RBC 2014

REGIONAL BIOPHYSICS CONFERENCE

BOOK OF ABSTRACTS

15-20 MAY 2014
SMOLENICE CASTLE, SLOVAKIA

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REGIONAL BIOPHYSICS CONFERENCE 2014

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The Regional Biophysics Conference is organised by Slovak Biophysical Society. This is traditional biannual scientific meeting etablished by biophysical societies of Austria, Croatia, Hungary, Italy, Serbia, Slovakia and Slovenia. However, the conference is open for all biophysicists. The first RBC meeting took place in March 2005 in Terme Zrece (Slovenia), the second one was held in August 2007 in Balatonfüred (Hungary), the third one in February 2009 in Linz (Austria), forth took place in September 2010 in Primošten (Croatia) and the fifth one in September 2012 in Kladovo (Serbia). The principal aims of the conference are: 1) to strengthen the collaboration between biophysics groups in the region, 2) to present the cutting-edge achievements from a broad range of biophysics disciplines to students and 3) to promote the field of biophysics. Following previous conferences, RBC 2014, features a combination of top level senior researchers and aspiring young scientists (postdoctoral fellows and graduate students). The conference is focused on the following topics:

1. Molecular biophysics
2. Membrane and cellular biophysics
3. Ionic transport
4. Modelling, bioimaging and instrumental techniques in biophysics
5. Bioinspired nanotechnologies and biosensors
6. Neurobiophysics
7. Medical biophysics

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May 16, 2014

Section S1: Molecular biophysics

Chairs: Mauro Dalla Serra, Gerhard J. Schütz

09.00-09.45 **Plenary lecture: László Zimányi** (Hungary): Homology modeling of cytochrome *b*561 proteins: structural basis for substrate binding and the transmembrane electron transfer
09.45-10.05 **Péter Závodszky** (Hungary): The role of noncatalytic domains in the regulation of complement proteases
10.05-10.25 **Maria Grazia Ortore** (Italy): Advances in the characterization of protein fibrillogenesis by small angle X-ray scattering
10.25-10.45 **Katalin Tóth** (Germany): Nucleosome structure and dynamics studied by bulk and single molecule FRET

10.45-11.15 Coffee break

11.15-11.35 **Ranieri Bizzarri** (Italy): Engineering the excited state of fluorophores: towards new probes for high-resolution imaging and sensing in living cells
11.35-11.55 **Andras Lukacs** (Hungary): Functional dynamics of BLUF domain proteins revealed by ultrafast spectroscopy transient absorption and time resolved multiple probe spectroscopy
11.55-12.15 **Géza I. Groma** (Hungary): Effect of Hofmeister salts on the conformation of FAD coenzyme studied by femtosecond time-resolved fluorescence
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12.35-12.55 **Győző Garab** (Hungary): Elements of structural-functional plasticity of thylakoid membranes in vivo. Nature, physical mechansisms and physiological significance
12.55-13.15 **Antonio Šiber** (Croatia): Condensing DNA in viruses: the shapes and the energies

13.15-14.30 Lunch

Section S2: Membrane and cellular biophysics

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15.15-15.35 **Renata Ünnep** (Hungary): The ultrastructure and flexibility of multilamellar photosynthetic membranes under physiological conditions - revealed by small-angle neutron scattering

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16.45-17.05 **Nenad Pavin** (Croatia): Dynein, microtubule and cargo: a ménage à trois

17.05-17.25 **Jure Derganc** (Slovenia): Membrane shaping by proteins and lipids: between energy and entropy

17.25-17.45 **Petra Pullmannová** (Czech Republic): The comparison of short and long acyl ceramides in the model skin lipid membranes

17.45-18.05 **Katarína Štroffeková** (Slovakia): Comparison of oxidative stress effects on metabolic profile and apoptotic intrinsic mitochondrial pathway in endothelial (HAEC) and tumor (U87/MG) cells

18.05-18.25 **Želko Vučinić** (Serbia): Redox systems, ROS and metabolism of the plant cell apoplast

18.30-20.00 Dinner

20.00-21.30 Poster sections P1 and P2

May 17, 2014

Section S3: Ionic transport

Chairs: Michael Poteser, György Panyi

09.00-09.45 **Plenary lecture: Gerald J. Obermair** (Austria): Emerging evidence for specific neuronal functions of auxiliary calcium channel subunits

09.45-10.05 **Michael Poteser** (Austria): Store operated ion channels and calcium-transcription coupling in mast cells

10.05-10.25 **Klaus Groschner** (Austria): From a homology model of TRPC3 structure towards novel therapeutic concepts

10.25-10.45 **Lubica Lacinova** (Slovakia): Identification of new proteins modulating N-type $Ca_V2.2$ channels

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12.15-12.35 **Alexandra Zahradníková** (Slovakia): Ryanodine receptor gating: Insights from structural models
12.35-12.55 **Marta Gaburjakova** (Slovakia): The functional communication among cardiac ryanodine receptors is not mediated by Ca^{2+} current
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Section S4: Modelling, bioimaging and instrumental techniques in biophysics

Chairs: Vladimir Baumruk, Janez Štrancar

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17.15-17.35 **Vladimír Baumruk** (Czech Republic) Chiroptical properties of a cyclic antimicrobial peptide Lasiocepsin and its analogs
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May 18, 2014

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09.45-10.05 **Amir Fahmi** (Germany): Nanofabrication via self-assembled hybrid nanomaterials into low dimensional structures

10.05-10.25 **Wlodzimierz Kutner** (Poland): Recent advances in electrochemical molecular imprinting for biomimetic sensing

10.25-10.45 **Miroslav Fojta (**Czech Republic): Labelled and functionalized DNA for novel bioassays

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11.30-12.15 **Plenary lecture: Ilia Ivanov** (USA): Bio-inspired sensors for bionic applications: FILMskin

12.15-12.35 **Maria Minunni** (Italy): Trends in affinity sensing

12.35-12.55 **Giuseppe Spoto** (Italy): Detection of microRNAs by droplet microfluidics

12.55-13.15 **Yannis F. Missirlis** (Greece): Modular bioreactors in tissue engineering: the role of mechanical signals

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14.00-18.00 Excursion

19.00-21:30 Conference dinner

May 19, 2014

Section S6: Neurobiophysics

Chairs: Pavle Andjus, Martin Kopáni

09.00-09.45 **Plenary lecture: Mirjana Pavlovic** (USA): The use of stem cells in the treatment of neurological diseases

09.45-10.05 **Pavle Andjus** (Serbia): Revealing excitotoxicity mechanisms in amyotrophic lateral sclerosis by biophysical means

10.05-10.25 **Marija Stanojević** (Serbia): Magnesium increases membrane input resistance of leech Retzius neurons during Ni^{2+} - induced epileptiform activity

10.25-10.45 **Sanja Josef Golubic** (Croatia): Topology and neurodynamics of the auditory M50 gating network

10.45-12.15 Coffee break and Posters P6

12.15-12.35 **Ján Jakuš** (Slovakia): Neuronal control of breathing and airway reflexes: new ideas and our experience

12.35-12.55 **Martin Kopani** (Slovakia): Iron biomineralization in *globus pallidus* of human brain

13.00-14.30 Luch

Section S7: Medical biophysics

Chairs: Ján Jakuš, Alexandra Zahradníková

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15.45-16.05 **Zuzana Garaiová** (Slovakia): Carbosilane dendrimers as potential delivery vehicles for HIV-derived peptides to dendritic cells

16.05-16.25 **Marcela Morvová jr.** (Slovakia): PMCA activity and membrane fluidity in patients with chronic kidney disease

16.25-16.45 **Karol Ondrias** (Slovakia): H_2S-NO interaction and consequent biological effects

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17.15-17.35 **Katarína Kozlíková** (Slovakia): The time dependency of the extrema in the QRS isopotential maps of young adult controls

17.35-17.55 **Vito Šimonka** (Slovenia): Role of nitric oxide in pathogenesis of atherosclerosis: modelling approach

17.55-18.05 **Janez Urevc** (Slovenia): Numerical prediction of hemodynamic response of an atherosclerotic blood vessel

18.05-18.25 **Tadej Emeršič** (Slovenia): Dynamic model of eicosanoid production with special reference to aspirin-exacerbated respiratory disease (AERD)

18.25-18.45 **Andreas Ebner** (Austria): Single molecule force spectroscopy and recognition imaging investigation of blood clotting relevant receptors on living platelets

18.45-19.00 Closing ceremony

19.00-20.30 Dinner

May 20, 2014

07.30-09.00 Breakfast

09:00 Departure

PLENARY LECTURES

Coherent quantum transport in biomolecules

Vlatko Vedral*

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A growing body of evidence suggests that biological processes could be utilising quantum coherence, superpositions, and even, in some cases, quantum entanglement to perform some tasks with higher efficiency [1]. I will first briefly summarise the existing evidence, including two of the most famous examples of biological processes: photosynthesis and magneto-reception [2]. I will then present the key features of modeling the flow of energy in complex systems [3]. The main challenge is to obtain experimentally a handful of parameters believed to be important for describing the interplay between coherence (within the system) and noise (arising due to the interaction with the system's environment). I will present single molecule spectroscopy experiments we are currently undertaking in our laboratory to obtain a better understanding of quantum effects in biomolecules. Finally, I will discuss how these experiments can be scaled-up, as well as how we can design artificial and hybrid biomimetic structures that mimic the underlying fundamental behavior [4].

Acknowledgements. This work is supported by the Oxford Martin School, National Research Foundation (Singapore), the Ministry of Education (Singapore), the EPSRC (UK), the Templeton Foundation and the Leverhulme Trust (UK).

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Homology modeling of cytochrome *b*561 proteins: structural basis for substrate binding and the transmembrane electron transfer

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Cytochrome *b*561 (Cyt-*b*561) proteins are 6 transmembrane helix proteins with the four inner helices holding two *b*-type heme cofactors via two pairs of conserved histidine residues. The first crystal structure of a Cyt-*b*561 has recently been published [1]. This structure confirms general expectations about the positions of the hemes and about the ascorbate and monodehydroascorbate substrate binding sites on the opposite membrane surfaces, as derived from experimental evidence and also from an earlier modeling study [2]. We have performed homology modeling [3] based on this crystal structure of an *Arabidopsis thaliana* Cyt-*b*561 to obtain the structure of four other representative family members *in silico*: *A. thaliana* tonoplast, bovine chromaffin granule, murine duodenal and tumor suppressor Cyt-*b*561s. The extent of homology with the crystallized protein decreases in this order. A very good agreement of the backbone folding has been obtained for all four targets and the template. Transmembrane electron transfer pathways and efficiency have been calculated [4] from one bound substrate through the hemes to the other substrate. Little homology is displayed in the protein matrix along the putative electron transfer path connecting the two hemes, whereas most of the conserved amino acids are found at the heme pockets, close to the two surfaces and the substrate binding sites.

Acknowledgements
This work was supported by the Hungarian Scientific Research Fund (OTKA K108697).

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Single molecule biology - studying movements and meetings within the plasma membrane

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Current scientific research throughout the natural sciences aims at the exploration of the *Nanocosm*, the collectivity of structures with dimensions between 1 and 100nm. In the life sciences, the diversity of this Nanocosm attracts more and more researchers to the emerging field of Nanobiotechnology. In my lecture, I will show examples how to obtain insights into the organization of the cellular Nanocosm by single molecule experiments. Our primary goal is an understanding of the role of such structures for immune recognition. For this, we apply single molecule tracking to resolve the plasma membrane structure at sub-diffraction-limited length-scales by employing the high precision for localizing biomolecules of ~15nm. Brightness and single molecule colocalization analysis allows us to study stable or transient molecular associations *in vivo* [1]. In particular, I will present results on the interaction between antigen-loaded MHC and the T cell receptor directly in the interface region of a T cell with a mimicry of an antigen-presenting cell [2, 3].
Moreover, we developed a method for *in vivo* micropatterning of plasma membrane proteins to measure molecular interactions [4]. This technology brings together our interest in immune signaling, and the capability for ultra-sensitive readout of large biochip surfaces. Cells transfected with a fluorescent fusion protein ("prey") are grown on micropatterned surfaces functionalized with specific antibodies to the extracellular domain of a membrane protein ("bait"); the fluorescence copatterning is used as readout for the bait-prey interaction. We applied this technology for the study of the interaction between CD4 – the major coreceptor for T cell activation – and Lck, an important tyrosine kinase in early T cell signaling. In addition to the well-known zinc-clasp structure, we found strong contributions of Lck membrane anchorage to the binding of the two proteins.

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Emerging evidence for specific neuronal functions of auxiliary calcium channel subunits

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In the central nervous system the second messenger calcium regulates neurotransmitter release, gene regulation, and neuronal plasticity. Voltage-gated calcium channels provide the major regulated calcium entry pathway in the membrane of neurons. They operate in a heteromultimeric complex between a pore forming α_1, and the auxiliary β and $\alpha_2\delta$ subunits. The cytoplasmic β and the extracellular membrane-attached $\alpha_2\delta$ subunit are required for the proper functional expression of the entire calcium channel complex. Moreover, the auxiliary subunits modulate the gating properties of the calcium channel and serve as scaffolds for upstream regulators and downstream effectors. Any of these properties affect the size of the calcium signal and in the synapse lead to changes in the functional coupling to neurotransmitter release. Beyond their classical role as auxiliary calcium channel subunits, β and $\alpha_2\delta$ have recently been implicated in cellular and neuronal functions independent of the channel complex. Certain β subunits, for example, are targeted into the nucleus, where they directly interact with the epigenetic machinery. $\alpha_2\delta$ subunits, on the other hand, may be involved in synaptic functions independent of the pore forming α_1 subunit. However, the specific roles of the distinct isoforms and splice variants of auxiliary calcium channel subunits in neurons are still elusive. Here I will present novel experimental evidence for redundant and specific functions of β and $\alpha_2\delta$ subunits in presynaptic calcium channel targeting, synapse formation, and the activity-dependent regulation of gene expression.

Acknowledgements
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Protein interaction and transport maps of live cell nuclei using a single plane illumination microscope with fluorescence correlation spectroscopy

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Proteins acting on DNA need to penetrate a dense network of chromatin and associated macromolecules in the cell nucleus to access their target sites [1]. Intracellular mobility of proteins is characterized by diffusion coefficients of the order of 1-100 $\mu m^2/s$, leading to millisecond time scales for movement on the submicrometer scale.

Here we show results from single plane illumination microscopy based fluorescence correlation spectroscopy (SPIM-FCS), a new method that combines the fast time resolution of FCS with the possibility of acquiring the mobility data in parallel on an entire two-dimensional cross-section [2]. This will then provide diffusion coefficients, flow velocities and concentrations in an imaging mode. Extending this technique to two-color fluorescence cross- correlation spectroscopy (SPIM-FCCS) also allows measuring molecular interactions in an imaging mode [3].

As a model system for protein-protein interactions and DNA binding we studied c-Fos and c-Jun, two components of the AP-1 transcription factor, which form a heterodimer and whose interaction and diffusion behavior we had studied earlier by point-focus FCS [4]. We obtained mobility maps of c-Fos-EGFP and c-Jun-mRFP fusion proteins in HeLa cells and simultaneously recorded interaction maps that show heterodimer formation. We find that the regions of the cell nucleus where the proteins show strong dimerization strongly correlate with the regions of immobilization, i.e. DNA binding. This shows that dimerization is a prerequisite for target binding and that most of the dimerized transcription factor is also bound to DNA.

In a second example, we investigated the dynamical changes in binding and mobility of the retinoid acid receptor (RAR-RXR) upon activation by ligand binding [5]. Activation shifts the population of the fluorescently labeled receptor into a slower-moving state, both increasing the fraction of the receptor population in this state and decreasing its diffusion coefficient.

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BioInspired & DNA nanotechnology

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COST TD1003: BioInspired Nanotechnologies: from concept to applications.
http://www.bioinspired-nano.eu/en/

DNA based nanostructures built on a long single strand DNA scaffold, known as DNA Origamis [1], appears as one of the most important step driving the emergence of the field of BioInspired Nanotechnologies. DNA Origamis act as a nano platform creating a wealth of paths to design new self assembled and self organised structures and to conceive sophisticated Chemical Reaction Networks with modular approaches. Based on our experimental results and modelling of DNA folding Origamis we showed that cooperativity is a key feature that ensures the robustness of the Origami structure. Then, we end the presentation with an analogy between biological systems that built robust regulation networks and functions and the construction of DNA structures [2-7].

Acknowledgements
We acknowledge the support and funding of the VIBBnano project.

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Bio-inspired sensors for bionic applications: FILMskin

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Small building blocks of nano materials have potential for bionic applications. In this presentation we will review a family of allotropic forms of carbon, including graphene, single wall carbon nanotubes, carbon quantum dots and others in their applications to mimic functionality of human skin. A concept of bionic Flexibke Integrated lightweight sknin (FILMskin) will be described and recent development of its components using carbon nanotube for design of artificial neural bundle, temperature and pressure sensors presented.[1]
The artificial neural bundle was designed using glass drawing technique and carbon nanotubes-silicate powder (CNT-p). The CNT-p was placed in the glass tube and a set of tubes was assembles and fused together into a bundle. The bundle was vacuum sealed to protect nanotubes from oxidation and drawn at temeprature close to the glass softenning temperature. The resulting fiber contained 160 electrocal insulated channels with carbon nanotubes. The glass drawing was repeated three time improving the alignemnt and conductivity of carbon nanotubes inside the channels. The final artificial neuron bundle had 0.47 mm diameter with 18,900 electrically insulating conducting channels.[2]
The pressure and temperature sensors were designed using vertically aligned carbon nanotubes and singlewall carbon nanotube membranes. We fond that the response of the temperature sensor is within the neuron firing frequency, suggesting potential application for bionic sensors.[3]
We will also present results on the tunability of mechancial and thermal properties of the FILMskin using carbon nanomaterials.

Acknowledgements
This research was conducted at the Center for Nanophase Materials Sciences, which is sponsored at Oak Ridge National Laboratory by the Scientific User Facilities Division, Office of Basic Energy Sciences, U.S. Department of Energy with partial support from the DARPA's on Revolutionary Prosthetics and Laboratory Directed Research Development program.

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The use of stem cells in the treatment of neurological diseases

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The aim of the work: The aim of this work is to give a review on the stem-cell treatment of neurological diseases and its efficacy in the research and clinical arena. The goal of cell therapy is to replace and/or repair dead or diseased neural cells.

