranes are the largest and most active organelles/organs in every cell/organism. They perform energy-conversion, e.g., oxidative phosphorylation in mitochondria and photosynthesis in chloroplasts, as well as signal-transduction, but are also involved in many diseases as primary trigger and/or target. Our research interest is especially devoted to **mitochondrial membranes**. Why mitochondria? They are enveloped even by two structural and functional different membrane systems. The surface area of the inner mitochondrial membrane in a human is 14000 m^2. By OxPhos protein complexes residing therein ~70 kg ATP per day are synthesized. In addition, mitochondria are involved in apoptosis (process of programmed cell death), regulation of cellular metabolism including synthesis of certain steroids and lipids, processing and storage of calcium and copper [1]**,** but are also the main generators and targets of free radicals and reactive oxygen species [2]. Therefore, *mitochondria are central for the cell in almost all organisms*!

Ageing is usually defined as the progressive loss of function accompanied by decreasing fertility as well as increasing morbidity and finally mortality with advancing age. The causes and mechanisms of ageing are still secrets. Therefore, understanding molecular processes underlying ageing remains a challenge. The process of ageing is controlled by a complex network of molecular pathways in which those related to mitochondrial functions are of superior importance. Age-dependent changes in the cellular proteome are currently considered as targets and even triggers of ageing (and of age-associated diseases such as AD and PD) [2,3]. We are applying a variety of techniques to unravel the molecular mechanisms of ageing as well as of Alzheimer's and Parkinson's disease, and the involvement of biological membranes [2]. Prolongation of health- and life-span by controlled reduction of the intake of calories results in defined alterations of mitochondrial energy metabolism, e.g., lowers complex I assembly and complex IV activity as well as promotes the formation of OxPhos supercomplexes [4,5]. This is suggested as a molecular mechanism to minimize ROS production by mitochondria.

It is well established that mitochondria are one of the most adversely affected organelles during ageing. There is increasing evidence that mitochondria even have a key role in causing ageing. There are many, too many theories trying to explain the cause of ageing. Contradictory

to the “Free Radical Theory of Ageing” postulated by D. Harman in 1956 [6], currently the most popular ageing theory, mitochondrial proteins of rat cortex exhibit less oxidative modifications in aged rats than in young rats (Monika Frenzel, NAD, unpublished results 2011). Supporting this initial observation, we do not observe massive increase in protein oxidation during ageing both in brain and heart mitochondria, challenging the Mitochondrial Free Radical Theory of Ageing (Carina Ramallo Guevara, Ansgar Poetsch, NAD, unpublished results 2016).

Alzheimer’s disease (AD) is the most common form of dementia. Since the number of patients will triple to estimated 120 million until 2050, it is important to understand the molecular mechanisms leading to this currently incurable neurodegenerative disease. It is most likely that AD is caused by amyloid-β (Aβ) peptides with 38 to 43 amino acids that are derived from the amyloid precursor protein (APP). Contrary to the extracellular plaque hypothesis, still favoured by most researchers in the field, even Aß monomers are bioactive via insertion into membranes [7,8]. As mitochondrial dysfunctions are early events in disease progression, there might be a connection that has to be investigated. In order to demonstrate cellular and organelle trafficking of Aß, to identify its target(s), and to analyse its deleterious effects on cell and membrane function, in our current studies the Aß1-42 peptide is disaggregated to form monomers/small oligomers that are externally applied to mammalian cells (human neuroblastoma cell line and rat oligodendroglia cell line). In contrast to plaques composed of aggregated Aβ fibrils, the monomeric/oligomeric peptide is able to enter cells. This is proven by confocal microscopy as fluorescence labelled Aβ1-42 peptide can be located at mitochondria and other organelles. In addition, cells treated with Aβ1-42 peptide exhibit decreased mitochondrial membrane viscosity demonstrating direct peptide intercalation into the mitochondrial membrane. We are able to track in time and space the pathway of the Aß peptides from the outside of the cell across the plasma membrane to the internal target membranes of specific organelles (Victoria Decker, Tamara Dzinic, Tobias Meckel, Ivan Okhrimenko, NAD, unpublished results). These pathways and concomitant alterations in cellular, mitochondrial and membrane function will be illustrated. Obviously, intracellular monomeric /oligomeric Aβ1-42 peptides influence mitochondrial physiology. Therefore, mitochondrial dysfunction might be the initial event in AD pathology. Overall, all our results are in line with and do indicate at the molecular level that Aß peptides intercalate into organelle membranes, including mitochondrial membranes, perturb the structure of lipid bilayers [7], modulate lipid dynamics [8] and in this way can prevent neurons from functioning normally. The Aß peptide induced changes in the biochemical and biophysical properties of membranes [2] will contribute to the understanding of the pathology of Alzheimer’s disease as well as being a clue for early diagnosis and therapy.

Parkinson's disease (PD) is as well a progressive neurodegenerative disorder manifesting its motor symptoms in the late age, after most of the affected dopaminergic neurons in the substantia nigra (SN) have been already lost. Interestingly, until the loss of approximately 70% of dopaminergic neurons in SN and subsequent 80% loss of dopamine (DA) in its target structure, striatum (STR), no major motor problems are visible, only small, diffuse, often peripheral symptoms [9]. This makes the diagnosis of PD very difficult at early stages when progression of degenerative processes could still be prevented. Ageing and oxidative stress are the main factors in PD pathogenesis. Discovering markers of early degeneration in central nervous system, when changes are still very small, is an important issue and therefore investigated by us [9,10,11]. We show for the first time that changes at the level of mitochondrial membrane viscosity influence function of OxPhos supercomplexes after dopaminergic system degeneration [9]. Dopaminergic lesion induces changes in activity and supramolecular assembly states of mitochondrial OxPhos complex I and IV. The specific activity of complex I and IV are much higher in supercomplexes than as individual complex

I and IV, up to 6 and 16 fold, respectively [9]. Disease-induced viscosity changes will influence assembly/ disassembly of OxPhos supercomplexes. This implicates that altered mitochondrial membrane viscosity could play an important role in regulation of mitochondria functioning and pathomechanisms of PD, supporting the notion that mitochondrial dysfunction is one of the main underlying causes for the disease pathology. On the avenue to drug development, in an animal model of Parkinson's disease restorative effects of 9-methyl-ß-carboline could be demonstrated that improve the effectiveness of the respiratory chain and promote the transcription and expression of neurotrophin-related genes [11]. Native DIGE proteomic analysis of mitochondria from substantia nigra and striatum during neuronal degeneration and its compensation reveal crucial processes involved in degeneration and its compensation in these both dopaminergic brain structures. Changes in biomarker proteins analysed indicate structural remodelling, cytoskeleton rearrangement, organelle trafficking, axon outgrowth and regeneration. Altered expression of carbohydrates metabolism and oxidative phosphorylation proteins are observed, including their protein–protein interactions and supercomplex assembly [10].

In general, for meaningful translation of knowledge gained on cell cultures to tissues, especially in respect to metabolism and to oxidative stress responses, the oxygen conditions in the cell culture should be comparable to levels in human body tissues, e.g. 5% in brain and not the atmospheric 21% oxygen usually administered [12].

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Biomembranes: results of computer studies

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Motivation and aim. Cell membranes attract a growing attention as very perspective pharmacological targets. Rational design of new efficient and selective compounds modulating activity of biomembranes, requires atomic-scale information on their spatial structure and dynamics under different conditions. Because such details resist easy experimental characterization, important insight can be gained *via* computer simulations.

Results. The polypeptide-membrane recognition reveals a prominent "self-adapting" character. Namely, the membrane active agents employ a wide arsenal of structural/dynamic tools in order to insert into the lipid bilayer and to accomplish their function. Importantly, lipid bilayer of biological membranes plays an essential role in the recognition and binding events [1]. In particular, the membrane surface reveals highly dynamic lateral heterogeneities (clusters), which differ in their packing and hydrophobic properties from the bulk lipids. Such a mosaic nature of membranes is tuned in a wide range by the chemical nature and relative content of lipids, presence of ions, etc. [2]

Conclusion. Polepeptide-bilayer interactions represent a fine-tuned process, which requires the two active players – the polypeptide and the membrane. Interplay of the factors determining such a process assures efficient and robust binding of peptides and proteins to cell membranes. Understanding of such effects creates a basis for rational design of new physiologically active molecules and/or artificial membranes with predefined properties.

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Two component signalling systems

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The strategies of signal propagation in Eukaryotes and Prokaryotes display significant differences. Whereas in eukaryotic cells amplification occurs mainly via spacial gradients in prokaryotes – because of their small size – temporal gradients have been evolved. One of the best elucidated example of such a system concerns chemotaxis signalling networks in bacteria. Chemotactic receptors (Methyl-accepting chemotaxis proteins, MCPs), forming dimers, consist of an extracellular receptor domain, the membrane domain and a long rod-shaped cytoplasmic domain. After receptor activation the signal travels along the dimeric complex, featuring a largely α-helical coiled-coil structure, via HAMP domains, responsible for signal conversion and inversion, to the adaptation domain, which undergoes reversible methylation/demethylation which increases the dynamic range of signal reception considerably. Finally, the signal is transmitted to the highly conservative kinase-activating domain. These membrane proteins form trimers of dimers which assemble to networked arrays providing a template to which His kinase CheA and coupling protein CheW are recruited with a functional stoichiometry of two trimers of dimers: one CheA dimer: two CheW. Amplification of the incoming signal occurs on the level of these arrays probably governed by cooperative mechanisms.

Conversely to the enterobacterial system which has been thoroughly investigated, only few detailed information about archaeal taxis signal transduction systems is available with *Halobacterium salinarum* the best studied system. It has been shown for SRII-transducer complex from Natronomonas pharaonis (NpSRII-NpHtrII) that light excitation triggers defined conformational changes at the protein interface between NpSRII and NpHtrII. It appears that signal transduction is governed by alternative alteration of dynamics which in an unknown mechanism modulates the activity of CheA. This model of alternating sequence of dynamic shifts has also been proposed for signal transmission in chemoreceptors. It seems that not only structural but also functional similarities exist between enterobacterial and archaeal chemoreceptors.

Emerging pharmacoloy of Trace Amine-Associated Receptors

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Trace amine-associated receptors (TAARs, 6 functional receptors found in humans - TAAR1, TAAR2, TAAR5, TAAR6, TAAR8 and TAAR9) comprise a family of recently identified G protein-coupled receptors that are clustered in a chromosomal region associated with schizophrenia. The best studied trace amine-associated receptor 1 (TAAR1), that is expressed in part within the dopaminergic neuronal circuitry, can be activated by a variety of monoaminergic compounds including trace amines, amphetamines and monoamine metabolites. TAAR1 is emerging as a promising new target for psychiatric disorders. Recent progress in identifying selective ligands for TAAR1 led to the possibility of evaluation of the functional consequences of stimulation/ blockade of TAAR1. By using these compounds in an experimental paradigm developed in our laboratory that involves mouse models of hyperdopaminergia and dopamine deficiency, as well as mice lacking TAAR1 (TAAR1-KO mice), we explored the role of TAAR1 in modulation of dopaminergic and glutamatergic transmission. Pharmacological or genetic targeting of TAAR1 revealed that stimulation of TAAR1 suppressed dopamine-dependent behaviors, while TAAR1 deficiency potentiated them. Apparantely, this modulation involves regulation of striatal D2 dopamine receptor function via D2R-TAAR1 heterodimerization. TAAR1-selective ligands have shown potential antipsychotic, antidepressant, and pro-cognitive effects in several experimental animal models suggesting that TAAR1 may affect Prefrontal Cortex (PFC)-related processes and functions as well. Recently, we documented a distinct pattern of expression of TAAR1 in the PFC, as well as altered subunit composition and deficient functionality of the glutamate N-methyl-D-aspartate (NMDA) receptors in the pyramidal neurons of layer V of PFC in mice lacking TAAR1. The dysregulated cortical glutamate transmission in TAAR1-KO mice was associated with aberrant behaviors in several tests, indicating a perseverative and impulsive phenotype of mutants. Conversely, pharmacological activation of TAAR1 with selective agonists reduced premature impulsive responses observed in the fixed-interval conditioning schedule in normal mice. These studies indicate that TAAR1 plays an important role in the modulation of dopamine-related processes in the basal ganglia and NMDA receptor-mediated glutamate transmission in the PFC. Furthermore, these data suggest that the development of TAAR1-based drugs could provide a novel therapeutic approach for the treatment of neuropsychiatric disorders related to aberrant frontostriatal circuitry.

The mechanism underlying amyloid polymorphism is opened for Alzheimer's disease Aβ peptide

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The number of diseases associated with incorrect folding of proteins – amyloidosis – has been ever growing during recent 30 years. In particular, these diseases include many neurodegenerative pathologies. In short, fibril depositions may occur in any human organ. It was established that incorrect folding of proteins leads frequently to their aggregation and generation of fibrils, which at adequate concentration form large aggregates in the form of plaques. The problem of fibril formation and disclosure of the mechanism of amyloid formation has been in the focus of attention. But this is complicated because amyloid fibrils display strong polymorphism. Under similar conditions, the same protein/peptide can form fibrils of different morphology. Moreover, the same proteins from different sources (for example, from different patients) or of different methods of sample preparation demonstrate morphologically different fibrils.

It has been demonstrated using Aβ40 and Aβ42 recombinant and synthetic peptides that their fibrils are formed of complete oligomer ring structures (Fig.1). Such ring structures have a diameter of about 8-9 nm, the oligomer height of about 2-4 nm and the internal diameter of the ring of about 3-4 nm. Oligomers associate in a fibril in such a way that they interact with each other, overlapping slightly. There are differences in the packing of oligomers in fibrils of recombinant and synthetic Aβ peptides. The principal difference is in the degree of orderliness of ring-like oligomers that leads to generation of morphologically different fibrils. Most ordered association of ring-like structured oligomers is observed for a recombinant Aβ40 peptide. Less ordered fibrils are observed with the synthetic Aβ42 peptide. Fragments of fibrils the most protected from the action of proteases have been determined by tandem mass spectrometry. The C-terminus is not accessible to proteases for both Aβ(1-40) and Aβ(1-42) fibrils [1]. It was shown that unlike Aβ40, fibrils of Aβ42 are more protected, showing less ordered organization compared to that of Aβ40 fibrils. Thus, the tandem mass spectrometry data agree with the electron microscopy data and structural models presented here (Fig. 2). X-ray data support our model. Using structural model for Aβ(1-40) fibrils (2m4j.ent) we constructed the possible model for Aβ(1-42) (Fig. 2). This study was supported by the Russian Science Foundation (14-14-00536).

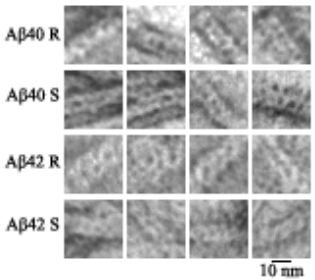

Fig. 1. Electron microscopy images of morphology of fragments Aβ40 and Aβ40 fibrils for recombinant (R) and synthetic (S) samples.

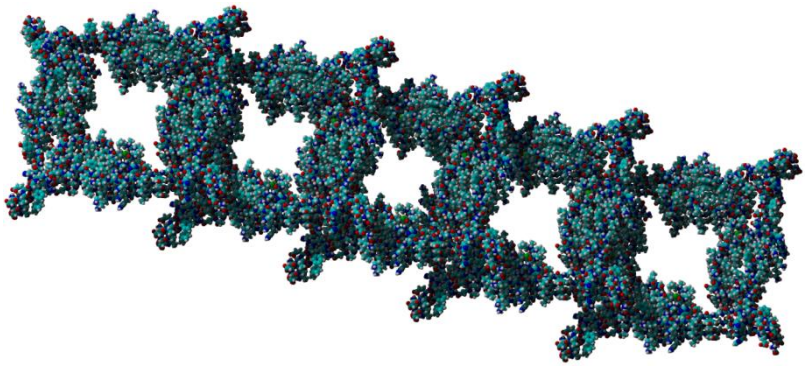

Fig. 2. Regular spacing of a ladder-type arrangement for Aβ(1-42) oligomers.

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Dielectric spectroscopy of biological materials

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The high-frequency conductivity $\sigma_{ac}(\nu,T)$ of disordered materials demonstrates the well-known Universal Dielectric Response (UDR) $\sigma_{ac}=\sigma_{dc}[1+(\nu/\nu_{co})^s]$ [1] (ν_{co} is the frequency, where the σ=const Drude transport changes to the hopping regime $\sigma_{ac}\sim\nu^s$, $s<1$; σ_{dc} is the dc conductivity). At low temperatures and high frequencies, the nearly constant loss (NCL) response is frequently observed in disordered, crystalline and ionically conducting compounds [2]. With the broad-band (1 Hz – 30 THz) dielectric spectroscopy we study the transport and molecular dynamics in extracellular filaments of *Shewanella oneidensis* MR-1 (pili), bovine heart cytochrome C (CytC) and bovine serum albumin (BSA). The spectra of complex ac conductivity $\sigma_{ac}(\nu)$ and dielectric permittivity $\varepsilon(\nu)$ are measured at temperatures 5 K to 300 K. The spectra of pili reveal both, UDR and NCL universalities. We associate the conductivity observed in pili above ≈250 K with the ionic transport in liquid bound water; the conductivity and permittivity obey the scaling relations $\sigma(\nu)/\sigma_{dc}=F_1(\nu\sigma_{dc}^{-1}T^{-1})$, $\sigma(\nu)/\sigma_{dc}=F_2\left(\frac{\Delta\varepsilon}{\sigma_{dc}}\nu\right)$, $\frac{\varepsilon'(\nu)-\varepsilon_{inf}}{\Delta\varepsilon}=F_3\left(\frac{\Delta\varepsilon}{\sigma_{dc}}\nu\right)$ (ε_{inf} is the high-frequency dielectric constant and $\Delta\varepsilon$ is the contribution from hopping conduction). In CytC, signatures of conductivity on delocalized carriers (presumably electrons or holes, not ions), are detected at room temperatures. The heat capacity of pili and of CytC reveal signatures of a boson peak - an excitation typical of disordered systems; signs of the boson peak are also seen in the low-temperature terahertz absorption spectra. The spectra of CytC and BSA show NCL regimes within the range of room temperatures down to liquid helium temperatures. The pili samples demonstrate the UDR-NCL crossover happening in the range 200-300 K while cooling down. The obtained results are analyzed from the viewpoint of microscopic models of dynamical response developed for inorganic materials.

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Using machine-learning, artificial intelligence and integrative approaches for protein structure predictions

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Although the fundamental forces between atoms and molecules are almost fully understood at a theoretical level, and computer simulations have become an integral part of research activities in academic and industrial chemistry (cf. 2013 Nobel Prize in Chemistry), the application of these methods to large biomolecules still faces important practical difficulties due to the combinatorial explosion of possible interactions involved. For example, performing a molecular dynamics (MD) folding simulation on a single small protein domain can involve several months of computation. As a result, folding simulation of a multi-domain protein is currently numerically intractable. Developing efficient protein structure prediction algorithms thus remains a major scientific challenge in computational biology.

Here we give an overview of computational methods for protein structure predictions developed in our group at Inria Grenoble together with MIPT Moscow. In particular, we demonstrate how machine-learning [1] and artificial intelligence can be used to facilitate the predictions. Also, we demonstrate the usefulness of integrative approaches with additional SAXS [2] and Cryo-EM [3] information. Finally, we discuss the performance of our methods in blind computational structure prediction experiments CASP [4], CAPRI [5], CSAR [6], and D3R [7].

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Crystallographic studies of membrane proteins

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Membrane proteins (MPs) are among the most important constituents of every biological cell's wall. Due to their partial hydrophobicity and lower stability relative to soluble proteins, MPs are notoriously difficult to study and, in particular, to crystallize. Here, we overview the in meso approach to MP crystallization and discuss recent examples of successful structure determination efforts [1–3]. We show that in many cases a single structure of a membrane protein is not sufficient for structural characterization and elucidation of its mechanism of action, and thus search for new crystallization conditions, new crystals belonging to different space groups, and new conformations is required. Finally, we discuss the stages of qualitative and quantitative structural analysis and comparison of MP structures.

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Molecules relevant for cardiovascular aging – specific role of mitochondrial p27

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Cardiovascular functionality decreases with age. Interestingly, recent studies have shown a protective effect of caffeine on the cardiovascular system. We have shown that concentrations of caffeine detectable in serum after moderate coffee consumption enhances the migratory capacity of endothelial cells, which critically depends on mitochondrial function. Therefore, we wanted to identify the molecular link between caffeine, mitochondrial energy metabolism and migration.

Surprisingly, we found that caffeine induces the translocation of p27/Kip1 (p27), a protein previously only known as a nuclear cell cycle inhibitor, into the mitochondria. Downregulation of p27 levels by RNA interference inhibited the caffeine-induced migration. To investigate the effects of p27 localization on mitochondrial energy metabolism and migration we expressed mitochondrially- and nuclear-targeted p27 in endothelial cells. While expression of nuclear p27 decreased basal migration, mitochndrial p27 enhanced migration, mitochondrial ATP production and the mitochondrial membrane potential. Similarly, only overexpression of mitochondrial p27, but not nuclear p27, rescued the complete loss of migratory capacity induced by knockdown of p27.

To investigate the link between caffeine, mitochondria and p27 *in vivo*, we performed microarray analysis of hearts from wildtype and p27-deficient mice treated with caffeine in their drinking water. Caffeine induced the expression of genes involved in mitochondrial energy metabolism and biogenesis only in wildtype mice demonstrating a crucial role for p27 in enhanced mitochondrial function.

In conclusion, caffeine seems to have a dual function explaining its protective functions in the cardiovascular system, a short term translocation of p27 to the mitochondria improving their function and in the long run a change in gene expression leading to mitochondrial biogenesis.

Aged isoform of β-amyloid as biomarker and drug target of Alzheimer's disease

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Alzheimer's disease (AD) is closely associated with ageing. In view of the amyloid hypothesis, the key molecular event of AD is structural transition of β-amyloid (Aβ) from the physiologically normal monomer state to soluble neurotoxic oligomers accumulating in the form of insoluble extracellular aggregates (amyloid plaques) in brain tissues. Zinc ions as well as 'aged' Aβ species present in the plaques are known to play a crucial role in triggering pathological conversion of endogenous Aβ. Isomerization of Asp7 is the most abundant age-related spontaneous non-enzymatic modification of Aβ. Our *in silico*, *in vitro* and *in vivo* studies have shown that Aβ species with isomerized Asp7 (isoAsp7-Aβ) significantly differ in their properties from healthy (non-modified) Aβ molecules [1]. We have found that isoAsp7-Aβ might constitute a nucleation seed and initiate formation of the neurotoxic zinc-dependent Aβ oligomers [2], thus inducing development of cerebral amyloidosis and other pathological processes characteristic of AD [3]. Moreover, the role of the Aβ metal-binding domain (the N-terminal region 1-16) as the minimal necessary and sufficient pathogenic unit of isoAsp7-Aβ has been strongly suggested [4]. These findings allow to link the emergence of isoAsp7-Aβ due to Aβ ageing with the onset of age-related pathology, and to use isoAsp7-Aβ species as potential biomarkers [5] and drug targets of early diagnosis and therapy of AD [6].

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Structural and functional studies of Human GPCRs

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Cell surface receptors and their related intracellular proteins are responsible for human cellular communications with each other and their environment, and are involved in a wide range of physiological activities. Such a central role in human biology makes cell signaling the target for intervention for tuning physiological responses and fighting numerous conditions and diseases. G protein coupled receptors (GPCRs) are involved in a wide range of physiological systems where they are responsible for around 80% of transmitting extracellular signals into cells.

In humans, GPCRs signal in response to a diverse array of stimuli including light molecules, hormones, and lipids, where these signals affect downstream cascades to impact both health and disease states. Yet, despite their importance as therapeutic targets, detailed molecular structures of only ~30 unique GPCRs have been determined to date. A key challenge to their structure determination is adequate protein expression and crystallization. Here we report the quantification of protein expression in an insect cell expression system for all 826 human GPCRs using two different fusion constructs. Expression characteristics are analyzed in aggregate and among each of the five distinct subfamilies. These data can be used to identify trends related to GPCR expression between different fusion constructs and between different GPCR families, and to identify and prioritize lead candidates for future structure determination efforts.

New concepts for the treatment of myopathies

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About one-third of cases of familial hypertrophic cardiomyopathy (HCM), a genetically inherited heart disease that affects 1 in 500 people worldwide, are caused by mutations in a component of β-cardiac myosin [1]. In regard to the therapeutic modulation of myosin function in the failing heart of these patients, strategies aiming to improve cardiac performance have focused on the compensation of impaired contractility by activating energy release and force production by β-cardiac myosin. However, this focus on increasing the energy efficiency and force output of the sarcomere by activating an ever decreasing number of functional contractile units is unlikely to result in more than short-term benefits. In the complex scenario of the failing cardiomyocyte, allosteric trigger events such as disease-causing mutations and various stress factors lead to a vicious cycle of deteriorating mechanical function and compensatory changes in signalling pathways, intracellular ion fluxes, and gene expression patterns.

Based on recent advances in our understanding of structure-function relationships in regulated actomyosin complexes [2-5], it appears to be more promising to focus therapeutic strategies on another frequent effect of mutations, which is an accompanying decrease in protein stability and reduced tolerance against mechanical and oxidative stress. The resulting increase in the abundance of misfolded myosin can play an important role in the pathophysiology of cardiac diseases leading to heart failure [6]. Here, the restoration of normal myosin homeostasis by pharmacological chaperons promises to provide an efficient means to interfere with the progression of heart failure. Small molecules that bind misfolded proteins and stabilize them or act as templates that encourage the proteins to fold correctly are termed pharmacological chaperones. Most of the pharmacological chaperones that are currently under development are active site ligands [7, 8]. Getting the therapeutic dosing regimen right is extremely difficult for this sort of compound and extrapolation from tests in animal models is not straightforward. Therefore, this promising approach has been applied so far only to a small range of diseases such as lysosomal storage diseases. Therapeutic applications of pharmacological chaperones that bind to allosteric sites, such as the compounds identified by my team [9], are predicted to be less problematic [7, 10]. In addition, the targeting of allosteric sites with non-inhibiting strategies extends pharmacological approaches to proteins that were previously regarded as non-druggable targets. The advantages of small-molecule chaperones include that they bind only to specific sites, thus acting only on a small number of protein targets and causing fewer side effects. We assume that their actions are mostly beneficial [11]. Their binding to high-energy folding intermediates can increase the rate of folding or promote refolding of misfolded protein. The spectrum of associated activities includes the stabilization and refolding of target proteins, prevention of the formation of toxic aggregates, and the maintenance of a steady-state level of their target protein at or above a minimal required concentration.