Essential results:
The results of stem cellular therapy in different neurological tissues are measured with respect to pattern optimization, successful engraftment, functional impairment, and continual symptomatic improvement. In humans, one would expect that the transplanted cells will: 1) GRAFT permanently into the region where they were transplanted, 2) REBUILD circuitry with the patient motor neurons, and 3) PROTECT patient neurons from further ravages of the disease.
With respect to pattern optimization, accumulated research data indicate that so far there are three phenotypically and functionally distinguished adult stem cell types that could be used in the cellular treatment of neurological diseases: hematopoietic stem cells (HSCs), very small embryonic-like stem cells (VSELs) and Mesenchymal dental pulp stem cells (MDPSCs). Despite these promising individual patterns, heterogeneous population of adult stem cells is still used in some cases. Not all of the neurological diseases are treatable, but degree of efficacy depends on whether it is: inflammatory, degenerative, cancerous disease or the injury. The cancer stem cell concept and targeted cancer stem cell therapy has dragged into cellular treatment different technological novelties such as nanoparticles, magnetotherapy, etc.

Conclusion: There are still controversies about stem cell treatment within the spectrum of neurological diseases. However, in some of them such as Parkinson's disease, certain cases of stroke, macular degeneration and some types of neural tumors a significant improvement has been achieved. Therefore, although not the answer to all the questions, stem cell treatment can be used safely at least as an adjuvant therapy in some neurological diseases.

Ultrasound-mediated delivery of nanoparticles to tumour cells

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Nanotechnology has started a new era in engineering multifunctional nanoparticles (NPs) for improved cancer diagnosis and therapy, incorporating both contrast agents for imaging and therapeutics. Encapsulating the drugs into NPs improves the pharmacokinetics and reduces the systemic exposure due to the leaky capillaries in tumours. A prerequisite for successful cancer therapy is that the therapeutic agents reach their targets and limit the exposure to normal tissue. The delivery depends on the vasculature, the transport across the capillary wall, through the extracellular matrix, and across the cell membrane. Although the NPs may pass the tumour capillaries rather easily, the uptake and distribution of NPs and the released drugs are low and heterogeneously distributed in the tumour tissue.

In order to improve the distribution of NPs the delivery should be combined with a treatment facilitating the delivery. Ultrasound has been reported to be able to improve drug delivery, and is especially efficient in the presence of microbubbles. We have developed a novel multimodal, multifunctional drug delivery system consisting of microbubbles stabilized by polymeric NPs. The NPs contains drugs, fluorescent dyes and iron oxide for optical and magnetic resonance imaging. In the talk the obstacles for successful delivery of NPs will be discussed and the promising results using the novel multifunctional drug delivery system in combination with focused ultrasound presented. The delivery system is used both to improve delivery of drugs to prostate cancer cells in athymic mice and to penetrate the blood-brain barrier in rats thereby allowing the treatment of various diseases in the central nervous system.

SECTION LECTURES

S1-1
The role of noncatalytic domains in the regulation of complement proteases

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The complement system plays an essential role in both the innate and adaptive immune responses. The complement system is a proteolytic cascade that can be activated through three different routes: the classical, the lectin, and the alternative pathway. Modular serine proteases cooperate to provide the activation signal, to do the regulation and complete the biological function. These proteases have highly similar modular structure, but have different specific functions; they consist of several distinct domains of various protein families of different size and fold. The general domain sequence is: CUB1-EGF-CUB2-CCP1-CCP2-SP, where SP is the catalytic domain. The noncatalytic modules provide both high specificity and elasticity required for the catalytic properties. In such a context, the enzyme function can be interpreted only having detailed knowledge of the structure and function of the individual regulatory modules. To reveal the role of noncatalytic modules in detail, domain combinations were constructed and expressed in baculovirus insect cell system. Our major statement is that the CCP2 module significantly increases proteolytic activity of the catalytic domain of C1r on its natural substrate, C1s. Therefore, we propose that CCP2 module provides accessory binding sites. Differential scanning calorimetric measurements demonstrated that CCP2 domain greatly stabilizes the structure of SP domain, and is responsible for dimerization. Our studies on the CUB-CCP1 domain pair of C1r provided the first direct evidence that CUB2 domain binds calcium, and binding of calcium induces the folding of the CUB2 domain, regulating the flexibility of the entire serine protease in a calcium dependent way. In contrast to the CUB2 domain the CUB1 proved to be totally insensitive to calcium. Our combined functional and structural studies by NMR, CD, DSC and ITC helped us to reveal the functional role of noncatalytic modules in complement proteases, indicating that they are responsible for the regulation of the enzyme function regarding the localization, timing, and recognition of interacting partners.

S1-2
Advances in the characterization of protein fibrillogenesis by small angle X-ray scattering

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Amyloid protein fibrillogenesis is a research issue that attracts the interest of a wide scientific community. First and foremost amyloid fibrils are a common component in many debilitating human neurological diseases such as Alzheimer's (AD, the most common form of senile dementia). With the size of the elderly population rising dramatically, the development of new methodologies for diagnosis and treatment of these diseases represents a fundamental challenge. Furthermore amyloid fibrils are stable and resistant to degradation, and a certain number of nonpathogenic, functional forms of amyloid have been identified [1].
Despite the huge interest in these amyloid structure, the proteins' early aggregation steps as well as the molecular mechanisms that determine the fibrillogenesis are still not completely resolved [2]. We present a few examples proving how the combination of time-resolved Small Angle X-ray Scattering (SAXS) and other biophysical techniques (Dynamic Light Scattering, Thioflavin T fluorescence, Fourier Transform Infrared Spectroscopy) can shed light on fibrillization.
Firstly a model protein, apomyoglobin mutant W7FW14F, has been investigated as fibril former evidencing that oligomerization in solution happens in less than 100 ms after the pH jump from 4.0 to 7.0., while the resulting pattern of protein prefibrillation reveals the simultaneous presence of worm-like species and of cylindrically-shaped aggregates [3]. Hence, the study of amyloid β-peptide fibrillization pattern in solution and at a protein concentration quite resembling that in vivo was performed [4]. The combined use of experimental techniques enables the characterization of the prefibrillar species that form at the beginning of the aggregation process, that are the crucial species in the onset of the disease and in neuronal cell degeneration. To develop basic knowledge about the molecular mechanisms at the basis of this aggregation, aims to lay the groundwork for the development of new therapeutic approaches for AD treatment and prevention.

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S1-3
Nucleosome structure and dynamics studied by bulk and single molecule FRET

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The nuclosome is the basic packaging unit of genomic DNA in eukaryotes. It consists of a core of two of each histone proteins (H2A, H2B, H3, H4), around which about 150 base pairs of DNA are wound in two turns. Since this protein-DNA complex must be disassembled to allow access to DNA, the opening and closing of the nucleosome is an essential step in eukaryotic transcription. In particular, covalent modifications of histones by acetylation, methylation and other variations are essential to determine gene activity during development, differentiation and transformation into cancer cells.
Here we studied the effects of histone acetylation and point mutations on nucleosome structure and dynamics by bulk and single molecule FRET measurements on mononucleosomes with donor/acceptor dye pairs either on the DNA or on DNA and histone proteins. The nucleosomes were reconstituted on different 170bp long DNA sequences. We characterized the dependence on histone origin, DNA sequence and histone modification and demonstrated intermediate states during salt induced nucleosome disassembly [1,2]. Regardless of the state of acetylation, origin of histones or DNA sequences nucleosomes disassemble via an intermediate state which is suppressed at higher nucleosome concentration, confirming our proposed model of step-wise disassembly [3]. Our results also suggest that H3 and H4 acetylation have partially opposing effects in regulating nucleosome architecture and that distinct aspects of nucleosome dynamics might be independently controlled by selective histone acetylation [4].
Posttranslational modifications or single amino acid mutations in chromatin gain more and more attention. We are investigating the role of such modifications using the same methods, looking for the effects of these small structural variations on the stability of the nuclesosome. We report about the effect of the acetylation of lysine 16 in the tail of histone H4, and on the mutation of lysine 27 in the H3 tail.

Acknowledgements
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S1-4

Engineering the excited state of fluorophores: towards new probes for high-resolution imaging and sensing in living cells

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High-resolution optical imaging techniques make now accessible the detection of submicron features in living cells. Here, we shall demonstrate how the excited state can be engineered in proteins and synthetic chromophores to obtain novel photochromic and viscosity sensitive probes.
Photochromic fluorescent proteins. The introduction of the E222Q mutation in the primary sequence confers good photochromic properties to otherwise photostable *Aequorea Victoria* fluorescent proteins. We demonstrated that at physiological pH the photoswitching of the anionic *cis* protein chromophore yields a neutral non-fluorescent *trans* state, providing the optical basis for *on*↔*off* cycling of fluorescence . The significance of these mutants for high-resolution cell imaging will be shown by means of photochromic FRET experiments and OLID imaging.
Polarity/viscosity probes. We recently developed two new green-emitting probes , characterized by *push-pull* hyperconjugated structures, which display significant emission and lifetime increase by increasing local microviscosity. These probes allow for efficient intracellular viscosity measurements by means of the phasor approach to fluorescence lifetime imaging, a fit-free method that overcome the main drawbacks of conventional lifetime imaging . One probe can be used to monitor membrane organization, whereas the other reports on the local viscosity in plasma membrane, lysosomes, and mitochondria .

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S1-5

Functional dynamics of BLUF domain proteins revealed by ultrafast spectroscopy transient absorption and time resolved multiple probe spectroscopy

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Nature has many elegant ways for sensing the light, using photoactive proteins like rodhopsins, xanthophins, photototropins or flavoproteins having very distinct pathways to regulate the photoresponse.
AppA is the most known BLUF (Blue light sensing using FAD) protein that serves as both sensor of light and oxygen. Its role is a transcriptional antirepressor of photosynthesis genes. In low light and low oxygen conditions AppA binds to the transcription factor PpsR enabling transcription of photosystem biosynthesis genes. When AppA is irradiated by intense light or exposed to high oxygen conditions it will release PpsR which then binds to DNA shutting of production of photosystem biosynthesis genes.
The mechanism of photoactivity in the BLUF domain has not yet been definitively established. In this work we present ultrafast transient absorption measurements in the visible and mid-infrared region as well as time resolved multiple probe spectroscopy performed on WT AppA, AppA mutant Y21W and PixD, another BLUF domain protein. With these methods we are able to identify the individual steps of the photocycle.

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S1-6

Effect of Hofmeister salts on the conformation of FAD coenzyme studied by femtosecond time-resolved fluorescence

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The Hofmeister series represents the relative effectiveness of different salts on the aggregation and conformation of proteins and other colloids. The aggregated states are facilitated by the kosmotropic anions (SO_4^{2-}, F^-), while the open structures by the chaotropic ones (ClO_4^-, SCN^-) [1]. Here we show that the conformation states of a small coenzyme, flavin adenine dinucleotide (FAD) also follow this rule unexpectedly well. It is known that in water the two groups of FAD are arranged in a stacked conformation, and in the presence of alcohol an open structure is also populated. The stacked and the open forms are characterized by short- and long-lived fluorescence, respectively [2]. We measured the time-resolved fluorescence of FAD in aqueous solution in the presence of characteristic kosmotropic and chaotropic anions in the 100 fs – 10 ns region. The kinetics was analyzed by a sophisticated method optimized for multicomponent decay [3], making possible to sensitively monitor the population of the different conformational states of FAD. As we observed earlier [3], even in pure water a considerable amount of molecules is in open conformation, and the fluorescence kinetics distinguishes three different closed states. In the presence of anions, the populations of these states are markedly changed, in the directions according to the Hofmeister's rule.

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S1-7

SALS, a WH2 domain protein in sarcomeric actin organization

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Striated muscle functioning is orchestrated by the basic myofibrillar contractile units, the sarcomeres. One of the essential components of the sarcomeric protein networks are the actin-tropomyosin based thin filaments. The structural features of the thin filaments are well resolved, however, the mechanisms governing the assembly and organization of sarcomeric actin filaments are not completely understood. Recently, SALS (*sarcomere length short*) was identified in *Drosophila melanogaster* as an important regulator of sarcomeric actin organization. The sequence analysis of the protein revealed that SALS possesses two WH2 (*Wiskott-Aldrich syndrome homology region 2*) domains and an upstream proline-rich region. Disruption of *sals* by RNAi leads to lethality at the embryonic stages. This phenotype results from muscle defects caused by the shortening and disorganization of thin filaments, suggesting that SALS' functions are related to the proper organization of sarcomeric actin filaments [1].

To decipher the mechanism by which SALS contributes to the establishment of sarcomeric actin structures first we investigated the interactions of the WH2 domains of SALS (SALS-WH2 and the proline-rich region containing fragment; SALS-Pro-WH2) with actin using fluorescence spectroscopy and microscopy based approaches. We found that the WH2 domains inhibit actin filament assembly as well as enhance their disassembly *in vitro*. Consistently, the overexpression of the WH2 domains in *D. melanogaster* results in flightless phenotype and shorter sarcomere length. The proline-rich sequence element does not affect these activities. We also show that SALS-WH2 interacts with tropomyosin, which interferes with the disassembly activity of SALS-WH2.

In conclusion, our results demonstrate that the WH2 domain-actin interaction is not sufficient for the biological function of the full length protein. To understand the molecular mechanisms underlying the biological function of SALS we aim to analyze the functional properties of other regions of SALS and its interactions with other sarcomeric proteins.

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S1-8

Elements of structural-functional plasticity of thylakoid membranes in vivo. Nature, physical mechansisms and physiological significance

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In the thylakoid membranes the protein complexes are packed in ordered arrays and the membranes are assembled into highly organized multilamellar systems, an organization warranting a substantial degree of stability. At the same time, the photosynthetic machinery possesses the ability for reorganizations in response to rapidly changing environmental conditions. In my talk I will pay special attention to the nature, physical mechanisms and physiological significances of the flexibility of thylakoid membranes at three different levels of the structural complexity: (i) lipid membrane: focusing on the role of non-bilayer lipids and lipid phases in the (bilayer) thylakoid membranes and testing our hypothesis on their involvement in the structural dynamics of thylakoid membranes [1,2]; (ii) the light-harvesting antenna complexes: dealing with the role of previously unknown photophysical (photon energy dissipation-assisted) feedback mechanism, thermo-optically driven reorganizations [2,3]; (iii) the multilamellar membrane system: focusing on small but well discernible rapid reorganizations in the periodic membrane ultrastructure in living cyanobacterial and algal cells and in whole leaves - revealed by the non-invasive technique, small angle neutron scattering [4-6]. These reorganizations are (or likely to be) correlated with different short-term regulatory mechanisms, such as light adaptation, photoprotection, heat stress and state transtions, and also affect the operation of some enzymatic functions of the photosynthetic apparatuses.

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S1-9
Condensing DNA in viruses: the shapes and the energies

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When condensed in free space, long DNA typically assumes a shape of a toroid. The geometry of the toroid, its inner and outer radii and the shape of its cross-section can be understood from a phenomenological model, describing condensation as an interplay between the (unfavorable) surface energy of the toroid and the (unfavorable) bending energy of the DNA strands in it – the only favorable contribution to the free energy is the volume term, requiring that the DNA strands be next to each other [1]. When condensed in confinement, e.g. in virus capsids, it is known that the, sufficiently short, DNA also assumes toroidal conformations, but the free energy balance is in that case additionally complicated by the adsorption energy (DNA-capsid interaction) and by the capsid confinement [2]. It has been proposed in the literature that the, sufficiently long DNA, may condense in conformations which are non-toroidal, i.e. which do not have the cylindrical axis of symmetry [3]. Nevertheless, such propositions were never tested in a suitable model, explaining the free energies of all the conformations that can be envisioned. I will show a generalization of the previously proposed models [1] to account for non-toroidal conformations of DNA condensed in spherical confinement. Such conformations may occur in viruses when they are completely filled. The models that I will present reproduce conformations that were previously predicted [3], but also several intriguing conformations that were never predicted in the context of viruses.

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S2-1

The ultrastructure and flexibility of multilamellar photosynthetic membranes under physiological conditions - revealed by small-angle neutron scattering

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To monitor ultrastructural changes in the thylakoid membrane system in vivo during photosynthesis under variable environmental conditions the non-invasive technique small angle neutron scattering (SANS) has been shown to be of special value. It has provided accurate, statistically and spatially averaged information on the repeat distances (RDs) of multilamellar thylakoid membranes in live algal cells and isolated plant thylakoid membranes under physiologically relevant conditions with time resolution of seconds and minutes [1-3]. In the present work, we studied the periodicity of granal thylakoid membranes in higher plant leaves and isolated chloroplasts and protoplasts – using SANS and thin section electron microscopy. Our SANS investigations have revealed, for the first time, thylakoid membranes in leaves respond dynamically with small but well discernible RD changes to moderately strong illumination; further we show that the RD of isolated thylakoid membranes depends strongly on the nature of osmoticum – the most widely used medium appears to perturb significantly the organization of membranes [4].

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S2-2
Long-range lipid-water interaction as observed by ATR-FTIR spectroscopy

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It is commonly assumed that the water structure at a lipid-water interface is influenced mostly in the first hydration layer. However, recent results from different experimental methods show that perturbation extends through several hydration layers. Due to its low light penetration depth, attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy is specifically suited to study interlamellar water structure in multibilayers. Results obtained by this technique confirm the long-range water structure disturbance. Consequently, in confined membrane environments nearly all water molecules can be perturbed. It is important to note that the behavior of confined water molecules differs significantly in samples prepared in excess water and in partially hydrated samples. We show in what manner the interlamellar water perturbation is influenced by the hydration level and how it is sequentially modified with a step-by-step dehydration of samples either by water evaporation or by osmotic pressure. Our results also indicate that besides different levels of hydration the lipid-water interaction is modulated by different lipid headgroups and different lipid phases. Therefore, modification of interlamellar water properties may clarify the role of water-mediated effects in biological processes.