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Role of membrane domains and juxtamembrane regions in p75 neurotrophin signaling

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Type I integral membrane proteins are one of the most interesting objects in modern structural biology. These proteins contain large intra- and extracellular domains, while their transmembrane (TM) domain is composed of a single α-helix. The majority of type I membrane proteins are cell receptors and are active inside the homo- , heterodimeric or oligomeric complexes. Full-size type I proteins cannot be crystallized, therefore, their activation mechanisms are described on the level of spatial structures of separate domains. For instance, more than 10 spatial structures of the TM domain dimers of type I proteins were determined in our laboratory. However, the assignment of the obtained structures of separate domains to the certain functional states of the full-size receptor is an exigeant task, which is usually accomplished based on the indirect data, such as the effects of point mutations. To establish the relationship between the states of various domains of a type I membrane protein, it is necessary to investigate the structure of constructs, containing several domains of the protein. Such a work was performed with the p75 neurotrophin receptor (p75NTR). The construct, corresponding to the p75NTR with deleted extracellular domain was produced, refolded and dissolved in a variety of membrane mimetics to study its spatial structure and intramolecular mobility by solution NMR spectroscopy. As a result, the motions and conformation of the intracellular “death domain” of p75NTR were shown to be independent on the state of the TM domain in both monomeric and dimeric states of the protein in all tested membrane-like environments. These data reject the previously suggested mechanisms of the p75NTR activation and allow to formulate the alternative hypothesis. The work resulted in the development and approbation of novel technologies for the studies of constructs composed of several domain of type I integral membrane proteins, including the design of new membrane mimetics for solution NMR studies.

Cryo electron microscopy in protein synthesis investigation

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mRNA translation is one of the fundamental processes in a cell, synthesizing proteins, the building blocks of life. We investigated this process, biochemically and structurally, at different levels of complexity and here I will discuss some of our interesting findings.

Ribosomes, the molecular machinery that catalyses translation has been the focus of my research. The monomeric 80S ribosome were studied using the method of single particle analysis (SPA) combined with a new way for image data collection and processing. This led to the first near-atomic structure of the human ribosome at an average resolution of 3.6Å (1). This structure provides unprecedented insights into rRNA entities and amino acid side-chains of the ribosomal proteins. Also, we have recently used this 80S structure as a reference for the first structure of the human 80S ribosome with a eukaryote-specific antibiotic - cycloheximide. We showed its anti-proliferative effect on several cancer cell lines. The structure sheds light on the detailed interactions, in a ligand-binding pocket of the human ribosome, that are required for structure-assisted drug design [Myasnikov A.G. at al, *Nature Communication*, 2016, accepted].

Alongside, the higher-order entities of ribosomes polyribosomes (polyRs) from wheat-germ extract were studied using velocity sedimentation and cryo electron tomography (CET). The conformations of eukaryotic polyRs formed in a long-term cell-free translation system were analyzed over all the active system lifetime. Three distinct types of the conformations were observed: (i) circular polyRs, (ii) linear polyRs and (iii) densely packed 3D helices (2, 3). At the beginning, during the first two rounds of translation, mostly the circular (ring-shaped and double-row) polyRs and the linear (free-shaped and zigzag-like) polyRs were formed. The progressive loading of the polyribosomes with translating ribosomes, induced the opening of the circular polyRs. Presumably, this led to the transformation of a major part of the linear polyRs into the dense 3D helices (4). Functional tests showed a reduced translational activity in the density gradient fraction of the 3D helical polyribosomes.

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Protein folding at the speed of life

Niemi A.

We argue that the Landau free energy of a protein backbone coincides with the Hamiltonian of a discrete non-linear Schroedinger equation (DNLS) with an additional helicity contribution. The solitons of the DNLS have a geometric correspondence with super-secondary structures such as helix-loop-helix. Entire folded proteins are modelled in terms of multi-soliton solutions of the DNLS, with sub-Angstrom root-mean-square precision. When we combine the Landau free energy with Glauber dynamics we obtain a model how the dynamics of a protein folding proceeds. As an application, we simulate the folding process of a myoglobin under varying ambient temperature, and show that the approach produces the experimentally observed pathway.

Regulatory RNAs in Bacteria and Archaea: their interaction with Sm-like (LSm) proteins

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Regulatory RNAs are very important molecules of the cells participating in many processes. They belong to a large family of non-coding RNA (ncRNA) that is not translated into proteins. The exact number of ncRNA in the cells is unknown. Identification of the ncRNA by transcriptomic and bioinformatics studies suggests existence of several hundred various ncRNA in bacteria and archaea and thousands ones in eukarya [1]. The regulatory RNAs are often called “small RNA”, or sRNA, because of rather small length in comparing with large ncRNA as ribosomal RNA.

Bacterial sRNA involved in gene expression regulation of many proteins. An important player in sRNA control is protein Hfq (host factor for Q-beta phage replication). It is an abundant homohexameric RNA chaperone which stabilizes the sRNAs and promotes their interactions with mRNAs leading to altered stability and/or translation of these targets [2]. Hfq belongs to Sm/LSm family of proteins containing so-called Sm-fold comprising an N-terminal α-helix followed by five antiparallel β-strands. Extensive structural and biochemical studies reveals three different RNA-binding sites on the protein surface. First of them possesses affinity to U-rich RNA sequences of mRNA and sRNA. The second RNA-binding site binds adenine-rich RNA sequences including poly(A) RNA 3’-terminus. These two sites locate at the opposite sides of the ring-shaped Hfq hexamer. Recently, the third, lateral RNA-binding site has been discovered by biochemical [3] and structural methods [4]. It seems that it is a meeting point of mRNA and sRNA.

At this moment, we cannot say much about sRNA and function of LSm proteins in archaea. Our studies are concerned of the comparison of the structures and RNA-binding properties of LSm proteins from *Methanococcus jannaschii, Methanococcus vannielii, Sulfolobus solfataricus* and *Haloarcula marismortui*. We used surface plasmon resonance, fluorescent anisotropy measurements and X-ray crystallography in our studies.

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FLInChR: a new optogenetic tool and its application for neural circuit dissection

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The discovery of a wealth of microbial rhodopsins that convert light-dependent isomerization of the bound chromophore into conformational changes enabling transport of ions across cellular membrane has led to the generation of a great toolkit for optogenetics. The most productive avenue for finding variants with particularly useful properties has been genome mining and molecular engineering. In addition to specific mutations, changes in membrane topology are hypothesized to have contributed to the expansion of membrane protein families in evolution, as well as to the refinement of protein function. In one striking example, ionotropic glutamate receptors are thought to have arisen following the insertion of an inverted potassium channel between two parts of a glutamate binding protein.

We describe a novel light-gated inhibitor of neural activity, FLInChR, generated through topological inversion of a Channelrhodopsin variant. In brain slices, FLInChR (**F**ull **L**ength **In**version of **ChR**) is able to sustain suppression of action potential generation for prolonged periods, yet displays enough precision for the "deletion" of individual action potentials in high frequency trains. In behaving rats, FLInChr can be used for optical tagging of specific neuronal populations, and when tested in a context where suppression of neuronal activity is thought to perturb behaviorally-relevant motor output, produces reliable impairment of task performance. The generality of the topological engineering approach by creating, through an inversion of Cs-Chrimson, a cation channel that displays significantly greater selectivity for K^+ over Na^+. Our findings argue that membrane topology provides a useful orthogonal dimension for molecular engineering of rhodopsin molecules with new functionalities, and suggest that topological inversion is not prohibitively energetically expensive, thus supporting the notion that changes in membrane topology have contributed to the evolution of membrane protein families.

Metagenomics of aquatic microbes, a window into the diversity of rhodopsins

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Aquatic environments, both continental and marine, represent likely the oldest ecosystems on Earth and have preserved similar conditions for eons. Their photic zones are playgrounds of the major solar energy biological harvesting machineries, namely chlorophyll and rhodopsin based bioenergetics. Along these lines, prokaryotic cells have been using solar energy for at least 2.2 billion years. This time span makes the rise of vascular plants and significant terrestrial ecosystems very recent events. Accordingly, prokaryotic rhodopsins have had time to diversify into an astounding variety. Hence, it is not surprising that this diversity is being used to develop optogenetic tools. Presently, the rhodopsins that are being studied and used in optogenetics come from pure cultures of microbes. However, it is a well-known fact that culture is a rather inefficient (slow and unreliable) tool for obtaining novel microbes. Direct sequencing of prokaryotic microbial genomes by metagenomics or single cell genomics are now the most efficient tools to get insights about microbes that, if not replacing culture, are allowing major advances. We have studied aquatic prokaryotes by metagenomics including two classic rhodopsin-field environments: hypersaline and marine and also a less exploited one (freshwater lakes). By using purely metagenomic approaches we have been involved in the discovery of several novel actinobacterial rhodopsins: macrhodopsin and acidirhodopsin, both marine and actinorhodopsins and proteorhodopsins from freshwaters. We also described novel xhantorhodopsins and xenorhodopsins from hypersaline environments and novel marine euryarchaea rhodopsins from the Mediterranean. All this wealth of new rhodopsins can be screened for novelty based on sequence and then candidates can be analysed for function and even structure by surrogate expression in *E.coli*.

Anti-ageing effect of dietary restriction

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Restriction of dietary intake with essential nutrients, referred to as dietary restriction (DR) here, reduces morbidity and mortality in a range of organisms including invertebrates and mammals. Accumulated evidence indicate presence of evolutionarily conserved mechanisms that regulate aging and longevity in animals in response to reduction of dietary energy intake. Those hypothetic mechanisms, some of them are already proved, are summarized into the layered structure composed of levels of the hypothalamus, circulating blood hormones, and cells including mitochondria (Figure 1). Animals adapt to DR with alterations of circulating hormones and the neuroendocrine system, i.e., 1) reduction of plasma concentrations of IGF-1, insulin, and leptin, 2) elevation of plasma ghrelin and adiponectin, 3) activation of neuropeptide Y (Npy) and/or agouti-related protein (Agrp) neurons in the hypothalamus, 4) inhibition of proopiomelanocortin (Pomc) and/or cocaine amphetamine related transcripts (Cart) in hypothalamic neurons, 5) finally, halts of growth, reproduction, and thyroidal activities; by contrast, activation of adrenal glucocorticoid axis. These changes alter cellular signaling, such as attenuation of mTor and insulin/IGF-1 pathways. Mitochondrial bioenergetics and cellular stress responses could be also modified.

To test a role for Npy, which is supposed to be a key molecule initiating the neuroendocrine response to DR, we conducted a lifespan study using Npy-knockout mice (1). The results indicate the necessity of Npy for the life-extending effect (1). We also conducted lifespan studies using knockout heterozygotic mice for Foxo1 or Foxo3 gene, each of the Foxo family transcription factor stays downstream to IGF-1 signaling. Our studies indicate that Foxo3 is required for the life-extending effect of DR (2); by contrast, Foxo1 is needed for the anti-neoplastic effect of DR (3). These findings suggest that aging and cancer are differentially regulated by Foxo3 and Foxo1 in mice. Subsequent studies suggest an involvement of Foxo3 but not Foxo1 in the regulation of mitochondrial bioenergetics. Recent studies using genetically engineered animals have been dissecting mechanisms that regulate lifespan and thus aging.

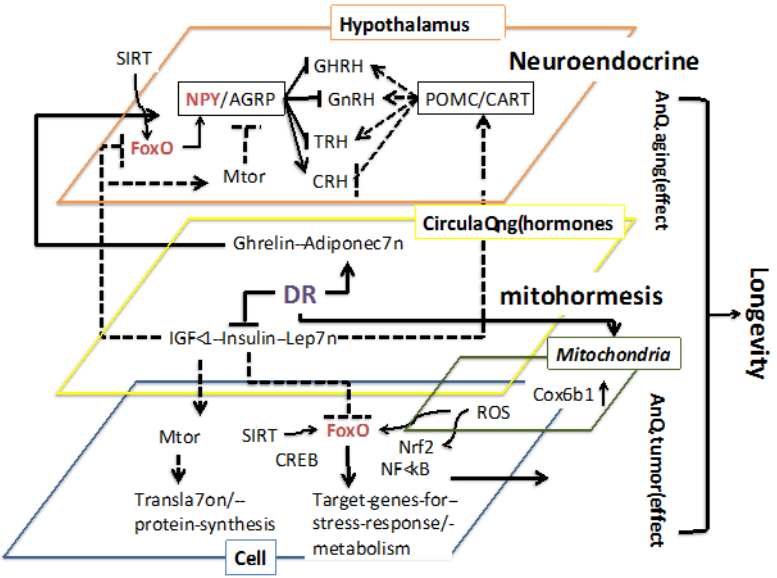

Fig. 1. Hypothetic mechanisms of the effect of dietary restriction (DR).

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Pushing back frontiers: in-house GPCR crystallography in structure-based drug design

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Structure-based drug design (SBDD) organizations working on soluble protein targets continue to utilize and rely on in-house x-ray sources to generate structural information due to their convenience. However, membrane protein structural biology has remained almost exclusively reliant on synchrotron light sources. Diffraction data collection on-site can provide fast turnaround times to medicinal chemists, enabling key decisions to be made quickly and efficiently in real time.

Over 40% of prescription medications target G protein-coupled receptors (GPCR) a superfamily of protein receptors which are notoriously difficult to crystallize due to their instability when removed from the cell membrane, so remaining intractable to most SBDD platforms. The challenges are huge and yet so are the potential rewards. Heptares proprietary StaR® technology generates thermostabilized receptors containing a small number of point mutations, homogenous and in a natural pharmacologically relevant conformation (agonist or antagonist) that matches the drug product profile. These can then be readily crystallised in both classical vapour diffusion using harsh short-chain detergents and lipidic cubic phase (LCP) to drive SBDD even with weak early stage compounds / fragments.

Together, Heptares and Bruker have performed feasibility studies to assess if in-house sources have reached a level that would have a role in SBDD pipelines specifically dedicated to GPCRs. We find that Heptares StaR technology alongside Bruker's state-of-the-art instrumentation enables high resolution structures to be obtained in-house in a realistic timeframe using the D8 VENTURE. The D8 VENTURE x-ray diffractometer consists of state-of-the art technology; the METALJET source is the only source available that can deliver small, high intensity x-ray beams and is coupled with the newly launched PHOTON II CPAD detector.

Here, we present the results obtained using the combination of both technologies yielding a 2.8Å dataset for the human Orexin-1 StaR in under 120 minutes on the D8 VENTURE. To the best of our knowledge the structure represents the first atomic resolution GPCR structure to be determined without the use of synchrotron radiation. Human Orexin-1 has been strongly implicated in the treatment of cocaine addiction with potential broader applications in substance addictions (nicotine, alcohol) and compulsive disorders (binge eating, gambling).

Conformational states underlying autoregulation of F-BAR domain membrane remodeling

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Growth factor signaling in neurons controls the expansion of synaptic arbors in response to activity and external stimuli, leading to long-lasting changes in synapse strength and connectivity that underlie learning and memory. Membrane deforming proteins cooperate with the cytoskeleton to sculpt lipid bilayers into complex and dynamic geometries, but we still do not understand how their activities are temporally and spatially regulated in cells.

Here we use single particle electron microscopy to show that the neuronal membrane remodeling protein Nwk is autoinhibited by intramolecular interactions between its membrane remodeling F-BAR domain and its C-terminal SH3 domains. These autoinhibitory interactions control both F-BAR-mediated membrane remodeling, and also unexpectedly inhibit SH3-mediated actin cytoskeleton assembly. Uncoupling these dual autoregulatory mechanisms in the fruit fly leads to excess neuronal synapse growth.

Membrane remodeling by F-BAR proteins is regulated by autoinhibitory interactions between their SH3 and F-BAR domains. We determined the structure of the F-BAR protein Nwk in both soluble and membrane-bound states. Upon membrane binding, Nwk SH3 domains do not completely dissociate from the F-BAR dimer, but instead shift from its concave surface to positions on either side of the dimer. The coordinated autoregulation couples membrane remodeling and SH3 domain activities, and is critical for proper control of neuronal shape and size.

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Shaping strategies in healthy longevity science

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Aged population has rapidly advanced in industrial countries, such as Japan and Germany. For instance, in 2030 the population of the aged-aged society (over 75 years old) is estimated about 20% in Japan. However the epidemiological data show that there is the gap between life expectancy and healthy life expectancy not only in these described countries, but also in most other industrial countries. Thus, elderly people gain more social and political impact in the society and require more medical support because of expansion of dys-health and disability.

The aim of our studies is therefore to elucidate the key factors of successful-, healthy-, and smart ageing, instead of prolongation of solely life span. Experimental data are still disputed because of the complex networks controlling ageing and age-related neurodegenerative diseases (1, 2). Thru all these theoretical and experimental data at least three important determinants are implicated that might induce healthy ageing, namely nutrition (3, 4), exercise, and social activities. To gain insight into the molecular mechanisms of ageing we have focussed on involvement of mitochondria as trigger and/or target of ageing in hippocampus, in comparison with other brain regions as well as heart and liver (5). The hippocampus plays various important roles such as in learning and memory (6,7). Also hippocampal disruption is one of the earliest diagnosis symptoms of *Alzheimer's disease (AD)*.

Second part of my talk deals with the strategies of future projects in ageing studies among Germany, Japan, and Russia.

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Rapid analysis of fluorescence microscopy images

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Genome sequencing, gene expression profiling, proteomics and metabolomics have facilitated the analysis of new layers of biological processes. However, efforts to relate large volumes of genetic information to cellular behavior have revealed the limitations in our understanding of cellular complexity. Fluorescence-based high-content screening technology was developed to meet the challenge of integrating these large volumes of information to model accurately increasingly complex cellular behavior. Applicability of fluorescence methodology made a leap forward with introduction of quantitative approaches. With the help of quantification, it became possible to interpret fluorescent observations objectively and analyze them statistically.

IN Cell Analyzer 2200 high-content screening platform from GE Healthcare Life Sciences allows researchers to visualize individual cells and measure multiple cellular processes over time. High-content screening microscopy experiments generally require at least five independent steps: sample preparation, image acquisition, image analysis, image data management and image analysis. The success of any high-content screening-imaging experiment relies on thoughtful assay design and appropriate image analysis approaches. We have created a number of methods for evaluating cell toxicity to determine nuclear area and cell number, and plasma membrane permeability. Methods developed for the characterization of bone marrow fractions obtained by density gradient centrifugation, comprising determining cell size, toxicity, assessment of the Hoechst dynamics absorption by living bone marrow cells. To investigate the influence of various factors on cell migration and cell-cell interaction we developed high-content analysis wound healing assay leading to increased assay precision and accuracy. Methods for the intracellular reactive oxygen species dynamic have evaluated for drug-screening procedure for photosensitizing agents used in photodynamic therapy.

Microscopy has always been the method of choice for cell biologists. With recent advances in automated microscopy platforms and computational image-analysis techniques, we can expect this technique to be instrumental in answering diverse biological questions.

Structural studies of the mechanism of translation

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Ribosome is the largest and most complicated RNA-protein assembly in the cell that translates the genetic code into proteins. Bacteria ribosome consist of a large and a small subunit, which together compose the 2.5 mega dalton (MDa) 70S ribosome. Their eukaryotic counterpart is the 80S ribosome (from 3.5 MDa in lower eukaryotes to 4.5 MDa in higher). Many ribosomal key components are conserved across the three kingdoms of life: bacteria, archaea, and eukarya and constitutes a common core undertaking the fundamental processes of protein biosynthesis.

First functional complexes of the ribosome with messenger RNA and transfer RNA have been detemined by X-ray analysis on ribosomes of extrim termophilic bacteria *Thermus thermophilus* [1, 2]. These complexes allow to explain mecanism of protein synthesis on atomic lavel including mechanism of decoding, mechanism of pepide bond formation and mechanism of translocation of mRNA-tRNA duplex in the ribosome.

The complete structure of the full 80S ribosome from *Saccharomyces cerevisiae* at a resolution of 3Å have been determined 10 years later [3]. The eukaryotic 80S and bacterial 70S ribosome shares 34 common proteins and eukaryotic ribosome has additional 45 unique proteins and bacterial ribosome has 22 additional unique proteins. The eukaryotic ribosome has additional expansion segments of ribosomal RNA which are located on the solvent side of the ribosome structure. Interface between ribosomal subunits responsible for mRNA and tRNA binding are found conservative. Our crystals capture the ribosome in two different conformations which are believed to reflect intermediate states in course of mRNA and tRNA translocation.

Bacteria and yeast ribosome experimental models have been used for structure determination of complexes with antibiotics and eukaryotic inhibitors including anticancer drugs.

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Posters

Activity and selectivity tests of light-driven pumps incorporated into lipid vesicles

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Optogenetics is nowadays one the most perspective field of science. Finding new optogenic tool and their characterization is still very challenging problem. For such studies we have developed method of determination of the pumping efficiency and selectivity of retinal light-driven transporters, incorporated into lipid vesicles.

We have successfully incorporated several proteins into small unilamellar lipid vesicles (proteorhodopsin, bacteriorhodopsin, KR2). All proteins were preliminary purified by Ni-affinity chromatography and SEC.

Liposomes were constructed in unbuffered salt solution (NaCl or KCl). pH changes in liposomes suspensions were monitored at protein concentration about 0.7 mg/ml. Experiments were performed also upon the addition of 30μM of protonophore carbonylcyanide m-chlorophenylhydrazone (CCCP).

pH changes reached absolute value of 0.2, which is extremely high for the pure system, containing only protein of purpose. Addition of CCCP abolished pumping activity in case of proton pumps and increased it in case of KR2. This is also an evidence of oriented incorporation of proteins into vesicles.

Using optimized method gives us a possibility for fast activity and selectivity screening of new promising optogenetic tools.

Liposomal formulation of a methotrexate lipophilic prodrug: interactions with cells in vitro

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Methotrexate (MTX)—a folic acid antimetabolite—is a cytostatic agent widely used in treatment of cancer and autoimmune diseases. Methotrexate competitively inhibits dihydrofolate reductase thus depriving the cell of tetrahydrofolate, an essential co-factor for biosynthesis of purine nucleotides and thymidylate. The efficiency of treatment with MTX is limited by systemic toxicity and frequent development of drug resistance. Methotrexate encapsulation in a nanoparticulate carrier is a promising means to improve its pharmacological properties and reduce side effects.

Previously, we developed a liposomal formulation of methotrexate incorporated in the fluid lipid bilayer (ePC:PI = 8:1 by mol) in the form of a tailored lipophilic conjugate, i.e. dioleoylglyceride ester of γ-COOH in the glutamate moiety of MTX (MTX-DG) [1]. A short hydrophilic spacer (N-methylenecarbonyl-β-alanine) between the bulky MTX moiety and diglyceride membrane anchor minimizes the disruption of the bilayer packing to load as much as 10 mol. % prodrug (to total lipids) into 100-nm liposomes. In the cell, MTX-DG should be easily hydrolyzed by esterases with the release of MTX. MTX-DG loaded liposomes surmounted the resistance of human T-lymphoblastic cells towards MTX [1].

To study a mechanisms of interaction with tumor cells and intracellular traffic of MTX-DG loaded liposomes, a fluorescent probe was synthesized – analog of MTX-DG with BODIPY-label at the ω-position of the aliphatic chain of diglyceride [2]. With this probe we showed that, after binding with tumor cell, MTX-liposomes localize in glycocalyx for 1.5 h and during next 2-4 h migrate to cytoplasm and perinuclear region. This process is accompanied by disassembling of MTX-liposome components (matrix lipids and prodrug), that is by liposome unloading. To quantitatively assess accumulation of liposomes by cells, we used flow cytometry. Tumor cells (A549, CoLo357) accumulated two times more MTX-liposomes as compared with “normal” cells (3T3, HEK296T), while accumulation of empty liposomes was approximately four times lower in all cell cultures. With the help of a number of inhibitors of endocytosis we showed that MTX-liposomes enter the cell mainly via clathrin-dependent pathway and to a lesser extent by other mechanisms.

This work was supported by the RFBR grants no. 16-34-01237, 13-04-00069.

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Investigations of biological active substances (BAS) actions to models of biomembranes

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Two models that correctly mirrored of biomembranes structure and functions were applauded for BAS actions study: 1) dimyristoilphosphatidylcholine (DMPC) synthetic saturated phospholipid was used for formation of model multilammelar liposomes; 2) erythrocytes ghost as experimental object with lipid-protein composition was used for study BAS effects to membrane's protein-lipid interactions. As a method, corresponding of this models possibilities differential scanning calorimetry (DSC) was used. The first order of phase transitions of DMPC were heated and cooling was registered when several rates of models heating with constant pressure [1]. The multiple repetitions of melting and cooling processes were held for consecutive the analyses of impact of BAS on restructuring in membrane, dependent on temperature changes. The model of large multilammelar liposomes, formed from DMPC or egg phosphatidylcholine (EP), reflects the structure of multilayer membranes in the cell. Some examples are: endoplasmic reticulum, the Golgi apparatus, mitochondria. Stowage period of membranes in big multilayer liposome formed from (EP), ~ 6,9 nm [2]. Same the distance in the muscle cells exists between sarcoplasmic reticulum and plasma membranes. Bilayer thickness of liposome membrane formed from egg phosphatidylcholine was ~ 4 nm [2] that also was corresponded of nature bilayers sizes. The using of DMPC melting variations are the essential DSC method modifications that permitted us to simulate of temperature changes, which may exist both in individual compartments of cells, and in whole cells. Besides of local cell changes in the rate and temperature repetitions at membrane compartments, the great lowering of temperature occurs when hibernation, when stepping out of hibernation condition the temperature on membranes, on the contrary, are going up. The phase state of proteins microdomains at bilayers of erythrocytes ghost has been examined by means of DSC under BAS. Changing of thermodynamic parameters was registered, and was compared with parameters of microviscosity.

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IPSC-derived neurons from patients with Down syndrome as in vitro cell model of Alzheimer's disease

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Alzheimer's disease (AD) is the most spreaded form of dementia [1]. There are 27 million people suffering from AD all over the world. Individuals with Down syndrome (DS) is the biggest risk group of AD (more than 7 million people). Up to 80% of individuals with DS acquire AD-like symptoms to age 50. These symptoms include beta-amyloid (bA) accumulation and neurofibrillar tangles formation in brain tissues [2].

In this report, we studied possibility of use cells derived from individual with DS as a base for cell model of AD. According to this aim, we reprogrammed to pluripotent state three cell lines of amniotic fluid derived from individuals with DS. Then we differentiated these cells in neural state.

We observed pathological metabolism of bA in the neural cells derived from individuals with DS in contrast with the cells derived from individuals with normal karyotype. Pathological metabolism of bA was expressed in upregulated secretion and accumulation of bA in the cells derived from individuals with DS. Gene expression levels of APP, BACE2, TMED10 and RCAN showed confident differences between cells derived from DS and normal karyotype groups. These genes are involved in bA processing [3][4].

Thereby we consider IPSC-derived neurons from individuals with DS as a reliable cell model of Alzheimer disease.

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Influence of dimerization and glycation of alpha-synuclein on amyloid transformation

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The toxic effect of alpha-synuclein oligomers is known due to their participation in the formation of pores in the cell membrane of neurons and the subsequent changes in ion transport. As a result of these processes, an increase in the content of calcium ions in the cytosol could activate basic forms of NO-synthase and thereby increase the production of NO. In turn, nitric oxide can increase the production of superoxide by inhibiting the mitochondrial respiratory chain, which might initialize the processes of nitration and oxidation of other proteins. We studied the effect of point substitution of a tyrosine residue for a cysteine residue, generating dimeric forms of alpha-synuclein, and its posttranslational modifications (glycation and oxidation) on the amyloid transformation of alpha-synuclein. The wild-type and Cys136-containing fractions of alpha-synuclein were separated using thiol-Sepharose. In the absence of reducing agents, Cys136-AS forms dimers due to the disulfide bonding. Both wild-type and Cys136 alpha-synuclein preparations are prone to aggregate during prolonged incubation under shaking at pH 4 and 37°C, but the aggregates produced by either monomeric or dimeric Cys136-AS do not exhibit amyloid properties according to the test with Thioflavin T. Moreover, an admixture of dimeric Cys136-AS prevents the amyloid transformation of the wild-type alpha-synuclein.