S2-3

Human perforin forms arcs that are associated with the granzyme translocation into target cells

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Cytotoxic cells eliminate malignant or virally infected targets through interplay of pore forming protein Perforin (PFN) and serine proteases (Granzymes). How PFN assists transport of granzymes across the bilayer is unclear. Granzyme delivery can occur without evidence of PFN mediated cylindrical pore formation. Susceptibility to granzyme B (GzmB) induced death correlates with rapid PFN-induced phosphatidylserine externalization in non-permeabilized targets. These observations suggest that PFN may form limited membrane oligomers that encourage plasma membrane coalescence and flip-flop of anionic phospholipids from the inner leaflet, namely toroidal pores. Addition of increasing concentrations of a monoclonal antiperforin antibody (pf-80) to PFN treated targets rescues them from necrosis caused by cylindrical pores while increasing phosphatidylserine associated flip-flop and GzmB induced apoptosis. By atomic force microscopy (AFM), PFN can produce arc-like structures having measureable conductances in planar lipid bilayers, that are augmented by pf-80. These arcs represent toroidal pores that offer sites for granzyme translocation through the bilayer.

S2-4
Dynein, microtubule and cargo: a ménage à trois

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To exert forces, motor proteins bind with one end to cytoskeletal filaments, such as microtubules and actin, and with the other end to the cell cortex, a vesicle or another motor. A general question is how motors search for sites in the cell where both motor ends can bind to their respective binding partners. In this study, we focus on cytoplasmic dynein, which is required for a myriad of cellular functions in interphase, mitosis and meiosis, ranging from transport of organelles and functioning of the mitotic spindle to chromosome movements in meiotic prophase. We discuss how dynein targets sites where it can exert a pulling force on the microtubule to transport cargo inside the cell.

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S2-5
Membrane shaping by proteins and lipids: between energy and entropy

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Lipid membranes are remarkably soft structures, with a bending modulus which is not much larger than the thermal energy at physiological temperatures. Shaping of cellular membranes therefore typically involves a tug-of-war between enthaplic and entropic effects. I will review some experimental and theoretical studies of membrane shaping by proteins and lipids. First I will describe the curvature-driven lateral sorting of membrane components and its implications for the stability of flattened membrane compartments such as Golgi cisternae [1,2]. Next, I will present a recently discovered cellular mechanism where membrane bending is significantly influenced by entropic repulsions among crowded membrane proteins with large extra-membrane domains [3]. Finally, I will describe a novel microfluidic method for controlled and reversible changes of the solution around flaccid giant lipid vesicles, which allows for a detailed analysis of membrane shape changes induced by membrane-interacting agents [4].

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S2-6

The comparison of short and long acyl ceramides in the model skin lipid membranes

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Stratum corneum (SC) is the outermost epidermal layer responsible for the skin barrier function. It consists of corneocytes embedded in the extracellular lipid matrix, which forms an organized lamellar structure and contains ceramides (Cer), cholesterol (Chol), free fatty acids (FFA) and cholesteryl sulfate (CholS). An increased level of Cer with a shorter chain length (C16 compared to C24) in SC of patients with atopic eczema (AE) was recently published [1]. The presence of short acyl Cer was proposed to act as one of the factors, which are responsible for the impaired skin barrier in AE. Therefore we investigated permeability and microstructure of membranes containing NS-type Cer either with long acyl chain (CerNS24) or with shorter acyl chain (CerNS16). Further variation was achieved using either lignoceric acid (LIG) or mixture of various SC fatty acids (mFFA; C16-C24 acyl chains).

The permeability was assessed in Franz-type diffusion cells using following permeability markers: water loss [$g/h/m^2$], flux of theophylline (TH) and indomethacin (IND) [$\mu g/cm^2/h$] and electrical impedance [$k\Omega \times cm^2$]. The microstructure was revealed by X-ray powder diffraction (XRPD).

Both, the water loss and the flux of IND were increased in membranes containing CerNS16 relative to membranes containing CerNS24, reporting their less efficient barrier. This effect was apparent in the membranes with either LIG or mFFA. Higher permeability to TH was detected in membranes CerNS16/Chol/LIG/CholS in comparison to CerNS24/Chol/LIG/CholS. The electrical impedance did not reveal a clear barrier-impairing effect of CerNS16. The CerNS24/Chol/mFFA/CholS membranes formed regularly arranged lamellar phase with the repeat distance d = 5.3 nm. It was much longer than the lamellar phase of corresponding CerNS16/Chol/mFFA/CholS membranes with the d = 4.1 nm. These results confirmed that shorter acyl Cer directly contributes to the increased permeability in model membranes and this finding can be relevant also for increased skin permeability in AE patients.

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S2-7

Comparison of oxidative stress effects on metabolic profile and apoptotic intrinsic mitochondrial pathway in endothelial (HAEC) and tumor (U87/MG) cells

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An oxidative stress with the generation of radical oxygen species (ROS) is a common mechanism causing cellular dysfunction and apoptosis. Deregulation of cell apoptosis can contribute to diverse pathologies, and understanding its regulatory mechanisms has great importance for the development of novel therapy strategies for cancer and cardiovascular diseases. The mechanisms by which ROS can cause apoptosis typically include receptor activation, caspase activation, Bcl-2 family proteins, and mitochondrial dysfunction. The generation of ROS can be triggered by various stimuli including photodynamic therapy (PDT). PDT leads to the formation ROS causing selective damage to the target tumor tissue, however the ROS vascular damage plays also significant role [1]. The molecular mechanisms underlying PDT, and specifically Hypericin PDT (HypPDT), are not completely understood. Cell responses to HypPDT are highly dependent on the Hyp intracellular accumulation [2, 3]. We were particularly interested in cell response to oxidative stress triggered by HypPDT in endothelial (HAEC) and tumor (U87-MG) cells. We investigated role of Bcl-2 family proteins [4] in HypPDT triggered apoptosis of HAEC and U87-MG cells. In the present work, we show that the presence of Hyp itself has an effect on the -distribution of Bcl2 family members, without affecting the mitochondria structure and function. The presence of Hyp triggers the translocation of Bax into mitochondria, and the translocation of Bax and Bcl2 into nuclei. In addition, presence of Hyp resulted in increased superoxide production measured by MitoSoxRed. HAEC respiration with Hyp present was similar to control cells, where in U87-MG cells with Hyp respiration was significantly decreased. The HypPDT resulted in oxidative stress via mitochondrial superoxide production causing mitochondria fission. HypPDT resulted primarily in apoptosis in U87-MG cells, whereas in HAEC cells there was a necrosis. HypPDT significantly deteriorated mitochondria in intact HAEC cells and resulted in diminished respiration. In contrast, HypPDT in U87-MG cells improved respiration.

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S2-8

Redox systems, ROS and metabolism of the plant cell apoplast

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The apoplast is a complex plant compartment, delimited from the symplast by the plasma membrane. It consists of the rigid cell wall fibrillar polymer network, the external surface of plasma membrane, and the liquid- and gas-filled spaces within this network, which provide interactions between the environment and the plasma membrane enclosed cytoplasm. All of these three components of the apoplast are rich in various organic molecules, enzymes and proteins attached, dissolved, or embedded in or to them. Our EPR studies have demonstrated that all of these components are capable of producing different reactive oxygen species. In this review we shall present the results of our analysis of the plasma membrane and cell wall bound redox and antioxidative enzymes (malate dehydrogenase, peroxidases and SOD) participating in the oxidative metabolism in the apoplast and their mutual interaction and coupling to organic acids and phenolics present in this cellular compartment.

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S3-1
Store operated ion channels and calcium-transcription coupling in mast cells

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Mast cells respond quickly to immunological stimulation by Ca^{2+}-dependent degranulation and release of inflammatory mediators. In addition to and independent of degranulation, Ca^{2+}-signals can also trigger the activation of transcription factors in mast-cells, resulting in the production cellular mediators of inflammation. However, this cell type does not respond to immunological activators in a uniform way, but shows distinct pathways connecting specific initial activating stimuli with cellular downstream responses. Explanations for this selective activation of cellular transduction pathways were found in the concept of spatially limited sub-plasmalemmal Ca^{2+}-signals that originate at ion channels and are sensed by specific intracellular protein complexes. Nevertheless, little is known about how Orai-, TRPC- and intracellular Ca^{2+}-channels, the main sources of Ca^{2} in mast cells, are functionally and spatially linked to elements of downstream pathways like FKBP12, calcineurin and the Ca^{2+}-dependent transcription factor NFAT.
We investigated the role of TRPC3 and Orai1 channels in NFAT signaling of RBL-2H3 mast cells using electrophysiology in combination with TIRF/FRET fluorescence microscopy and heterologous expression of mutant channel proteins as well as genetic knockdown by siRNA. Ca^{2+} entry via Orai1 but not TRPC3 was found to be required for store depletion-induced NFAT activation. Nonetheless, overexpression of the FKBP12 binding deficient mutant TRPC3P704Q substantially inhibited Orai1-dependent NFAT translocation in RBL-2H3 cells. Association of TRPC3 with FKBP12 was demonstrated by TIRF/FRET microscopy and the FRET signal was strongly reduced by replacing TRPC3 by TRPC3P704Q. Interestingly, association of Orai1 to FKBP12-containing signalosomes was observed in TIRF/FRET experiments and overexpression of TRPC3 facilitated the association of Orai1 with FKBP12 as well as calcineurin. Moreover, these interactions were reduced by expression of TRPC3P704Q.
We suggest that TRPC channels enable linkage of Orai-mediated Ca^{2+}-entry to activation of calcineurin/NFAT by targeting FKBP12 and calcineurin into Orai1-containing plasma membrane microdomains.

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S3-2

From a homology model of TRPC3 structure towards novel therapeutic concepts

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Transient receptor potential canonical 3 (TRPC3) represents one of the closest relatives of the Drosophila TRP protein and a potential key player in human (patho)physiology. Constituting a nonselective cation channel, TRPC3 has been recognized as an important Ca^{2+} signaling molecule, while little is known about the structural basis of this function.

Utilizing recently available structural information on the voltage-gated sodium channel from *Arcobacter butzleri* (Na_VAB[1]) and the transient receptor potential vanilloid 1 (TRPV1[2]), we set out to identify structural elements that control ion permeation and gating by homology modeling. Using a structure-guided mutagenesis approach, we identified a glutamate residue (E630) within the putative pore loop as a determinant of divalent permeation[3]. Mutations in a hydrophobic cluster at the C-terminal end of transmembrane domain 6, corresponding to the S6 helix bundle crossing in the template channels, disturbed channel gating. Replacing a large hydrophobic residue (I667) by alanine or glutamate resulted in substantial constitutive channel activity, suggesting I667 as part of the dynamic structure occluding the conduction pathway[4]. Mutational destabilization of the gate was associated with reduced Ca^{2+} permeability, altered cysteine cross-linking in the selectivity filter and enhanced sensitivtiy to block by ruthenium red. Screening for further pivotal determinants of gating revealed G652 as a hinge-point essential for specific modes of channel activation. Based on our molecular model we will discuss pharmacological strategies to selectively activate certain cellular functions of TRPC3.

In summary, we elaborated a novel molecular model of the TRPC3 permeation pathway and localize the channel's selectivity filter as well as the physical gate. We provide evidence for allosteric coupling between the gate and the selectivity filter and for divergent functional modes of TRPC3 activity. Our findings provide insight into the molecular organization of TRPC channels and reveal novel pharmacotherapeutic concepts for TRPC channel modulators.

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S3-3
Identification of new proteins modulating N-type $Ca_V2.2$ channels

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Activity of neuronal calcium channels is tightly regulated by a multitude of adjacent proteins. We used the yeast split-ubiquitin system to search for and characterize so far unknown interaction partners of Ca_V2 Ca^{2+} channels [1]. We identified Solute carrier family 38, member 1 (Slc38A1), Reticulon 1 (RTN1) and Glutamate receptor, ionotropic, N-methyl D-aspartate-associted protein 1 (Grina) proteins as interaction partners of the α_1 subunit of N-type $Ca_V2.2$. Direct interaction of these proteins with the $Ca_V2.2$ α_1-subunit was verified by performing yeast confirmation assays.
For electrophysiological analysis we used a CHO cell line stably transfected with rat $Ca_V2.2$ □$_1$-, $\alpha_2\delta$- and β-subunits. These cells were either mock-transfected with pEGFP-N1 vector or transfected with the same vector with full length Slc38, RTN1 or Grina cDNAs. All three putative interaction partners modulated inward Ba^{2+} current measured by a whole-cell patch clamp. The current amplitude was significantly suppressed by Grina, slightly enhanced by Slc38 and was unaffected by RTN1.
Voltage- and activity dependent inactivation of the $Ca_V2.2$ channels was modulated by interacting proteins in complex way. Grina slightly accelerated initial voltage-dependent inactivation and attenuated cumulative inactivation caused by a high-frequency train of depolarising pulses. Slc38 attenuated initial inactivation and enhanced total cumulative inactivation caused by a pulse train. RTN1 did not affect initial inactivation and accelerated cumulative inactivation during a pulse train.
All three investigated proteins affected activity of the $Ca_V2.2$ channels in a unique way. These results suggest that i) observed modulation was not caused simply by the presence of a new protein in $Ca_V2.2$-expressing CHO cells; ii) analysed proteins may modulate presynaptic transmitter release, which is being controlled by $Ca_V2.2$ channels.

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S3-4

Ryanodine receptor gating: Insights from structural models

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Ryanodine receptors (RyR2s) of cardiac myocytes are tetrameric calcium release channels of the sarcoplasmic reticulum (SR) membrane that open during the systole to provide calcium for contraction. Their activity during the diastole is very low. Over a hundred RyR2 mutations cause an increase of diastolic calcium release, resulting in cardiac arrhythmias. It has been postulated that RyR2 mutations affect the strength of intramolecular interactions ("domain switch") in the RyR2 molecule. To understand the structural basis of the effect of mutations on RyR gating, we have constructed homology models of three mutation-rich domains (domains NTD, RIH-2 and the central domain) implicated in the "domain switch". We have also constructed peptides from mutation-prone regions of NTD (DP_{cpvtN2}, aa 410-438) and the central domain (DP_{cpvtC}, aa 2380-2411) and studied their effect on reconstituted rat RyR2 channels in planar lipid bilayers. Both peptides dose-dependently increased RyR2 open probability by induced long openings. Docking of homology models of the NTD and the central domain into the cryo-EM map of the closed (EMD-1606) and open (EMD-1607) skeletal RyR showed that the sequences corresponding to DP_{cpvtN2} and DP_{cpvtC} are accessible from the cytosol, which explains their easy replacement by domain peptides. Binding of domain peptides will introduce changes into the tertiary structure of RyR. However, the N-terminal region and the central domain, although located close to each other, cannot directly interact. Docking of the homology model of the RIH-2 domain into the cryo-EM maps has shown that this domain may serve as the mediator of the "domain switch" between NTD and the central domain. Based on these data we propose a new model of the "domain switch".

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S3-5

The functional communication among cardiac ryanodine receptors is not mediated by Ca^{2+} current

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The contraction of cardiomyocytes is triggered by the release of Ca^{2+} from the lumen of intracellular stores via ryanodine receptors (RYR2). RYR2 channels spontaneously assemble into clusters with "checkerboard-like" organization. This specific arrangement enables a functional communication among channels that is manifested by simultaneous openings and closings of multiple RYR2 channels (coupled gating). Although, the physiological relevance of this phenomenon has not been established yet, several model studies highlighted that even a small change in the channel communication could trigger severe cardiac arrhythmias. Molecular mechanism of coupled gating is not understood in detail; hence, we focused on the role of Ca^{2+} current as a source of a transient accumulation of Ca^{2+} on the cytosolic channel face. Using the method of ion channel reconstitution in a planar lipid membrane, we found that luminal Ca^{2+} as a source of Ca^{2+} current in lumen-to-cytosol direction did not increase the sensitivity of coupled RYR2 channels to cytosolic Ca^{2+} when compared with the single RYR2 channel. In addition, we tested the effect of luminal Ca^{2+} on the RYR2 sensitivity to caffeine. We revealed that luminal Ca^{2+} exhibited similar stimulating effect on the single as well as coupled RYR2 channels. The gradual elevation of Ca^{2+} current via RYR2 pore by applying voltage also did not stimulate the channel activity. Our results collectively indicate that coupled gating of RYR2 channels is not mediated by Ca^{2+} current. RYR2 channels likely communicate through the direct transfer of conformational changes that represents faster and more effective way to spread various signals among proteins.

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S3-6

Locked-open activation gate impedes recovery from inactivation in Shaker K^+ channels

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In the absence of N-type inactivation Shaker potassium channels display slow (C-type) inactivation. It has been shown earlier that Cd^{2+} traps the V476C *Shaker* channels in the open state, even at very negative voltages, by forming a metal bridge between a cysteine in one subunit and a native histidine (H486) in a neighbouring subunit. The current experiments tested the hypothesis that locking the activation gate in the open configuration prevents recovery from inactivation. To address this hypothesis we compared the extent of recovery from inactivation for control conditions and in the presence of 20 μM Cd^{2+}. V476C/IR channels contained an alanine in position 449 to facilitate the entry of the channels into the slow-inactivated state. 2.0-s-long depolarizing pulses from a holding potential of −120 mV to +50 mV were applied and when applicable, 20 μM Cd^{2+} was added to the fully inactivated channels. Under control conditions the channels completely recovered from inactivation within 60 s, whereas upon Cd^{2+} application less than 10 % of the current recovered under identical conditions. The protonation of the interacting histidine prevented the Cd^{2+} modification. Our interpretation is that the locked-open state of the channel prevents the gating transitions necessary for recovery from inactivation suggesting that the closure of the activation gate is essential for the recovery from slow inactivation.

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S4-1

First evaluations of a hybrid quantum-chemical approach to determine free energy differences and their impact on experiments

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The correct representation of solute-water interactions is essential for the accurate simulation of most biophysical phenomena, including catalysis and ligand binding. Several highly accurate quantum methods are available to deal with solvation by using both implicit and explicit solvents. So far, however, most evaluations of those methods were based on a single conformation, which neglects entropy. Here, we present the first test of a novel approach to determine hydration free energies that uses molecular mechanics (MM) to sample phase space and quantum mechanics (QM) to evaluate the potential energies. Free energies are determined by using re-weighting with the Non-Boltzmann Bennett (NBB) method [1]. Based on snapshots from MM sampling and accounting for their correct Boltzmann weight, it is possible to obtain hydration free energies that incorporate the effect of solute entropy. We evaluate the performance of several QM implicit solvent models, as well as explicit solvent QM/MM for the blind subset of the international SAMPL4 hydration free energy challenge [2]. While classical free energy simulations with molecular dynamics give root mean square deviations (RMSD) of 2.8 and 2.3 kcal/mol, the QM/MM hybrid approach yields an improved RMSD of 1.6 kcal/mol. By selecting an appropriate functional and basis set, the RMSD is reduced to 1 kcal/mol. Based on the theoretical predictions, three erroneous experimental values were identified and retracted from the competition. Thus, simulations of solvation have reached a level of quality where they can identify and inform experimentalists about potential problems and mistakes.

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S4-2

Conformational imaging in membranes by fluorescence microspectroscopy

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Bleaching corrected, polarization-dependent fluorescence microspectroscopy (FMS) with nanometer spectral resolution was applied to characterize motional patterns of two alkyl chain-labeled 7-nitro-2-1,3-benzoxadiazol-4-yl (NBD) phospholipids in three different model membranes, representing the three main biomembrane phases. By comparison to a dynamic model, two main conformations with preferential fluorophore dipole orientations roughly parallel and perpendicular to membrane normal, and with characteristic peak positions (λ_{MAX}) separated for 2–6 nm, were identified. Their relative populations and respective λ_{MAX} were especially affected in liquid-ordered phase, indicating a specific sensitivity of the two probes to molecular packing with cholesterol.

S4-3

Optically trappable SERS probes prepared by silver photo-reduction to SU-8 microstructures

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Optically trappable SERS (surface enhanced Raman spectroscopy) probes can be used for localized Raman measurements in combination with different laser tweezers techniques. Trapped dielectric spheres partially covered with metal colloids [1] were utilized by Petrov and co-workers to detect the recoil effect (momentum transfer) of Raman and fluorescence photons emitted by molecules adsorbed on metal surfaces [2, 3]. The biophysical potential of such probes (even if immobilized) was proved by SERS studies of single DNA molecules [4] and diffusion measurements of drugs inside live cells [5]. Working with spherical probes necessitates spatial overlap of the trapping and excitation laser beams, which can cause unwanted sample heating. Moreover, to assure transparency of the spheres the extent of metal coverage is limited. These problems are eliminated by application of trappable 3D structures, where the active SERS area is spatially separated from the trapping positions [6]. Here we report on trappable SU-8 microstructures prepared by two-photon polymerization. The structures are functionalized by silver photo-reduction while being trapped in a simple micro-fluidic device [7]. The new probes were tested by detecting SERS spectra of emodine.

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S4-4

Chiroptical properties of a cyclic antimicrobial peptide Lasiocepsin and its analogs

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We have investigated electronic and vibrational chiroptical properties of the novel antimicrobial peptide (AMP) lasiocepsin (LAS) containing two disulfide bridges and of its three analogs. The set of selected compounds included the natural LAS (H-Gly-Leu-Pro-Arg-Lys-Ile-Leu-**Cys**-Ala-Ile-Ala-Lys-Lys-Lys-Gly-Lys-**Cys**-Lys-Gly-Pro-Leu-Lys-Leu-Val-**Cys**-Lys-**Cys**-OH), two its analogs with just one native disulfide bridge and the remaining two cysteine residues replaced by alanines (Las[Cys17-Cys27,Ala8,25] – LAS A; Las[Cys8-Cys25, Ala17,27] – LAS B), and a completely linear, open analog having all four cysteines substituted by alanines (Las[Ala8,17,25,27] – LAS C). The analogs retain reduced (LAS B) or almost none (LAS A, LAS C) activities against common pathogens[1]. The effect of changing the disulfide bridge pattern on the LAS's secondary structure was studied by electronic circular dichroism (ECD), vibrational circular dichroism (VCD) and Raman optical activity (ROA). A combination of these techniques helped us to clarify a role of disulfide bridges in stabilization of LAS's conformation. Moreover, ECD and particularly ROA spectroscopy provided us with details on disulfide conformation. ECD indicates comparable conformation of the disulfide bridge for analogs containing one disulfide (LAS A, LAS B), while ROA enabled us to determine sense of disulfide torsion, even in the more complicated case of natural LAS containing two disulfide groups.

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S4-5

Electric cantilevers, small optical cantilevers and advanced cantilever functionalization as improvements in bio AFM techniques

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Since its invention in the middle of the 1980´s the atomic force microscope has developed to one of the key techniques in nanoscience. Especially in life science the main benefit is the ability to take images of biological samples under aqueous, near physiological conditions at nanometer resolution. Enhancement of the cantilever to a molecular biosensor yields to techniques which allow the detection of forces in the pico newton range [1]. The combination of recognition and topographical measurements (TREC) has been developed to determine receptor distributions on surfaces [2].

Nevertheless there are several limitations for a much wider use of these techniques in applied life science. Topographical imaging is time consuming, molecular recognition force spectroscopy (MRFS) and TREC suffer from complex cantilever functionalization and also the handling limits these techniques to experts in basic science.

But exchanging the conventional cantilevers to smaller, faster and more sensitive ones increases the imaging speed by a factor of ten and the sensitivity by a factor of five [3]. Using DNA building blocks in combination with DNA aptamers to functionalize cantilevers for MRFS and TREC simplifies the complex standard functionalization to simple incubation steps [4]. To improve the general tip handling and stability, active cantilevers for electronic, instead of optical readout have been developed. The newest generation of active cantilevers can be used for bio imaging in liquid and are nearly as sensitive as commercial bio cantilevers [5].

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S4-6

Inhibition of myosin light chain phosphatase (MLCP) accounts for the additional delayed contraction of airway smooth muscles (ASM)

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Our measurements of force in ASM show that force develops in two distinct phases after cholinergic and phorbol-ester stimulation. In the first phase the force reaches 70-80 % of the total contraction within one minute, whereas in the second phase the force progressively increases by additional 20-30 % and finally reaches plateau after 30 minutes. It is hypothesized that several enzymes (PKC, RhoK, ZIPK) modulate MLCP activity on a slow and a fast time-scale, however the models that would elucidate these processes still do not exist. The present semi-theoretical model considers these molecular mechanisms and predicts the delayed phase and proposes a mechanism for it:

i) according to our previous experimental and theoretical study RhoK is implicated only in the fast contractile response and might be implicitly related with increases in $[Ca^{2+}]_i$;
ii) active RhoK phosphorylates CPI-17, which then inhibits the catalytic subunit (PP1c) of MLCP;
iii) rate of phosphorylation at sites Thr-696 and Thr-853 of the MLCP regulatory subunit (MYPT1) is according to measurements slow and is involved in auto-inhibition of the PP1c;
iv) occupied phosphorylation sites of myosin light chain (MLC), MYPT1 and CPI-17 coexist in their position and compete between each other for binding with PP1c.

Phosphorylation of CPI-17 is explicitly modeled and calculated, whereas the input function for the time-dependencies of phosphorylation at Thr-696 and Thr-853 are still a missing part in the mechanism. For this reason, the fits to measured data are considered in modelling. The kinetics of the competitive reversible inhibition of all phosphorylated sites for the PP1c is considered, whereby equilibrium dissociation constants are determined for each particular inhibitor from the IC_{50} values. The activity of myosin light chain kinase (MLCK) is modeled by the 8-state kinetic scheme of interactions between Ca^{2+}, calmodulin (CaM) and MLCK and force is expressed from the 4-state latch bridge model. The model could be ascribed as semi-theoretical model since it still considers a direct measured input, but, nevertheless, it elucidates the underlying mechanism of reversible competitive inhibitions between different phosphorylation sites as a possible candidate for the explanation of the delayed development of force.

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S5-1
Nanofabrication via self-assembled hybrid nanomaterials into low dimensional structures

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Tuning the physical properties of low dimensional structures via control the assembly of hybrid building blocks to construct (multi)functional materials is a key challenge in nano(bio)technology. In this presentation, the bottom-up nanofabrication based on the assembly of functional building blocks will be discussed as an alternative to conventional top-down methods to fabricate well-defined functional nanostructured hybrid materials [1]. We explore the potential of nanostructured hybrid materials based on varieties of inorganic components developed in-situ within self-assembled soft polymeric matrix into low dimensional structures [1-3]. The main advantage of the in-situ preparation is that the size and the size-distribution of the inorganic moieties can be controlled within the soft (bio)polymeric matrix [3-5]. Moreover, the ability of the polymeric matrix to self-assemble into one dimensional nanostructures can be exploited to direct the spatial arrangement of the inorganic components [5-7]. A mechanism will be proposed with respect to direct the self-assembly process under ambient conditions. As well as a description of the driving forces leading to the fabrication of ordered domains will be discussed.

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S5-2
Recent advances in electrochemical molecular imprinting for biomimetic sensing

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As continuation of our attempts to devise a reliable nicotine (Nic) chemosensor [1], two new bis(2,2'-bithienyl)methane derivatives, i.e., that with the zinc phthalocyanine (ZnPc) substituent and that with the 2-hydroxyethyl (EtOH) substituent (ZnPc-S8 and EtOH-S2, respectively), were synthesized to serve as functional monomers for biomimetic recognition of Nic by molecular imprinting [2-4]. Formation of a pre-polymerization complex of the Nic template with ZnPc-S8 and EtOH-S2 was confirmed by both the high negative Gibbs free energy gain, ΔG = -115.95 kJ/mol, calculated using the density functional theory at the B3LYP/3-21G(*) level, and the high stability constant in chloroform, $K_s = 4.67 \times 10^5$ M^{-1}, determined by UV-vis spectroscopic titration. A Nic-templated molecularly imprinted polymer (MIP-Nic) film was deposited by potentiodynamic electropolymerization onto a quartz crystal resonator of EQCM from solution of this complex. The imprinting factor was as high as ~9.9. Complexation of Nic molecules by the MIP cavities was monitored with X-ray photoelectron spectroscopy (XPS), as manifested by a shift of the binding energy of the Zn $2p^3$ electron of ZnPc-S8 after Nic templating. For sensing application, simultaneous chronoamperometry (CA) and piezoelectric microgravimetry (PM) measurements were performed under flow-injection analysis (FIA) conditions. The limit of detection (LOD) of the CA and PM chemical sensing was as low as 40 and 12 μM, respectively. Among them, the CA chemosensing was more selective with respect to the cotinine and myosmine interferences due to the discriminating potential of 1.10 V vs. Ag/AgCl applied. Differences in selectivity to the analyte and interferences were interpreted with modeling of complexation of Nic and, separately, each of the interferences by a "frozen" MIP-Nic molecular cavity.

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S5-3
Labelled and functionalized DNA for novel bioassays

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Labelled nucleic acids (NA) are widely applied in studies of NA interactions with other molecules as well as in various diagnostic techniques as tools offering highly sensitive and selective analysis. Being interested primarily in application of electrochemical techniques in biomolecular research, we have developed different strategies of DNA modification with redox-active moieties (reviewed in [1]). One of them consists in chemical modification of natural DNA components, such as modification of thymine residues by osmium tetroxide reagents to form stable electrochemically active osmium adducts. The other strategy is based on enzymatic reactions with modified (labelled) deoxynucleotide triphosphates (dNTPs) resulting in attachment of the labelled nucleotides at 3'-end of a primer oligodeoxynucleotide (ODN) using terminal deoxynucleotidyl transferase (TdT) enzyme or incorporation of the modified nucleotides into a DNA stretch sequence specifically according to a DNA template [2]. Simple primer extension reactions allow incorporation of base conjugates with relatively bulky moieties, while efficient exponential amplification of modified DNA fragments by the polymerase chain reaction (PCR) is more feasible through incorporation of small moieties bearing reactive groups (such as 5-formylthiophene) followed by conjugation with the ultimate label on DNA (such as hydrazine derivatives bearing redox active groups to convert them into corresponding hydrazones in reactions with the incorporated aldehyde groups). Besides redox DNA labels useful in analysing DNA sequences and sequence polymorphisms [2], we introduced also novel fluorescent DNA labels [3] responding by fluorescence changes to DNA-protein interactions and reactive vinylsulfonamide and acrylamide groups [4] for efficient cross-linking and "fishing-out" specific DNA binding proteins.

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S5-4
Trends in affinity sensing

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Affinity-based biosensore (ABBs) are innovative and compact analytical devices that incorporate a biological, or biologically derived, sensing element (biochemical receptor) combined with a physico-chemical transducer. They are able to provide selective, sensitive and reproducible responses to a sample in a very short time and, furthermore, they make sample pre-treatment unnecessary (or at least greatly reduce any pre-treatment). During the past two decades they proved very successful in a wide range of fields, including food, environmental and clinical analysis.
Here, recent developments and open problems in the field will be discussed [1,2]. The ABBs suitability for molecular diagnostic applications from protein to nucleic acids analysis, with examples on real matrice such as human blood or serum will be presented [3].

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S5-5
Detection of microRNAs by droplet microfluidics

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Digital microfluidics has emerged as a key technology for DNA and RNA amplification and detection [1]. Compared with the macroscale, the microfluidic environment offers important advantages in biomolecular detection, such as reduced sample volumes required for the analysis, shorter analysis time, and potential for automation and integration. Such advantages are particularly desirable in nucleic acid amplification and detection.
The most renowned and today largely used nucleic acid amplification technique exploits the polymerase chain reaction (PCR). The method is simple, sensitive and cost-effective. However, it is prone to sample contamination and suffers from biases in the template to product ratios of the amplified target sequences. The method relies on thermal cycling in vitro to reach a maximum of twofold amplification in each cycle. The repeated heating and cooling represents an important limitation, particularly when the full exploitation of advantages associated with the integration of nucleic acid amplification protocols in microfluidic-based devices is going to be achieved. In order to overcome such a limitation, several alternative isothermal amplification methods have been developed [2]. These methods do not require thermal cycling, which greatly simplifies point-of-care diagnostics. In addition they are often simpler and more tolerant to inhibitors present in real matrices than PCR. Recently, the isothermal circular strand-displacement polymerization (ICSDP) has emerged as a new and promising method for nucleic acid amplification and detection.
In this work, the combined use of digital microfluidics and MB-assisted ICSDP amplification for the selective detection of miR-210 in nanoliter droplets is presented. miR-210 is an important microRNA target that is associated with endothelial cell hypoxia and erythroid differentiation of leukaemic K562 cells. Possibilities offered by digital ICSDP in amplifying and detecting nucleic acid sequences were preliminarily investigated by detecting a Roundup Ready Soybean (RR-soybean) related oligonucleotide sequence.

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S5-6

Modular bioreactors in tissue engineering: the role of mechanical signals

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The highly interdisciplinary area of tissue engineering, by its nature, involves several fields of research from basic materials development to stem cell handling to clinical applications.

While the need for quick applications is driven by necessity we are still far away from understanding how the hybrid system of material scaffolds-cells-biomolecules operates optimally either in-vitro (in a bioreactor) or in-vivo.

In our effort to monitor some basic responses of particular cells to specific environments we have developed a bioreactor able to supply a multitude of mechanical cues, singly or in combination to endothelial cells.

In this presentation we will provide evidence of the importance of substrate stretching and frequency of stretching, of the shear rate of the flowing feeding medium on top of the cells, and of a simulated microgravity environment to the morphological adaptation of the cells and the rearrangement of its cytoskeletal proteins for each particular adaptation. It will also be shown how the combination of these signals correlates with specific gene expressions.

S6-1
Revealing excitotoxicity mechanisms in amyotrophic lateral sclerosis by biophysical means

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Amyotrophic lateral sclerosis (ALS) is an adult onset fatal neurodegenerative disease characterized mainly by the progressive loss of upper and lower motor neurons resulting in wasting, paresis and death from respiratory failure. Approximately 5-10% of ALS cases are familial (fALS), while the rest are sporadic (sALS) with unknown cause of disease. Driven by early studies demonstarting that transfer of purified immunoglobulins G from ALS patients (ALS IgGs) could cause the disease in mice we have shown that these immune factors also induce excitotoxic synaptic activity in different *in vitro* setups, not only consisting of motoneurons. In light of the newly established theory that the disease is non-cell autonomous we have checked the effect of ALS IgGs on calcium homeostasis and mobility of endosomes/lysosomes in cultured rat cortical astrocytes. ALS IgGs evoked calcium transients (Ca^{2+}-waves) in astrocytes the effect not requiring extracellular Ca^{2+}. Pharmacological dissection revealed the role of inositol 1,4,5-triphosphate, phospholipase C, phosphatidilinositol-3-kinase, as well as of the transient receptor potential cation channels. On the other hand, experiments on intracellular vesicles labeled with lysotracker, demonstrated that ALS IgGs increased their mobility in a Ca^{2+}-dependent manner. It is yet to be proven if this increase is also related to excitotoxicity and glutamate release from astrocytes.

Another studied ALS specific humoral factor of excitotoxicity was the egsogenous mutant SOD1 – G93A (expressed in some fALS cases). In cultured astrocytes it also disrupted Ca^{2+} homeostasis and changed electrophysiological properties of membranes.

Thus, we have revealed IgGs and mSOD1 as specific humoral excitotoxic factors in ALS affecting not just neurons but non-neuronal glial cells as well. While mSOD1 is probably released by the astrocytes themselves, IgGs can actually cross into brain parenchima through a hampered blood-brain barrier in ALS (as revealed by our MRI studies on the ALS G93A rat).

S6-2

Magnesium increases membrane input resistance of leech Retzius neurons during Ni^{2+} - induced epileptiform activity

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The aim of this study was to examine the effect of 20 mM Mg^{2+} on membrane input resistance (IR) of leech Retzius neurons (LRNs) during epileptiform activity induced by nickel. Magnesium is used therapeutically as anaesthetic, antiarrhythmic and anticonvulsant. *In vitro* it is shown to suppress Na^{+} currents in mammalian neurons.

Intracellular recordings were made from LRNs of isolated ganglia of the leech *Haemopis sanguisuga*, using glass microelectrodes filled with 3M KCl. Epileptiform bursting activity was induced with 3 mM Ni^{2+} - Ringer solution (Ni^{2+} - Ri). After its stabilization, 20 mM Mg^{2+} - Ni^{2+} - Ri was applied (Mg^{2+} - Ni^{2+} - Ri). For measurements of the IR of directly polarized membrane, a high input impedance bridge amplifier was used to inject a current through the recording microelectrode. The amplitude of the recorded voltage produced by rectangular hyperpolarizing current pulses was used to calculate IR.

In standard Ri resting membrane potential of LRNs was -38.00 ± 1.93 mV (n=6). Bath application of Ni^{2+} - Ri induced epileptiform activity. In Ni^{2+} - Ri IR was 11.35 ± 1.06 MΩ (n=5). Superfusion with Mg^{2+} - Ni^{2+} - Ri suppressed epileptiform activity and significantly increased IR to 14.88 ± 0.55 MΩ (n=3, $p<0.05$).