Glycation of alpha-synuclein by methylglyoxal stimulates protein aggregation, but prevents it amyloid transformation. We suppose that the effect of point substitutions of amino acids stimulating the formation of dimers as well as post-translational modifications of alpha-synuclein should be taken into account to avoid erroneous interpretation of experiments on amyloid transformation of this protein.

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Palmitate-induced pore in the cycling of ions across the inner mitochondrial membrane

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Earlier we found that in the presence of Ca^{2+}, palmitic acid (Pal) promotes a non-classical permeability transition in mitochondria, which has been identified as the opening of a novel cyclosporin A-insensitive Ca^{2+}-dependent pore (PalCaP). It was shown that Pal in complex with Ca^{2+} increases the nonspecific permeability of artificial (planar and liposomal) membranes and has higher affinity to Ca^{2+} among a large number of other lipids and fatty acids. The assumption was made that the mechanism of Pal/Ca^{2+}-induced membrane permeabilization relates to the Ca^{2+}- induced phase separation of Pal and can be considered as formation of fast-tightening lipid pores. One of the main features of lipid pores is their ability to close spontaneously, with rapid restoration of membrane integrity, providing for the possibility of ion cycling across the mitochondrial membrane [1].

In this study, we continue to investigate the phenomenon of transient Pal/Ca^{2+}(Sr^{2+})-induced permeabilization and demonstrate that it can be involved in the cycling of ions across the inner mitochondrial membrane. It was found, that mitochondria, when exposed to a pulse of Sr^{2+} ions in the presence of valinomycin (a potassium ionophor) under hypotonic conditions, demonstrated a prolonged cycling of K^+ and Sr^{2+} ions, reversible cyclic changes in the membrane potential, respiration rate, and mitochondrial matrix volume. The pre-incubation of mitochondria with Ca^{2+}-dependent phospholipase A_2 inhibitors (aristolohic acid, AACOCF$_3$, p-trifluoromethoxyphenylhydrazone, et al.) considerably suppressed all spontaneous Sr^{2+}-induced cyclic changes in mitochondria. A new Sr^{2+} cycle can only be initiated after the previous cycle is finished, indicating a refractory period in the mitochondrial sensitivity to Sr^{2+}. The effects of Ca^{2+} and Sr^{2+} were observed in the presence of cyclosporin A (a specific inhibitor of mitochondrial permeability transition pore (MPT) opening). We suppose that the mechanism of these oscillations may be explained by opening of the mitochondrial short-living lipid pore which can be induced by fatty acids (mainly, palmitic acid) and Sr^{2+}. A possible physiological role of short-living lipid pores in maintaining of cell ion homeostasis is discussed. The work was supported by RFBR grant (№ 15-04-03081-a).

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The plant CLC channels: the diversity of functions, membrane localizations, structures and mysteries

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The membrane proteins of the CLC family have been found in all forms of life. The family is comprised of anion channels and anion/proton antiporters with as little as a single amino acid substitution converting an antiporter to a channel. Its members transport nitrate and/or chloride across a membrane either passively (channels) or in exchange to protons (antiporters). The latter also compensate the positive charges resulting from electrogenic H+ATPase operation in organelle membranes. These proteins are involved in many physiological processes, for instance, protein turnover and maintaining bone plasticity in animals. In eukaryotic cells, CLC proteins were only found in intracellular membranes. In plants, the CLC proteins play roles in mineral nutrition, ion homeostasis and turgor regulation. In our work, we look at the presently little-known role of the CLC antiporters in plant tolerance to high concentrations of chloride. We isolated a membrane fraction enriched with Golgi membranes from roots of a halophyte Suaeda altissima and demonstrated the electrogenic anion/proton exchange in isolated membranes. These data present evidence of anion/proton antiporter(s) functioning in Golgi of S. altissima root cells. We also cloned a CLC protein gene expressed in roots of S.altissima that we believe is responsible for this exchange. The protein structural similarities and differences with members of the family from other species as well as the ways to functionally complement its function and to further study its structure are presented and discussed.

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Mutated alpha-synuclein induce mitochondrial dysfunction and neuronal cell death

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Parkinson's disease (PD) is a common neurodegenerative disease characterized by the progressive loss of dopaminergic and non-dopaminergic neurons, and the accumulation of intracellular inclusions containing aggregated alpha-synuclein. The majority of PD is sporadic (>90%) but approximately 5-10% of cases are familial and due to mutations in PD-related genes, principally alpha-synuclein, parkin, PINK1, DJ-1, and LRRK2. A wealth of genetic and pathological data suggests that misfolding of protein alpha-synuclein is a primary step in both sporadic and familial form of PD. Using the live cell imaging we unravel the mechanism of toxicity of most common mutated form of alpha-synuclein A53T, A30P and E46K. We have found that monomeric A53T (100-300 nM) but not WT or A30P and E46K induced slow and progressive mitochondrial depolarisation (by 13%) in primary neurons and astrocytes. The same concentration of the monomeric A53T also induced increase of mitochondrial NADH that in combination with decrease in mitochondrial membrane potential strongly suggests inhibition of the mitochondrial complex I. Inhibition of the mitochondrial respiration by A53T induced increase in mitochondrial ROS production (measured with MitoSox).

Nanomolar concentration of monomeric A53T but not WT alpha-synuclein induced significant increase in the number of dead neurons measured using Hoechst 33342 + Propidium Iodide staining. Importantly, pre-incubation of the cells with Nrf2 activators was protective against cell death induced by A53T mutated alpha-synuclein. Thus, monomeric A53T alpha-synuclein induced neurotoxicity by inhibition of the mitochondrial complex I and ROS overproduction.

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Spontaneous formation of lipid rafts as a result of specific sphingomyelin-cholesterol interactions probed by molecular dynamics

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Membrane rafts are small (10-200 nm), heterogeneous, highly dynamic, cholesterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Rafts are implicated in membrane trafficking, signal transduction, including immune response, and entry and budding of pathogens. At the same time small rafts could be stabilized forming larger platforms through protein-protein and protein-lipid interactions.

In this work, we focused on large-scale molecular dynamics (MD) simulations of raft formation in model membranes and quantitative description of interactions between different types of lipids. We simulated bilayer systems containing binary and ternary mixtures of saturated and unsaturated lipids and cholesterol (Chol) and performed metadynamics simulations to determine free energy profiles of interaction of lipid species forming rafts. We observed spontaneous raft formation in ternary system with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), cholesterol and D-erythro-n-palmitoyl-sphingomyelin (SM) in ratio 2:1:1. The coexistence of both the L_o/L_d phases in ternary system was reproduced. Also we found specific interaction mode between sphingomyelin and cholesterol providing insight into raft microstructure.

This work was supported by grant № 16-34-01047 by Russian Foundation for Basic Research. All simulations were provided with the facilities of Lomonosov Supercomputer.

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Optimization of *H. salinarum* bacteriorhodopsin overexpression in *E. coli*.

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Heterologous overexpression of functional membrane proteins is one of the major bottlenecks of structural biology. Bacteriorhodopsin is a good example of the difficulties that appear when trying membrane protein overexpression. We reported a general approach [1] with a limited number of steps which allows to localize the reason of poor expression of a membrane protein. This complementary protein approach allowed us to increase bacteriorhodopsin expression by almost two orders of magnitude through the introduction of two silent mutations into its coding DNA.

The present study was undertaken to optimize the expression of the optimized gene in *E. coli*. We have examined the expression of the gene in different strains of *E. coli* that are widely used for the expression of membrane proteins, namely C41 and C43, as well as SE1 strain. SE1 strain turned out to give the highest yield of the functional bacteriorhodopsin that was at least 2 times higher than for the other strains. We have also demonstrated that one of the main variables that influences the protein expression level is the chemical nature of the inductor. We have analyzed the level of bacteriorhodopsin expression in SE1 strain when expression was induced with IPTG and lactose. We have obtained the bacteriorhodopsin yields to be 0.70±0.02 and 4.73±0.96 mg per liter of culture, respectively. We suppose that the reduced rate of protein synthesis results in the accumulation of the correctly folded protein.

Combining the complementary protein approach with the optimized protocol for bacteriorhodopsin expression we have obtained the final average yield 2.4±1.3 mg of functional bacteriorhodopsin per liter of culture. Using the same protocol we have also produced the D85N, D96N, and other mutants of bacteriorhodopsin. After crystallization trials for the first time the highly ordered crystals of bR expressed in *E. Coli* were obtained using the produced protein. The crystals obtained with *in meso* nanovolume crystallization diffracted to 1.67 Å.

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Principal component analysis of lipid molecule conformational changes in molecular dynamics simulations

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Every living cell is surrounded by a lipid membrane, and molecular dynamics simulations provide atomic-level information about the processes occuring there. Typically, lipid molecules exhibit multiple local and global conformational changes occuring on the timescales from femtoseconds to hundreds of nanoseconds. Most structural analyses of molecular dynamics simulations of lipid molecules either focus on average properties of the whole membrane or on specific conformational changes such as head group isomerization.

Recently, we applied principal components analysis (PCA) to study the conformations of lipid molecules. The aproach leads to identification of major collective motions and results in comprehensive quantitative characterization of the molecule's conformational space and characteristic time scales of motions in molecular dynamics simulations. Furthermore, it provides a simple framework for comparison of different simulations of membrane systems.

While the approach can be used to analyze the influence of external factors and additives on the conformation and dynamics of lipids, we illustrated it by comparing the simulations of DOPC bilayer using eight commonly used force fields and found the similarities and dissimilarities between them. Analysis of dissimilarities should lead to overall improvement of force fields and, consequently, simulation results.

The molecular dynamics simulations were performed using the JUROPA supercomputer at the Research Center Jülich. The work was supported by the CEA(IBS) – HGF(FZJ) STC 5.1 specific agreement, Russian Science Foundation research project 14-14-00995 and the 5top100-program of the Ministry for science and education of Russia. The work was also supported by FRISBI (ANR-10-INSB-05-02) and GRAL (ANR-10-LABX-49-01) grants within the Grenoble Partnership for Structural Biology (PSB).

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About supercomplex of OXPHOS system in heart mitochondria

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It is well known that complexes of oxidative phosphorylation (OXPHOS) system could operate in two states – in state of supercomplex and in dissociated state. Earlier on liver mitochondria it was found that supercomplex functioning regime of OXPHOS is realized under conditions of normal and low tonicity, while in high tonicity OXPHOS operates in dissociated state [1].

But in present work it was found that in heart mitochondria OXPHOS system operates in state of supercomplex in a wide range of tonicities of incubation media (120-600 mOsm). This was obtained by polarographic method using double inhibition technique by Baum. The investigation of mitochondrial ultrastructure by methods of electron microscopy (EM) and small-angle neutron scattering (SANS) under conditions of normal tonicity (300mOsm - isotonia) and low-amplitude swelling (120mOsm - hypotonia) proved the existence of two types of mitochondrial ultrastructure.

By the method of EM the difference between shape and thickness of cristae in isotonia and hypotonia was found. By the method of tomography the folded configuration of cristae was shown. The investigation of ultrastructure of heart mitochondria by methods of EM and inhibiting analysis was carried out on intact functioning mitochondria in presence of respiration substrates.

SANS was carried out on dense mitochondrial suspension. It was shown that both in isotonia and in hypotonia the highly-organized lamellar structures of lipid-protein bilayer form [2-4]. Data obtained reveal the difference of cristae thickness in isotonia and in hypotonia. The formation of lipid structures was shown using variation contrast method.

Thus the comparision of data, obtained from the investigation of function and structure of mitochondria let us suggest the existence of two types of supercomplexes with different structural parameters.

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Unique structural organization of prokaryotic membranes probed by high-performance computing

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Motivation and aim. Each domain of life possesses individual lipid composition of plasma membranes. *Bacteria* and *Eucaryota* feature zwitterionic and anionic phospholipid (PLs) bilayers, respectively. *Archaea* exhibit "bolalipid" monolayer membrane with each molecule possessing two O- or Θ-linked isoprenoid chains and two polar "heads". Most probably, individual membrane structure determines unique ecological portrait of *Archaea*, which are extremophiles, and plays an important role in growth and division of *Bacteria*. Prokaryotic membranes appear to be a unique target for antibacterials, in case of pathogenic bacterial strains, and promising bionanotechnological material, in case of *Archaea*. Here, we study the fine dynamic organization of bacterial and archaeal membranes with their microenvironment using high-performance molecular dynamics (MD) simulations.

Methods and Algorithms. Accessible simulation time for common supercomputers is 0.5–1 μs, which is long enough to model biomembranes. Our "bacterial" membrane was anionic and consisted of 75% POPG and 25% POPE PLs, with additional lipid-II molecule (the key player in bacterial cell wall synthesis; stands for promising target for novel antibacterial therapy). "Archaeal" membrane was built of a series of bolalipid "mimetics". In this series, acyl chains contained different number of characteristic for *Archaea* methyl or cyclopentanyl groups. Analysis of MD trajectories revealed local heterogeneities in bacterial membrane with lipid-II and complex phase behavior of archaeal membranes.

Results. For bacterial membrane, our analysis revealed that lipid-II molecule substantially disturbs lipid bilayer (as compared to the membrane without lipid-II) and introduces unique amphiphilic pattern into the membrane surface. Potentially, this complex pattern constitutes the "moving target" for many natural antibiotics, and may be employed in design and/or discovery of novel antibiotics [1].

For archaeal membranes, MD uncovered the role of "side" groups in bolalipids' hydrophobic chains: "straight" acyl tails form completely rigid "gel" phase, which is incompatible with life. Introduction of "side" groups gradually "melts" the membrane, finally arriving to "liquid crystal" state, which is believed to be native for the membrane [2].

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The influence of thromboxane A_2 receptor antagonists on the platelets activation

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The interaction between potential synthetic antiplatelet agents and human platelets must be investigated for the study of their action on the platelets signal pathways and their action mode. The results are needed for the understanding of cell responses formation and the possibility of cell response controlling. The platelet main signaling pathways are activated by thrombin, ADP and thromboxane A_2, but the cross point of these pathways is not revealed yet. It was shown that the use of large concentrations of thromboxane A_2 was not influenced on the quantity of activated platelets formed under thrombin action [1].

The main goal of our research was the study of influence of 5-substituted 3-pyridylisoxazoles as thromboxane A2 receptor antagonists on the platelet activation. We used the flow cytometry method for the platelets subpopulations formation and the laser aggregometer "Biola" (Russia). The washed human platelets suspensions were prepared from healthy men donors blood samples with gel-filtration and double precipitation method [2, 3]. We investigated antiaggregatory activities of four 5-substituted 3-pyridylisoxazoles together with thromboxane A_2 receptor selective antagonist GR32191B and their abilities to inhibit the formation of activated platelets subpopulations induced by thrombin and CRP. All compounds partly inhibit the formation of serine-positive activated platelets subpopulation (to 40%), but they not affect the serine-negative activated platelets subpopulation formation. All synthetic compounds showed the high antiaggregatory activities when arachidonic acid was used as the aggregation inductor. The influence of tested compounds on the pro-coagulating serine-positive activated platelets subpopulation demonstrates their availability as new drugs.

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Fold recognition using deep convolutional networks

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Deep learning (DL) is a popular approach in the field of machine learning, which recently gained momentum and sparked a lot of interest in the research community [1], particularly in computer vision and image recognition. Unlike previous 'shallow' approaches, DL tries to learn hierarchical representation of the data in hand. It alleviates the need for feature extraction and the 'curse of dimensionality' that limited performance of 'shallow' approaches.

Recently, DL was applied to biological data and yielded remarkable results in the human splicing code prediction [2], identification of DNA- and RNA-binding motifs [3] and predicting the effects of non-coding DNA variants at single nucleotide polymorphism precision [4].

We present the first application of deep convolutional networks to the problem of protein fold recognition. In this work, we generated the data set consisting of 1250 protein folds and their misfolded structures using the 3DRobot decoy generation method [5]. We used deep 3D convolutional network to assign score to a structure. We assessed the prediction quality on three popular benchmarks for fold recognition and demonstrate that our approach significantly outperforms other knowledge-based potentials. More specifically, our scoring function ranks 77% of native structures in Top 5 from their corresponding decoys on three 3DRobot benchmarks.

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ATR-FTIR spectroscopy: a powerful method for biomembrane study

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Here we report about the application of FTIR spectroscopy as a powerful and highly informative method to study the physicochemical properties of biomembranes: liposomes and membranes of cells. ATR-FTIR spectroscopy allows determining the fine structure and physico-chemical parameters of biosystems in aqueous solution, which is in contrast to common IR methods (e.g. absorption or transmitting IR-spectroscopy), which lack the necessary sensitivity.

ATR-FTIR revealed a number of intensive bands of a typical spectrum of lipid system: they comprise bands of asymmetric and symmetric valence oscillations of methylene groups CH_2 as and CH_2 s (2915 – 2930 cm^{-1} and 2850 – 2855 cm^{-1}, respectively), which are sensitive to changes of lipid package in the bilayer. Here, we emphasize that these bands are very sharp and conserved with no significant shifts: e.g. the phase transition of PC (egg phosphatidylcholine) /CL (cardiolipin) (80/20 w/w) liposomal gel-like bilayer – fluid-like bilayer is accompanied by a shift of CH_2 as band from 2919 cm^{-1} to 2924 cm^{-1} and CH_2 s band from 2851 cm^{-1} to 2853 cm^{-1}. Bands of carbonyl groups (1720 – 1750 cm^{-1}) and phosphate groups (1220 – 1270 cm^{-1}) are multicomponent bands. High-frequency components correspond to low-hydrated groups; low frequency bands correspond to highly hydrated groups. A decrease of hydration degree is usually caused by formation of electrostatic interactions with charged ligands. In combination with curve-fit procedure analysis of shape and position of peaks allows determining fine changes in the state of some functional groups e.g. phosphate groups of cardiolipin.

This process has been demonstrated on the examples of interaction of liposomes of different composition and polymers (PVP, chitosan MW 15 kDa, 90 kDa, glycol-chitosan, PEG-chitosan, chitosan – folic acid) and drugs: doxorubicin and its lipophilic prodrug, sirolimus. Combination ATR-FTIR spectroscopy with DLS and HPLC allows determining the fine structure and physico-chemical parameters of liposomal systems: K_{dis} of non-covalent complexes liposome - polymer, mechanism of drug incorporation etc.

Cell membrane can be also studied by ATR-FTIR spectroscopy in combination with microscopy control. This study is based on changes in the adsorption area of CH_2 as and s, because these peaks are not overlaid with peaks of proteins and molecules. We have studied influence of doxorubicin and sirolimus on Caco-2 and A549 cell lines and found changes not only in the adsorption area of CH_2 as and s but also in the area of phosphate groups (1150 – 1270 cm^{-1}) indicating changes in the DNA. This fact correlates with well-know mechanism of drug action.

Mechanisms of Aging and Age-Related Diseases: bibliometric and patent analyses

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Bibliometric and patent analyses have been applied in a wide variety of fields. The objective of this research is to assess such scientific field as "Mechanisms of Aging and Age-Related Diseases". In different bibliometric databases such as Web of Science Core Collection and Scopus we observe exponential growth of publication activity over the last 20 years in this field, citation activity also increase exponentially. These facts indicates the highest research community interest in problems associated with aging at the cellular and lower levels. The United States of America (36,31%), Italian Republic (10%), and Japan (8,87%) are leaders by the number of publications. Russian Federation is seventeenth in the list with 1,72% of publications (according to Web of Science Core Collection). Almost 10% of the studies in this area are funded by National Institutes of Health (NIH) - USA (according to InCItes). We found 33 Highly Cited Papers (one of them was written in collaboration with Russian investigators) and 1 Hot Paper in this field (according to Essential Science Indicators).

In patent database Thomson Innovation we observe irregular linear growth of patent activity over the last 20 years. The United States of America (66, 44%), People's Republic of China (15,11%) and Republic of Korea (3,81%) are leaders by the number of patents in which they are priority countries.

Eight major projects which were funded by different Resolutions of the Government of the Russian Federation and Federal Target programmes during 2013-2014 years was found in information system of Directorate of State Scientific and Technical Programmes (sstp.ru). All above indicates that in Russian Federation the basis for development in this scientific field is forming.

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Generation of single-domain antibodies to membrane proteins, using novel CYB5 fusion partner and phage display technology

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Antibodies have recently became an essential tool being a part of immunodiagnostics, therapeutics and as a valuable instrument in life science research [1]. Nevertheless, development of universal recognition modules still a big challenge for academia and pharma. The lack of universal and easy-to-use tools for recombinant antibodies characterization and reliable quantification in complex mixtures also causes some difficulties.

In order to monitor recombinant antibodies expression in real time and to control their folded state we developed a novel antibody cytochrome b_5 fusion protein and proposed its implementation for antibody discovery.

The combination of redox dependent absorbance spectrum of red-colored cytochrome b_5 with its high value molar extinction coefficient allows us to monitor antibody fusion proteins localization, redox state and quantify them in reliable way in turbid solutions. Moreover, it was revealed that due to outstanding stability and solubility, cytochrome b_5 significantly enhances expression level of antibody fragments in *Escherichia coli* periplasm.

We utilized synthetic single-domain antibody phage display library as the source of specific binders. The library had a diversity of 10^9 clones and was constructed by random diversification of CDR loops of stable human VH domain by PCR mutagenesis. Such approach make it possible to raise antibodies with desired properties in desired conditions [2].

A number of specific single-domain antibodies with moderate affinities to various cytokines and pharma targets (human growth hormone, erythropoietin, cytochromes P450) were isolated from synthetic antibody libraries and characterized by physicochemical and immunochemical methods.

In order to elucidate molecular mechanisms responsible for different functional activity of CYP11B2 and CYP11B1 – membrane proteins that possesses 93% sequence homology, we performed a phage display library screening for non-cross-reactive single-domain antibodies against CYP11B2 and CYP11B1. Utilizing a negative selection approach, we succeeded in generating specific binders that has substantially different binding kinetics to membrane proteins CYP11B2 (aldosterone synthase) and CYP11B1 (steroid 11β-monooxygenase).

As the result, a complete pipeline for antibodies discovery and design was created. Starting from hybridoma cloning or *de novo* isolation from phage display libraries to expression and downstream applications, it allows to us to obtain a recognition modules with desired properties.

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PARP overactivation causes mitochondrial dysfunction in Parkinson's Fbxo7-deficient cells

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Evidence indicate that mutations in F-box only protein 7 (*Fbxo7*) gene cause early-onset autosomal recessive Parkinson's disease. Although the mechanism is not fully understood, recent findings showed that PD-associated mutations in *Fbxo7* disrupt mitochondrial autophagy (mitophagy), suggesting a role for Fbxo7 in modulating mitochondrial homeostasis.

Here, we have analyzed the mitochondrial function in two models of Fbxo7 deficiency: fibroblasts of patients carrying the homozygous R378G mutation in *Fbxo7* and neuroblastoma SH-SY5Y *Fbxo7* knockdown cells. Results indicate that loss of Fbxo7 is related with the overactivation of poly(ADPribose) polymerase (PARP), which causes cellular NAD^+ depletion. Reduced NAD^+ availability leads to reduced mitochondrial NADH pool, increased NADH redox index and impaired activity of complex I in the electron transport chain. Under these conditions of compromised respiration, mitochondrial membrane potential and ATP contents are reduced and cytosolic ROS production is increased in Fbxo7-deficient cells. Treatment of the cells with the PARP inhibitor DPQ, restores cellular NAD^+, mitochondrial NADH homeostasis and rescues complex I activity in *Fbxo7* deficient cells, showing that PARP overactivation is the cause of the respiratory impairment in these cells.

These findings bring new insight into the mechanism of Fbxo7 deficiency in Parkinson's disease, emphasizing the importance of mitochondrial dysfunction in neurodegenerative disorders.

Artificial membrane receptors of Kv1-channel blockers: design and applications

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Some disorders are associated with overexpression and/or increased activities of voltage-gated potassium Kv1-channels such as Kv1.1 (multiple sclerosis, spinal cord injury), Kv1.3 (psoriasis, multiple sclerosis, rheumatoid arthritis and type-1 diabetes) and Kv1.6 (epilepsy). In these cases, blocking of Kv1-channels alleviates disease symptoms and can be achieved with peptide toxins acting at sub-nanomolar concentrations. Potential pharmacological applications require advanced (selective) blockers of the target channels.

Artificial KcsA-Kv1.x (x=1-6) receptors were recently designed by inserting the ligand-binding site from human Kv1.x channels into the appropriated domain of the bacterial KscA channel [1]. On the basis of these receptors we have developed bioengineering cellular systems that have two main components: *E.coli* spheroplasts with membrane-embedded KcsA-Kv1.x (x=1,3,6) channels and fluorescently labeled ligand [2-5]. Confocal laser scanning microscopy is used to visualize channel-blocker interactions in these systems. The cell-based systems facilitate considerably a search for Kv1.x (x=1,3,6) ligands in venoms of scorpions and other invertebrates [3-5], which are natural and often unexplored libraries of high affinity blockers of Kv channels. Also these systems are a convenient tool to study Kv1.x channel affinity and selectivity of natural and designed recombinant peptides [3-5]. The developed analytical “mix and read” systems are reliable alternative to radioligand and electrophysiology techniques in the search and construction of Kv1.x blockers that are of scientific and medical importance.

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Ion channels formed by cytochrome *c* in planar bilayer lipid membranes in the presence of hydrogen peroxide: effect of membrane composition

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Interaction of cytochrome *c* with mitochondrial cardiolipin is known to convert this electron transfer protein into peroxidase, which is supposed to play an essential role in apoptosis. Cytochrome *c*/cardiolipin peroxidase activity has been found previously to cause permeabilization of liposome membranes, in particular, leakage of fluorescent probes, such as carboxyfluorescein, calcein, sulforhodamine B and 3-kDa (but not 10-kDa) fluorescent dextran from liposomes [1]. Studies of the effect of the combination of cytochrome *c* with hydrogen peroxide on the conductance of planar bilayer lipid membranes (BLM) [2,3] have shown the ability of this electron-transfer protein to form ion channels under the oxidative stress conditions.

Here, we examined the impact of membrane lipid composition on the activity of ion channels formed by cytochrome c in planar BLM in the presence of hydrogen peroxide. According to our results, the addition of cytochrome c with hydrogen peroxide to the bathing solution at both sides of the BLM led to the appearance of regular fluctuations of the electric current across BLM with the amplitude of about 0.8 nS under the conditions of low ionic strength. The presence of about 20% cardiolipin in the BLM composition was mandatory for the formation of these ion channels. The conductance of the cytochrome c ion channels was higher with equine heart cardiolipin, than with synthetic tetraoleoyl cardiolipin. Apart from the presence of cardiolipin, the activity of the cytochrome c ion channels was sensitive to the composition of the bulk membrane-forming lipid. In particular, no ion channels were observed, if soybean phosphatidylcholine (asolectin), comprising 80% of the membrane lipid in the above experiments, was replaced by dilinoleoyl phosphatidylcholine or dierucoyl phosphatidylcholine. The induction of ion channels by cytochrome c with hydrogen peroxide in planar BLM was suppressed by antioxidants, such as Trolox.