Suppression of Na^{+} - dependent Ni^{2+} - induced epileptiform activity by Mg^{2+} in LRNs is accompaned by an increase in membrane IR. We conclude that the most probable mechanism responsible for both effects is blockade of Na^{+} channels by Mg^{2+}.

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S6-3
Topology and neurodynamics of the auditory M50 gating network

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Magnetoencephalography (MEG) with its excellent time resolution and reliable source localization techniques offers a valuable approach for assessing neurodynamics of sensory processing. One of the strongest manifestations of early sensory processing is the amplitude variability of the M50 component of neuromagnetic responses that is commonly used to explore the brain's ability to modulate its response to incoming stimuli, a process conceptualized as the gating mechanism. Although numerous studies have explored the modulation of the auditory P50/M50 components and suggested multiple generators, there is still no agreement on the underlying network. To identify the spatial and temporal characteristics of the cortical sources underlying the gating processes, auditory evoked field (AEF) responses were evoked by tones of a passive oddball paradigm (1 kHz, p=0.8, standards; 1.2 kHz, p=0.2, deviants) in 10 healthy elderly and 10 amnestic mild cognitive impaired (aMCI) or Alzheimer's disease (AD) patients. AEFs were recorded with the 275 channel CTF system in a magnetically shielded room at the Mind Research Network, Albuquerque, NM. Using multi-start simplex nonlinear spatio-temporal localization approach three cortical regions underlying the auditory M50 network were identified: prefrontal cortex (PF) in addition to bilateral activations of superior temporal gyri (STG). However, network topology was affected by neurodegeneration. i.e., the PF source was localized for 10/10 healthy subjects, whereas 9/10 demented patients were lacking the PF source for both tone conditions. In addition, this study provided the first characterization of the cortical dynamics of the identified M50 generators and found significantly enhanced activity of the STG sources in response to both tone conditions for all subjects who lacked a PF source. The reported results provide novel insights into the topology and neurodynamics of the M50 auditory network and suggest an inhibitory role of the PF source that normally suppresses activity of the STG sources.

S6-4

Neuronal control of breathing and airway reflexes: new ideas and our experience

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Almost 35 years of our research on central control mechanisms of breathing and respiratory reflexes data from our laboratory have contributed to world-wide increase of knowledge in the field of respiratory neurobiophysics and neurophysiology. However, the brainstem structures and mechanisms of the cough, sneeze aspiration and expiration reflexes, are still poorly understood. Nowadays there is generally accepted that the underlying rhythmic pattern of respiratory neural activity consists not of two phases (i.e. inspiratory and expiratory), but of three phases: inspiratory, post-inspiratory, and expiratory. Basically, there are three types of respiratory neurones corresponding to the three neural phases of breathing. A number of animal experiments have proved that the respiratory neurones are concentrated in five main areas of the brainstem reticular formation, the dorsal (DRG) and ventral (VRG) respiratory groups, the Bötzinger (BOT) and pre-Bötzinger (pre-BOT) complexes and finally the Pontine respiratory group-PRG. Respiratory rhythmogenesis is driven by complex interactions that reflect both the synaptic interactions between the synaptic inputs to brainstem neurones as well as the intrinsic voltage-dependent membrane properties of those neurones. Our older transection experiments in cats showed that the neurones in the rostral pons may have modulatory effects on the cough reflex. Later, we also recorded the single-unit activity of Inspiratory and/or Expiratory neurones from medullary VRG during quiet breathing and coughing in non-paralyzed cats. Also the expression of the immediate-early gene c-Fos, a marker of neuronal activation, was employed in our experiments on anesthetized cats. Some findings and peculiarities being obtained during the authors experimental work on the central control of breathing, coughing and related motor behaviours are discussed.

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S6-5
Iron biomineralization in *globus pallidus* of human brain

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The basal ganglia contain the highest levels of iron in the brain. Some studies indicate a disruption of iron metabolism in patients with neurodegenerative disorders. It is known that glycoconjugates (mucopolysaccharides) have capacity to chelate iron. As a result, transformation of iron to various iron oxides may occur. Their presence was confirmed by our Mössbauer spectroscopy measurements. Our results of SQUID magnetometry showed the presence of magnetite/maghemite in some samples. It is suggested that if the magnetite in the brain is a by-product of iron metabolism its presence may be important in pathologic conditions commonly associated with aging. Since magnetite was found in samples without clinic-pathological diagnosis of neurodegenerative disease we suggest that magnetite is not a mark of neurodegeneration. The presence of crystalline, well defined solid structures support a biologically controlled mineralization pathway. From the size of particles it can be suggested that iron accumulation and crystals formation should be a long-term process. Magnetic resonance imaging could help to clarify neuropathologic processes in vivo. Processes focus on decreasing brain iron levels may offer novel ways to delay the rate of progression and possibly defer the onset of neurodegenerative diseases.

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S7-1

Carbosilane dendrimers as potential delivery vehicles for HIV-derived peptides to dendritic cells

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Dendrimers - synthetic polymers possessing unique hyperbranched architecture are widely studied due to their potential to improve disease treatment by efficient delivery of therapeutically active compounds to the site of action. The AIDS research is currently focused to delivery of antigen – HIV derived peptide across the plasma membrane of dendritic cells (DCs). As an antigen carries, we have proposed to use carbosilane dendrimers of second generation (CBD, G2) [1]. We used CBD of two types: CBD of either carbon-silicon bonds (CBD-CS) or oxygen-silicon bonds (CBD-OS). Three types of HIV-derived peptides were selected: Nef, P24, Gp160. These peptides complexed with CBDs dendrimers (dendriplexes) were then studied for interactions with membranes of various lipid compositions that model plasma membrane of DCs.

We prepared Langmuir monolayers (LMs) and large unilamellar vesicles (LUVs) composed of zwitterionic dimyristoylphosphatidylcholine (DMPC) and negatively charged dipalmitoylphosphatidyl glycerol (DPPG). Interactions between LUVs and peptides itself or dendriplexes were studied by size and zeta potential measurements; measurement of surface pressure was applied in the case of LMs. HIV-derived peptides itself in the concentration range 0.3 – 6 μM didn´t change the hydrodynamic diameter of DMPC LUVs, but zeta potential slightly decreased. These parameters were not affected for DMPC/DPPG LUVs upon the peptides treatment. HIV-derived peptides complexed with CBD-CS moved the zeta potential of DMPC/DPPG LUVs from -31,4 mV to 21,1 mV; hydrodynamic diameter increased from 104,7 nm to 141,1 nm at the highest dendriplex/lipid molar ratio of 0.1. Interactions between CBD-OS dendriplexes and LUVs had similar behavior to those of CBD-CS dendriplexes. However, the presence of CBD-OS caused more pronounced changes in the measured parameters for both the pure DMPC as well as DMPC/DPPG LUVs. Surface pressure of lipid monolayers has increased for system studied in the following order: CBD-OS dendriplexes > CBD-CS dendriplexes > peptides.

Acknowledgement
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S7-2
PMCA activity and membrane fluidity in patients with chronic kidney disease

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Chronic kidney disease (CKD) is accompanied by increased intracellular calcium concentration [1]. There are several mechanisms of regulation calcium concentration, involving calcium entry by way of various types of calcium channels in the plasma membrane, calcium influx from intracellular compartments and also active transport by Ca^{2+}-ATPase and the sodium-calcium exchanger witch transport calcium into the intracellular pools or pumped it out of the cells. In this study we concentrate our interest on activity of plasma membrane calcium ATPase (PMCA) of red blood cells (RBCs) and its relation to membrane fluidity. Twenty-five patients with stage 2-3 of CKD and the same number of healthy volunteers with normal hematological and biochemical values were included in the study. Isolation of erythrocyte membranes was carried out by using standard method Hanahan&Ekholm [2], protein content of isolated membranes were established by Lowry method [3]. PMCA activity was determined spectrophotometrically as amount of inorganic phosphate, which is generated by the decomposition of ATP into ADP at the transition of calcium ions across the plasma membrane. Membrane fluidity was appreciated using fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH). PMCA activity in human RBCs was significantly decreased in the group of patients with chronic kidney disease (stage 2-3) as compared to the control group of healthy volunteers (43.81 ± 20.41 nmol Pi/mg/h vs. 59.97 ± 18.16 nmol Pi/mg/h, $n = 25$, $P < 0.01$). In contrast, there was no significant difference of fluorescence anisotropy. The difference between groups was only 2.7 % (0.258 ± 0.022 vs. 0.265 ± 0.017, $n = 25$). Therefore, we conclude that the reduced PMCA activity is not caused by the surrounding environment of membrane lipids, which might affect its function via lipid – protein interactions.

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S7-3

H_2S-NO interaction and consequent biological effects

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The H_2S endogenously produced in mammals affects many of their physiological and pathological functions including modulation of the NO signalling pathway, neuro-modulation, cellular division, apoptosis, heart regulation, ischaemia/reperfusion, hypertension, vaso-relaxation, atherosclerosis, septic and haemorrhagic shock, inflammatory processes, erection and hibernation. To contribute to the understanding of the cross-talk between H_2S and NO signaling pathways, we studied H_2S induced NO release from nitroso-compounds *in vitro* and the biological effects of the reaction products. H_2S released NO from NO-donor S-nitrosoglutathione (GSNO) and cultured cells, indicating that biological systems contain nitroso-compounds from which H_2S can induce NO release. H_2S donor Na_2S decomposes GSNO, which results in the formation of polysulfides (S_n^{2-}) and ($SSNO^-$). Intravenous application of S_n^{2-} and H_2S mixture had significantly pronounced effect on rat hemodynamic parameters compared to H_2S alone. $SSNO^-$ relaxed phenylephrine induced aortic and mesenteric ring contraction. Its effect was more pronounced in comparison to GSNO. As detected by spin trap EPR technique, $SSNO^-$ released NO faster than GSNO and scavenged the cPTIO and Tempol radicals. S_n^{2-} and $SSNO^-$ modulated the activities of calcium RyR2 and chloride channels. In conclusion, the exogenously produced products of H_2S-GSNO interaction, S_n^{2-} and $SSNO^-$, had biological effects *in vivo* and *in vitro*. The results contribute to the understanding of the biological effects of H_2S which are important in the exploration of H_2S donors as new drugs in the treatment of "H_2S related diseases".

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S7-4

The time dependency of the extrema in the QRS isopotential maps of young adult controls

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The electrical activity of the heart ventricles is represented by the QRS complex that can be recorded and analysed using different procedures. The aim of this retrospective study was to analyse the sequence of isopotential maps in young adult controls when using time normalisation of the QRS complex.

The QRS complex of each subjects was divided into 20 equidistant parts. We constructed 21 isopotential maps from 24-lead system after Barr in 90 controls (42 men) without cardiovascular diseases, aged (18.6 ± 0.4) years. We analysed values and the time course of extrema: maximum (MAX), minimum (MIN), peak-to-peak value (PEAK=MAX-MIN).

The mean QRS complex duration was (92 ± 12) ms. The potential values ranged from – 4.3 mV to 3.5 mV in individual maps. We always found significantly lower mean values of extrema (flatter maps) in women than in men, except for 2/21 MAX comparisons. The absolute maximum appeared in the middle of the QRS complex preceding the absolute minimum in average by 2 maps (about 9 ms). The time courses of extrema were fitted by polynomial curves of the 6^{th} order against the sequential map number $x = 1, 2, ..., 21$ with corresponding correlation coefficients $r > 0.98$.

The obtained values of extrema reached higher values than the published data from the standard chest leads (lower values in women than in men) [1], which, however, do not allow evaluating of the time course and cover only a small part of the chest.

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S7-5

Role of nitric oxide in pathogenesis of atherosclerosis: modelling approach

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We are integrating several autonomous models into one complex unity in order to achieve deeper understanding of the links between shear stress, nitric oxide (NO) production, cyclic guanosine-monophosphate (cGMP) production, Ca^{2+} dynamics and regulation of vascular tone. All these mechanisms are important for hemodynamic regulation, which plays a decisive role in the pathogenesis of atherosclerosis – complex chronic inflammatory disease of the large and medium-sized arteries. The flow of blood at vessel bifurcations is classified as disturbed flow and is associated with vortices and consequently lower shear stress, which has significant influence on the activation of endothelial nitric oxide synthase (eNOS) to produce NO. Free NO activates soluble guanylate cyclase (sGC) in smooth muscles, which produces cGMP. cGMP activates enzyme protein kinase G (PKG), which phosphorylates many important proteins that participate in intracellular mechanisms of down-regulation of cytosolic Ca^{2+}. Consequently, vascular smooth muscle cells relax. Increased diameter of vessel changes blood flow into more laminar, which consequently leads to decrease in NO production. This auto-regulation feedback loop is studied in terms of changes of different system parameter values.

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S7-6
Numerical prediction of hemodynamic response of an atherosclerotic blood vessel

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Atherosclerotic plaques occur in well-recognized arterial regions such as areas of flow separation, curvatures and bifurcations, which suggest that fluid dynamics and vessel geometry contribute to plaque formation [1]. Vessel segments with low wall shear stress or highly oscillatory wall shear stress (WSS) seem to present the highest risk for atherosclerosis. Moreover, the relationship between intraluminal stresses and the development as well as the progression of atherosclerosis is not yet understood [2]. Significant contribution to deeply understand the development of plaque and the role of WSS in the progression of the disease could give relevant mathematical models. In our work, we examine the hemodynamic conditions and the intraluminal mechanical response of the vessel by using a fluid-structure interaction (FSI) approach [3]. In this way, the WSS acting on the vessel wall can be accurately predicted by taking into account the pulsatile nature of blood flow as well as the deformability of the vessel wall. The time dependent variation of WSS and the intraluminal stresses are observed for a vessel with a different degree of stenosis. Our results demonstrate that the WSS is significantly affected by the degree of stenosis. If combined with clinical observations on the progression of atherosclerosis, these results enable further elucidation of the role of altered hemodynamic conditions.

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S7-7

Dynamic model of eicosanoid production with special reference to aspirin-exacerbated respiratory disease (AERD)

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A theoretical model for the dynamic prediction of absolute and relative levels of prostaglandins and leukotrienes after oral NSAID dosing is worked out to account for the aspirin-exacerbated respiratory disease (AERD) or aspirin-induced asthma (AIA). It is based on the modeling of enzymatic driven conversion of arachidonic acid to eicosanoids in inflammatory cells which is a target of NSAIDs. Coupled kinetic and pharmacokinetic models include 15 variables and more than 50 parameters, all with references. Model results and analyses focus on two ratios, $[PGE_2]/[PGD_2]$ and $[PGE_2]/[LTC_4]$, hallmarks of AERD due to hypersensitivity to NSAIDs (e.g. aspirin). Four model populations (non-asthmatic, aspirin-tolerant and two aspirin-intolerant asthmatic) are defined for simulations in states of no-inflammation and inflammation. Model simulations performed at rest and after oral dosing of NSAID elucidate hypothesis originating from experiments that inflammation mimicked in experiments with cytokine-mix increases expression of prostaglandin E synthase less in aspirin-intolerant than in aspirin-tolerant and non-asthmatic patients. The sensitivity of the system elucidates also some other possible scenarios. Model results considerably agree with measured ones and account for several measured imbalances of eicosanoids after NSAID dosing: significantly lower production of PGE_2 in aspirin-intolerant case, esp. in inflammation; several orders larger $[PGE_2]/[PGD_2]$ in inflammation than in no-inflammation and no impact of NSAID on that ratio; up to 1000-fold lower $[PGE_2]/[LTC_4]$ in aspirin-intolerant compared to non-asthmatic cases. The risk for bronchoconstriction is tested for different NSAIDs, i.e. ibuprofen, indomethacin and aspirin (irreversible inhibitor of cyclooxygenases) as well as for selective inhibitors of cyclooxygenases - COXIBs. The model reveals the origins of several imbalances in ratios of eicosanoids, whereby these ratios are the main measured variables for diagnosing NSAID-triggered hypersensitivity. Thus, the applicable target of the model is the development of new metabolomic-based diagnostic tools and methods for measurements of arachidonic-acid-metabolites in blood or other body fluids. Even more, the model can also be applied to any other pathology, e.g. osteoarthritis, rheumatoid arthritis, NSAID-related gastric complications etc. by proper case- and cell- specific adaptations.

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S7-8

Single molecule force spectroscopy and recognition imaging investigation of blood clotting relevant receptors on living platelets

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Blood clotting is one of the most prominent properties of platelets. This process is accompanied by platelet activation, a process were smooth non-adherent platelets are inverted to sticky speculated particles expressing end releasing biologically active substances. Although main parts of this highly complex mechanism are well understood some details are still under discussion. Here we tried to enlighten one part in platelet activation by high resolution methods based on the atomic force microscope (AFM). AFM imaging and Single Molecule Force Spectroscopy [1, 2] were used to explore both, morphological changes as well as receptor activities of platelets. Imaging time series was accomplished with and without fixation steps at the single platelet level. Hereby the response of mechanical stimulation of the platelet by the AFM cantilever tip was directly observed. We demonstrate that living and fixed platelets develop filopodia after a short activation time followed by their disappearance including cellular bleb formation. Thereafter a second filopodia formation (filopodia extrusion) was observed; those filopodia subsequently disappeared again, and finally platelets detached from the support due to cell death. We determined the influence of mechanical stress on the chronology of morphological changes of platelets and demonstrated shear force induced filopodia formation. Through recordings over several hours, topographical AFM images over the full platelet lifetime – from early activation up to apoptosis – are presented. SMFS measurements on living platelets [3] allowed determining the activation state of the most prominent membrane receptor integrin αIIbβ3 at all different phases of activation. αIIbβ3 was fully activated, independent of the morphological state. In addition Topography and RECognition imaging [4] allowed to localize the integrins at the cellular surface.

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POSTERS

P1-1
Biophysics based approach for inferring bacteriophage infection strategies

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Understanding bacteriophage infection strategies has recently came in focus in the context of increased resistance of pathogenic bacteria to antibiotics, since these viruses can specifically destroy a given pathogenic bacteria. A common way of analysis represents a labor-intensive combination of experimental and bioinformatic approaches. We here investigate to what extent we can understand gene expression strategies of lytic phages, by directly analyzing their genomes through bioinformatic methods based on theoretical biophysics techniques. We address this question on a recently sequenced bacteriophage 7-11 that infects bacterium *Salmonella enterica*, which is homologous to another, experimentally characterized, lytic bacteriophage *phiEco32*.