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Far Infrared and Terahertz Spectroscopy of electrogenic bacteria extracellular matrix

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The studies of dielectric phenomena in biological materials have been in focus of researchers' attention over decades, however, the origin of the observed responses is still under discussion. Current report is devoted to the study of *S.oneidensis* MR-1 extracellular matrix and filaments (EMF) and two reference proteins, cytochrome C and bovine serum albumin, by means of infrared and terahertz spectroscopy and differential calorimetry. The measurements were conducted in a wide frequency and temperature range. The results obtained at room temperature show the predominance of the absorption processes caused by the bound water response in EMF. Three groups of resonant lines are picked out of the spectra of EMF and cytochrome C, including the Debye relaxation at low frequencies, translational and librational vibrations of water and a band of undetermined origin. The temperature dependences of dielectric permittivity at low frequencies and of EMF heat capacity demonstrate a peculiarity, which is explained by freezing of bound water in the samples. Furthermore, the boson peak – an excitation inherent in disordered materials – is detected in dielectric spectra of EMF and in temperature behavior of the specific heat of all three materials.

SAXS data analysis parameters of M-state of Bacteriorhodopsin D96N

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In this work we present the results of SAXS measurements of changes of gyration radius of bR D96N containing purple membranes during light-induced M-state compared with its ground state. SAXS was done on the X-ray instrument Rigaku, MIPT, Dolgoprudny, Russia [1].

It was found that the strongest changes could be seen at alkaline pH values, higher than 8.5 [2-3]. In all our measurements we keep pH at 9.0 value. The concentration of purple membranes in samples was varied between ~20 mg/ml and ~2.5 mg/ml.

For high concentrations (10-20 mg/ml) changes of the gyration radius were not noticed, possibly, due to the strong light absorption on the surface of capillary. In the middle of the capillary there is no absorption and bR remains in the ground state, consequently there is fewer percentage of M-state induced BR molecules in relation to the whole amount.

For lower concentrations (5-2.5 mg/ml) the gyration radius changes were seen between 1.5-2.0Å. This indirectly approves the hypothesis of surface light absorption of bR in the capillary, but the reasons of the effect are being discussed.

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Benchmark for validation SAXS profile calculators

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Small-angle scattering is one of the fundamental techniques for structural studies of biomolecules. In order to interpret experimental SAXS curves it is necessary to have tools that allow to calculate SAXS curves for a known structure with a great precision. A fair number of programs for calculating SAXS profiles exists [1][2][3], however it is not clear how to verify their results. Chi (χ) value is often used as a measure of goodness-of-fit of a modeled to an experimental profile, but we should always keep problem of overfitting in mind. Rambo and Tainer [4] in 2013 suggested to create a benchmark consisting of 3 structures with experimental SAXS profiles and sets of their non-native structures (decoys), because it seems reasonable to assume that a good SAXS profile calculator should be able to distinguish the native structure from its decoys. In order to compare performance of our new SAXS profile computation program Pepsi-SAXS with the existing analogues (Crysol and FoXS), we made some steps in that direction. However, from the structures suggested by Rambo and Tainer we took only Glucose Isomerase, because creating decoys for structures containing not only aminoacids, but also DNA and RNA represents certain problem. On the other hand, we created sets of decoys for several other structures. To create decoys we used CONCOORD, 3DRobot, and ZDOCK methods. Also, we consider it is better to choose structures whose experimental SAXS profiles match well the theoretically calculated SAXS profiles, so we mainly choose structures for which all SAXS-computation methods that we compare gave Chi values less than 2.0. We created sets of several thousand decoys for more than 10 structures and studied performance of most popular SAXS-profile calculators: FoXS and Crysol and our new program Pepsi-SAXS.

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Steroidogenic cytochrome P450s: protein-membrane and protein-protein interactions

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Cytochrome P450s (CYPs) are ubiquitous hemeproteins involved in biosynthesis of bioactive molecules and metabolism of drugs. In mycobacteria and fungi CYPs play an essential role in metabolic functions. Thus P450s are important targets for drug development for the treatment of endocrine disorders and drug-resistant forms of tuberculosis and mycosis.

We applied a protein family-based approach in structural studies of human and *Mycobacteria tuberculosis* CYPs to understand molecular mechanisms of substrate recognition, binding and catalysis. We provided the evidence for alternative pathway of steroid hormone biosynthesis using 7-dehydrosteroids (precursors of secosteroids) [1]. Similarly, in *M.tuberculosis* these enzymes responsible for metabolism of 7-dehydrocholesterol and vitamin D was identified suggesting that pathogenic mycobacteria can modulate local immunoresponse [4].

We also provided the first structural insight for the complex of cytochrome P450 with ferredoxin revealing a precise manner of interaction for inter-protein electron transfer from a 2Fe-2S cluster to a heme iron cofactor [2]. Suggested mechanism of protein complex formation is likely common for all mitochondrial P450s. The structures with all intermediates of a three step reaction of pregnenolone synthesis demonstrated a mechanism for sequential regio- and stereoselective hydroxylations and suggest how the C-C bond cleavage occurs. Combining our findings from series of structures of cholesterol metabolizing CYPs with previously published data, we propose a model for cholesterol binding from the membrane [3].

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The cell-free expression of TM and CNBD domains of human HCN1 channel

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Membrane proteins (MPs) play a crucial physiological role in the life of cell. MPs carry out a number of functions such as signal transfer between the extracellular and intracellular environment and import and export of various substances through the membrane. Also, MPs allow the cells to identify each other and provide interaction between them. Dysfunction of MPs leads to development of diseases in humans and can lead to drug resistance in cancer cells and bacteria. Therefore, these proteins are of great interest for medicine and pharmacology, and determination of the MPs' structures will simplify search and developments of new drugs.

In order to obtain the structure of a protein it's necessary to go through several difficult stages of protein production, which include: protein production in preparative amounts, isolation and purification of the protein in the functional state, and finally long-term stabilization of non-aggregated protein [1]-[2].

Among the tools for protein production are cell-free expression systems (CFES), which have several advantages over other systems such as possibility of production of large amounts of cell-toxic proteins and synthesis of membrane proteins in soluble form by adding various membrane modulating media [3].

Here, we used CFES based on bacterial lysate extracted from *E. coli*. We have produced different fragments of channel HCN1 (transmembrane and cyclic nucleotide-binding domains), using previously developed protocols. Efficiency of the cell-free expression of these domains and effect of various detergents on protein stability was analyzed.

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Patch-clamp-spectroscopy of ros effects and role of the redox-patch-clamp-spectroscopy in the aging and age-related disease

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It has been known for a long time that reactive oxygen species are chemical agents of aging [1-4] based on ROS-induced DNA damage [5], tissue chondriome damage [3,6,7] (and, consequently, respiratory function destroy [8]) and membrane lipid peroxidation [9]. Well known, that membrane electrophysiology regulated by redox-signaling and, consequently, by ROS. Good method for study of ROS effect with synchronal correlation analysis of ROS content in a biological media is patch-clamp or voltage-clamp [10-12]. This has led several authors to propose the use of patch-clamp spectroscopy [13,14] as a method for analyzing of membrane effects of ROS and one of powerful instruments for searching of biochemical descriptors of aging and some age-related diseases.

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Chitosan derivatives and spidroin as electron beam lithography resist for conductivity study

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Bio-compatible electron beam lithography for either substrate modification or charge transfer investigation is developing within the last 30 years. First successful steps were done with the study of polyethylene glycol modification (cross-linking) by electron radiation[1]. Authors were able to control cell-adhesion according to the figure created on the substrate by electron beam lithography technique. The most out-standing result was published in 2014[2]. The group has investigated properties of fibroin solubility under electron radiation (both cross-linking and destruction) and has shown the ways to modify the substrate for the use in fluorescent microscopy. However some problems still do not allow this method to be used for direct study of charge transfer. Mainly they are:

1. Wash-off of exposed and unexposed areas.
2. High doses required for the properties change.

Here we report the study of two candidates for bio-compatible electron beam lithography resist. Recombinant spidroin has shown close to the fibroin results, with much better wash-off. Chitosan derivatives have shown low-dose treatment needed for the substrate modification and more bio-friendly recipe[3]. We've studied the dependence of irradiation dose for properties change on chitosan derivative molar mass and the acid used for the formation of derivative.

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Expression, purification and stability assays of human cysteinyl leukotriene receptor type 2

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Cysteinyl leukotrienes are the family of potent inflammatory mediators derived from arachidonic acid via lipoxigenase pathway. Their interaction with the receptors belonging to the g-protein coupled receptors (GPCRs) superfamily called cysteinyl leukotriene receptors leads to a strong physiological response. At least two types of human cysteinyl leukotriene receptors are known: cysteinyl leukotriene receptor type 1 (CysLT1) and type 2 (CysLT2)[1]. Having a different expression pattern in human organism, they are also involved in different pathological processes: CysLT1 is usually associated with asthma[2] and allergy[2], while CysLT2 is believed to be connected mostly with allergy[2] and some types of cancer like uveal melanoma[3], etc.

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[4]. Thus, several genetic constructions of target protein CysLT2 with small fusion partner proteins were created to obtain a required quality of protein and improve possible crystal contacts. These proteins were expressed using baculovirus expression system in Sf9 insect cell line and expression conditions were set up. Receptor purification conditions were optimized. To achieve the best protein stability in presence of ligands an extensive ligand screening was made with the most stable constructs. Thermostabilizing point mutations were designed and applied and then several rounds of point mutagenesis were performed. Crystallization in lipidic cubic phase (LCP) was set up, diffusion rate was measured for different crystallization precipitant screens by fluorescence recovery after photobleaching method (FRAP).

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The recombinant domain of the telomere-binding protein TRF2 with unknown functions and antibodies to it

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Telomeres are nucleoprotein structures required for the protection of chromosomes ends. Telomeres are likely to play a crucial role in the processes of aging. The telomere-binding protein TRF2 has a domain with functions still unclear. Computer analysis of TRF2 sequence has shown that this domain has a limited similarity to several sequences of rod domains of intermediate filaments. Intermediate filaments, particularly lamins, are the components of nuclear envelope, so the domain of TRF2 with unknown functions is likely to be responsible for the interaction of telomeres with the nuclear envelope. Investigation of this interaction may shed light to the processes of ageing, because mutation in *LMNA* gene encoding lamin A leads to Hutchinson-Gilford progeria syndrome which is characterized by premature ageing, abnormalities of nuclear membrane and telomeres shortening.

In this study a recombinant protein udTRF2 corresponding to the domain of TRF2 with unknown functions was obtained. The fragment of TRF2 gene encoding udTRF2 was amplified by PCR. The plasmid vector pCDNA3hTRF2Full containing TRF2 sequence was used as a matrix for PCR. Primers for PCR contained restriction sites for restriction enzymes *Fau*ND I and *Hin*d III. The fragment of gene encoding udTRF2 was cloned into the cloning vector pTZ57R/T and subcloned into the expression vector pET32a(+). Presence of the insert in the recombinant plasmid was verified using DNA sequencing. The construction named pET32a-udTRF2 was transferred to the expressing host strain *E. coli* RosettaBlue(DE3). Expressed polypeptide was detected by SDS-PAGE of the total cell protein and western blotting with antibodies to TRF2. An apparent molecular weight of the protein is about 25 kDa and roughly corresponds to the calculated molecular mass (21 kDa). The recombinant protein udTRF2 was extracted from the soluble fraction of bacterial lysate using ammonium sulfate precipitation and ion-exchange chromatography with Q-sepharose. Protein was used to obtain polyclonal antibodies against the udTRF2 domain. The antibodies were shown to interact with TRF2 in mouse liver nuclear extract and U937 cell line nuclear extract that points out the possibility of using them for investigations of udTRF2 functions.

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Evaluate the effect Lactobacillus lactis on AKT signaling pathway in HT29 cancer cell line and Huvec normal cell line

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Introduction: Probiotics induce production of antimicrobial substances by the intestinal host cells, reinforce the epithelial barrier integrity, and compete with pathogenic microorganisms for enterocyte binding sites. Probiotic micro-organisms in the intestine led to increase in the amount of useful bacteria, which in the effectiveness of common cancer in the colon. The aim of this study was the best time of cytotoxicity for extracted cell wall from Lactobacillus lactis as probiotic bacteria on AKT gene expression in HT29 cell line and Huvec cell line.

Methods: The Lactobacillus lactis were cultured in specific medium (MRS broth) at anaerobic condition for 24-48 hour. After incubation period culture medium was centrifuged, then the cells were washed twice with PBS buffer to remove additional medium. After that, different concentrations of cell walls were prepared in RPMI medium.HT29 cancer cell line and Huvec normal cell line (RPMI) cultured in specific mediums. To evaluate the toxicity of these bacteria on HT29 cancer cell lines and Huvec normal cell lines MTT [3-(4, 5-Dimethylthiazol-2-yl) 2, 5- Diphenyl tetrazolium Bromide] assay was perform. Total RNA was extracted from HT29 and Huvec cell line and cDNA synthesized, followed by preformation the Real time PCR.

Results: Our finding in MTT assay revealed that HT29 cell line decreased than cultured primary cells of after 24 hours. Real time PCR in AKT gene expression analysis showed that the impact of these bacteria on HT29 apoptosis through the AKT gene.

Conclusion: Taken together our results demonstrated that proliferation reduction of HT29 cancer cell lines related to the effects of Lactobacillus lactis in AKT gene expression, so have inhibit effect in proliferation cells, because AKT gene is responsible increase proliferation.

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The role of mitochondrial ATP synthesis mechanism in aging and age-related diseases

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The human body receives the main energy in the form of ATP, mainly by oxidative phosphorylation in the mitochondria, and a small part from glycolysis. It has been recognized a long ago that several major human diseases (2 type diabetes, cancer, heart diseases, etc.) and aging are associated with mitochondrial dysfunction. Mitochondrial dysfunction causes a dramatic fall of ATP production and an increase in ROS production. It is still unclear role of structural changes of mitochondria in ATP synthesis mechanism. Therefore we propose a mechano-chemiosmotic model of ATP synthesis [1]. According to this model, an asymmetric contact of dimers of opposite cyt bc_1 complexes is formed in intracristal space during shrinkage of organelles [2], which is a mechanical regulator of electron transfer from [2Fe-2S] cluster to heme c_1 and ROS production. Moreover, we offer a new mechanism of ATP synthase functioning, where the lysines and arginines of ATP synthase play an important role. In addition, arginine is a stimulator of growth hormone, and the organism stops the synthesis of arginine in order to limit the growth after the human age of 28 years [3]. The shortage of arginine in the body primarily results in decrease a synthesis of ATP by oxidative phosphorylation in the mitochondria, and the ATP synthase complex, too. Therefore, to compensate for the energy loss, the body shifts to a low-efficiency glycolysis which is accompanied by the occurrence of 2 type diabetes. In this case, mitochondria are in a swollen state. It promotes excess ROS production by cytochrome bc_1 which causes a cancer. Thus, the change in the structure of the inner membrane of mitochondria, causing swelling-shrinkage of intracristae space, is electron transfer regulator, of ROS (reactive oxygen species) production, ATP synthesis, therefore, aging and aging-related diseases [Animation http://www.youtube.com/watch?v=PgKoKnVvBi4].

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Interaction of cytolytic peptides with lipid membranes: coarse-grained and full-atom MD-studies

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The venoms of some insect and snake species are rich in broadly cytotoxic peptides, otherwise called cytolytic polypeptides [1]. They exhibit membrane activity in the experiments with model lipid membranes *in vitro*, and display cytotoxicity against a variety of living cells [2]. The well-known examples of linear cytolytic peptides are latarcins from spider venom [2]. Their cysteine-rich counterparts are full beta-sheet proteins from snake venom, called cardiotoxins (CTs), or cytotoxins [3]. Up to now, interaction of CTs with lipid membranes has been poorly investigated with MD-methods and only single work devoted to this subject has been published [4].

In this work, we investigated interaction of cardiotoxins and latarcins with model lipid membranes of different composition, using a combination of full-atom (FA) and coarse-grained (CG) MD-simulations. The CG-approximation allows significant extension of the lengths of MD-trajectories (up to tens of microseconds), compared to the FA-variant, at a relatively similar amount of computation. Then, FA-simulations of certain fragments of CG-trajectories allow delineation of atomic details of the interactions and reveal distinct modes of the lipid/peptide interactions. As a result, we were able to derive characteristics of the peptides, responsible for the membrane perturbation, and elucidate the role of certain lipid species in attenuating activity of the peptides. The current results contribute to development of novel anticancer and antibacterial drugs, based on the venom-derived polypeptides.

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Comparative analysis of the silver behenate parameters by diffraction and small-angle scattering

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Silver behenate is very often used for q-scale calibration of X-ray and neutron small-angle instruments. In spite of numerous measurements [1-3] and suggestion to use this sample for nanoscale objects measurements questions of accuracy and value parameters still exist separately for each instrument [4].

In our work the silver behenate powder was studied by different methods: small-angle X-ray (Rigaku, MIPT, Dolgoprudny, Russia) [5], X-Ray diffraction method (PANalytical, JINR, Dubna, Russia), small-angle neutron scattering (YuMO spectrometer, JINR, Dubna, Russia) [6]. It was found that lattice parameters extracted from X-ray diffraction (XRD) curves give slightly different results for SAXS and SANS methods. Due to XRD curve, at least, the 15 peaks were obtained. The doubt in peaks existence due to low intensity level for SAS curves was resolved. It is shown that the right border of the q-range can be extended up to 1,5 A^{-1} for Rigaku instrument. The parallax problem in this case is discussed.

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The role of calcium-dependent signal pathway in glial cell death after axotomy of the crayfish stretch receptor

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Traumatic nerve injury, in particular, axotomy (AT), leads to neuron death. Satellite glia plays an important role in survival of neurons, but its role in protection of AT-injured neurons is not well known. We studied the role of Ca^{2+}-dependent pathway in responses of the crayfish stretch receptor (CSR) neuron and satellite glia to axotomy. As a control, we used CSR isolated so that its axon remained connected to the crayfish abdominal ganglion [1].

The obtained results have shown that AT increased necrosis and apoptosis of glial cells at 8 hours, when death of neurons was still not detected. 3-fold increase in external calcium concentration, $[Ca^{2+}]_o$, induced glial apoptosis in axotomized but not control samples. 3-fold decrease in $[Ca^{2+}]_o$ did not influence glial apoptosis. Unexpectedly, glial necrosis decreased in the presence of $3[Ca^{2+}]_o$, but increased in $(1/3)[Ca^{2+}]_o$. Thapsigargin (Tg), an inhibitor of endoplasmic reticulum Ca-ATPase (SERCA), induced glial apoptosis after AT but not in control. Ionomycin (Im), a calcium ionophore, induced apoptosis both after AT and in control samples. Fluphenazine, an inhibitor of calmodulin (Flu) had no effect on apoptosis. Im and Tg were also pronecrotic in intact samples, while Flu induced glial necrosis only after AT. The application of toxic concentrations of dantrolene, an inhibitor of ryanodine receptors, and ochratoxin A, an activator of SERCA, induced glial apoptosis but not necrosis after AT. The blockage of mitochondrial permeability transition pores (MPTP) with cyclosporine A, which thereby prevented Ca^{2+} release from mitochondria, decreased cell death in all samples.

Thus, the combination of AT with high $[Ca^{2+}]_o$ and different pathways which increase $[Ca^{2+}]_i$ induced apoptosis of glial cells. Decrease in $[Ca^{2+}]_o$, inhibition of SERCA, Ca^{2+} ionophoresis, and inhibition of calmodulin promoted glial necrosis, while MPTP blockage protected glia from axotomy-induced death. Apparently, Ca^{2+} is involved in the detrimental action of AT. Thus, death and survival of glial cells can be managed by modulation of intracellular and extracellular Ca^{2+} concentration.

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Investigation of chromophoric group modification on *exiguobacterium sibiricum* rhodopsin (ESR) properties

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A new retinal protein (ESR) - light driven proton pump from the psychrotrophic bacterium *Exiguobacterium sibiricum* has a Lys96 as a proton donor moiety to the Schiff base that distinguishes ESR from related retinal proteins – bacteriorhodopsin (BR), proteorhodopsins (PRs), and xanthorhodopsin (XR), in which the donor function is performed by Asp residues with a carboxyl side chain. Light-induced changes in retinal proteins are associated with charge redistribution in the excited retinal chromophore and are driven by isomerization of the chromophore moiety around a "critical" double bond. Thus, the chromophore molecule modification is a promising approach to the structure-function relationship study in such pigments. In the present study, for the estimation of the influence of the chromophoric group structure on the functional properties of ESR, expressed in *E. coli*, we investigate the effect of replacement of natural chromophore (retinal (AR1)) with its ring-modified analogs: 4-oxoretinal (AR2), 3,4-didehydroretinal (AR3), 5,6-dihydro-5,6-epoxyretinal (AR5), which differ in electronic and conformational properties. Other types of retinoid molecule modifications included acyclic derivative (AR6) and analog with polyenic chain modification - 13-desmethylretinal (AR4).

We have shown that ESR easily forms a photoactive artificial pigments from the recombinant ESR-rhodopsin membrane fraction and retinals (AR1-AR6) in dodecylmaltoside micelles. The protonated Schiff base bond between retinal analogs and the Lys residue in the binding site of ESRh is moderately stable against hydrolysis by hydroxylamine in the dark, but very slowly undergoes replacement by excess *all-E*-retinal (except (AR4)). Artificial pigments ESRh1-5 have an expressed cycles of photoconversions, but details of these processes will be published in the nearest future. Both of BR6 and ESRh6 analogs undergone the irreversible destruction under light illumination.

It was found that electronic and conformational properties of the retinoid analogs molecules influenced in a similar way on the optical parameters of ESRh and BR pigments families.

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The creation of molecular dynamic model of the outer membrane of Gram negative bacteria

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Gram-negative bacteria are the causative agents of numerous diseases. Their cell walls with additional outer membrane protect cells from external factors. Lipopolysaccharides (LPSs) of the outer membrane form the first line of bacterial defense. Released from the cell wall, LPS molecules exhibit endotoxic activity. Along with that, these negatively charged molecules serve as potential binding centers for cationic antimicrobials. However the search of antimicrobial agents is impossible without understanding the structure of the outer membrane of the cell wall of Gram-negative bacteria.

The goal of the work was to create molecular dynamics model of the outer LPS asymmetric membrane of Gram-negative bacteria on the basis of minimal LPS structure and to investigate its properties. The asymmetric membrane consists of the outer monolayer including LPS of Re-mutants and the inner monolayer of phospholipids.

LPS molecules include three domains: lipid A, inner and outer core oligosaccharides, and O-specific polysaccharide (O-antigen). LPSs' negative charges originate from phosphate, pyrophosphate and carboxyl groups located mainly in the inner core region. Re mutant is a strain without O-antigen chain and with the minimal structure of core part required for bacterial survival. We characterized MALDY mass spectrum of Re mutants and resolved its precise molecular structure. On the base of this Re mutant structure we created molecular dynamic (MD) model of LPS membrane of Gram-negative bacteria. The MD method makes possible calculation of basic features of the model bacterial membrane such as thickness of hydrophobic part, surface area per lipid, electron density, membrane fluidity. We performed 1200 ns MD simulation. Over the time span of 1200 ns the surface area per lipid holds constant value in the simulation. Electron density study of Re mutant membrane revealed almost symmetric profile, which confirms stability of the model membrane. MD simulation is performed using a software package GROMACS.

In the following we will study interaction of MD model of LPS membrane with the model of antimicrobial agent, oktakis cholonyl zinc phtalocyanine (Pc), which was created previously. It will allow interpreting of experimental data of Pc adsorption on the cell walls of Gram-negative bacteria.

Influence of space flight conditions on the phicobilisomes of *Synechocystis*

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Phycobilisomes are supramolecular protein complexes, which consist of phycobiliproteins and perform the function of light harvesting in the photosynthetic apparatus of cyanobacteria. During this work we show that phycobilisomes of the cyanobacteria *Synechocystis* are sensitive to ionizing radiation which simulates conditions of space flight. Modeling the behavior of biological objects in conditions of high radiation and attenuated geomagnetic field is important scientific problem. Because it is known that these conditions often affect vital activity of biological objects. However the mechanism is faintly studied and requires further investigation.

In this study we compare the effects of "space flight" *in vivo* on the cells of *Synechocystis* and *in vitro* on isolated phycobilisomes. For modeling "space flight" conditions we used the cyclotron U-120 SINP MSU, which yields accelerated helium nuclei with energy of 30.3 MeV for irradiation of biological objects. Changes in the structural and functional state of the phycobilisomes of cyanobacteria was evaluated by using the optical methods - absorption spectroscopy, spectrofluorometry (Fluoromax-4, Horiba Jobin Yvon) and spectrofluorometry with picosecond time resolution (Simple-Tau 140, Becker & Hickl).

It was found that the optical density of phycobilisomes and corresponding fluorescence intensities are decreased after irradiation and the effect is dose dependant. Probably, this effect can be explained by reducing concentration of the phycobilins and changing their states after irradiation. It was shown, that fluorescence lifetime of isolated phycobilisomes is reducing, at the same time in cyanobacteria cells the corresponding lifetime is increasing after irradiation with the same dose. We suppose that differences in dependencies of the lifetime can be explained by dissociation of phycobilisomes on to phycobiliproteins and interruption of the excitation energy transfer processes *in vivo*, while *in vitro* we observe denaturation of phycobiliproteins.

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Cellular tight Junction Opener protein: gene expression, purification and characterization

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One of the key tumors feature is the presence of intercellular tight junctions hiding oncomarkers and acting as barriers for the penetration of drug molecules with a molecular mass greater than 400 Dalton. That hampers cancer therapy, especially in the case of monoclonal antibodies and chemotherapeutics. Recently it was shown that adenovirus serotype 3 (Ad3) is able to open transiently tight cellular junctions. The effect is associated with the fiber knob protein. The protein exists as a homotrimer. Establishing connection between the trimers makes the protein ability to initiate a cascade of events leading to DSG-2 shedding and eventually to opening of tight junctions. This type of protein is known as a Junction Opener protein (JO).

To further study JO and to put it into practice as a co-therapeutic agent the high-yield JO production technology was developed. The gene encoding JO was collected from chemically synthesized oligonucleotides by PCR and was cloned into the pGEMEX1 plasmid under T7 promoter control. The optimal conditions for recombinant strain growth were selected. The JO purification method was developed that included metal-chelate affinity chromatography and anion exchange chromatography. The JO yield was more than 50 mg/L. The purified protein preparation was characterized by such methods as SDS-PAGE gel electrophoresis, gel filtration chromatography on calibrated column and dinamic light scattering. The biological activity was demonstrated by confocal microscopy on eukaryotic cell line SKBR3 using fluorescently tagged JO. The binding constant to DSG-2 was determined by the plasmon resonance method. In summary, three variants of JO were produced and characterized.

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Reprogramming of microbial immune interference activity by artificial protein assembly

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Tools on a basis of Crispr/Cas system type 2 is widely used for the directed engineering of chromosomal sequences, gene activity regulation and etc. Now the Crispr/Cas functionality modification is reached by covalent joining to its protein components of various functional blocks, for example, transcription activators or repressors. Obvious drawback of such approach is the lack of flexibility. Considerable efforts are put for the creation of the hybrid constructions, capable to carry out only a highly specialized task. Application of affinity tags allows realizing a modular principle of Crispr/Cas complex construction, that potently simplifies a problem of its functionality change. The tag availability in one of Crispr/Cas protein component allows to attach other functional blocks supplied with a complementary tag. In this report the idea of Crispr/Cas modular construction has been realized on an example of luciferase reporter gene activity regulation in mammalian cells. The Crispr/Cas complex lacked the nuclease activity (dCas9) has been reprogrammed for the reporter gene promoter recognition. The estrogen receptor ligand-binding domain (LBD) was used as transcription factor. dCas9 and LBD have been supplied with the affinity tags specifically interacting with each other.