Our main result is identification of novel promoters for the bacteriophage-encoded sigma factor in the 7-11 genome. Interestingly, standard methods for promoter recognition, based on Monte-Carlo procedure, fail to correctly identify the promoters, but a simpler procedure, based on pairwise alignments of intergenic regions, correctly identifies the desired motifs; we argue that such search strategy is more effective for promoters of bacteriophage-encoded sigma factors that are typically well conserved, but appear in low copy numbers. The same efficiency of the standard and newly-proposed search strategies was obtained when two additional genomes (*phiEco32* and *Xp10* phages) are analyzed. We also identified promoters for bacterial-encoded sigma factor in the 7-11 genome, by using a recently improved model of bacterial promoters specificity. Identification of all the promoters allows clustering the genes in putative early, middle and late class, which in consequence reveals the bacteriophage infection strategy. We therefore find that direct analysis of bacteriophage genomes is a plausible first-line approach for understanding bacteriophage transcription strategies.

P1-2
Low energy THz vibrational studies from amino acids to polypeptides

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Terahertz spectroscopy is a powerful technique to provide information on low energy molecular state transitions, intermolecular bonding and intramolecular vibrations. The received *time domain terahertz spectroscopy* (TDTS) data is converted by Fast Fourier Transformation (FFT) to transmittance spectrum and to absorbance spectrum on interest.
The structure and as a consequence the vibrations of proteins depend on the physico-chemical properties of the environment and the molecular interactions that they are involved. The amount of collective modes increasing by molecule size and complexity hinder characteristic spectroscopic responses to THz radiation, therefore spectroscopic comparison of structural changes requires specific techniques. We investigated biologically relevant molecules to study their spectral response for THz radiation and found matching spectral lines with predicted ones by theoretical molecule modelling. Characteristic absorbance peaks were determined for some amino acids and specific proteins of our interest.
This fundamental research will help to discover the relationship between the complex spectrum of polypeptide chains and its building elements; the amino acids.

P1-3
Distinct polymerization properties of actin isoforms due to various assembly factors

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The actin filament system is one of the fundamental components of the cytoskeleton. Multiple well conserved actin isoforms had been described but the different functions of these isoforms is not completely understood. In vertebrates six different actin isoforms are expressed in a tissue-specific fashion, which can be divided into two major groups, as muscle and non-muscle isoforms. In vertebrates muscle α1-skeletal, α-cardiac and two smooth-muscle (α2 and γ1) isoforms can be found. The cytoskeletal actins are exclusively β and γ1 isoforms.

Actin isoforms exhibit highly conserved amino acid composition. Their sequences differ only in a few amino acids found at the N-terminus. Despite these minor sequence differences the actin isoforms compose functionally different cellular actin structures. β-actin preferentially localizes to filopodia and stress fibres, whereas γ1-actin composes the cortical and lamellipodial meshworks. The generation of these diverse actin networks is catalysed by different assembly factors, like formins and Arp2/3 complex machinery.

We investigated the properties of different actin isoforms using biophysical and biochemical approaches. The cytoskeletal actin isoforms were produced in baculovirus/SF9 expression system. To form higher-order actin structures, the monomers assemble into filaments upon polymerization. The polymerization kinetics of actin isoforms was monitored either by light-scattering or by the changes in fluorescence of pyrenyl probe bound to Cys-374 of actin.

Our results revealed that the spontaneous polymerization properties of actin are isoform dependent. The polymerization rate of β-actin is the highest, which is followed by α1-skeletal and γ1 isoforms. We found that assembly factors, such as mDia1 formin and the Arp2/3 complex catalysed the assembly of actin isoforms in an isoform specific manner.

P2-1
Kinetics of lipoplexes formation: stopped - flow fluorescence experiment

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The interaction of DNA with liposomes charged positively by cationic amphiphiles, cationic lipids or divalent cations results in supramolecular aggregates with internal ordered microscopic structure, so called lipoplexes. Lipoplexes are tested as nonviral vectors for transfer and expression of DNA in cells. The ability of phospholipids to form these structures in the presence of divalent metal cations is also of importance for the DNA arrangement in viruses, in chromosomes, in the processes of DNA translocation through membranes, etc.

During the process of lipoplex formation DNA polyanion interacts electrostatically with cationic liposomes. The interaction is rapid, and takes place during the aggregation process, both phospholipid and DNA undergoing a complete topological transformation into compact quasispherical particles reaching up to ~ 1-2 mm diameter, with ordered internal microstructure. Information about kinetics of lipoplex formation is scarce in literature. We performed stopped-flow fluorescence measurements to study kinetics of the interaction between DNA (herring testes) and dipalmitoylphosphatidylcholine (DPPC) unilamellar liposomes in presence of divalent cations (Ca^{2+}, Mg^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+}). DNA and DPPC (1:1 vol/vol) were mixed rapidly with solutions of metal cations. This mixing process was followed by a significant decrease of fluorescent emission intensity of ethidium bromide (EB). EB probe intercalates between stacked DNA bases and is excluded from the DNA molecule due to its binding to the lipid bilayer. Aggregation process was followed at several concentrations of cations. The binding followed first-order reactions and appeared to occur in three steps. The reaction rate constants and the ratio of the released EB in the individual steps were determined. Small angle neutron scattering experiments (SANS) were performed for selected samples to follow structural changes in lipid bilayer.

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P2-2

The effect of PKC δ on the cell death after photoactivation of hypericin

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In U87 MG cells (human glioma cell line), Hyp is predominantly localized in endoplasmic reticulum and partially in mitochondria, lysosomes and Golgi apparatus [1]. Cell death induced by its photoactivation is triggered via mitochondrial apoptotic pathway. A member of lipid-regulated serine/threoninePKC family, protein kinase C □□δ functions as a proapoptotic protein during DNA damage-induced apoptosis. It also has a critical role in the regulation of various cellular processes including cell proliferation and tumor promotion [3].

We used small interfering RNA (siRNA) to find out how would post-transcriptional silencing of PKC δ gene influence the cell death pathway. Before addition and photo-activation of Hyp, U87-MG cells were pre-incubated with siRNA for ~ 24 hours. Flow cytometry was used to determine cell survival, type of cell death and production of reactive oxygen species (ROS). By fluorescence microscopy sub-cellular distribution of Bcl-2 family protein members (Bak and Bax) and PKC δ were monitored.

Decreased expression of PKC δ did not result in notable changes of ratio of apoptotic/necrotic cells after Hyp photo-activation. However the production of ROS observed after Hyp photosensitization in the cells treated with si-RNA δ was fundamentally decreased. Moreover, incubation of cells with si-RNA δ leads to the translocation of Bak and Bax from cytosol to plasma membrane. This specific plasma membrane localization tends to slowly disappear after Hyp photo-activation when cells undergo cell death process-24 hours after irradiation. Taking into account that si-RNA□ treatment leads to the decrease of PKC δ and that its translocation toward plasma membrane can be induced under certain conditions, we suggest that Bak and Bax are directed to plasma membrane in order to target some possible PKC δ.

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P2-3

Lateral pressure changes in lipid bilayers induced by variation of polar region composition: excimer fluorescence study

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The lipid composition can strongly affect the behavior of membrane proteins. The cause can be a non-specific modulation of protein conformational equilibria via changes in the lateral pressure profile [1]. These equilibria are often sensitive to variation of lipid molecular characteristics, such as lipid headgroup repulsion and curvature elastic stress, which can be modulated by incorporation of charged and nonlamellar lipids, respectively.

In the present study excimer fluorescence dipyrenyl phosphatidylcholine lipid probes ($dipy_nPC$, pyrene moieties are attached to the n-th carbons of both acyl chains), sensitive to lateral pressure, were used. Fluorescence measurements were performed using FluoroMax-4 spectrofluorimeter (HORIBA Jobin Yvon, France). Excitation was at 345 nm. The ratio of excimer to monomer fluorescence intensities ($\eta=I_{exc}/I_{mon}$) obtained at 375.5 and 475 nm, respectively, was used as a measure of the lateral pressure as described in [2].

Effects of headgroup charge were studied on dioleoylphosphatidylcholine:dioleoyl phosphatidylserine (DOPC:DOPS) = 100:0 – 0:100 mol% system. With increasing DOPS mol%, a systematic decrease of η for $dipy_4PC$, however no significant changes for $dipy_{10}PC$ were obtained. This indicates that the peripheral lateral pressure decreases with increasing charge of the polar region, while the lateral pressure in the central region of the bilayer is not affected. Effects of non-lamellar lipid were studied on palmitoyloeoylphosphatidylcholine:palmitoyl oleoylphosphatidylethanolamine (POPC:POPE) = 100:0 – 0:100 mol% system. Up to 60 mol% POPE, η for $dipy_4PC$ is constant, at higher concentrations η decreases. For $dipy_{10}PC$ a systematic increase of η was obtained up to 90 mol % POPE, for pure POPE system the η suddenly decreases reaching the value of pure POPC. This indicates that increasing the amount of a non-bilayer lipid increases the lateral pressure in the central core of the bilayer, however above 60 mol% it exerts an opposite effect near the water interface.

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P2-4

Changes in anti- and pro-apoptotic Bcl2 proteins distribution and metabolic profiles in human aorta endothelial cells caused by oxidative stress triggered by either Hyp-PDT or hypoxia

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Dysregulation of apoptosis can contribute to diverse pathologies. Diminished apoptosis of vascular endothelial cells (ECs) in angiogenesis is crucial for tumor growth and ischemic disease [1], while excessive apoptosis impairs the integrity of the endothelial barrier and is associated with infection, inflammatory and autoimmune disease [2], and tumor-related metastasis [3]. Therefore, understanding apoptosis regulatory mechanisms in ECs has great importance for the development of novel therapy strategies. An oxidative stress with the generation of radical oxygen species (ROS) is a common mechanism causing ECs' dysfunction and apoptosis. The ROS generation can be triggered by various stimuli including photodynamic therapy (PDT) and hypoxia. The mechanisms by which ROS can cause ECs apoptosis typically include receptor activation, caspase activation, Bcl-2 proteins, and mitochondrial dysfunction. The molecular mechanisms underlying PDT, and specifically HypericinPDT (HypPDT), are not completely understood [4]. We particularly investigated mitochondrial dysfunction and role of Bcl-2 proteins in apoptosis of ECs triggered by HypPDT. Our results indicate that HAEC cells use primarily oxidative phosphorylation pathway for energy production. HAEC respiration is similar in the absence or presence of Hyp without irradiation. The presence of Hyp itself renders HAEC cells to be more apoptotic, and significantly affects distributions of Bcl2 proteins (Bax translocation into mitochondria, and Bax and Bcl2 into nuclei), without affecting the mitochondria function. This effect is most likely due to hydrophobic interaction of Hyp with Bcl2 similar to the interaction between Bcl2 and ABT263 (Bcl2 inhibitor) [5]. HypPDT has severe effects on HAEC cells: abolishes mitochondria function; further significantly affects Bcl2 proteins distributions; and causes primarily necrosis. Effects of hypoxia on Bcl-2 proteins distribution and mitochondria functions are under investigation.

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P2-5
Interaction of antimicrobial peptides and biological membranes

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Antimicrobial peptides are small proteins that exhibit a broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as fungi. Biological activity of antimicrobial peptides is intensively studied. Their chemical structure allows them to interact (attach and insert) with membranes. The most general mode of their action is the disruption of microbial membranes or induction of pore formation resulting in the lysis of the cells. Nevertheless the fine details about how these peptides interact with biological membranes are not fully clarified yet. In order to better understand this mechanism we have performed *in situ* atomic force microscopy studies using different antimicrobial peptides on artificial model membranes (dipalmitoylphosphatidylcholine) and on *Escherichia coli* bacteria. The antimicrobial effect of two types of peptides was tested: (1) indolicidin, a cationic 13-residue peptide-amide isolated from cytoplasmic granules of bovine neutrophils and (2) nodule-specific cysteine-rich peptides that represent a group of plant antimicrobial peptides. We were able to detect concentration-dependent interaction of these peptides and membranes, which resulted in the destruction of the model membrane. To get detailed structural information about the bacterial cell surfaces, high resolution images were taken of individual bacteria before the treatment with antimicrobial peptides (under quasi-physiological conditions) The changes in the surface roughness were followed in time during the treatment. Besides imaging, atomic force microscopy was used to determine the elastic properties of bacterial cells as well.

P2-6
Interaction between bacterial populations grown in coupled microchambers

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Heterogeneity is a typical characteristics of natural microbial habitats. Nutrients and various resources form spatial gradients have an impact on the life of the microbes. Bacteria use swimming motility and chemotaxis to explore such heterogeneous environments and find better living conditions. On the other hand microbes typically live in complex multispecies communities. Inter- and intraspecies communication through chemical signaling plays a crucial role in the functioning of these communities. We have studied the interplay between chemical signalling and chemotaxis as a form of complex interactions between bacterial populations. Microtechnology offers tools to create microenvironments where the distribution of chemical compounds is spatially heterogeneous. We have fabricated a microfluidic device that creates temporally stable chemical gradients in a flow-free environment. The device consists of two large reservoirs and a narrow observation channel between them separated by a porous membrane. This membrane acts as a physical boundary for cell populations grown in the chambers, nevertheless it enables chemical coupling between them. The device therefore makes it possible to culture physically separated, but chemically coupled bacterial populations and study the interactions between them. Throughout the experiments we can study how cells change the chemical composition of their environment and how they react to these changes. We were able to observe attractive and repulsive interactions between bacterial populations (*E. coli* and *P. aeruginosa*) and showed that chemotaxis and likely intercellular signaling play a fundamental role in these phenomena. The effect of signal molecules secreted by bacteria during cell-cell communication (e.g. quorum sensing) on the motility of *P. aeruginosa* and *E. coli* have also been investigated.

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P2-7
Electrical impedance spectrum of geranium root during growth

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Electrical impedance spectroscopy can be a successful method for observing the growth of root without the remove of plant from soil [1-3]. Impedance spectrum of young geranium root cuttings in pot filled with vermiculite – as an artificial soil was determined with precision LCR meters of HP 4284A and HP 4285A in frequency range from 30 Hz up to 30 MHz. The soil electrode – a stainless still rod – was inserted into the soil 3 cm far from plant, the plant electrode – an Al foil with conducting gel – was fixed on the stem a few mm above the ground surface. From the measured spectra the impedance of soil was eliminated. The corrected impedance spectrum of root was approached by two distributed circuit elements in serial connection with a resistance. For the curve fitting a non linear least square method of MathLab program was used. The resistance parameter of the second distributed element showed good correlation with the length of root and the relaxation time parameter increased as the resultant cross-section of root increased.

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P3-1
Characterization of luminally located Ca^{2+} binding site on the cardiac ryanodine receptor

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Contraction of cardiomyocytes is tightly coupled with the transient elevation of cytosolic Ca^{2+} that activates the muscle contractile apparatus. Ca^{2+} ions are mobilized from intracellular stores through ryanodine receptor (RYR2). The main regulatory part of the RYR2 channel is a gigantic cytosolic domain where well-characterized Ca^{2+} activation site is localized. However, the RYR2 luminal regulation has also become important. It has been clearly shown that dysregulation of the RYR2 channel from the luminal side was accompanied with the appearance of specific cardiac arrhythmias. Localization as well as detailed characteristics of the RYR2 luminal site is not known. The aim of our study was to characterize this binding site with testing a functional effect of various luminal X^{2+} ions on the channel response to cytosolic caffeine. Native RYR2 channels were isolated from the rat heart and reconstituted into a planar lipid membrane. The interaction of luminal X^{2+} ions (selected from the group of earth metal cations: Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}) with the RYR2 channel was monitored by comparing EC_{50} values for the caffeine activation. We found that RYR2 channel exhibited the strongest response to cytosolic caffeine in the presence of luminal Ca^{2+}. Furthermore, the effect of luminal X^{2+} ions on the caffeine sensitivity decreased in the following order: $Ca^{2+} > Sr^{2+} > Ba^{2+} \sim Mg^{2+}$. Our results suggest that the interaction of X^{2+} ions with both cytosolic and luminal binding sites of the RYR2 channel is comparable from the qualitative point of view and it is likely govern by the ion size. In addition, we can speculate that 3D-architecture of Ca^{2+} sites localized on both cytosolic and luminal faces of RYR2 channel might be similar.

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P4-1
Gold nanoparticle-coated microtools for localized fluorescence enhancement

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There is an increasing interest in functionalized complex microstructures for micro-and nanotechnology applications in biology. Particularly in imaging, metal-enhanced fluorescence (MEF), achieved by microscopic surfaces coated with metal nanoparticles (NPs) has been applied recently on cells observing otherwise weakly detectable signals [1-2]. We introduce microstructures made of SU-8 photoresist by two-photon polymerization into various shapes that were coated with gold nanoparticles (NP) of 70 nm [3]. We demonstrated localized MEF by microstructures equipped with tips of sub-micron dimensions. Using green and red absorbing fluorophores we showed that the method may be applied in different ranges of the VIS spectrum. The enhancement factor was as high as 6 in areas of several square-micrometers and more than 3 in the case of the local enhancement. With further development the microtools can be prepared to be actuated by optical tweezers and position to any fluorescent micro-object, such as single cells to realize localized, targeted fluorescence enhancement.

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P4-2

Application of surface-enhanced Raman spectroscopy for the detection of trace quantity of pesticides aldrin, α-endosulfan, and lindane

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Surface-enhanced Raman scattering (SERS) is extremely sensitive analytical technique due to its high sensitivity and selectivity in molecular identification [1]. SERS enhancement is mainly based on giant electromagnetic (EM) field induced by nanostructured noble metal surfaces and associated with the localized surface plasmon resonance. In relation to normal Raman scattering SERS provides an enhancement of up- to 10^6, in certain cases it leads to an enhancement of up- to 10^{14}-10^{15}, what corresponds with single molecular detection. Most currently employed SERS substrates are metal (Ag, Au, Cu) nanoparticles (NPs) in suspension (colloids). The EM intensification occurs mostly in localized regions of the metallic surface called hot spots (HS), such as interparticle junctions or gaps between NPs [1]. An ideal situation for building interparticle HS is the use of bifunctional molecules which act as NPs linkers [2]. Many molecules adsorbed on metal surfaces are not only able to induce the formation of the HS but can also act as molecular hosts of specific analytes.