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The interaction of water-soluble derivatives of [60]fullerene with molecular therapeutically important targets related to Alzheimer's disease

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Alzheimer's disease (AD) is one of the most important medical and social problems for the modern society. Patients suffering from AD face constantly progressing dementia leading to a complete loss of memory and intelligence. There

are no pharmaceuticals that can be used for the successful treatment of AD. It is known that [60]fullerene and many of its functional derivatives exhibit a range of promising biological activity; in particular, antioxidant and neuroprotective properties.

We studied a few different water-soluble polysubstituted derivatives of [60]fullerene (WS[60]FDs), in which 5 or more ligands attached to the fullerene spheroid. It is shown that these compounds penetrate the bilayer phospholipid membrane by the fluorescence techniques. In order to compare the radical scavenging activity of the selected WS[60]FDs, we studied the kinetics of the luminol chemoluminescence in the subcellular fraction of the homogenized mouse brain tissue in the presence of tBuOOH as a radical source. It is shown that two compounds suppress monoamine oxidase B (MAO-B), which are major AD therapeutic target. At the same time, they are positive modulators of the ionotropic AMPA glutamate-type receptors in the mammalian central nervous system. Thus, WS[60]FDs enhance the synaptic signal transduction that might be correlated with their ability to stimulate the cognitive processes in mammals. At the same time,

positive modulators of AMPA receptors triggers gene expression mechanism, which responsible for the synthesis of neurotrophins (growth factors regulating the survival and function of neurons). Moreover, these WS[60]FDs do not exhibit any neurotoxic effects, because they do not activate the ionotropic NMDA glutamate-type receptors in the mammalian central nervous system.

The investigated WS[60]FDs have influence on the spatial memory of mice, increase their research activity and have no psychoactive side effects. These findings suggest that the investigated WS[60]FDs can be considered as potentially promising lead compounds for treatment of Alzheimer's disease.

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Changing of spectral and physiological properties of a light-driven sodium pump

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Recently a light-driven sodium pump has been discovered, characterized and tested as an inhibitory optogenetic tool. Sodium pumping rhodopsin from *Dokdonia eikasta* KR2 has an absorption maximum at 523 nm at pH 7.5 and to create more red-shifted variants we analyzed available structures of the KR2 (PDB codes: 4xtl, 4xtn) and did the rational mutagenesis of residues in the retinal proximity region (i.e. M149, G153 and S254).

The mutants of KR2 under investigation were: M149A, G153V, M149A/G153V, S254A, S254F, S254G, S254L, S254M, S254N, S254T, S254V, S254Y.

The protein mutants were expressed in Escherichia coli C41 strain, expression was induced by the addition of 1mM isopropyl β-D-1-thiogalactopyranoside. The cells were then washed trice with unbuffered 100 mM NaCl or KCl solution. Finally, the pH changes in cell suspensions (OD600 = 8.0) were monitored. Experiments were performed also upon the addition of 30μM of protonophore carbonylcyanide m-chlorophenylhydrazone (CCCP). The following mutants completely abolished the protein function and were not used for further characterization: S254F, S254L, S254M, S254N, S254V. The remaining mutants have shown sodium pumping activity and S254A mutant has gained an additional potassium pumping activity. All functionally active mutants were purified using Ni-affinity chromatography and the absorption spectra were collected for them at pH 7.5 (50 mM Na/Na-Pi, 100 mM NaCl).

Based on structural analysis of KR2 we discovered another potassium pumping variant and provided the variants with absorption maximum blue-shift up to 53 nm and red-shift up to 22 nm.

S254A was not only red-shifted to 22 nm, but also demonstrated significant potassium pumping activity.

The substitution of Zn by Fe in Langmuir film of Parkin protein

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Age-related Parkinson disease (PD) is characterised by the loss of dopaminergic neurons of the substantia nigra (SN). Apart of well known accumulation of insoluble protein inclusions known as Lewy bodies it is widely accepted the association between PD progression and dyshomeostasis of some metals such as iron and zinc in brain. The elevated brain iron generally with aging is considered at risk of neurodegeneration [1-3].

Parkin protein harbours eight zinc-binding clusters by sulfur atoms of cysteine residues, thiolate group of which are ideal targets for nucleophilic attack by oxidants or nitrosative agents major . The oxidation and reduction of the Sulphur ligand are coupled with the binding and release of zinc ion and serve as redox zinc switches at Zn-S sites.

The present study established the ability of Parkin protein to form Langmuir-Blodget films at the air/glycerol interface. The films were examined by the x-ray standing wave (XRSW) method. Fitting of measured angular dependence of fluorescence yield of Zn K_a with the numerically calculated one unequivocally verify the participation of parkin protein molecules in the formation of monomolecular layer of 90Å thickness. The only minor variations of surface pressure of Parkin protein film were found for a day. Nevertheless long-time (12 hours) observations of elemental composition have revealed considerable alternation of the elemental composition of Parkin film: while the fluorescence intensity of Zn K_α peak substantially decreased with time, the intensity of Fe K_α peak on the contrary increased in the same order of mafnitude. Important to note that the intensities of K_α peaks of the other elements presented in film (e.g. sulfur) are remained unchanged.

Since the oxidative stress has been proposed as one of the major contributing factors in the elevation Fe levels in the SN, and taking into account known vulnerability of Parkin in cell to the same type of stress due to its abundance of cysteine residues , the presented data let to assume that loss of Zn ions by proteins containig the impairing Zn-Cys clusters could lead to binding of Fe ions.

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Proteoforms of lymphocyte phosphatase-associated phosphoprotein

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Lymphocyte phosphatase-associated phosphoprotein (LPAP) is a lymphocyte transmembrane protein that is associated with the phosphatase CD45. It is able to bind to the coreceptor CD4 and the kinase Lck. These data give some hint that LPAP may participate in the regulation of the activation signaling cascade downstream of the antigen-specific receptor. However, the details of this process are unknown, especially since LPAP has no protein homologues and its three dimensional structure is unresolved.

Using 2D-Difference gel electrophoresis (2D-DIGE), we have shown that LPAP exists as several proteoforms. In peripheral blood lymphocytes (PBL) LPAP is represented by at least five proteoforms, four of which are phosphorylated. LPAP from thymocytes is phosphorylated but has the only major phosphoform. In CEM and Jurkat cell lines the phosphorylation pattern is similar with that of PBL.

It was previously established that LPAP phosphorylation status depends on the cell activation state. We have demonstrated that upon the stimulation of cells with phorbol myristate acetate (PMA) LPAP undergoes partial dephosphorylation. In particular, the lower spot train disappears in LPAP from CEM, and the acidic spot in the upper train becomes fainter. Another pattern of dephosphorylation has been observed after the stimulation of Jurkat cells and PBL.

Association of LPAP with its binding partners may affect LPAP phosphorylation. To test that, we have analyzed LPAP proteoforms from CEM knockout (KO) lines without either CD4 or CD45, generated via CRISPR/Cas9 technology, as well as from the Jurkat cell line without Lck kinase (J.CaM1.6).

The knockout of CD4 and CD45 molecules resulted in a drastically decreased amount of LPAP. In the absence of CD45, LPAP phosphorylation patterns were in general similar in wild type and KO cells both before and after the stimulation. In contrast, in CD4 KO cells only two LPAP proteoforms were detected. In Lck KO Jurkat cells LPAP phosphorylation pattern was unaffected.

Further studies of LPAP phosphorylation and its connection with the binding partners will help to reveal the function of this protein.

The comparison of bacteriorhodopsin and rhodopsin by multiple sequence alignment

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Microbial and animal rhodopsins, also termed type-I and type-II rhodopsins, have a great significance due to the development and application of optogenetics. Until now, the theme of the origin of the animal rhodopsins is the subject of debate.

The theme of the work is a comparison of protein sequences of microbial and animal rhodopsins, as well as comparison of conserved motifs by the spatial disposition of amino acid residues in the protein relatively retinal. Alignment of more than 100 protein sequences of rhodopsins made using the MUSCLE Multiple Alignment Tool from bioinformatic toolkit Unipro UGENE 1.24. The spatial disposition of conserved motifs found on the high resolution structures from the Protein Data Bank, results presented using Maestro, version 10.7, Schrodinger Suite.

As a result of multiple alignment was constructed phylogenetic tree, which confirm the correctness of rhodopsin dividing into two classes - microbial and animal rhodopsins [1]. Microbial rhodopsins including proteins of bacteria and archaea, were on one branch of the phylogenetic tree with proteins of dinoflagellates and green algae. On the other branch, visual rhodopsin of mammals and cephalopods separated from the light-sensitive proteins of ctenophores. Analysis of the spatial disposition of conserved motifs was done for bacteriorhodopsin and rhodopsin.

It was found that conserved motifs is mainly located near the retinal. Especially conserved is aromatic residues from “Trp – – Tyr” motif, which in bacteria and archaea takes the form “W – – Y P – – – W” and in animal takes the form “W – P Y – – – A”. Likely explanation is that these aromatic amino acids essential for protein catalysis of photoisomerisation reaction of retinal.

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Glycation modulates amyloid transformation of prion protein

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A prion protein is a membrane-bound protein of neural tissue, which plays a crucial role in the development of a number of amyloid diseases, such as syndrome Kreutzfeldt-Jacobs, fatal familial insomnia, Kuru, scrapie and others. The interaction of the infectious prion with native prion protein amyloid causes its transformation and the subsequent accumulation of amyloid fibrils that causes the development of neurodegeneration. The aim of our work was to study the effect of glycation of prion protein to its ability to form toxic oligomers and fibrils to clarify the relationship between disorders of carbohydrate metabolism (first of all, occurring in diabetes) on the development of prion amyloidoses. We modified recombinant ovine prion protein by glucose and methylglyoxal. Most effective glycation of lysyl residues occurred in case of methylglyoxal. In itself, glycation does not lead to the aggregation of prion protein; however, it causes an increase in fluorescence thioflavin T, which is specific dye for detection of amyloid structure. We studied the effect of glycation on the processes of oligomerization and aggregation of prion protein.

We also investigated the effect of prions (native, glycated, and also oligomers and fibrils) on the system of chaperones, using bacterial chaperonin complex GroEL/GroES. Chaperone-dependent reactivation of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was blocked by monomeric form of the prion. PrP oligomers slow down chaperone-dependent reactivation of GAPDH, and the PrP fibrils do not affect this process. Chaperonin complex GroEL/GroES can simultaneously bind GAPDH and different forms of prion protein PrP. Thus, we have shown that glycation of prion protein, which can occur at elevated concentrations of glucose and other sugars in the blood, significantly alters the efficiency of amyloid transformation and can affect the development of prion amyloidoses.

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Small angle neutron scattering spectrometer (YuMO) on IBR-2 reactor

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Nanometer scale structures include sizes from 10-20 up to 1000 angstrom. Small-angle neutron scattering (SANS) is a well-established characterization method sample inhomogeneiries in various materials in the nanometer scale[1]. SANS instruments are reactor-based using monochromatic neutron beams or time-of-flight instruments at pulsed neutron sources. Our reactor (IBR-2) combine reactor and pulsed sources due to pulsed character of power.

In 2011 the IBR-2M pulsed reactor (JINR, Dubna, Moscow region) will face new challenges. The unique small angle neutron scattering spectrometer YuMO allows to measure samples and obtain I(q) curves in absolute scale (time scale varies from minutes to hours). This spectrometer is used for many scientific directions. Main features of the small angle neutron scattering spectrometer (called YuMO) are as follows: high flux, absolute scale, two-detector system, very good resolution and circle geometry. Due to direct view of the surface of the moderator from the reactor and time-of-flight method for wavelength determination we have higher flux (in comparison with pulsed sources) [2], [3] and higher penetration.

Most of the objects of small-angle neutron scattering (SANS) experiments require the measurements of a studied sample in a wide range of momentum transfer (Q-range). Larger Q-range means more reliable determination of parameters of the investigated material as well as higher accuracy of its calculated structural parameters. The dynamic range (a ratio of Qmax to Qmin measured simultaneously) of SANS instruments is normally determined by the size of the detector, which is limited mainly by technical reasons and by the wavelength range of available thermal neutrons in the neutron beam. It was shown[4], [5] that the time-of-flight method provides necessary conditions for a considerable increase of the dynamic range of a SANS instrument by using two or more detectors of scattered neutrons with central holes placed at different distances from the sample. A very good resolution (up to 1% of module scattering vector) of YuMO spectrometer realised by using new type of position sensitive detector PSD [6] and time-of-flight method due to pulsed IBR-2 reactor [7]. Nevertheless, the requirements to our SANS spectrometer are increased. First of all, the requirements to the sample environment. We present here the possibilities of YuMO spectrometer, namely: magnetic field up to 2.8 T, high pressure setup up to 4 kbar, light illumination of the samples [8].

The problems concerning specific features of experiment realization at a small angle neutron scattering spectrometer located at the 4-th beam-line of IBR-2, namelly: two detectors system, vanadium standart in front of each detectors, high flux on a sample, central hole of detectors and geometry of beam line are described. The scheme and mathematical background of the experiment are discussed.

The short review of results of small angle neutron scattering spectrometer YuMO modernization was presented. Base of modernization is two detectors system. As a result the dynamical q-range is twice increased and the data acquisition time has been reduced at least twice. Detail description of YuMO spectrometer has been given.

The short review of realized on spectrometers investigations in field of biology, polymers, material science and physical chemistry are given. These investigations has the methodical aspect.

It was shown that spectrometer has the **world level** of submolecular structure investigations.

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The new model of nuclear pores and their role in transcription

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The author proposed hypothesis about the involvement of direct DNA-lipid interactions in the nuclear pore and nuclear envelope assembly. Only taking into account interactions between DNA and cellular membrane lipids one can explain the origin of such structures as nucleoid, nuclear pore and nuclear matrix.

DNA acts as zwitterionic liposomes fusogen during ternary complexes (TC): DNA-zwitterionic liposomes-Mg2+ formation. TC arise in the chromatin areas with three-stranded hybrids: DNA - low molecular weight RNA (lmwRNA) at their interactions with two membrane vesicles (~70 nm in diameter). The melting temperature of DNA/lmwRNA triple helix is considerably lower than the same sequence of double-stranded DNA. That can specify preferential attachment of triple-stranded hybrids to the membrane vesicles. The triple helix unwinding during fusion of two nuclear membrane vesicles results in pre-pore structure: DNA/lmwRNA hybrid and a single-stranded DNA (ssDNA) located on the outer diameter of fused “big vesicle”. The “big vesicle” during interaction with double nuclear membrane can form nuclear pore channel, fusing two membranes.

Author proposed that the DNA-induced fusion of zwitterionic liposomes in vitro may involves in assembly of nuclear envelope and pore in vivo. (Kuvichkin VV., (2011), J. Membr. Biol., 241,109-116).

The structure of interphase chromatin cannot be considered without taking into account its interaction with nuclear membrane in the region of nuclear pores. This is the reason for the enhanced transcriptional activity of the genes neighboring the DNA-membrane contacts, appearing of transcription factories, chromosome territories, chromosome kissing effect etc.

Lipids around protein transmembrane domains: what drives a dimer formation

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Transmembrane (TM) α-helical domains are very common structural elements in membrane proteins. They anchor proteins and support their orientation in membrane, but more important is that in some cases they take active part in protein functioning. Receptor tyrosine kinases represent a large family of cell receptors involved in many regulatory processes. Their activation is connected with dimerization of TM domains that consist of single α-helices, so the question of helix-helix interactions in membrane is of a crucial importance. However, this process is not well understood because the role of membrane is often understated compared to direct protein contacts. Also, there is a growing interest in lipid binding sites on membrane proteins. We assess the free energy of helix association along with protein/lipids structure and dynamics via atomistic MD studies of TM helices of human glycophorin A (GpA) and model peptides – poly-alanine and poly-leucine. We conclude that the dimerization process displays prominent entropic character, and association of α-helices is governed by the membrane [1]. We performed a detailed analysis of the bilayer properties in the vicinity of the monomers and dimers of selected peptides and found significant bilayer perturbations near the latter ones. The formation of regular patterns of density minima and maxima was detected in the hydrophobic part of the bilayer in the presence of all peptides with the characteristic size of 1.5 nm, but for GpA these perturbations were organized in a special way. A conclusion was reached that the natural sequences, like GpA, are evolutionary adopted to maximize not only the entropy gain, but also to avoid unfavorable protein-protein contacts on the dimerization interface. We propose that the membrane serves as a peculiar dynamic "Aether", which helps proteins to find each other and to form a stable dimer.

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Lynx1 competes with Aβ1-42 for the binding with multiple nicotinic acetylcholine receptor subtypes in the brain

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Lynx1 regulates synaptic plasticity in the brain by regulating nicotinic acetylcholine receptors (nAChRs). It is not known to which extent Lynx1 can bind to endogenous nAChR subunits in the brain or how this interaction is affected by Alzheimer's disease pathology. We apply affinity purification to demonstrate that a water-soluble variant of human Lynx1 (Ws-Lynx1) isolates α3, α4, α5, α6, α7, β2, and β4 nAChR subunits from human and rat cortical extracts, and rat midbrain and olfactory bulb extracts, suggesting that Lynx1 forms complexes with multiple nAChR subtypes in the human and rodent brain. Incubation with Ws-Lynx1 decreases nicotine-mediated extracellular signal-regulated kinase phosphorylation in PC12 cells and striatal neurons, indicating that binding of Ws-Lynx1 is sufficient to inhibit signaling downstream of nAChRs. The effect of nicotine in PC12 cells is independent of α7 or α4β2 nAChRs, suggesting that Lynx1 can affect the function of native non-α7, non-α4β2 nAChR subtypes. We further show that Lynx1 and oligomeric β-amyloid$_{1-42}$ compete for binding to several nAChR subunits, that Lynx1 prevents β-amyloid$_{1-42}$-induced cytotoxicity in cortical neurons, and that cortical Lynx1 levels are decreased in a transgenic mouse model with concomitant β-amyloid and tau pathology. Our data suggest that Lynx1 binds to multiple nAChR subtypes in the brain and that this interaction might have functional and pathophysiological implications in relation to Alzheimer's disease.

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Effect of *Lactobacillus lactis* on PTEN signaling pathway in Human cancer cell line HT29 and Human normal cell line Huvec

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Introduction: Studies have been shown that genetic and epigenetic changes in colon cancer have a very important role. Probiotics are bacteria of the normal flora in adequate amounts, in addition to its nutritional benefits. Presence of probiotic bacteria in the intestine directly related to reduce colorectal cancer, they may be involved in several cell signaling in this phenomenon. In order to determining role of Probiotics bacteria in cancer suppression, we studied the effect of *Lactobacillus lactis* in PTEN gene expression as an important oncogene.

Materials and Methods: *Lactobacillus lactis* cultured in MRS Broth and human cancer cell line HT29 and normal human cell line Huvec cultured in RPMI1640. Evaluation of *Lactobacillus lactis* cytotoxicity on HT29 and Huvec, bacteria lysis and medium supernatant added to cell culture respectively, then viability of cell lines measured by MTT [3-(4, 5-Dimethylthiazol-2-yl) 2, 5- Diphenyl tetrazolium Bromide]assay. In addition, RNAs isolated and next cDNA synthesis by means of Real time PCR, PTEN gene expression evaluated.

Results: The results of MTT assay indicated that *Lactobacillus lactis* has toxic effect on HT29 cell line and reduced its proliferation. Analysis of the results of the Real time PCR shown that there was positive effect on PTEN gene expression.

Conclusion: Overexpression of PTEN gene in the presence of *Lactobacillus lactis* reduced the proliferation of HT29 cancer cell line, the possibility that this could be done through inhibition of AKT gene.

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New rhodopsin found in *Sphingomonas paucimobilis*: promising tool for optogenetics

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Promising medical applications of optogenetics require expanding its toolbox for controlling cell processes with high specificity and spatio-temporal precision. Among the most perspective approaches today there is changing properties of existing tools (particularly spectral characteristics, conductivity, selectivity) by mutations in conservative amino acid residues, and also searching for naturally occurring opsins with required features. Bioinformatic search conducted in open databases of proteins and genomes of microorganisms have shown about 7000 microbial rhodopsins which are not studied yet. Gene encoding one of them belongs to the genome of *Sphingomonas paucimobilis*, Gram-negative bacterium featuring a unique composition of lipid membrane. While in most of other Gram-negative bacteria outer membrane is mainly composed of lipopolysaccharides, in *Sphingomonas paucimobilis* it consists of glycosphingolipids [1]. This peculiarity of native lipid environment of the chosen microbial rhodopsin gives us good ground to expect new interesting properties.

The alignment of the studied rhodopsin with bacteriorhodopsin (bR) has shown that they have the same key residues involved in ion transfer except one in the position 96: the protein of interest has serine instead of the aspartic acid. With regard to theoretical data based on alignment with other well-known perspective opsins, and also on preliminary 3D-structure analysis [2], we suppose that the *Sphingomonas paucimobilis* rhodopsin may be sensory rhodopsin similarly to previously described *Anabaena* sensory rhodopsin [3]. However, it is possible that this novel protein may function like the light-driven H^+-pump because of the similarity with bR. The definite answer can be given only by experimental characterization of the protein in living cells by biophysical methods such as fluorescent microscopy and patch-clamp, and also by X-ray structure solution. We expect that the *Sphingomonas paucimobilis* microbial rhodopsin will take worthy place among new generation optogenetic tools.

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X-ray standing waves as a tool for element-specific density profiles in biomembranes

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X-ray standing waves (XSW) is a well established method used for probing of atomic structure in bulk crystals and at interfaces. In this work, we employ standing wave formed by Bragg reflection above the surface of a periodic multilayer (ML) to analyze light atoms distribution in organic layers deposited onto the solid substrate. This approach allows to tailor the period and phase of the x-ray standing waves according to the object properties: the probed distance can vary freely in a range from several angstrom to several tens of nanometers and the wavelength of the radiation can be matched to the absorption edges of the atom species present in the sample [1, 2]. It is also possible to choose a wide range of top-most layer materials covering the solid substrate, thus enabling a functionalization of the surface.

We demonstrate that standing-wave X-ray fluorescence enables the label-free, element-specific structural investigation of molecular layers at atom-scale resolution perpendicular to the interface [3]. As an application example, we show an investigation of element-specific distribution of light atoms within planar experimental models of interacting membrane surfaces. In particular, it yields specific density profiles of the chemical elements P and S belonging to lipid headgroups and polymer chains.

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New geroprotectors based on macrocyclic compounds

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Geroprotectors are interventions that aim to prevent, slow, or reverse the processes of aging in model organisms or humans. High throughput screening of 41 new macrocyclic compounds as geroprotectors was carried out on model system of nematode *Caenorhabditis elegans*. As a result, 10 compounds were found to possess biological activity and extend lifespan of nematodes up to 20%.

Some compounds under investigation have beta-lactame fragments. Such molecules possess neuroprotective activity and thus can be widely used in treatment of Parkinson disease and in antitumor therapy.

Some of the molecules were successfully tested by computer modeling as selective ligands of 5-HT6R receptor, mediated with G-proteins. It's an important target of serotonine involved in mitosis. 5-HT6R receptor activates mTOR signaling pathway which is associated with the problems of aging.

General types of macrocyclic compounds that can act as perspective geroprotectors were found out during this model investigation.

Staining of halobacteria for fluorescent microscopy by cationic (rosamine) dye

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Extremophilic halobacteria are though to be an attractive research object, on the one hand, due to ancient biomechanisms they possess and, on the other hand, due to their extraordinary viability and ability to survive in hostile conditions. The combination of these properties make these organism a target for search of extraterrestrial life.

Importance of halobacteria research encourages the attempts of application of the most up-to-date methods, such as fluorescent microscopy, which could enable scientists to study halobacteria in most natural conditions at up to single molecule resolution. The main challenge for fluorescent microscopy application to halobacteria research is the lack of dyes appropriate for staining in high ionic strength medium. Another obstacle is low permeability of halobacterial cell wall.

The experiments conducted with MitoTracker Orange dye show that dyes from rosamine family can be suggested as useful tool for halobacteria research. Positively charged molecules of MT Orange are rapidly evolved into the cell by its membrane potential. They easily permeate cell wall of Halobacterium Halobium and cause bright stable staining. Staining retains washing procedures and causes no cytotoxicity. It is inherited, remains bright during long incubation periods and has no significant effect on cells' growth rate, which is essential advantage compared to the closest competitors (DAPI, LIVE&DEAD).

MitoTracker staining was applied to observe transformation of *Halobacterium Salinarum* to spheroplasts in real time and at single cell scale.

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Pre-crystallization preparation of human endothelin receptor type B

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Human endothelin receptors belong to β-branch of A class of the G-Protein Coupled Receptors (GPCRs) superfamily. GPCRs are extremely pharmacologically important, with more than 40% drugs targeting them. The human endothelin system, which includes endothelin receptors types A & B (ET_B and ET_A), plays a highly important role in the blood pressure regulation. Endothelium cells produce peptides, known as endothelins 1-4, which activate endothelin receptors and launch cascades of reactions that lead to vasoconstriction or vasodilatation depending on the receptor subtype and the tissue. Additionally, endothelin receptors take part in such processes as transmission of nerve impulses, development of neural crest, and regulation of acid-base-salt balance in kidneys. For future crystallization trials we developed some different genetically engineered constructs. In order to stabilize ETB receptor we introduced a compact soluble protein, apocytochrome b_{562}RIL (BRIL), in the third extracellular loop of the receptor or on the N-term. BRIL is known to be an effective crystallization driver for GPCRs [2] .Also these constructs have different truncations of C-term. Expression level and quality for these created constructs was determined by flow-cytometry and confocal microscopy. Homogeneity was studied using SEC. Thermal stability of the protein in the presence and absence of ligands was measured by the Thermal Shift Assay. Finally, the mobility of the receptor in Lipid Cubic Phase (LCP) at many different conditions was probed by the LCP-FRAP (Fluorescence Recovery After Photobleaching) assay. These tests allowed us to determine effectiveness of various constructs for crystallization. The best constructs showed good SEC, thermal stability, mobility in LCP and expression level.

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Structural behavior of bicelle-based crystallization system: small-angle X-ray scattering study

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One of the methods for crystallizing membrane proteins is the bicelle-based crystallization: proteins are placed in a mixture of long-and short-chain lipids (or detergents). The question about the behavior of the lipidic phase during crystallization remains open.

In our study we used the small-angle X-ray scattering (SAXS). Since the scheme of a SAXS experiment includes a horizontal X-ray beam and a transmission mode, the usage of standard crystallization plates is impossible. To overcome this problem, we have developed the technique of protein crystallization in glass capillaries.

The samples were composed of DMPC/CHAPSO matrix and the prototypical membrane protein bacteriorhodopsin embedded into the lipidic bilayer.