Aldrin, α-endosulfan, and lindane are pesticides belonging to organochlorine family. These pesticides break down slowly in the environment and can accumulate in the fatty tissues of animals. They may cause grave damage to living organisms by changing the growth rate of some plants and animal species. Moreover, pesticides also represent certain risks for human health (cancerogenecity). With respect to these facts, the detection and identification of these chemicals (even at very low concentration) is very important.

Using various types of metal colloids and aliphatic α,ω-dithiols as bifunctional linkers, we have determined the fingerprint region (300-400 cm^{-1}) for the SERS detection of the above mentioned pesticides and the limit of detection for these molecules (aldrine ($50x10^{-9}$ M), α-endosulfan ($285x10^{-9}$ M) and lindane ($64x10^{-9}$ M)). The most suitable substrate for SERS detection of the pesticides appears to be citrate silver colloid particles covered by 1,8-octanedithiol.

Our recent results confirm the high sensitivity of SERS for the detection of low quantities (~10^{-9}M) of some organochlorine pesticides which provide solid basis for the construction of suitable nano-sensors for the detection and identification of this type of chemicals.

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P4-3

Nanomechanical study of the planar lipid systems by using electrochemical PeakForce QNM AFM

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The objective of the presented results is to reveal the influence of the cholesterol on nanomechanical properties of model cell membranes using PeakForce QNM Atomic Force Microscopy. We will present planar lipid systems (supported phospholipid bilayers) which resembles lateral organization of phospholipids in cellular membranes. Lipid bilayers were transferred onto atomically flat Au(111) substrates by fusion of small unilamellar vesicles (SUVs). Model membranes with compositions similar to those of the eukaryotic and bacterial cell membranes were prepared using neutral DMPC and negatively charged phosphatidylglycerol (DMPG). Electrochemical PeakForce QNM AFM was used to characterize morphological and mechanical changes of the phospholipid bilayers induced by the presence of the cholesterol at different potentials.

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P4-4
Indirect optical manipulation of live cells with functionalized polymer microtools

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Cells can be trapped and manipulated with optical tweezers. However, due to the high laser intensity inside an optical trap, a cell is likely to get damaged by the trapping laser itself. Previous studies confirmed a dosage dependent viability for trapped cells. Therefore, even in traps with low laser power, a trapped cell will die over time. To overcome this limitation we designed and fabricated two-photon polymerized microtools, which can be bound to target cells, and manipulated by holographic optical tweezers. The binding is achieved by functionalizing the surface of the cells with biotin molecules, and that of the microtools with biotin and streptavidin. Thus, when a holographically trapped microtool is brought to contact with a target cell, biotin-streptavidin-biotin bridges form between them, providing a force much greater than an optical trap can exert. Therefore bound cells can be dynamically manipulated in 3D by rotating and translating the microtool with holographic optical traps. With this indirect manipulation the target cell is separated from the trapping laser in space, thereby its exposure by the optical field can be greatly reduced.

P4-5

DMD simulations of folding and early stages of oligomerization of stefins

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We simulated folding and early stages of oligomerization of stefin B [C3S, E31Y] at various strengths of effective electrostatic interactions between sidechains with discrete molecular dynamics approach. We compared calculated and experimentally obtained oligomer size distributions and selected the value of strength of electrostatic interactions that gave the best fit. At the same value we calculated secondary structure, potential landscape, solvent-accessible surface area per amino, contact maps, and chain flexibility.

We obtained a reasonable agreement between simulated and measured oligomer size distribution which surprisingly shows a higher propensity of dimers than monomers in solution. The compactness as determined by N-to-C terminal distance, and SASA increase with oligomer size. Also remarkable is the increase in β-strand contents and high protection of the part from 35th to 70th amino - as if we would be observing prefibrillar oligomers forming a core which would be on the way to fibrils and appearing in the partially denaturing environment.

P5-1
Electrochemical aptamer-based biosensor amplified by PAMAM dendrimers for detection of aflatoxin B_1

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We present an aptamer-based electrochemical biosensor for detection of aflatoxin B1 (AFB_1), a mycotoxin identified as a poisonous contaminant of food products. Mycotoxins are secondary toxic metabolites produced fungi genera under specific conditions of moisture and temperature, which are generally associated with diseased or mouldy crops. Among mycotoxins, the most abundant group found in food is aflatoxin, a hazardous and carcinogenic metabolite mainly produced by the flavus and parasiticus species of the genus Aspergillus [1].

The sensor is assembled in a multilayer framework that utilizes cyclic voltammetry and impedance spectroscopy for acquiring the sensor response by means of the redox indicators $K[Fe(CN)_6]^{-3/-4}$. Poly(amido-amine) dendrimers of fourth generation (PAMAM G4) immobilized on a gold electrode covered by cystamine, were employed as signal amplifiers for immobilization of single stranded amino modified DNA aptamers specific to AFB_1.

The system cystamine-PAMAM was compared with other sensing platforms such as cystamine-aptamers, 11-mercaptoundecanoic acid (MUA)-aptamers and MUA-PAMAM-aptamers, being the cystamine-PAMAM-aptamers the best approach for producing more sensitive and reproducible signal in the range of concentrations 0.1-10 nM of AFB_1. The limit of detection (LOD) for this system was 0.5 ± 0.07 nM. The sensor was additionally validated in certified contaminated samples of peanuts as well as in spiked peanuts solutions showing optimal response. Non specific interaction was verified by testing the sensor in other mycotoxins such as aflatoxin B_2 (AFB_2) and ochratoxin A (OTA). The sensor was regenerable in 0.2 M glycine-HCl and did not lose its stability up to 60 h storing at 4 ºC. Atomic Force Microscopy (AFM) studies were also performed for illustrating individual steps of biosensor assembly.

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P5-2
High-speed all-optical logic operations with bacteriorhodopsin

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In data-processing applications requiring high speed and wide bandwidth, photonic devices – where logic operations are processed on an all-optical basis – represent a promising alternative of their electronic counterparts. Besides in/organic active optical crystals, dyes and polymers, molecules of biological origin with suitable nonlinear optical properties can also find applications in integrated optical – biophotonic – devices.
The principle of all-optical logical operations utilizing the unique nonlinear optical properties of a protein was demonstrated by a logic gate constructed from an integrated optical Mach–Zehnder interferometer as a passive structure, covered by a bacteriorhodopsin (bR) adlayer as the active element. Logical operations were based on a reversible change of the refractive index of the bR adlayer over one or both arms of the interferometer. Depending on the operating point of the interferometer, we demonstrated binary and ternary logical modes of operation. Using an ultrafast transition of the bR photocycle (BR-K), we achieved high-speed (nanosecond) logical switching. This is the fastest operation of a protein-based integrated optical logic gate that has been demonstrated so far. The results are expected to have important implications for finding novel, alternative solutions in all-optical data processing research.

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P5-3
Singlet oxygen generation in reaction center/carbon nanotube bio-nanocomposites

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Photosynthetic reaction center proteins (RCs) are the most efficient light energy converter systems in nature. The first steps of the photosynthesis take place in these proteins. Due to their unique properties, combining RCs with nano-structures promising applications can be predicted in optoelectronic systems. The aim of our work is to create a system for efficient light energy conversion (e.g. photovoltaics), integrated optoelectronic devices or biosensors (e.g. for specific detection of pesticides). We bind RC, purified from *Rhodobacter sphaeroides*, to different carrier matrices like carbon nanotubes (CNT), conductive polymers and porous silicon. In this work we demonstrate the stability of different RC/CNT complexes. One of the most determining factors seems to be the singlet oxygen generation accompanying the photochemistry of the system. After photo excitation reactive oxygen species (ROS, including, e.g., singlet oxygen ($^1O_2$), superoxide anion (O_2^-), and hydroxyl radicals (OH·)) are formed with large probability. In our work we used 1,3-diphenylisobenzofuran (DPBF) to detect the arising singlet oxygen. As our system is very complex and inhomogeneous, the singlet oxygen generation was measured under different parameters, using different binding methods, concentrations and spectral intervals. It is important to reduce the formation of the ROS components because they decrease the efficiency of the photochemical energy conversion. In addition, they react with the intracellular components resulting in their degradation (the RC itself as well).

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P5-4

Spontaneous and microwave assisted formation of casein stabilized gold-nanoparticles – possible use in sensor applications

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Milk quality can be strongly affected by proteolytic enzymes present either endogenously in the milk or exogenously as a result of bacterial contamination giving rise to a food quality risk in otherwise safe storage conditions. Proteolytic enzymes can degrade casein, the major protein component of milk, by releasing degradation products with unfavorable organoleptic qualities. Proteolytic activity in milk is usually determined indirectly by bacterial count or directly by spectrophotometric enzymatic assays. These methods are either time consuming or impeded by the turbidity of raw milk sample. Development of an accelerated economic test for casein degrading capability could be beneficial for the dairy industry.

Casein stabilized gold nanoparticles have been synthesized in green-chemistry based spontaneous or microwave assisted way from technical grade casein. The effect of solution pH, reaction time and microwave power on nanoparticle stability and size distribution has been studied and compared with results obtained with reducing agent aided synthetic ways.

Proteolytic sensitivity of differently prepared casein stabilized nanoparticle layers in comparison with casein immobilized on gold electrode has been measured by electrochemical means in order to assess the possibility of accelerated test.

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P5-5
Detection of plasmin activity at surfaces modified by casein and short peptides using various methods

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Detection of enzyme activity is of great importance for understanding the mechanisms of its action as well as for practical purposes. Plasmin is a protease present in the blood under micromolar concentration range. The plasmin from the blood is infiltrated into the cow milk, where cleavage of protein – casein takes place. Higher activity of plasmin causes extensive casein cleavage which results in bitter taste of the milk. Such milk is not suitable for cheese production. We performed studies focused on plasmin activity at surfaces composed either of β-casein or short peptides containing specific site for plasmin cleavage. The casein or peptide layers were formed on gold surfaces by self-assembly and their cleavage was detected by cyclic voltammetry (CV) and thickness shear mode acoustic (TSM) methods. In the CV method, we used short peptides specific for plasmin modified at one side by ferrocene and at the other side by thiol groups for facilitating the formation of chemisorbed layer on the gold surface. The addition of plasmin resulted in the cleavage of ferrocene-peptide fragment causing decrease in redox current. This method revealed high detection limit of approx. 0.1 nM and it was validated in a milk samples with satisfactory recovery up to 87 %. TSM method allowed detection of changes in resonant frequency and motional resistance of quartz crystal with protein layer. The sensitivity of detection of short peptide cleavage by plasmin was insufficient, however we obtained significant changes of motional resistance that is evidence of the viscoelastic contribution. Significant changes of frequency and motional resistance took place already at 1 nM plasmin. AFM experiments performed with casein at gold or mica confirmed the plasmin activity at the surface. The surface of casein was rather rough. The mean roughness reached up to 10 nm. The incubation of the protein layer with plasmin (10 nM) during 30 min resulted, however, in decrease of the roughness to the value similar for naked gold or mica.

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P5-6
Real-time sensing of hydrogen peroxide by carbon nanotube/horse radish peroxidase/ITO enzyme electrode

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The determination of H_2O_2 is of great interest because it is a product of reactions catalyzed by several oxidase enzymes in living cells and it is essential in environmental and pharmaceutical analyses. In our experiments horseradish peroxidase (HRP) was bound to carboxyl-functionalized multiple walled carbon nanotubes (MWCNT-COOH) in the presence of coupling reagents, N-Hydroxysuccinimide (NHS) and 1-[3-dimethylaminopropyl]-3-ethyl-carbodiimide (EDC). A fluorescence method was worked out to determine the amount of decomposable H_2O_2 by our bio-nanocomposite in a sensitive way. Fluorescence measurements proved that the immobilized HRP maintained its oxidative activity for guaiacol when H_2O_2 was added. We applied succesfully fluorescamin (a chemical specific to primary amine groups) to determine the concentration of the HRP bound to the complex and the enzyme activity. An ITO/HRP/MWCNT bio-nanocomposite electrode was fabricated. SEM images proved that the assembly of the composite was successful. The electrochemical characteristics of the biosensor were studied by cyclic voltammetry which confirmed the electron transfer between the immobilized HRP and the enzyme electrode in the presence of H_2O_2. The biosensor displayed a sensitive electro-catalytical response to the reduction of H_2O_2.

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P5-7
Structure-biological function correlations for Q_B site inhibitors of bacterial photosynthetic Reaction Centres

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Many herbicides employed in agriculture (e.g. triazine compounds) and some antibiotics (e.g. stigmatellin) bind to a specific site of the Reaction Centre protein blocking the photosynthetic electron transport. Crystal structures showed that all these inhibitors bind at the secondary quinone site albeit in slightly different ways. It has already been demonstrated that various herbicides have different binding affinities, which are usually estimated as inhibition constants K_i (in turn intended as binding thermodynamical constants) and, consequently, binding enthalpy values, ΔH_{bind} (the entropy component ΔS_{bind} being about the same for every herbicide). Therefore, the action of inhibitors depends fundamentally on: (i) the herbicide molecular structure; (ii) interactions between the herbicide and binding site; (iii) protein environment.

In our studies we obtained K_i and ΔH_{bind} values from experimental measurements with several common triazine inhibitors. Moreover, molecular structure modeled calculations were optimized and their intramolecular charge distributions (*Mullikan charges*) were estimated by *ab initio* computer calculations. Experimental and modeled data were compared to those from available databank crystal structures.

We can state that herbicide inhibition efficiency (K_i and ΔH_{bind}) is mainly influenced by steric (geometry of interactions) and electrostatic factors (herbicide molecular charge distribution).

Steric reasons make apolar bulky groups on the herbicide N-7 nitrogen atom (like t-buthyl in terbutryne) preferable for establishing stronger interactions with Q_B site, while such substituents are not recommended on N-8 atom (an i-propyl group would be better, like in promatryne).

Electrostatic factors instead make a $-SCH_3$ group, less electrophylic and polar, more suitable for optimal interactions on the C-1 carbon, as it is found in terbutryne and contrary to the case of terbumeton (where the same position is occupied by a more polar $-OCH_3$). Finally, in terbutryne the N-4,7,8 nitrogens maintain a larger electron density so that more effective hydrogen bonds may be formed between the inhibitor and the surrounding amino acids of the protein.

As preliminary results, these data may help in understanding at a molecular detail which factors are critical in discriminating good herbicides form „evil" ones towards a specific biological action and designing biosensors for detection of pesticides in a sensitive way.

P5-8

AFM as a tool to image the interactions between cytochrome c and calixarenes in supported lipid membranes

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AFM method was used as a very useful tool in studying the surface topography of the supported bilayer lipid membranes (sBLM) composed of 1,2-sn-glycerodimyristoylfosfatidylcholine (DMPC) with incorporated calixarenes ^{t}Oct[6]CH2COOH (CX) specific to cytochrome c (cyt c). It is supposed that cyt c interacts with CX through amino groups of lysine residues at its surface. Therefore we also applied single molecule force spectroscopy (SMFS) to the binding between cyt c immobilized at AFM tip and CX incorporated into the lipid film. In later case cyt c or individual NH_2-group have been connected to the AFM tip through special linker. The topography of bare sBLM in a gel state (T=19 ^{o}C) revealed relatively smooth surface (R_{RMS} = 0.18 nm) and thickness ~5.1 nm which agrees well with previous studies. Incorporation of CX into DMPC bilayer resulted in increase of the surface roughness (R_{RMS} = 0.39 nm) and in increase of thickness in average by 0.5 nm. The incubation of the layer with 30 nM of cyt c resulted in a surface smoothing (R_{RMS} = 0.32 nm) and in a further increase of the thickness between 0.7 to 1.2 nm. The SMFS experiments with cyt c modified AFM tip approved its specific binding to CX and allowed us to determine binding parameters k_{off} (1.14±0.59 s^{-1}) and x_{β} (3.98±0.63 Å). The SMFS experiments with an amino-ended linker also resulted in highly specific interactions with comparable values for k_{off} (2.74±0.66s^{-1}) and x_{β} (5.91±2.55 Å). This suggests that both electrostatic and amino group specific interactions between cyt c and CX cavity exist.

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P5-9
Immobilization of DNA aptamers on tetrahedrons surface for thrombin binding analysis

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In order to use aptamers as molecular recognition elements in biosensing it is important to develop strategies for their immobilization to maintain their proper folding and binding abilities. A thickness shear mode acoustic method (TSM) has been used for study the binding of thrombin to the DNA aptamers immobilized at the gold surface covered by DNA tetrahedrons. The binding of thrombin to the conventional aptamers sensitive to fibrinogen (FBT) and heparin (HPT) exosite as well as HPT in a loop configuration (HPTloop) allowed us to determine constant of dissociation (K_D) and limit of detection (LOD). The sensing system composed of HPTloop has been characterized by K_D = 66.7±22.7 nM, which was almost twice lower in comparison with that of FBT and HPT. For this biosensor also the lower LOD has been obtained (5.2 nM), while for conventional HPT aptamers it was 17 nM. Less sensitive sensor based on FBT aptamers revealed LOD 30 nM which agree with less affinity of this aptamers to thrombin in comparison with that of HPT. We applied also AFM method to analyze topography of the gold layers stepwisely modified by DNA tetrahedrons, DNA aptamers and thrombin. The height profile of the layers were in reasonable agreement with the dimensions of the adsorbed molecules. The results indicate that DNA tetrahedrons are efficient platform for aptamers immobilization.

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P5-10
Electrochemical biosensor for vascular endothelial growth factor using different aptameric configurations

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Vascular endothelial growth factor (VEGF) is a signal protein that has the ability of promoting angiogenesis and thus it is considered as a crucial marker of tumor growth and metastasis. VEGF is also responsible for certain blood vessel diseases such as neovascular glaucoma and macular degeneration on eyes [1]. Therefore, the development of an effective, accurate and non-invasive method of detection of VEGF is highly required. Additionally, aptamer-based biosensors constitute a viable approach with relative high accessibility, ease of implementation and direct response in sensing proteins.