We have observed that, just before the protein crystals appearance, the peaks arise in the scattering curves. These peaks correspond to the distance of about 85 Å (for several series of crystallization). This distance decreases by 5-10 Å in the course of crystal formation. The peaks remain after appearance of protein crystals. The ratio of peak positions is 1:2 that can indicate on presence of lamellar phase.

The second group of peaks we observed corresponds distances 500-600 Å (for several series of crystallization).

We also put the pure lipidic phase without protein in crystallization conditions and investigated its structural changes with time. We observed the same phase behavior of the system as for the lipid-protein system. The scattering curves contain two diffraction peaks with position ratio 1:2 as well. Their positions correspond to distance about 75 Å.

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Effect of zea mays l extract on hela cell membranes in vitro

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Background. Cancer is a leading cause of disability and death among the population. Cervical cancer occurs more often in younger patients today. Surgical treatment of cervical cancer has a number of implications both for the reproductive function and for the quality of life of patients. Therefore, the search for new treatments for cancer, including from plant material are relevant today. It is also essential that new drugs have been effective, selective and safe [1].

The aim of the study is to reveal the cytotoxic activity of Zea mays L. extract in relation to cervical cancer cells in vitro experiments using fluorescent staining.

Flavonoid-containing extract of diploid forms of *Zea mays L*. was used for the study. The extract was obtained by the copyright method [2]. The study was conducted on cell culture of cervical cancer cells (HeLa) from Cryobank Collection of Cell Cultures Institute of Cytology RAS. Cultivation was carried out in plastic flasks in RPMI 4. Cells in a RPMI4 without extract was used as a control. We analyzed the following concentrations of Zea mays L. extract: 0.28 mg / ml; 0.56mg / ml; 1.12mg / ml, 2.25mg / ml; 4.5mg / ml.

Propidium iodide and acridine orange were used as fluorescent dyes (colourants). Propidium iodide stains only dead cells, and acridine orange stains only living cells.

Results. At the concentrations of maize from 0.28 at 4.5 mg / ml, the cell death was not observed after one day of incubation, but we found a tendency to decrease the total number of cells as compared to the control. We observed cell death with increasing concentration beginning from 9 mg / ml.

Conclusion. At low concentrations the extract of Zea mays L has a weak cytostatic effect on HELA cells. At increasing of concentration of extract the cytotoxic effect is weak. Thus, corn extract can be studied as potential antitumor agent against cervical cancer, but it's necessary to study the spectrum of antitumor activity and mechanisms of extract action.

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Glutamate as a signaling molecule in rat heart mitochondria

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Amino acid glutamate is one of the major mitochondrial metabolites. It participates in malate-aspartate shuttle which transported NADH (complex I substrate) from cytoplasm to mitochondrial matrix. Recent studies show that glutamate is also an important regulator of mitochondrial functioning. In the hypoxic-like conditions (succinate 2mM, ATP hydrolysis, complexes I and III are blocked) relatively low addition of glutamate (100mkM) to isolated rat heart mitochondria significantly stimulates H_2O_2 production (measured fluorometrically with Amplex Ultra Red). Results of inhibitor analysis show that the H_2O_2 source is succinate dehydrogenase (SDH) FAD-center. The most important distinction of common ROS generation on SDH (for instance, described in [1]) from glutamate-dependent is that the latter is essentially Ca^{2+}- and potential-dependent. Possible reason of ROS synthesis stimulation by glutamate is the increase of $NADH/NAD^+$ ratio.

Glutamate is also an agonist of glutamate receptors, such as NMDAR (which is present in rat brain mitochondria according to [2]). In our study we show the presence of NMDAR (GRIN2B and GRIN1 subunits) in isolated rat heart mitochondria by immunogold labeling and protein immunoblot. There are no signs of receptor presence obtained in heart tissue lysate (lack of sensitivity), that indicates the receptor localization exactly in mitochondria. The possible receptor functions discussed are its participation in calcium transport and in excitation-metabolism coupling.

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Surface-enhanced Raman spectroscopy for the study of conformation of submembrane molecules

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Surface-enhanced Raman spectroscopy (SERS) provides a great enhancement of Raman signal from molecules on the surface of nanostructures with plasmonic properties. The distance between molecule of interest and nanostructures should be very small which makes it challenging to investigate molecules inside cells and membrane organelles.

We offer a protocol for investigation of cytochrome *c* floating between inner and outer membranes in intact mitochondria. That became possible due to design and application of silver-based nanocomposites with long-distance enhancement properties. We used inVia Raman microspectrometer (Renishaw, UK) with 514 nm and 532 nm lasers for SERS spectra recording. Our research provided information about cytochrome *c* heme conformation *in situ*, which is almost impossible to achieve by other methods. SERS spectra of mitochondria were sensitive to the distance between two mitochondrial membranes and to the functional state of mitochondria.

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The 3D structure of the human ion channel Kv10.2, microscopy and molecular modeling

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Voltage-gated potassium channel Kv10.2 belongs to the family EAG channels. Mutations in this channel leads to the development of neurological disease and certain tumors. Directed drug design requires knowledge of the details of the three-dimensional structure of the channel. EAG family members are characterized by long, with respect to other families, N- and C-terminal intracellular amino acid sequences which include structural and functional domains. N-terminal domain in PAS Kv10 plays an important role in the activation, and is believed to alter the rate of deactivation, possibly by binding to the linker S4-S5.

In this study, we have 3D structure of the human truncated channel Kv10.2 using microscopy of single molecules. In this channel there is no N-terminal cytoplasmic domain PAS (Kv10.2ΔPAS), but the channel forms a tetramer. Previously, we have shown that the cytoplasmic domains of full-length channel Kv10.2 form a structure of the type "hanging gondola." The cytoplasmic portion includes connecting PAS domains and cNBD. Removal PAS domain leads to a conformational change in the cytoplasmic portion of the channel as well as localization suggests certain cytoplasmic domains are part of the full-length channel.

For interpretation of the 3D structures we used homology modeling and molecular dynamics simulation. There are several templates available to the moment including eag domain-CNBHD complex of the mouse EAG1 channel, full-length Shaker potassium channel Kv1.2, C-linker/CNBHD of zELK channels and others. But there are still no templates for many fragments that led to necessity of partial de novo modeling. Analysis of molecular trajectory allowed estimating dynamical characteristics of channel, supposing interdomain interactions

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Structural and dynamic features of interaction between lantibiotic nisin and lipid-II in biomembrane

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Bacterial resistance to antibiotics is one of the biggest threats to global health today. Search for new antibiotics is as important as knowledge of already known antimicrobial compound's mechanism of action, that can be used in rational design of their properties to overcome bacteria's strategies for resistance.

One very well validated target of several classes of antibiotics is the membrane-imbedded peptidoglycan precursor – lipid-II. Nisin represents a group of lantibiotics, which specifically bind to conservative pyrophosphate group of lipid II. There is the NMR structure of nisin/lipid-II complex in solution, while its native structure in lipid bilayer is unknown. In order to predict spatial structure of such a complex in membrane, we investigated the conformational capabilities of the isolated molecules – lipid II and nisin – in their natural environments as well as their complex in solution by conducting a series of molecular dynamics simulations.

Our experiments revealed that pyrophosphate group of the individual membrane-bound lipid-II molecule may adopt the same conformation as in the complex. In this case, it forms a unique pattern of hydrogen bond acceptors on the membrane surface, that is not typical for other parts of the membrane. We expect this conformation to be theoretically suitable for capturing by the "trap" (nisin). Moreover, it was shown that lipid-II induces stable changes of topography and hydrophobicity of the membrane surface [1].

By contrast, conformations of nisin molecule do not correspond to those found in the complex, however, it's high flexibility allows a proposal that the target (lipid II) capturing is accompanied by structural changes.

In most cases, superposition of the resulting structures of the complex with selected lipid-II conformation yielded potential, sterically unbarred models of complex in membrane, which dynamic properties have to be explored.

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The ultrastructural changes in the brain in the farther period of closed craniocerebral injury are similar to age-related reorganizations during the natural aging process

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Closed traumatic brain injury (TBI), has underestimated complications in farther post-traumatic period. Molecular concussion caused by trauma , and shock wave that spreads throughout the brain energetically significant conformational changes in membranes are the main factors that determine the development and the outcome of the wound healing process.

Ultrastructural analysis of brain changes of patients with TBI and rabbits with experimental gunshot injury at different times after injury was conducted using standard electronic microscope method.

A few hours after the trauma most of the primary gap junctions spontaneously formed in the first minutes after the injury involuted, apparently due to a decrease of intracellular edema and diffusion of water into the intercellular space. The number of gap junctions, localized near the active zones with pathologically altered synapses increased. Strong perivascular and pericellular edema around hyperchromic cells were occurred. Basal membrane (BM) demonstrated thickening, loosening and sometimes "swelling". Signs of microcirculatory disorders (sludge erythrocytes , capillaristasis) were occurred in a few days after trauma along with "cerebral vasospasm" of great vessels, which is associated with a secondary wave of deaths among people in the post-traumatic period. Development of oxidative stress as a result of accumulation of toxic radicals lead to local and generalized destructive and degenerative changes of neurons and glial cells and cellular elements of blood-brain barrier. 1-3 months after the trauma the germination of collagen fragments and calcifications in the BM vessels and endothelium lining decay were observed. Huge centers of ischemic degeneration of parenchyma adjoining to such fragments of vessels, the proliferation of glia and fibrous astrocytes around neurons complicate trophicity, cell-cell communication and brain tissues drainage in general.

Thus, traumatic brain injury in many positions is similar to naturally accumulated age-related changes. Apparently they differ in the dynamic of pathological processes, the extent of the destruction changes and in the range of complications caused by the negative environmental factors. In our view TBI, even mild, without damaging the bones of the skull and the violation of the integrity of brain tissue, can possibly accelerate the aging process .

Computer Modeling Guided by NMR Restraints Reveals Binding Mode of tarantula toxin VSTx1 to isolated of voltage-sensing domain of KvAP Channel

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Voltage-gated K+ channels (Kv) play essential role in the cellular excitability and propagation of nerve signals. The spider toxin VSTx1 (tarantula *Grammostola spatulata*) inhibits activation of the prototypical K+ channel KvAP (archea *Aeropirum pernix*) by blocking voltage-sensing domains (VSD) in the depolarized (activated) conformation and delaying the domain return to the hyperpolarized (resting) conformation.

In the present work the interaction of VSTx1 with isolated VSD of KvAP channel having depolarized conformation was studied by combination of NMR spectroscopy and Computer Modeling. In the NMR study VSTx1 binds to the DPC/LDAO (2:1) micelle surface by large hydrophobic patch surrounded by the ring of the positively charged residues (Lys4, Lys8, Arg24 и Lys26). However, the Molecular Dynamics (MD) calculations using explicit POPC/POPG (3:1) membrane and Monte-Carlo simulations revealed the toxin fluctuations between several membrane bound topologies. To account the flexibility of the interacting molecules the VSD/VSTx1 complex was modeled using combined MD/protein-protein docking approach. Binding interfaces in the docking procedure were restricted to ones determined by NMR. Initial ensemble of 20000 structures was reduced to 34 models using several kinds of filters. Obtained models were additionally re-scored using NMR orientation data obtained with spin-labeled VSD. Finally we arrived to the two structures of VSD/VSTx1 complex, which have the quite similar binding mode. In the obtained complexes the toxin binds to the domain between S1 and S4 helices. The N-terminal part of VSTx1 interacts with S1-S2 helical hairpin, while the C-terminal toxin fragment (Ser22-Phe34) with S4 helix. In the resting conformation of VSD the S3b-S4 helical hairpin is probably buried and inaccessible to the toxin molecule bound at the membrane surface.

Summarizing the obtained results, we could propose that VSTx1 binds to the interface region of the lipid bilayer and from this state forms the complex with the VSD S1-S2 loop. After membrane depolarization and switch of the VSD into depolarized (activated) conformation, the toxin molecule captures the S4 helix, thus blocking the VSD in the depolarized state.

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Fast iodide-SAD phasing for membrane protein structure determination

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Membrane proteins participate in vital reactions such as signal transduction, solute transport, charge separation and conversion of energy. Understanding such fundamental processes at molecular level requires knowledge of structures of the proteins at high resolution. Each step of X-ray crystallographic pipeline from protein production to structure is not straightforward. One of these uneasy checkpoints is experimental phasing.

Here we present a fast, easy, non-toxic and universal method to solve structures of membrane proteins via iodide-single-wavelength anomalous diffraction (iodide-SAD). The universality of this method is expected to be based on a common feature of membrane proteins – availability at hydrophilic-hydrophobic protein interface of positively charged amino acids which an iodide strongly interacts with. To demonstrate the method we took four membrane proteins varying by sizes of membrane and soluble parts: light-driven sodium pump KR2 from the marine bacterium *Krokinobacter eikastus*, a light-driven proton pump MacR from marine bacterium *Candidatus Actinomarina*, a fragment of histidine protein kinase NarQ from *Escherichia coli* and human adenosine A2A G-protein coupled receptor. The structures have been solved by single-wavelength anomalous diffraction of iodide-soaked crystals.

The iodide-SAD method was shown to be highly efficient for different data collection strategies including both standard and serial X-ray crystallography.

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Knowledge of native interfaces of molecular complexes is sufficient to construct predictive models for the selection of binding candidates

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Selection of putative binding poses is a challenging part of virtual screening of molecular interactions, e.g. in protein-protein or protein-ligand complexes. Predictive models to filter out binding candidates with the highest binding affinities comprise scoring functions that assign a score to each binding pose. Existing scoring functions are typically deduced by collecting statistical information about interfaces of native conformations of molecular complexes along with interfaces of a large generated set of non-native conformations. However, the obtained scoring functions become biased toward the method used to generate the non-native conformations, i.e., they may not recognize near-native interfaces generated with a different method. We have demonstrated that knowledge of only native molecular interfaces is sufficient to construct well-discriminative predictive models for the selection of binding candidates for protein-protein [1] or protein-ligand [2] complexes. We have introduced a new scoring method that comprises a knowledge-based potential deduced from structural information about the native interfaces of crystallographic molecular complexes. We derive the knowledge-based potentials using convex optimization with a training set composed of native molecular complexes and their near-native conformations obtained using deformations along the low-frequency normal modes. As a result, our knowledge-based potentials have only marginal bias toward a method used to generate putative binding poses. The potentials are smooth by construction, which allows using it along with the rigid-body optimization to refine the binding poses. Furthermore, the obtained knowledge-based potentials could be directly incorporated into sampling stage of the molecular docking protocol [3]. Our potentials proved to be very successful in recent CAPRI [4], CAPRI/CASP [5], and CSAR [2] docking assessments. Our methodology can be easily adapted to the recognition of other types of molecular interactions.

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Diversity and evolution of four-domain voltage-gated ion channels revealed by bioinformatic analysis

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Changes in membrane potential, such as action potential and/or grade potential, represent a characteristic feature of eukaryotic excitable membranes. It is assumed that the emergence of signaling systems based on action potentials was tightly related to the evolution of voltage-gated sodium (Na_v) and calcium (Ca_v) channels which are the members of the four-domain voltage-gated ion channel (FDVGIC) family [1]. Genomic and electrophysiological studies show that FDGIC family is more diverse then it was considered before. Nowadays five FDVGIC subfamilies are well described: Na_v, high-voltage-activated Ca_v, low-voltage-activated Ca_v and voltage-insensitive sodium leak channels (NALCN) of animals, as well as voltage-insensitive calcium channels of fungi (Cch); however, their phylogenetic relationships remain unclear [2]. In this study, we provide taxonomically broad phylogenetic analysis of FDVGIC amino acid sequences that belong to animals, fungi and 16 other groups of eukaryotes (protists). The analysis revealed that most protists possess their own subfamilies of FDVGICs. To understand functional evolution of FDVGICs in eukaryotes, we searched for functionally relevant regions of these channels, i.e. selectivity filters, trans-membrane segments S4 (voltage sensors) and inactivation gate motifs responsible for fast inactivation of Na_v, and mapped these structural features on FDVGIC phylogeny. Our results agree with the hypothesis that the ancestral FDVGIC was a calcium selective channel [3]. Moreover, we argue that the ancestral FDVGIC was voltage-gated and possessed an inactivation gate motif. Funded by the Russian Science Foundation, project 16-14-10116.

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Designing of ratiometric fluorescent indicator for hydrogen peroxide based on genetically encoded indicator NeonOxE

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Major biological processes occurring in living organisms including cell proliferation and differentiation, tissue repair, inflammation, circadian rhythm, aging and others use hydrogen peroxide as signaling compound [1]. For detecting this low weight oxygen metabolite, different molecular probes have been created to date. One of them is a genetically encoded fluorescent indicator - NeonOxE, relating to line sensors of HyPer [2].

NeonOxE is an intensiometric sensor for detecting hydrogen peroxide inside biological objects, endowed with bright contrast and low pH-sensitivity. However, NeonOxE has a typical spectrum of green fluorescent proteins with a single excitation maximum that results in difficulties in correctly assessing of a signal magnitude in living systems. In this study, we tried to obtain ratiometric form of NeonOxE to eliminate aforementioned drawback.

Derived forms of NeonOxE was obtained by random mutagenesis followed by screening libraries with using a fluorimeter. We found a ratiometric version of NeonOxE with a pronounced cyan excitation maximum at 408 nm and determined the appropriate mutation, W157C. In addition, we measured some of its characteristics including quantum yield and pH-sensitivity. These values were comparable to those known for the original NeonOxE. The mutant was also expressed in HEK293NT where it has demonstrated reduced contrast response.

Work is in progress to increase current fluorescent contrast of derived mutant along with preservation of other properties. In prospect, this version of NeonOxE has all chances to become one of the most popular indicators of hydrogen peroxide known thus far.

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Correlation between oxidative stress, mitochondrial fragmentation and cell death in yeasts

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Expression “oxidative stress” is used to describe the increased intracellular generation of reactive oxygen species (ROS). Mitochondrion is the main source of ROS in the cell. Overproduction of ROS is generally associated with mitochondrial dysfunction and poses hazard to life of the cell.

The aim of the work was to identify the correlation between oxidative stress, mitochondrial fragmentation and cell death of aerobic yeasts. The model organism we used, yeast *Dipodascus magnusii* has relatively large cell size and demonstrates branched mitochondrial reticulum under normal conditions; thereby it appears to be perspective model for microscopic methods of mitochondrial fragmentation studies.

Oxidative stress induced by tert-butyl hydroperoxide was shown to destruct reticular structure of *D. magnusii* mitochondria. Lipophilic mitochondria-targeted antioxidants SkQ1, SkQT1 and SkQThy were purposed to minimize the consequences of prooxidant-induced oxidative stress. Preincubation with mitochondria-targeted antioxidants led to decrease of oxidative stress in yeasts. Mitochondria-targeted antioxidants treatment was found not only to prevent mitochondrial fragmentation in yeasts, but also to restore reticular structure of mitochondria, fragmented due to the oxidative stress. Higher doses of prooxidant were shown to induce yeast cell death with classical parameters of apoptosis. Preincubation with mitochondria-targeted antioxidants was shown to decrease the level of cell death.

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Phase transition features in lipid membrane mixture: A possible influence of lipid length tail?

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The investigation of lipidic membranes near the phase transition has a significant importance not only for theoretical findings but also from a biological point of view because the cell membrane plays a key role in biological processes [1]. In this respect, lipid membranes provide a good model for numerous biophysical studies. Recalling that the biological cell membrane is composed of many lipids. Therefore, in order to create a plausible model of the membrane, two lipids with very different temperature points of the main phase transition have to be used.

In the present work we study multilayer membranes (MLV) of lipid mixtures by the method of small-angle neutron scattering (SANS) and densimetry. We use two different lipids: DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) with the main phase transition temperature T_1=41.3°C and POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) with the phase transition temperature T_2=-4°C. SANS measurements were performed on the small-angle spectrometer YuMO (IBR-2, JINR, Dubna, Russia) [2]. Densimetry data were obtained on the densimeter Anton Paar 5000M with the temperature the repeatability of 0.001°C and the density accuracy of 0.000005 g/cm^3 [3]. We found that repetition period of the ripple phase of this mixture decreases when we increase the weight ratio DPPC:POPC (wt/wt). It is important to emphasize that the point of the main phase transition (from gel to La phase) did not exhibit any significant changes according to both the densimetry measurements and the SANS study. The comparison of our results with the previously reported data [4] is provided and the reasons for their differences are discussed.

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A genetically encoded photosensitizer miniSOG causes lysosomal membrane damage

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Genetically encoded photosensitizers are widely used as an optogenetic tool for oxidative stress. This approach allows targeting subcellular structures and induce light-mediated effects. In our research we tested phototoxic flavoprotein miniSOG fused with Rab7 localized on a cytoplasmic surface of late endosomes and lysosomes.

The HeLa Kyoto cells expressing miniSOG-Rab7 were effectively killedafter blue-light illumination.The mechanism of cell death was analyzed using caspase-3 activation test. HeLa Kyoto cells were transiently transfected with miniSOG-Rab7 and mKate2-DEVD-iRFP(red-shifted caspase-3 sensor based on FRET) [1].Twenty-four hours after transfection cells were illuminated with blue-light and the increaseof ratio mKate2/iRFP signals was observed which indicates caspase-3 activation. Furthermore illuminated cellsretained the level of fluorescence signals, thus it was concluded that the plasma membrane was intact (feature of apoptosis, not necrosis;as compared with KillerRed-Rab7) [2].

We suggested that cell death was caused by lysosomal membrane permeabilization (LMP).To confirm this assumption we conducted the LMP detection test based on translocation of fluorescently labeled Galectin 1 [3].We observed theformation of mDsRed-Galectin1 fluorescent punctawhich indicated LMP. To examine if lysosomal proteasescathepsins are involved in the process of cell death we performed inhibition analysis. We used cathepsin B-, L-, and H-specific inhibitor E-64 and the cathepsin D-specific inhibitor pepstatin A. It was revealed that E-64 decreased the amount of dead cells approximately 2-fold, whereas pepstatin A completely precluded miniSOG-Rab7-mediated phototoxicity.

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SAXS contrast variation method in structural studies of phospholipid nanodiscs

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Small Angle X-ray Scattering (SAXS) [1] is a powerful method for structural investigation of biological objects, such as proteins, membranes, macromolecules, etc.

In this work, we applied contrast variation method [2-3] to a SAXS experiment to obtain information about structure of phospholipid nanodiscs – membrane-mimicking systems composed of phospholipid bilayer fragment and two Membrane Scaffold Proteins (MSP) [4]. Nanodiscs were prepared from lipid DMPC and protein MSP1E3D1.

The SAXS measurements were performed at the BioSAXS beamline BM29, ESRF, Grenoble, France [5]. Solutions of nanodiscs in buffers with 0, 80, 160, 240, 320 and 400 mg/ml of sucrose were prepared.

For every sucrose concentration, Patterson functions, radii of gyration and intensities at zero angle were calculated. It is observed that dependence of form factor value at zero angle versus electron density of solvent is nonlinear. Changes of nanodisc structure in solution with sucrose observed.

Average electron density, partial volume and electron charge of nanodiscs were obtained using approximation of sucrose concentration to the zero value. Calculated volume is equivalent to volume of cylinder with height of 35 Å and diameter of 120 Å. Number of electrons in nanodisc was calculated, it has the same value as the number of electrons calculated for ratio MSP1E3D1/DMPC = 1/160 [4].

It was shown that the charge of nanodisc and, possibly, its partial volume, decrease with increasing of sucrose concentration. The possible reason is a decreasing of the number of water molecules associated with lipid bilayer. According to this explanation, dependence of minimal possible number of dissociated water molecules versus sucrose concentration was obtained.

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Novel lipid-dependent dimerization of human GLTP induced by point mutation in the recognition center

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Human Glycolipid Transfer Protein (GLTP) carries out the important function of non-vesicular transport of glycosphingolipids (GSLs) between membranes and may acts as a sensor of glycolipid levels. GSLs trafficking defects associated with number of neurodegenerative disorders including Parkinson's disease and Alzheimer's disease. Details of the all alpha-helical GLTP-fold mechanism of action remain unclear. Previously, reversible lipid-dependent dimerization was discovered for holo-GLTP [1]. Dimers are commonly involved in catalysis, regulation and structural assembly. Structural indicated a homodimer, reproducibly revealed in different crystal forms of GLTP bound with various GSLs. The homodimer is characterized by a 70-80 degree angle between wild-type monomers complexed with sulfatide, but the inter-monomer angle narrows to 63-66 degrees upon D48V mutation [2]. The inter-monomer contacts were found to mainly involve helix6-helix6, as well as helix2-helix2 at their C-termini. The X-ray structure of another mutant, K87Q, complexed with 18:1-glucosylceramide, reveals a novel homodimer with a different dimerization contact region that includes the mutation site, which was not involved in the original dimerization contact region. Fluorescence spectroscopy assays involving intrinsic Trp emission changes show that K87Q-GLTP retains the original binding capacities for sulfatide, glucosylceramide and galactosylceramide. The X-ray structure of K55F variant with galactosylceramide 18:1 also reveals another homodimer, different from homodimer discovered in [1] and homodimer of K87Q-GLTP. In opposite to last one K55F has low-affinity binding to the same GSLs. Thus, GLTP dimer design could provide a way to dissect certain steps of the glycolipid transfer process. The influence of dimer type on steps of lipid transport by GLTP needs further investigation.

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Point mutations of 'secondary' residues in the human GLTP recognition center induce novel cerebroside-dependent types of dimer

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Glycolipid homeostatic defects are associated with a number of neurodegenerative disorders including Parkinson's disease and Alzheimer's disease. Human glycolipid transfer protein (hGLTP) is involved in non-vesicular transfer of glycolipids and may act as a sensor of glycolipid levels. The exact molecular mechanism of GLTP action is still unknown. Reversible lipid-dependent dimerization of GLTP is proposed to be a part of transport mechanism. Many X-ray structures of GLTP complexed with different glycolipids reveal formation of moderately flexible homodimers with inter-monomer contacts mainly in area of helix6-helix6 and helix2-helix2 at their C-termini [1].

Here, we confirmed the dimerization specificity for holo-GLTP by X-ray structure of wild type complexed with sulfatide 12:0 in 'no-symmetry' P1 space group with four 'typical' dimers in the asymmetric part. We also found that point mutations of certain 'secondary' residues in the GLTP recognition center produce novel types of dimer. K87Q-GLTP and K55F-GLTP complexed with N-18:1 glucosylceramide and N-18:1 galactosylceramide, respectively, dimerization that differs from the 'typical' GLTP dimer and from each other. Both mutants use the 'sphingosine-in' binding mode for ceramide. In both cases, the mutation sites are involved in the inter-monomer contacts. Fluorescence spectroscopy assays involving intrinsic Trp emission changes show that K87Q-GLTP retains the original binding capacities for such GSLs, as sulfatide, glucosylceramide and galactosylceramide. However, the K55F-variant shows low-binding capacity to the same GSLs. Thus, GLTP dimer design could provide a way to elucidate some steps of the glycolipid transfer process or even serve as a tool for GLTP specificity modification. This work was supported in part by Russian Foundation for Basic Research project 14-04-01671 and 15-04-07415, NIH NIGMS GM45928 and NCI121493, and CIC bioGUNE research funds.