We present an electrochemical biosensor for VEGF based on different configurations of aptamers: monomeric, supported monomeric and dimeric arrangements. For this purpose we used V7t1 aptamer (5' TTT TTT TTT TTT TTT TTA GAT GGG CCG GGC AGG TGG GGG TGT 3´) modified by thiol group at 5' end and that containing complementary supporting part for formation of aptamer homodimer. Such a homodimer has 2 identical binding sites for VEGF. We also constructed aptamer with rigid supporting part by hybridization of V7t1 with short oligonucleotide dA_{15} complementary to the supporting part of V7t1. The sensitivity of the sensor has been compared with the sensor based on other thiolated aptamer sequence Veg (5´-TTCCCGTCTTCCAGACAAGAGTGCAGGG-3´). Electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used for sensor assembling and protein detection. Biosensors were assembled on gold support and aptamers were attached to the surface via chemisorption of thiols. Aptasensors based on V7t1 thiolated aptamers demonstrated to have a limit of detection (LOD) of 1.56 nM for dimer, 2.64 nM for supporter monomer and 2.34 nM for monomer alone. Veg aptamer-based biosensors showed a LOD = 2.1 nM. The aptasensors based on dimeric configuration were also tested in human plasma spiked by different concentrations of VEGF. The sensor recovery varied between 131.6 to 84,6 % for 1 nM and 100 nM VEGF, respectively.

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P5-11
Photocurrent generated by photosynthetic reaction center protein based nanocomposites

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Intensive studies have shown recently that photosynthetic proteins purified from plants (PS-I and PS-II) and from purple bacteria bind successfully to nanostructures while their functional activity is largely retained. Current researches are focusing on finding the best bio-nanocomposite sample preparations and experimental conditions for efficient energy conversion and for the stability of the systems. In our studies reaction center proteins (RC) purified from purple bacterium *Rhodobacter sphaeroides* were bound successfully to amine- and carboxy-functionalized multiwalled carbon nanotubes (MWCNTs) immobilized onto the surface of Indium tin oxide (ITO) by using specific silane crosslinker and conducting polymer. Structural (TEM, AFM) and functional (electrochemical measurements) techniques have shown that RCs can be bound effectively to the functionalized carbon nanotubes (CNT). The complexes have high stability and generate fotocurrent in wet and dry condition as well. An electrochemical cell with three electrodes (reference Ag^+/AgCl, counter platinum and the working sample) was designed especially for measuring the photocurrent generated by this composite material. Several hundreds of nA photocurrent was measured with fully active RCs while the current was missing when the RC turnover was disrupted by depleting the electron acceptor quinones. The study of possibility for generating photocurrent in organic solar cell based on RC protein is also under process.

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P5-12
Regenerable Functionalization of Biosensing Gold Sufaces based on genetically modified Avidins

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Quartz Crystal Microbalance (QCM) is an extremely sensitive technique allowing to detect mass changes in the nanogram/cm^2 range with a wide dynamic range extending into the 100 microgram/cm^2. An AT-cut quartz crystal which has one gold electrode deposited on each side gets excited to oscillate at its resonance frequency by a voltage controlled oscillator (VCO). According to Sauerbrey's equation, the observed change in oscillation frequency of the crystal is in relation to the mass change per unit area at the gold electrode surface of the quartz crystal. QCM can either be used in gas phase, under vacuum or in liquid environment, which allows a broad field of application like determining the affinity of molecules to surfaces functionalized with specific bait molecules.
For studying interactions between biomolecules such as the avidin-biotin-interaction, the quartz crystal has to get cleaned under harsh chemical conditions after each functionalization. Since it would be desirable to reuse a sensor chip after a measurement series for cost and time reasons, a regeneration method was investigated. To allow subsequent functionalization with new biotinylated bait molecules, a pH-sensitive avidin mutant (M96H) was used, which is very robust and remains fully functional in weak acids (pH > 3). However, this mutant can easily be partly denatured by a mixture of citric acid (pH 2.0) and SDS.
As QCM is able to detect the binding of avidin M96H to a biotinylated surface due to a drop in resonance frequency, the regeneration process can be observed as an increase to the initial value, too. For demonstrating the reversible functionalization of biosensing gold surfaces, QCM measurements as well as Surface Plasmon Resonance (SPR) measurements were deployed.

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P5-13
Assessment of enegy harvesting from the human body for personalised medicine and biosensor applications

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The development of methods for energy harvesting from the human body is a growing research field that can offer remarkable advances in medical and portable electronic devices. Proper functionality of these devices relies heavily on the continuous supply of a sufficient amount of electricity. Body motion is a useful energy source that can be transformed to generate sufficient electrical power in order to power these miniaturised electronic medical devices. Therefore, it is easily understood that energy harvesting from human body can lead to the advance of personalised medicine and offers great advantages in health care and treatment. In addition, it provides the users with the possibility of reducing the frequency or eliminating surgeries.

A pacemaker heart device and a Continuous Glucose Monitoring (CGM) system are two examples of such medical devices that can exploit energy harvesting technology. The pacemaker is a small device which is placed in the chest or abdomen to help control abnormal heart rhythms, which uses electrical pulse to prompt the heart to beat in a normal rate. Continuous glucose monitoring systems usually consist of a glucose biosensor, a transmitter and an external monitor to view glucose levels. Glucose biosensors are needle-type electrodes that are implanted under the skin of the abdomen and they are vital for continuous glucose monitoring, because they can constantly record blood glucose concentrations and signal a warning in case of hypoglycemia or hyperglycemia. In the search of methods that can improve the aforementioned portable medical devices, piezoelectricity, thermoelectricity and electromagnetism must be included.

In this contribution, an analytical overview and comparative assessment of the energy requirements of medical devices is presented; and, the methods of utilising the human body as an energy source to replace batteries and introduce medical devices with almost infinite lifetime are reviewed and critically assessed.

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P6-1
Functional MRI in evaluation of changes in motor cortex of patients suffering from myasthenia gravis

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Introduction Myasthenia gravis (MG) is an autoimmune neurological disorder which interferes neuromuscular transmission by blocking acetyl-choline receptors in synapses [1]. This results in weakening of muscles of eye,limbs and torax, and in the majority of cases bulbar muscles. Although, some neuropsychological studies had reported cognitive impairment in MG patients [2,3], the other alterations in brain function were not investigated. The aim of this study is to investigate changes in activation patterns in zones of motor cortex responsible for movements of bulbar muscles.

Patients and methods. The study included 26 patients (11 male, 15 female, median age 37 years) with myasthenia gravis, confirmed by clinical and immunological tests, and 10 age-matched control subjects. All MR examinations was performed using Philips Achieva 1.5 T scanner and 8-channel head coil. The protocol consisted of 3D-T1 weighted FFE, GRE field mapping and FFE-EPI sequences. Two block-designed paradigms were used: in the first subjects were instructed to repeatedly (with frequency 1 Hz) make o-shape with their lips and in the second subjects were asked to touch palate with the tip of tongue. In both cases as control, periods of rest were used. First and second level analysis fMRI were performed using FEAT and MELODIC routines in FSL v4.0 package.

Results and conclusions. Results showed that MG patients exhibit significantly higher ($p<0.05$) percent change of fMRI signal compared with healthy controls in parts of motor cortex responsible for both movements of tongue and facial muscles. In patients with acute deterioration of disease the observed change was twice as large as in control subjects. We speculate that the observed changes may be a kind of feedback to impaired signal transmission at neuromuscular junction.

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P6-2

The use of dose-volume-histogram for the evaluation of dose distribution in radiotherapy of uveal melanoma

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Background: Stereotactic radiosurgery (SRS) is a therapeutic modality which uses the targeted action of ionizing photon radiation for treating malignancies. It is characterized by the application of one single high radiation dose directed with high accuracy and with steep dose gradient to the target structures. SRS allows for excellent local tumor control while sparing adjacent surrounding tissues.
Methodology: 19 patients with uveal melanoma (stage T2 and T3) underwent CT and MRI to identify and map the exact area to be treated. Afterwards, the dose distribution in target (tumor) and critical structures (optic nerve, lens) using dose-volume histogram (DVH) was evaluated. DVH reduces 3D dose distribution in volume to a simple 2D curve. The most optimal plan keeps the dose in the radiosensitive structures minimal, extending only to a very small risk structure volume. Irradiation was performed on a linear accelerator Clinac 600C/D, the therapeutic dose was 35,0 Gy.
Results and Discussion: Our preliminary data suggest that despite of high efficacy and low toxicity of the SRS, there is still risk of adverse effects due to distributed dose to the critical structures, which may sooner or later affect the functionality of the irradiated tissue. Unfortunately, this issue has not been discussed in detail so far. This needs to be further investigated in parallel with continuing development of light-field techniques, such as proton beam therapy (not yet available in Slovakia) which has a comparatively good treatment success and optimally protects the risk structures due to the characteristic dose distribution according to the Bragg peak.

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P6-3

Chemotactic responses of *Escherichia coli* to different antibiotics in a microfluidic device

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Micro- and nanofabrication technologies can be used to create precisely engineered, physically and chemically defined bacterial habitats. In our laboratory we developed a novel microfluidic device which creates a temporally stable chemical concentration gradient in a flow-free environment. The device contains two microchambers and a channel which are separated by a porous membrane. This membrane is a semi-permeable barrier and it allows chemical coupling between the chambers and the channel. One of the chambers contained the antibiotic of interest and the other one motility buffer, thus in the observation channel bacteria were subject to a chemical concentration gradient. Recent publications [1, 2] based on microfluidics suggest that concentration gradients and motility may have a crucial role in the evolution of antibiotic resistance. As a result, antibiotic resistant mutant strains may appear much faster than it was predicted. To understand the mechanisms behind fast evolution of antibiotic resistance, systematic studies of cellular motility and chemotaxis in antibiotic gradients are crucial. We used our microfluidic device to create spatial gradients of antibiotics and study small populations of bacteria in these gradients for an extended period of time. We observed the chemotactic responses of Escherichia coli to different antibiotics e.g. ampicillin, in a wide range of concentrations, from 0,5 μg/ml to 250 μg/ml. Using Escherichia coli strains expressing green or red fluorescent proteins allowed us to monitor chemotactic and non-chemotactic mutants at the same time. After analysing the fluorescence microscopy data we could conclude that at certain concentrations, antibiotics could repel chemotactic bacteria while leaving the non-chemotactic mutants unaffected. These results have important implications regarding the evolution of antibiotic resistance. Beside using our microfluidic device we conducted single cell tracking experiments to determine how antibiotic concentration change the swimming speed and the tumbling frequency of bacteria.

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P6-4
Electrophysiological exploration of HeLa cells treated with ruthenium(II)-arene complex

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During the past decades, cancer treatment research was focused on developing alternative cytostatic compounds that will eventually replace cisplatin, as widely used cytostatic agent. Investigation of ruthenium(II) complexes as potential anti-cancer agents yielded promising results, thus future efforts will be focused on understanding a mechanism of action against tumour cells of these complexes[1].

Current recordings were obtained with the whole-cell configuration of the patch-clamp technique[2]. HeLa cells were voltage-clamped at the holding potential of -50mV before applying voltage steps protocols. Whole-cell currents were evoked by stepping the holding potential of -50 to -150 mV and up to +70mV in 20 mV increments. Current densities were calculated by dividing the whole-cell currents measured in the steady state by Cm. HeLa cells were continually treated with IC_{50} concentration of ruthenium(II)-arene complex (previously determined) for 2h and then the membrane currents were measured. Results showed significant reduction of current densities in the membrane of HeLa cells treated with ruthenium(II). Density of inward currents, measured at -150mV, were decreased from -6.3±1.6 pA/pF to -1.2±0.1 pA/pF, while density of outward currents, measured at 70mV, were decreased from 4.2±1.2 pA/pF to 1.2±0.3 pA/pF. Current reduction might be result of ion channels block caused by indirect effect of ruthenium(II) complex or by changing membrane fluidity, which is the known effect of ruthenium complexes.

The I-V profiles were examined by several approaches (factor analysis, independent component analysis, linear discriminant analysis), but the obtained results showed no difference between threated and non-threated cells, indicating that the mechanism of action was not the change in expression of ion channels.

Further studies will involve EPR spectroscopy for examination of membrane fluidity.

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P6-5

DSC analysis of blood plasma on patients with skin cancer, and psoriasis

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We discuss the thermal changes of human blood plasma components in melanoma malignum (MM) patients with or without regional lymph node metastases as well as in different stage of psoriatic disease by Differential Scanning Calorimetry (DSC).

Fifteen white adults had been operated with MM. From routine histopathological parameters tumor thickness according to Breslow were evaluated. Invasion value was between Clark level II and IV. Regional lymphatic infiltration was evaluated as a prognostic factor, and the sentinel lymph node was positive in 7 cases.

Our results showed that comparison of healthy controls with the cases with melanoma and local metastases, exhibited at least two marked different thermal domains during the denaturation. The first T_ms were only slightly influenced by the Breslow's depth and the Clark level, but they alteredd in the melting enthalpies. The second T_ms and the calorimetric enthalpy changes demonstrated a significant difference of the melanoma depth dependence in 0.95-8 mm range and in Clark levels of II-IV.

Next study included 76 white adult patients with diagnosed psoriasis. To define the severity of symptoms 3 groups were established based on PASI scoring system. PASI 0: symptoms free, PASI 1-9 showed mild symptoms, and severe symptoms observed if PASI were over 10. According to medication patients were divided into the untreated and systematically treated with drugs groups. For medical treatment **methotrexate**, acitretin, adalimumab, **methotrexate** and adalimumab, infliximab were applied.

Our results showed that DSC is an useable method not only during medical treatment cases, but to make a distinction between the efficacies of each drug therapy.

P6-6
Choroidal melanoma stage T1 - planning protocol for one day session stereotactic radiosurgery and proton beam irradiation

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Objective: Comparison of two methods of irradiation of patient with malignant choroidal melanoma in stage T1 by stereotactic radiosurgery and proton beam irradiation.
Case report: We performed stereotactic radiosurgery to treat female patient (born 1939) with malignant melanoma of the choroid stage T1 N0 M0 with TD 35.0Gy. Plan has been drawn up for stereotactic irradiation - model for linear accelerator Clinac. Patient characteristics were compared with the virtual plan for proton radiation therapy. We used the scheme in Physical parameters FIAN-Technical Center in the Russian Federation. We compared both planning protocols and assess in particular the extent of radiation surrounding non-tumor tissue.
Results: By comparing the two planning schemes in this patient with T1 stage intraocular melanoma irradiation levels of surrounding tissues and structures in both cases were corresponding to the required standard.
Conclusion: Therapy of uveal melanoma by proton beam irradiation in Slovakia is not available yet, although it has several advantages, such as fractionation and the possibility of achieving a higher dose of irradiation to deposit (more than 50.0 Gy). The dose to the tumor during irradiation can be optimized. The model device allowed us to make OPTMI - Therapy (Proton Treatment with Optimized Modulated Intensity).

Acknowledgement
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P6-7
Study of mechanisms of interactions between polyalkylcyanoacrylate nanoparticles and lipid monolayers

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Polymeric nanoparticles have been shown to be potential drug delivery vehicles able to load and carry active drugs to diseased cells, such as cancer cells. Focusing to cancer therapy, the drug can be delivered by nanoparticles using the unique pathophysiology of tumor - enhanced permeability and retention effect. In this context, we focused in elucidation of the molecular mechanisms of action of PEGylated polymer nanoparticles of hydrophobic polyalkylcyanocrylate suspended in water. We used polyalkylcyanocrylate nanoparticles of two types: polybutylcyanoacrylate (PBCA) and polyoctylcyanoacrylate (POCA) [1]. Both nanoparticles were PEGylated with Jeffamine M-2070 and Brij 35. The nanoparticle mean size was 180 nm and 160 nm, and zeta potential -20 mV and -18mV, for PBCA and POCA respectively.

The plasma membrane of cells was modelled by Langmuir monolayers composed of zwitterionic dimyristoylphosphatidylcholine (DMPC). Interaction between PBCA (or POCA) with DMPC monolayers were studied by surface tensiometry, where the change in surface pressure upon the addition of particles was monitored and analyzed [2]. Experiments were performed at three different initial surface pressure values of 10 mN/m, 20 mN/m and 30 mN/m. The lower surface pressure is typical for cells undergoing rapid mitotic division, while the surface pressure of 30 mN/m corresponds to the physiologic value in membranes of normal cells. The effect of addition of polyalkylcyanoacrylate nanoparticles was studied in the concentration range 1-80 μg/ml.

We have found that upon the sequential addition of PBCA into the water subphase, surface pressure of DMPC monolayer increased, but only at lower initial surface pressure values. The observed maximal surface pressure change was 9,3 mN/m and 3,7 mN/m at the corresponding initial surface pressure of 10 mN/m and 20 mN/m, respectively. At the initial surface pressure value of 30 mN/m, PBCA did not cause any significant increase in surface pressure. The similar behavior was observed for POCA nanoparticles.

To conclude, tensiometry study showed that polyalkylcyanoacrylate nanoparticles are able to penetrate into lipid monolayer only at subphysiological surface pressure (<30mN/m) and thus can be used as potential drug delivery vehicles into the cancer cells.

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P6-8

Tissular pO_2 measurements by time-resolved luminescence spectroscopy: Correlation of *in vivo* results obtained with Ru(phen) and protoporphyrin IX in different tissue compartments

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The partial pressure of oxygen (pO_2) in biologic tissues plays an important role in the mechanisms taking place during radiotherapy and photodynamic therapy (PDT). Therefore, monitoring this parameter is of high interest to predict the outcome of these treatment modalities. Since the triplet state of most photosensitizers (PS) used in PDT is quenched by molecular oxygen, the pO_2 level can be determined by measuring the luminescence lifetime resulting from the de-excitation of this triplet state. Although (Ru(phen)) exhibits a strong phosphorescence originating from this state, this is not the case for protoporphyrin (PPIX), an approved photosensitizer. This is the reason why the delayed fluorescence of PPIX is detected to measure the pO_2.[1,2] The relation between these luminescence lifetimes and the pO_2 level is given by the Stern-Volmer equation. As Ru(phen) and PPIX present different biodistributions at the microscopic and macroscopic scales, it is of high interest to study the correlation of pO_2 measurements performed simultaneously with these two molecular probes in the same sample. Such a correlation study will provide useful information regarding the tissue compartments in which oxygen plays the most critical role in the mechanisms involved in PDT and radiotherapy. Preliminary *in vivo* results obtained with the chicken egg's chorioallantoic membrane model after topical administration of Ru(phen) and aminolevulinic acid (a precursor of PPIX) demonstrated the feasibility of this correlation study. The different tissue compartments - involving blood vessels (arteries and veins) as well as the extravascular space - were probed with our optical fiber-based time-resolved (micro)-spectrofluorometer.

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