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Sodium-translocating rhodopsins as ancestors of G-protein coupled receptors

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Microbial rhodopsins (MRs) and G-protein coupled receptors (GPCRs, which include animal rhodopsins) are two distinct (super)families of heptahelical transmembrane proteins with a common structural scaffold, but no significant sequence similarity. Their similar roles as receptors have long prompted suggestions of the evolutionary relationship between MRs and GPCRs, but no convincing evidence of their common origin could be found. Several crystal structures of class A GPCRs contain a Na^+ ion in the middle of the transmembrane helical bundle [1]. The recently published crystal structures of a Na^+-transporting bacterial rhodopsin [2,3] prompted us to investigate the evolutionary relations between MRs and GPCRs by comparing their Na^+ binding sites. This approach was previously used to establish common origin of Na^+-translocating bacterial and archaeal ATP synthases [4].

The observed similarity of Na^+-binding sites of MRs and GPCRs allowed us to construct a structure-guided sequence alignment for the two (super)families, which highlighted their evolutionary relatedness. Our analysis supports a common underlying molecular mechanism that involves a highly conserved aromatic residue playing a pivotal role in the rotation of the transmembrane helix VI in GPCRs and helix F in MRs, respectively [5].

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Towards a comprehensive view of the eukaryotic plasma membrane: a dynamic interplay of lipids, ions, receptors, and cytoskeleton

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Existence of a living being is possible only when there is a precise coordination between cells in an organism and between molecules in the cells. Malfunction of this coordination contributes to age-related diseases such as loss of sensitivity to hormones and growth factors, amyloids overproduction and plaque formation, cardiovascular diseases and cancers. Events on a plasma membrane (PM) are coordinated as well. This coordination exists on a scale from the entire cell surface down to nanodomains, enabling the PM to transmit signals with the high fidelity and the cell to act as a whole. The examples are (but not limited to): coordination of protrusions at the leading edge with the loss of adhesion and retractions of the trailing edge during cell migration; prevention of egg fertilization by the second sperm immediately after the first sperm has come in contact with the egg's PM; long range directed movement of immune receptors during formation of immune synapse; synchronous all-or-nothing activation of tens to hundreds receptors in nanoclusters. The coordination implies regulation of receptor's localization and activity, but the mechanisms of such regulation are poorly understood.

Receptors on PM are regulated by multiple types of lateral interactions most of which are indirect, such as lipid-, cytoskeleton-, calcium-, transmembrane potential-mediated interactions. Moreover, each type of interaction has different modes. Lipid-mediated regulation, for example, may be exerted by: partitioning in certain lipid phase (lipid raft); membrane curvature; local production of active lipid components (diacylglycerol and lysophospholipids); competition for a limited lipid component required for receptor activation (inositol phosphates and gangliosides). All mediators of the lateral PM interactions are closely interrelated. So, a cytoskeleton is intimately associated with anionic lipids and multiple receptors on PM through adaptor proteins. It is obvious that besides shaping of PM, the cytoskeleton regulates activity of receptors. Different cytoskeleton structures impose different modes of regulation. Static structures composed from tubulin and actin fibers restrict movement of all PM molecules including receptors and lipids. Dynamic cytoskeleton structures of short actin filaments and myosin cause disorder of PM molecules by continuous chaotic myosin-mediated filaments movement, their growth and breakage. The dynamic cytoskeleton prevents lipid rafts from coalescence and receptors from spontaneous activation, while receptor clustering by a ligand promotes transition from chaotic into ordered cytoskeletal structures, directed actin and receptors flow and reshaping of PM.

I will present a view of PM that integrates essential factors of the membrane physiology known to date. It will aid in understanding of receptors disregulation in a disease and promote the development of relevant PM models.

Electrophysiological and modelling study of possible mechanism of potentiation of $GABA_A$ receptors by a non-steroidal anti-inflammatory agent, mefenamic acid

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$GABA_A$ receptors ($GABA_AR$) are chloride ion channels that mediate fast synaptic transmission and belong to a superfamily of pentameric ligand-gated ion channels. $GABA_AR$ are subject to modulation by endogenous neurosteroids, as well as plenty clinically important central nervous system drugs including general anesthetics, benzodiazepines and others. We have observer that mefenamic acid (MFA), a non-steroidal anti-inflammatory agents, strongly potentiates GABA-induced currents in Purkinje cells acutely isolated from rat cerebellum. At concentration of 3-100 μM MFA increased the amplitude of GABA (2 μM) current with EC_{50} 15.5 μM and maximal potentiation up to 700%. MFA also dramatically increased the deactivation time constant (τ_{off}): for currents induced by 2 μM GABA τ_{off} increased by 12.5 times in the presence of 100 μM MFA. In this study, we combined electrophysiological and modeling approaches to investigate the possible site(s) interaction of MFA with $GABA_AR$. In electrophysiological experiments, we investigated the putative interaction of MFA with the binding site of general anesthetic etomidate. We have found that the maximal effects of both MFA (10 μM) and etomidate (3 μM) in the potentiating GABA (2 μM) currents were nonadditive, suggesting that MFA acts through the same binding site in the $GABA_AR$ complex targeted by etomidate. There is growing evidence that etomidate binding site is located at the interface between the $GABA_A$ receptor's α and β subunits in the transmembrane domain [1]. We used computational techniques to investigate the mechanisms of MFA binding in the $GABA_AR$ α/β intersubunit interface. We have built homology model of the open $GABA_AR$ based on the X-ray structure of the glutamate-gate chloride channel and used powerful Monte-Carlo energy minimization approach to predict the MFA binding site. We have found that MFA have similar structural determinants of binding as was previously shown for etomidate. Thus, our in silico study supports the hypothesis that MFA acts through the transmembrane β(+)/α(–) subunit interface of the $GABA_AR$, targeting a site overlapping with that of the general anesthetic etomidate. These results suggest strategies for developing new drugs that positively modulate $GABA_A$ receptor and may be used in clinical practice.

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Divide and conquer approach in studies of multidomain membrane proteins: NMR investigation of isolated VSD of human Kv2.1 channel

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Voltage-gated potassium channels (Kv) play crucial role in functioning of cardiovascular, nervous and muscular systems of multicellular organisms. Despite their great importance, aspects of structural organization and mechanisms of voltage-dependent activation of Kv channels are insufficiently studied. Four symmetrically arranged α-subunits comprise the main functional group of a Kv channel. Each of four α-subunits contains six transmembrane helices that make up voltage-sensing domain (VSD, S1-S4 helices) and pore domain (PD, S5-S6 helices).

Here we present a study of isolated VSD of human Kv2.1 channel, which is the main potassium channel in human brain. Also, it takes part in glucose-dependent insulin secretion in pancreatic β-cells and in the process of apoptosis as both initiator and propagator. These particular features make Kv2.1 interesting as a potential drug target. It is known that blockers of Kv2.1 channels can enhance insulin secretion and reduce damage caused by a stroke or ischemia. Development of subtype-specific drugs requires atomic resolution structure of the individual domains making up the channel.

The milligram quantities of totally and selectively (on residue basis) ^{15}N- and ^{13}C/^{15}N-labeled VSD samples were produced by cell-free expression using S30 extracts of *E.coli* cells. To find optimal conditions for NMR study the following membrane-mimicking media were screened: micelles of zwitterionic and anionic detergents (FOS-10, DPC (FOS-12), FOS-14, LDAO, LPPG, DC_7PC); mixed DPC/LDAO micelles at 1:1, 1:2, 2:1 ratios; and DHPC/DMPC, Chaps/DMPC, Chaps/DMPG bicelles. The pH of the samples was varied in the 5.0 – 7.0 range. The best quality spectra were obtained for VSD solubilized in 1:1 mixture of DPC/LDAO detergents at pH 5.0. The VSD/DPC/LDAO sample was stable for longer than one-week period. The VSD samples in DC_7PC micelles and bicelles were unstable.

Almost 65% of VSD backbone assignment was obtained in the DPC/LDAO mixture using a set of 3D triple-resonance NMR experiments and special combinatorial labeling scheme with selectively labeled samples. The observed line-broadening for the subset of ^{1}H-^{15}N resonances revealed the presence of μs-ms motions in the S2 and S3 helices of VSD.

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Thermodynamics of membrane protein folding in-vitro. Is there cold denaturation of membrane proteins?

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Understanding of mechanisms of protein folding is very important problem of biophysics and structural biology. Although there is detailed theory of folding of the globular water-soluble proteins, the folding of membrane proteins is far less analyzed and understood.

Here using heteronuclear NMR we studied the transitions between folded and partially unfolded states of four-helical membrane protein - voltage-sensing domain (VSD) of KvAP channel (150 a.a.). VSD preserves its native fold in the micelles of zwitterionic detergents (DPC). In contrast, the anionic detergent LPPG lead to transitions of VSD to the partially unfolded state. Solubilization of VSD in LPPG moderately changed the secondary structure, but strongly influenced tertiary structure and backbone dynamics of the domain. NMR studies revealed the disruption of the interhelical packing and marked increasing of ps-ns backbone mobility as compared to the natively folded VSD. However our data revealed the quasi-native topology and compactness of the VSD/LPPG complex. The observed VSD structure is reminiscent of a "molten globule" state found in the folding pathway of soluble proteins.

Transitions between folded and unfolded ("molten") states were reversible and depended on LPPG fraction in the sample. In this case the influence of LPPG was similar to the chaotropic agents (such as urea or guanidine chloride), which are usually used for water-soluble proteins folding studies.

In addition, at the DPC/LPPG molar ratio ~1:1 we saw strong temperature dependence of the VSD state: it was unfolded at 20-30ºC and virtually fully folded at the 45ºC. Analysis of signal intensities of both forms in the NMR spectra permitted to quantify the energetic of the transition between the two states in response to change in LPPG/DPC molar ratio and temperature. The temperature dependence of the Gibbs free energy (ΔG^0) of VSD folding process revealed the presence of non-zero contribution of heat capacity change (ΔC_p).

Observed dependence explained the transition of VSD from the unfolded to the folded state during temperature increasing, and vice versa. In case of water-soluble proteins, similar effect known as "cold denaturation is well studied. Thus, we observed cold denaturation of membrane protein. According to our knowledge, this effect was not described before for membrane protein *in-vitro*.

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Ginger extract mitigates ethanol-induced changes of alpha and beta –myosin heavy chain isoforms gene expression and oxidative stress in the heart of male wistar rats

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The association between ethanol consumption and heart abnormalities, such as chamber dilation, myocyte damage, ventricular hypertrophy, and hypertension is well known. However, underlying molecular mediators involved in ethanol-induced heart abnormalities remain elusive. The aim of this study was to investigate the effect of chronic ethanol exposure on alpha and beta –myosin heavy chain (MHC) isoforms gene expression transition and oxidative stress in rats' heart. It was also planned to find out whether ginger extract mitigated the abnormalities induced by ethanol in rats' heart.Male wistar rats were divided into three groups of eight animals as follows: Control, ethanol, and ginger extract treated ethanolic (GETE) groups. After six weeks of treatment, the results revealed a significant increase in the β-MHC gene expression, 8- OHdG amount, and NADPH oxidase level. Furthermore, a significant decrease in the ratio of α-MHC/ β-MHC gene expression to the amount of paraoxonase enzyme in the ethanol group compared to the control group was found. The consumption of Ginger extract along with ethanol ameliorated the changes in MHC isoforms gene expression and reduced the elevated amount of 8-OHdG and NADPH oxidase. Moreover, compared to the consumption of ethanol alone, it increased the paraoxonase level significantly. These findings indicate that ethanol-induced heart abnormalities may in part be associated with MHC isoforms changes mediated by oxidative stress, and that these effects can be alleviated by using ginger extract as an antioxidant molecule.

Recombinant mambalgin-2 from the venom of *Dendroaspis polylepis* reduces proliferation of human glioblastoma U251MG cells

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Glioblastoma multiforme is the most common and aggressive of the primary brain tumors. Malignant glioma cells signify an amiloride-sensitive sodium conductance that is attributed to the acid-sensing ion channels (ASICs) or their complex with epithelial sodium channels (ENaCs). This amiloride-sensitive sodium conductance is present in high-grade (grades III and IV) tumors and is not observed in normal astrocytes or low-grade gliomas. Amiloride and its analogs were shown to reduce tumor growth in rodents. Spider venom toxin PcTx1 which is known as a potent antagonist of ASICs was reported to inhibit glioma cell migration and proliferation. Together these observations make ASICs or ASIC/ENaC heteromeric channels a potential therapeutic target.

Recently, two peptides called mambalgins have been isolated from the venom of the snake *Dendroaspis polylepis*. Mambalgins belong to the three-finger toxin family and are potent inhibitors of various ASICs. We have developed for the first time the high-efficient system for bacterial production of mambalgin-2 from *D. polylepis*. This system allowed us to obtain the recombinant toxin and characterize its physical properties by the variety of techniques including HPLC, MALDI-MS and NMR. Biological activity of recombinant mambalgin-2 was tested on *Xenopus* oocytes expressing rat ASIC1a channel and was similar to the activity of the wild type toxin. Incubation of human glioblastoma U251MG cells with 1 μM of mambalgin-2 during 48 h led to a reduction in the cell number down to ~ 60% relative to the control. An analysis of the dose-response curve revealed the concentration-dependent mode of the recombinant mambalgine-2 with EC_{50} in nanomolar range.

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Hypoosmotic conditions provoke structural and functional changes in membranes of phosphorylating mitochondria

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Background It is well known that the temperature of a lipid phase transition and its properties are controlled by hydration level of a lipid bilayer. [1] At high hydration levels, a pretransition and intermediate (P_β) phase can be observed on certain phospholipids. In [1] an equivalence of hydration level and osmotic pressure was proved. Our goal was to study an effect of osmotic stress on membranes of operating mitochondria, possibly having certain influence on mitochondrial functioning.

Methods Isolated rat liver mitochondria were examined under normal and hypoosmotic (120 mOsm) conditions. Hydrophobic fluorescent probe pyrene was used to indicate alterations in lipid (pyrene monomer to excimer intensity ratio) and protein (level of tryptophan fluorescence quenching by pyrene) membrane components. Phosphorylation was observed by polarografic method, with succinate as respiration substrate and rotenone.

Results Significant simultaneous changes in system parameters were found in a narrow temperature interval near 19 ˚C in hypoosmotic medium. First, Im/Ie ratio at 19 ˚C displays changes attributed [2] to lipid pretransition, with a main transition at 26 ˚C. Second, at 19 ˚C a quenching level is abruptly reduced, that implies suppression of protein-lipid energy transfer and indicates protein aggregation. Finally, a pronounced increase was detected in phosphorylating efficiency measured as ADP/O, although no sharp changes in respiration or phosphorylation rates were observed at 19 ˚C.

Conclusion Hypoosmotic conditions provoke both structural and functional changes in membrane of phosphorylating mitochondria. Lipid pretransition influence mitochondrial functioning in an intricate way. The effect may have physiological significance, for example, in hibernating organisms, or be a rudimental one, since 19 ˚C is an ambient temperature.

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Structural parameters of silver hydrosols: electron microscopy and small angle X-ray scattering

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Silver nano- and microparticles are widely used for biological and medical research including drug design. Modification of nano- and microporous membranes with silver hydrosols is a promising application aimed at fabrication of SERS substrates with high sensitivity to different analytes including biologically important molecules. Reproducible fabrication of SERS substrates requires detailed knowledge of nanoparticles' properties such as size distribution, surface charge, content of agglomerates, and others.

In this work we studied silver hydrosols, i.e. silver water suspensions, prepared using electric discharge technology [1]. The silver particles are formed due to the dispersion of the electrode metal in a high-current discharge between closely spaced silver electrodes and further transfer of dispersed silver particles in water. The obtained solutions were investigated on Rigaku [2] (MIPT, Dolgoprudny, Russia) and BM29 [3] (ESRF, Grenoble, France) instruments by small angle X-ray scattering method. SAXS curves were treated by Fitter [4] and SasView programs [5], where triaxial ellipsoids and chain of spheres models were shown as the best fits. For the chain of spheres model we obtained fit parameters 8.6, 7.1 and 5.0 nm as radii, with 20%, 30% and 50% polydispersity, respectively, for three different samples of suspension. For the Rigaku and BM29 instruments the fitting parameters were found different, and the reasons are discussed. The parameters of acquired silver particles were estimated. The dried samples of silver hydrosols were also analyzed by the methods of electron microscopy. Polyester track-etched membranes were modified with polyethyleneimine and then coated with Ag nanoparticles. Electron microscope examination was performed with a Hitachi SU8020 instrument. Electron microscope images of membrane surface at different magnification were recorded and analyzed. In summary, structural parameters of silver particles were obtained and polydispersity was estimated using two different analytical methods.

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Influence of water-soluble fullerene derivatives on a therapeutic target for type 2 diabetes and Alzheimer's disease

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Type 2 diabetes mellitus (T2DM) is a complex polygenic disease with many metabolic disorders. In recent years it has been found that type 2 diabetes may act as catalyst onset Alzheimer's disease (AD) [1].

This paper investigates the effect of water-soluble fullerene derivatives (WSDF) on the therapeutic target for T2DM: sorbitol dehydrogenase (SDG), aldose reductase (AR), monoamine oxidase A (MAO-A), as well as target for AD: monoamine oxidase B (MAO-B), the main target of the AD according to latest researches, and the process of lipid peroxidation (LP), since the increase of free radicals in the body plays a catalytic role in the development of both diseases. We have investigated eight WSDF.

It was found that all tested compounds except compounds 2 and 6 possess antioxidant activity. Fullerene derivatives 7 and 8 inhibit lipid peroxidation more effectively at a concentration of 10^{-5} M on 75% and 73%, respectively. Water-soluble derivatives 1,2,3,4 and 8 showed marked inhibitory effect on the functioning of MAO-A. All compounds except 6 inhibit the catalytic activity of MAO-B, however, the most effective inhibitor is 4 and 8. It was established that compounds 1, 5 and 8 inhibit the catalytic activity of the sorbitol dehydrogenase at 21, 32 and 59% respectively. These results suggest that fullerene derivatives 1, 5 and 8 have both antidiabetic and cognitive-stimulating effect on the animals, which makes it possible to consider these compounds as potential drugs for complex treatment of both diseases.

For further research on animals we selected compound-leader 8, which is an inhibitor of the catalytic activity of enzymes-target T2DM and exhibits cognitive-stimulating activity on animals that allows us to consider this compound as potential drug for the integrated treatment of T2DM and AD. Application of fullerene derivative 8 on experimental model T2DM rats led to the normalization of blood glucose level that testifies to its pronounced anti-diabetic effects.

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Mechanism of neurodegeneration in Parkinson's disease: from alpha-synuclein aggregation to oxidative stress-role of calcium and transition metals

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Protein misfolding and aggregation, as well as oxidative stress are key pathogenic processes in many neurodegenerative diseases, including Parkinson's disease (PD), Nevertheless, the exact mechanism by which alpha-synuclein induces oxidative stress and neuronal death in PD is still unknown.

In our study, we have found that only in oligomeric form alpha-synuclein is able to induce apoptosis through a calcium dependent mechanism. Electrophysiological experiments revealed increased conductivity of the plasma membrane by both monomeric and oligomeric alpha-synuclein. Interestingly, only oligomers were able to form a pore-like structure even in a very low concentration.

Alpha-synuclein in physiological concentrations and in its monomeric state does not generate ROS or lead to toxicity, but induces calcium signal. However, once the process of aggregation is initiated, only minute concentrations of toxic oligomeric species formed very early in the self-assembly process are sufficient to initiate massive ROS production, oxidative stress, and apoptosis. The mechanism by which the toxic oligomer generates ROS is directly associated with redox metal ions and we show that it can be prevented by the chelation of free iron or copper.

This work unravels the interaction between protein misfolding, metal homeostasis, and oxidative stress and highlights the rational basis for a novel therapeutic strategy in PD.

Identification high-affinity binding peptides for human thromboxane synthase

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Thromboxane synthase (TXAS, CYP5A1) is a protein bound to outer membrane of endoplasmic reticulum that catalyzes prostaglandin H_2 conversion to metastable reaction products (thromboxane A2, 12-hydroxyheptadecatrenoic acid). That fact assumes allosteric regulation, as well as presence of potential partner proteins responsible for the transfer of reaction products, nevertheless with no experimental evidence to date. Since TXAS plays an important role in the development of a number of cardiovascular diseases, interest in the research and development of inhibitors and modulators of its activity increases [1].

In this work molecular cloning and heterologous expression of the truncated form of the human thromboxane synthase in bacterial cells was carried out. Recombinant hemoprotein was purified to homogeneous state and has a molecular weight corresponding to the expected. CO difference spectrum of reduced recombinant TXAS and ligand binding properties corresponds to the same of the native enzyme isolated from platelets, which leads to the conclusion about the identity of the resulting recombinant and native TXAS.

Dodecapeptide motif was identified using screening random dodecapeptides phage library. This peptide has a great similarity (>60%) to thrombopoietin (THPO) peptidomimetic. Peptide was then synthetized and indicated high affinity (K_d=9,56*10^{-8} M) binding with TXAS (measured by biolayer interferometry kinetic assay). Molecular dynamics simulation (~50 ns) showed preferable binding region near FG loop domain, which plays role in interaction with membrane and formation of substrate access channel of most mammalian membrane-bound cytochrome P450 enzymes.

Possible mechanism of interaction between THPO and TXAS may consist in endocytosis of specific peptides after THPO proteolysis or in endocytosis of the hormone-receptor complex at elevated THPO concentration and, ultimately, its subsequent degradation in the lysosomes (which is typical for some peptide hormones). However, this does not exclude the possibility that non-membrane domains of TXAS interacting with the complex.

Thus, in the present work for the first time was obtained experimental evidence of possible interactions between human THPO and TXAS and suggests the role of THPO hormone in TXAS pathway regulation.

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Molecular dynamics reveals large inter-domain rotation in the major pneumococcal autolysin LytA

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Susceptibility to *Streptococcus pneumoniae* is remarkably age-related being greatest in children under 5 years old and adults over 65. Whilst the immaturity of the immune system is largely responsible for poor immunity in the former, the underlying causes of susceptibility in older adults is complex. The pneumococcal autolysin LytA is an essential virulence factor, which causes degradation of bacterial cell wall [1]. LytA homodimer comprises two amidase and choline binding domains. The amidase domain cleaves the bond between the glycan chain and the peptide stem of the peptidoglycan (PG), main component of bacterial cell wall. Teichoic acids hold this extracellular protein on the cell wall via several choline-binding sites.

The crystal structure of the full-length LytA dimer [2] does not fully describe the small angle X-ray scattering of this protein in solution [3]. Furthermore, docking of PG glycan chains on full-length LytA reveals that these chains are perpendicular to each other, which contradicts any existing peptidoglycan model. On the basis of these questions we hypothesized that the LytA structure is flexible and can provide inter-domain movement. In order to analyze inter-domain movements that may be is present in LytA, we performed molecular dynamics (MD) simulations on the LytA full-length dimer structure for 5 nanoseconds at 350K. MD simulations indicate that the LytA amidase domain can rotate up to 40° along the long axis of the choline-binding domain. The domain movement analysis via DynDom server [4] also reveals that the hinge region is located between residues Gly171 and Gly177, both strictly conserved among most streptococcal strains. Interestingly, the main chain of both glycine residues takes a conformation that is non-compatible with any other residues than glycine. Our results demonstrate that the two glycine residues can be essential for inter-domain rotation. In conclusion, the achieved modeling indicates that amidase domains can bind simultaneously to parallel PG chains, which provides novel insights into cell wall binding mechanisms by LytA.

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Molecular dynamics study of binding ATP and ADP analogous in the active and regulator sites of E.coli phosphorybosyl pyrophosphate synthetase

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E.coli phosphoribosylpyrophosphate synthetase (PRPPS, EC 2.7.6.1) belongs to the class I of the phosphoribosyl pyrophosphate synthetases. The enzymes of this family catalyze the synthesis of 5-phosphoribosyl pyrophosphate (5-PRPP) by transferring the β,γ-diphosphoryl group of ATP to the C1-hydroxyl group of ribose-5-phosphate (R5P). R5P is involved in a number of important biochemical processes associated with purine and pyrimidine metabolism and required at all times in the cell. Due to this the function of PRPP synthetases is central to life and the study of their structure and mechanism of action is of special interest. The three-dimensional structure of *E.coli* PRPPS in complex with Mg^{2+} ions was determined at 2.71 Å resolution (PDB ID: 4S2U) [1]. The activity of class 1 PRPPS is regulated not only by excess of the substrate and product of the reaction but by the binding of ADP in the allosteric regulatory site.

In this study the binding of substrate analogue mATP in the active site and analogue of allosteric inhibitor mADP in the allosteric site of the *E.coli* PRPPS have been studied based on the structure of the corresponding complexes of homologous protein from *B. subtilis* (PDB ID: 1DKU) using molecular dynamics method. The starting coordinates of ligands in *E. coli* enzyme were determined by superposition of both protein structures on Cα-atoms. For the simulation we used GROMACS software and AMBER99SB force field. 100ps potential energy minimization was performed to relax the structure and avoid steric clashes in further simulations. Pressure and temperature of the system were set to 1atm and 300K by running NPT and NVT simulations (100ps each). Parinello-Rahman barostat was used for pressure control. Berendsen thermostat was used to control the temperature. A 10 ns productive MD trajectory was obtained. This work was carried out using high-performance computing resources of federal center for collective usage at NRC "Kurchatov Institute", http://computing.kiae.ru/.

As a result the more precise positions of both ligands in the active and allosteric sites of *E.coli* PRPPS were found and the nearest surrounding of the ligands was described.

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Doxorubicin-induced apoptosis and autophagy in central nervous system: The protection of xanthone derivative of *Garcinia mangostana*

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Doxorubicin (DOX) is an anthracycline antibiotic that effectively treats a variety of cancers. Despite its effectiveness, it can generate free radical by NADPH-cytochrom P-450. Increased free radical may elevate the level of pro-inflammatory cytokine, tumor necrosis factor alpha (TNF), which can pass through the blood brain barrier. The present study extends our previous finding to demonstrate that DOX-induced central nervous system toxicity is preventable by a xanthone, derivative of *Garcinia mangostana*. Total phenolic content, total antioxidant capacity and free radical scavenging activity of xanthone were evaluated *in vitro* study. Intraperitoneal injection of xanthone prior to DOX administration in mice have been shown suppression the level of circulating TNF and oxidative injury markers, including protein carbonyl, nitrotyrosine and 4-hydroxy-2'-nonenal (4HNE), in brain tissue. The levels of p53, Bax and DRAM (damage regulated autophagy modulator) were increased in Dox-treated mice indicated that p53 may play a modulation role in both apoptosis and autophagy. The anti-apoptotic protein Bcl-xL was significantly increased in DOX-treated mice compared with the control groups. Interestingly, DOX treatment also increased the autophagy marker, Beclin 1 and LC3-II in brain tissues. Pretreatment with xanthone suppressed DOX-induced increases in all indicators of tissue responses tested and showed a potent free radical scavenging activity. Our results are the first to show that xanthone attenuated cell death both suppresses apoptosis and autophagy, most likely via its antioxidant property.

Keywords: Doxorubicin; xanthone; apoptosis; autophagy; neurotoxicity

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Full saturation of lipid bilayer with membrane-active peptide

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It was recently noted that antimicrobial peptides (AMPs) and cell-penetrating peptides (CPPs) have similar physicochemical properties and may exert same biological activity. The antimicrobial action was demonstrated for several CPPs, and the ability to translocate into the living cells with moderate toxicity was found for some AMPs [1].

The common physical features of such membrane-active peptides are their positive charge and generally amphipathic structure which determine strong binding to the negatively charged membranes [2]. The mode of action is largely determined by membrane-mediated or direct self-interactions of peptides [3]. These interactions may result in self-assembly of porous structures proposed for several AMPs, translocation through bilayer, detergent-like behavior or even formation of amyloid-like aggregates. Typically these effects arise with the increase of peptide concentration on a membrane.

Here we present a study of highly cationic amphipathic cell-penetrating peptide EB1 [4] in the crowded state on a model anionic lipid membrane. We employed CD spectrometry to analyze peptide partitioning and found that EB1 is helical upon binding to liposomes (POPC/POPG lipids), while unstructured in solution.

We found that there is a broad range of relatively high peptide to lipid ratios at which number of bonded peptides per lipid remains constant. This observation suggests a strong self-interaction of peptides on the membrane surface. Moreover, we demonstrate that such a saturation cannot be explained by the standard method of electrostatic repulsion factorization using Gouy-Chapman theory [5]. We suggest that the theory oversimplifies highly cationic peptides with point charge representation and propose to use coarse-grained molecular model.

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Novel protocols for biopolymer and bio particle purification by Free-Flow-Electrophoresis

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Free-flow electrophoresis (FFE), also known as "carrier-free electrophoresis", is an electrophoretic separation technique, which separates **preparative and semi-preparative** amounts of samples, ranging in size from metal ions to cells, according to minimal differences in charge and isoelectric point (pI). Because it is a **matrix-free** technique, that operates in standard buffer systems and allows for a cooled environment, it maximizes the preservation of protein activity and structural integrity of **protein complexes** and **bio particles**, leading also to **high recovery rates**. It is compatible with a wide range of electrophoretic modes, like isoelectric focusing (IEF) or interval zone electrophoresis (IZE), that allow for **high resolution** separation (< 0.04 ΔpI) with a **high throughput** (IZE: up to 3.5 mg protein/h, continuous methods up to 80 mg protein/h) in short amounts of time (less than 10 minutes separation time for IZE). In the past this technique has proven to be useful for the separation of a lot of different samples including protein complexes,[1] organelles,[2] liposomes/vesicles,[3] protein isoforms[4] and nanodiscs.[5]

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Structural studies of *Staphylococcus aureus* hibernation promoting factor homolog SaHPF by high-resolution NMR spectroscopy

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is any strain of *Staphylococcus aureus* that has developed resistance to beta-lactam antibiotics. One of the main target of antibiotic in bacteria is the ribosome. Recent development of cryo-EM allow to determine structure of the ribosome and its complexes with protein factors at high resolution and interpret mechanism of interaction and structure rearrangement at molecular level. Bacteria slow down protein synthesis during stress conditions by converting ribosomes into translationally inactive 100S dimers, enter a stationary phase. In this phase, bacterial cells are resistant to external stresses, which allows them to resist antimicrobial agents. Expression of stationary-phase proteins, such as ribosome hibernation promoting factor (SaHPF), results in formation of 100S dimer, which leads to "ribosome hibernation" that aids cell survival. Structure of SaHPF protein with molecular weight 22 000 Da is unknown. Sequence analysis has shown that this protein is combination of two homolog proteins obtained in E.coli - ribosome hibernation promoting factor HPF (11 000 Da) and ribosome modulation factor RMF (6,500 Da). Recently, crystal structures of E. coli 70S-EcHPF complex [1] and Thermus thermophilus 70S-EcHPF or 70S-EcRMF hybrid complexes [2] revealed that the binding of these two proteins to the intersubunit cavity overlaps with the tRNA binding sites or mRNA exit tunnel respectively. Cryo-electron microscopy studies have also demonstrated that neither EcHPF nor EcRMF are involved in the contacts between two ribosomes in the dimer. However, S. aureus specific SaHPF is almost twice bigger than its *E. coli* counterpart. It shares similarity with EcHPF only on its N-terminal region, while C-terminal domain suggested to be homologues to EcRMF protein, although there is no direct evidence for this. We produced a recombinant expression, synthesis and purification protocols of ^{13}C, ^{15}N-labeled SaHPF protein for structural studies by high-resolution NMR spectroscopy and made screening for appropriate buffer conditions for 3D NMR experiments of SaHPF protein. We believe that the structure of SaHPF will provide us new insights about the formation of ribosome dimers in staphylococci, which may bring new horizons in treatment diseases of this severe pathogen.

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Genetic engineering, expression and purification of human GPR17 (GPCR) constructs. Evaluation of protein stability depending on binding of ligands

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GPR17 is a G-protein coupled receptor (GPCR) expressed in cells of brain, heart and kidney. It is a rhodopsin-like receptor which is phylogenetically close to the leukotriene receptors (CysLT1 and CysLT2) and purinergic receptors (P2Y1 and P2Y12).

GPR17 interacts with both leukotrienes and purines and plays controversial role in brain and spinal cord cells recovery after injuries. It is assumed that GPR17 is one of the cell death regulators immediately after hurt but later, in contrast, it takes part in tissue regeneration.

Drugs targeted at GPR17 may help multiple sclerosis and ischemia treatment. The damage of rat's brain in artificially created ischemia disease decreased after GPR17 inhibition. In addition, GPR17 takes part in myelin sheath formation, the lack of which is known to be the reason of multiple sclerosis.

GPR17 was expressed in Sf9 cells using Bac-to-Bac expression system. We created a number of genetically engineered constructs in order to improve receptor stability and monomer percentage *in vitro*.

Surface expression of the receptor was defined as number of cells expressing GPR17 on their surface divided by total number of cells expressing given receptor. Surface expression was considered as the measure of GPR17 stability *in vivo*. Construct screening revealed two features which increase both surface expression *in vivo* and monomer percentage *in vitro*. Influence of ligands on purified GPR17 was also analyzed.

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Lynx1 affects the peak-amplitude of α7ACh-evoked current in L1 interneurons in the brain

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Lynx1 is one of endogenous peptides in the brain, belonging to Ly6/uPAR family [1]. Lynx1 has been shown to bind the different types of nicotinic acetylcholine receptors (nACh-receptors) [2]. But it is remained unclear how Lynx1 affects nACh-receptors function in the brain. Here we study the effect of water-soluble recombinant analogue of human Lynx1 (ws-Lynx1) on interneurons of the layer 1 responding to 1mM acetylcholine in the rat visual cortex using whole-cell patch-clamp configuration and fast drug-application system. It was revealed that ws-Lynx1 at 1μM concentration didn't affect the peak-amplitude ACh-evoked current, while application of 10μM ws-Lynx1 resulted in the ~ 30% enhancement of the peak-current amplitude. Using specific inhibitors 4μM MLA and 8mM DhbE, α7ACh-receptors was identified to be involved in the responses observed. Thus ws-Lynx1 at 10μM, but not at 1μM affects the α7ACh-receptors in L1 interneurons in the rat visual cortex increasing the peak-current amplitude of responses.

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Gaussian curvature for typical lipidic cubic phases: Energetic view on membrane proteins crystallization processes

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The materials based on lipidic cubic phases (LCP) are supposed to be the most intriguing and very perspective substances for many scientific problems, and in particular, for membrane protein crystallization. However, there is no valuable theoretical description of how membrane protein crystals start to grow in LCP.

In this work, we calculated Gaussian curvature in every point on infinite periodical minimal surfaces (IPMS) for typical LCP types of symmetries: Ia3d, Pn3m and Im3m. We draw a color map of Gaussian curvatures and visualized channels of light path of diffusion of membrane proteins on the surface of each LCP, using equation $f(x,y,z) = 0$ taken from [1] for IPMS for each LCP and Gaussian curvature $K(x,y,z)$ taken from [2].

As a result, we can observe pathways of membrane proteins diffusion and places of their probable clustering on the surface of LCP.

It is shown that for different symmetries the processes of membrane protein crystallization are going by different ways.

Acknowledgments

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Genes of retinal membrane proteins in psychrophilic microflora of the White Sea sediments

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Research on the genetic diversity of psychrophilic bacteria has developed rapidly with the recent applications of molecular biology. New species and genera are constantly being reported. Our research is dedicated to the identification of genes of retinal membrane proteins in conditions of the Arctic Circle. Retinal membrane proteins, is a family of proteins that use retinal as a chromophore for light reception.

To find genes of retinal membrane proteins we explored 10 samples collected from the bottom of the White Sea (from 3.5-35 meters depth) and at the seaside at various locations near the island Olenevsky. DNA was isolated from all sample using Wizard Genomic DNA Purification Kit. The polymerase chain reaction (PCR) with isolated DNA has been carried out with 4 different primers. Primers were designed based on the sequences of following proteins: Blue-light absorbing proteorhodopsin, Green-light absorbing proteorhodopsin, Exiguobacterium sibiricum rhodopsin (ESR), Candidatus Actinomarina minuta rhodopsin, Dokdonia eikasta Sodium pumping rhodopsin. Putative genes of retinal membrane proteins were detected by gel electrophoresis with PCR products. The PCR product with ESR-primers showed the presence of the searched genes in the sediments of the White Sea. The next step of our research is sequencing of eluted PCR products.

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Search of homologous genes of bacteriorhodopsin in psychrophilic microflora of the White Sea sediments

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Research on the genetic diversity of psychrophilic bacteria has developed rapidly with the recent applications of molecular biology. New species and genera are constantly being reported. Our research is dedicated to the identification of retinylidene protein coding genes in conditions of cold temperatures. Retinylidene protein, is a family of proteins that use retinal as a chromophore for light reception.

From the bottom of the White Sea and at the seaside at various locations near the island Olenevsky from depths various from 3.5 to 35 meters 10 samples were collected. DNA was isolated from all sample by using Wizard Genomic DNA Purification Kit, results were verified by electrophoresis. The polymerase chain reaction (PCR) with isolated DNA samples has been carried out with 4 different primers. Primers were designed based on the sequences of following proteins: Blue-light absorbing proteorhodopsin, Green-light absorbing proteorhodopsin, Exiguobacterium sibiricum rhodopsin, Candidatus Actinomarina minuta rhodopsin, Dokdonia eikasta Sodium pumping rhodopsin. The PCR reaction was presumably held for primer for Exiguobacterium sibiricum rhodopsin, which indicates the existence of the searched genes in the sediments of the White Sea. The next step of our research is sequencing of eluted PCR products.

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Modulation of ligand - receptor binding functional activity as a key point of peptides based drugs biological action molecular mechanism

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Brain cells plasmatic membranes contains a wide range of the different neuroreceptors. Modulation of ligand - receptor binding functional activity is often underlies the mechanism of pharmacological effects of many drugs. Semax, Selank and proglyprol are novel peptide drugs with a broad range of activities in central nervous system. Semax is particularly beneficial to individuals with ischemic stroke attacks and other forms of brain traumatic damage and also demonstrates good results for the treatment of cognitive disorders, optic nerve diseases, and peptic ulcers. Selank possesses strong anxiolytic properties, but it also has an immunomodulatory activity. Tripeptide Pro-Gly-Pro (proglyprol) is an inherent structural component of both previous drugs but its own ability to protect various cells from noxious factors. The specific binding of tritium labeled Semax and of peptides PGP and HFPGP (which are stable Semax metabolites) to various rat brain areas was investigated, and it was found that all of these compounds had different binding sites on neuronal cells plasmatic membranes. In this study we analyzed influence of peptides mentioned above on the specific binding of tritium labelled analogs of some endogenous neuromediators to Acetylcholine, GABA, Glutamate, Vanilloid, Dopamine, TRH (thyrotropin-releasing hormone) and other receptors of rat brain cells plasma membranes. We also tested the joint action of specific ligands of some crucial neuroreceptor systems in the system of peptide + non-peptide allosteric modulator, such as Gly, Pregnanolone (5α-Pregnan-3α-ol-20-one) or Ethanol (EtOH). We studied the influence of the peptides within a wide concentration range (from picoM to microM) and found that regulatory peptides were able to affect the specific binding of some different ligands to its own receptors. The influence (or the lack of effects) is strongly depends of combination of some factors, such as: peptide structure, peptide concentration, type and subtype of receptor. The obtained results suggest that neuropeptides (Semax, Selank and proglyprol) have an important role in the occurrence of direct and indirect changes in the basic parameters of the specific ligand-receptor interactions of endogenous neuroregulation systems in the brain. The peptides we studied are apparently allosteric modulators of wide range of receptors. The spectrum of possible interactions of Selank, Semax and proglyprol with the plasma membranes of nerve cells is dose-depend and can be much wider, including also the modulation of receptors of other types.

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Human Hsp70 structure and dimerization mechanism revealed by small-angle X-ray scattering

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Heat shock proteins (Hsp70) are a family of essential molecular chaperones, highly expressed in both prokaryotic and eukaryotic cells [1]. Hsp70 is a major regulator of stress response and plays a central role in protein homeostasis by regulating folding of damaged and newly synthesized proteins.

Hsp70 molecule consists of two domains, nucleotide-binding domain (NBD) and substrate-binding domain (SBD), those functional interplay determines Hsp70 cycle. The binding of ATP to NBD changes SBD affinity to substrate, and SBD regulates ATPase activity of NBD. The structures of NBD in complex with various ligands and inhibitors [2] have been reported, and recently, a nearly complete structure of SBD has been obtained [3]. However, allosteric inter-domain interactions crucial for Hsp70 mechanism are still poorly understood.

Here we present small-angle X-ray scattering (SAXS) of an intact Hsp70 protein, encoded by human HSPA1A gene. We used highly pure recombinant Hsp70 to prepare complexes with substrate peptides and nucleotides involved in the Hsp70 functional cycle. Calorimetry and spectroscopy were employed to assess whether these complexes represent thermodynamically and structurally distinct states. Then SAXS data obtained for the most functionally relevant states at DESY P12 and ESRF BM29 synchrotron beamlines were used to build the low-resolution models of Hsp70 complexes.

The rigid-body modeling of SAXS data for monomeric Hsp70 allowed us to define relative positions of NBD and SBD in solution, highlighting the possible determinants of inter-domain allosteric interaction. Analysis of the SAXS data for partially aggregated Hsp70 in the absence of substrate peptide showed that the protein exists in the form of dimers where the linker between domains plays a role of the substrate for another Hsp70 molecule. Multimeric Hsp70 complexes may represent a biologically relevant state for the storage of chaperones, and our results provide a structural basis for a previously proposed dimerization mechanism.

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Analysis of spherical shell model for SAXS-coupled to SEC of apoferritin and ferritin

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Small-angle scattering (SAS) is one of the soft matter investigation methods that can be used either for material science or biological objects. In this work, we investigated ferritin and apoferritin proteins, which play key role in iron metabolism and are used as "testing sample" of q-scale calibration for SAS instruments. These proteins are known for the specific spherical structures where ferritin have iron core while apoferritin contains cavity. In SAS experiments, apoferritin and ferritin curves are often fitted with spherical shell model, while these proteins have non-spherical surface[1,2]. In this study, Patterson function (pair-distribution function) for the spherical shell model was theoretically calculated and compared with the experimental and structural data. The investigations by small angle X-ray (SAXS) method were performed on-line with size exclusion chromatography (SEC) at the BioSAXS beamline BM29, ESRF, Grenoble, France[3]. Structural parameters of the system such as inner and outer radii (R_{in}, R_{out}) and scattering density of the core (ρ_{core}) were obtained by comparison of theoretical and experimental data. The curves obtained by Crysol program (ATSAS package) from available PDBs are given. The difference between experimental and theoretical curves and structural parameters was estimated and questions of the applicability and limits of the model are discussed.

The work was supported by the Russian Academic Excellence Project 5-100 for enhancing the competitiveness of MIPT among the world's leading research and education centers of the Ministry of education and science.

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Structural models of dimeric states of transmembrane domain in proteins of insulin receptor family

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Insulin receptor family refers to receptor tyrosine kinases and consists of three highly homologous proteins: insulin receptor (IR), type-1 insulin-like growth factor receptor (IGF-1R) and insulin receptor-related receptor (IRR). IR and IGF-1R take part in regulation of cellular metabolism, growth, division, differentiation and survival [1], while IRR regulates excess alkali excretion in the body [2]. Violations of normal signaling of these receptors contribute to pathological conditions and developmental disabilities.

These proteins represent $(\alpha\beta)_2$ heterotetramers with extracellular α-subunits and transmembrane (TM) β-subunits, that include intracellular kinase domains. Modern theories of activation of these receptors suggest autophosphorylation of the tyrosine kinase domains. Two major models of the activation process were presented and both include dimerization of TM domains [3, 4].

Activation dynamics of these receptors still is not clear because of necessary consideration of membrane environment, which is critical for their correct organization and functioning. In this study, we elaborated molecular models of TM domain dimers of IR, IGF-1R, IRR. Structural and dynamics properties of these systems were analyzed using molecular hydrophobicity potential approach and molecular dynamics simulations. The resulting models can be used to study the effects of membrane environment and point mutations on the dimers' stability and therefore on activity of the receptor.

This work was supported by the Russian Science Foundation (grant 14-14-00871).

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The price of auditory sharpness: model-based estimate of metabolic demands of the octopus cells

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Octopus cells (OCs) of the mammalian auditory brainstem cochlear nucleus (CN) precisely encode the timing of fast transient sounds and tone onsets [1, 2]. Among membrane mechanisms allowing for OCs high temporal fidelity, the low membrane resistance in the resting state should result in high energy demands. We thus provide model-based assessments of OC energy consumption in point [3] and distributed [4] models using the method suggested by Moujahid and co-outhors [5]. The results predict that resting OCs consume up to orders of magnitude more ATP than other CN (bushy and stellate) neurons and cerebellar granule cells, and times more than cerebellar Purkinje and cortical pyramidal neurons, while relative activity-related increase in energy consumption is close to that of efficient neurons [6]. We next analyze whether the morphology and biophysics of OCs are optimized with respect to metabolic rate, which leads to a question if there are specific mechanisms of energy delivery or local production to match the high energy demand of the OC.

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Highly sensitive coherent anti-stokes raman scattering imaging of protein crystals

Arzumanyan G.M.[1,2], Doroshkevich N.V.[1], Mamatkulov K.Z.[1], Shashkov S.N.[3], Zinovev E.V.[4], Vlasov A.V.[4], Round E.S.[4,5,6], Gordeliy V.I.[4,5,6]

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Serial crystallography at last generation X-ray synchrotron sources and free electron lasers enabled data collection with micrometer and even sub-micrometer size crystals which have resulted in amazing progress in structural biology.[1, 2] However, imaging of small crystals which although is highly demanded remains a challenge, especially in case of membrane protein crystals.

We describe here a new approach, based on polarized coherent anti-Stokes Raman scattering (P-CARS) imaging of *in meso* grown MP (and also water soluble protein) crystals. CARS microscopy provides an advanced nondestructive and label-free technique with high sensitivity and high lateral spatial resolution capable of selective chemical imaging of major types of macromolecules: proteins, lipids, nucleic acids, etc. Like spontaneous Raman, CARS probes vibrational modes in molecules and does not require exogenous dyes or markers, which is advantageous in imaging small molecules for which labeling may strongly affect their properties.

It is shown, that CARS, especially P-CARS, can be generally applied for fast, high resolution, high contrast and very informative imaging of protein crystals.

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Autotransporter based system for cellular display of the recombinant proteins at the surface of *Escherichia coli*

Zlobinov A.V.[1,3], Petrovskaya L.E.[1], Shingarova L.N.[1], Novototskaya-Vlasova K.A.[2], Dolgikh D.A.[1,3]

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The development of the cell surface display systems is one of the current trends in biotechnology. This strategy enables construction of whole cell biocatalysts, live vaccines and is an effective approach to the screening of combinatorial protein libraries. We have constructed a new system for the exposure of different proteins on the surface of bacterial cells in the form of hybrids with the transmembrane domain of a new representative of the autotransporter family from *Psychrobacter cryohalolentis* K5T (AT877).The gene encoding AT877 was amplified from the genome of *P. cryohalolentis* and expressed in *Escherichia coli*. The outer membrane localization of the expression product was demonstrated. We have combined the DNA sequences encoding α-helical linker and the translocator domain of AT877 with EstPc esterase from the same bacterium. Thus, we have obtained a new whole cell catalyst possessing high activity at low temperatures. In subsequent experiments we demonstrated the capability of the constructed system to provide cell surface display of various recombinant proteins, including 10th human fibronectin domain (10Fn3), and fluorescent protein mCherry. Successfull exposure of these proteins on the surface of *E. coli* cells was demonstrated with ELISA and confocal microscopy.

Furthermore, the selection of combinatorial library of TNF-binding 10Fn3 variants was performed with the use of the obtained system. Tumor necrosis factor (TNF) plays a crucial role in the development of rheumatoid arthritis, psoriasis, Crohn's disease, and other human disorders. Therefore, construction of TNF-binding proteins aimed to reduce its increased concentration in the body is an urgent task. After three rounds of selection, several variants were selected which demonstrated efficient interaction with TNF. Thus, the use of the developed system for the display of recombinant proteins on the cell surface of *E. coli* is a promising strategy for the selection of artificial binding proteins based on 10Fn3 and for the bacterial display of other recombinant proteins.

The work is supported by RFBR grant 16-04-00717 and the grant SS-8384.2016.4.

Optogenetic approaches to induce and monitor cell death

Zlobovskaya O.A.[1], Sarkisyan K.S.[1], Gorbachev D.A.[1], Mishin A.S.[1], Lukyanov S.A.[1], Lukyanov K.A.[1]

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Genetically encoded photosensitizers are a promising tool to induce oxidative stress in a tight, controlled in space and time manner. Depending on their intracellular localization, photosensitizers can lead to necrosis (i.e., when located on the cell membrane) or apoptosis (i.e., located in mitochondria) [1]. We compared the ability of different photosensitizer proteins to cause cell death at different illumination conditions and demonstrated that KillerRed and KillerOrange can be used as chromatically orthogonal probes.

Also, we developed a novel FRET-based red-infrared caspase-3 sensor. We took advantage of its red-shifted spertra to perform simultaneous imaging of EGFP-Bax translocation during staurosporin-induced apoptosis. Staurosporin is generally believed to cause apoptosis through intrinsic (mitochondrial) pathway, though its mechanism of action is poorly understood [2]. An important step during intrinsic apoptosis is the Bax translocation into the mitochondrial membrane, where it forms a special pore through which cytochrome c flows into the cytoplasm [3]. The release of cytochrome c from the mitochondria cleaves and activates caspase-3 and caspase-9. To our surprise, most cells in our experiments demonstrated a reverse order of events, namely caspase-3 activation followed by Bax translocation. Our data suggest that staurosporine can mediate activation of caspase-3 in a Bax-independent manner.

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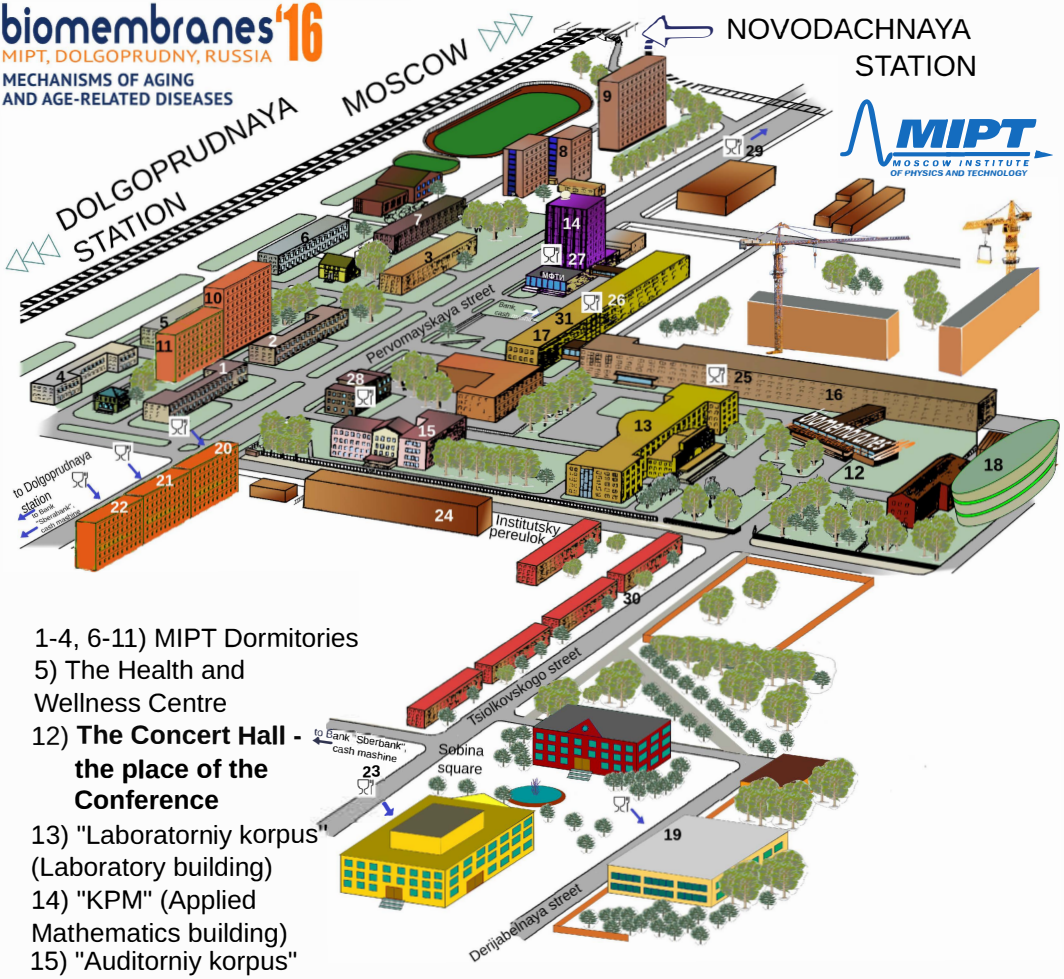

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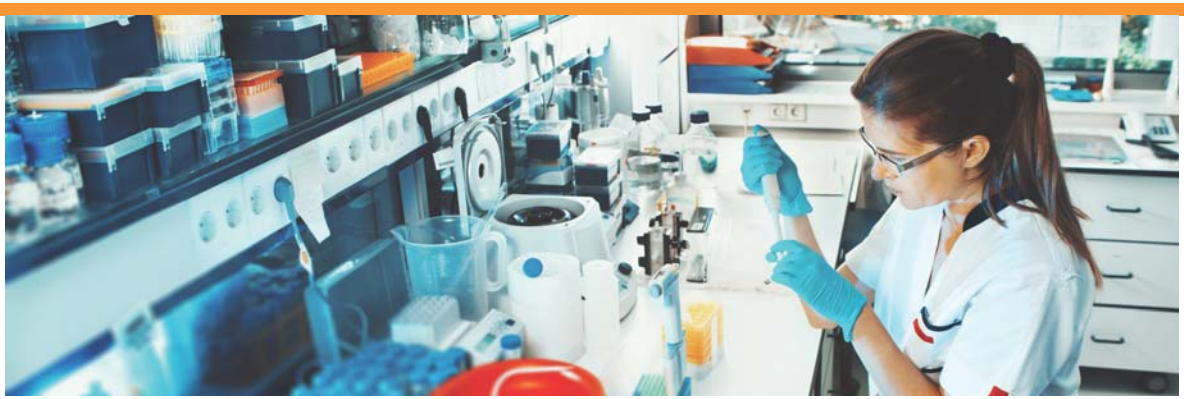

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