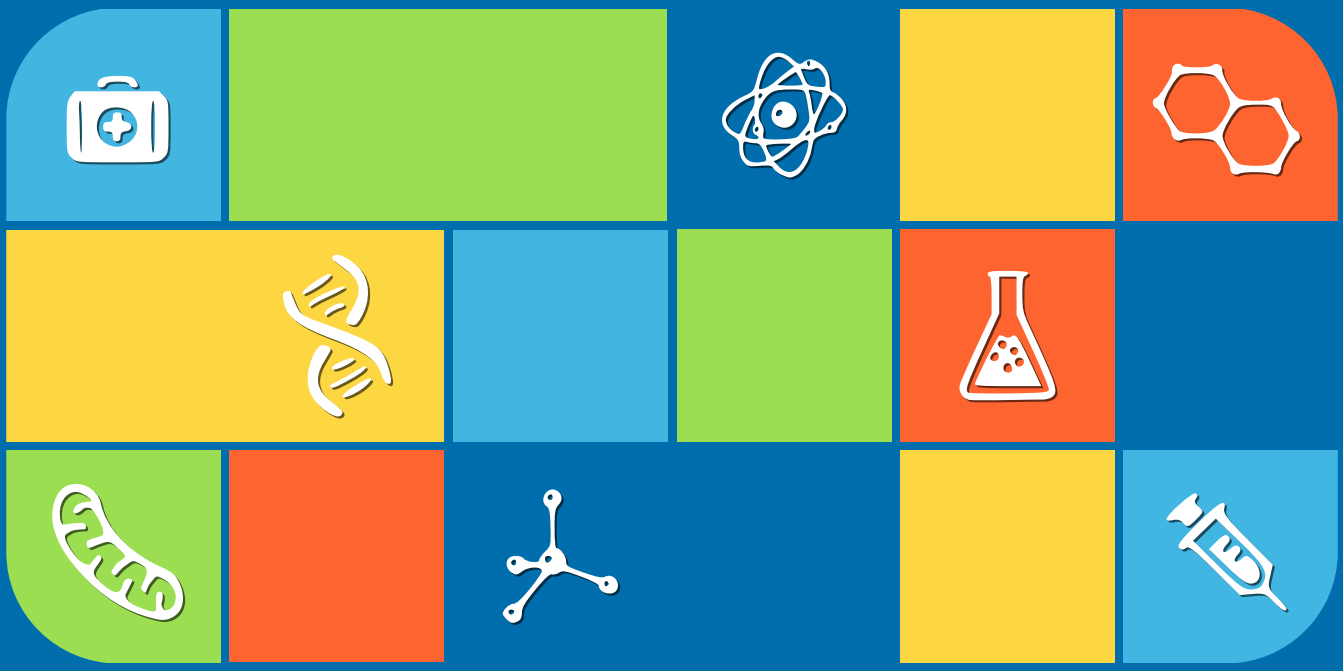
14th Greta Pifat Mrzljak International School of Biophysics



ABC of Physics of Life

BOOK OF ABSTRACTS

Split, Croatia, Aug. 23. - Sept. 01. 2018

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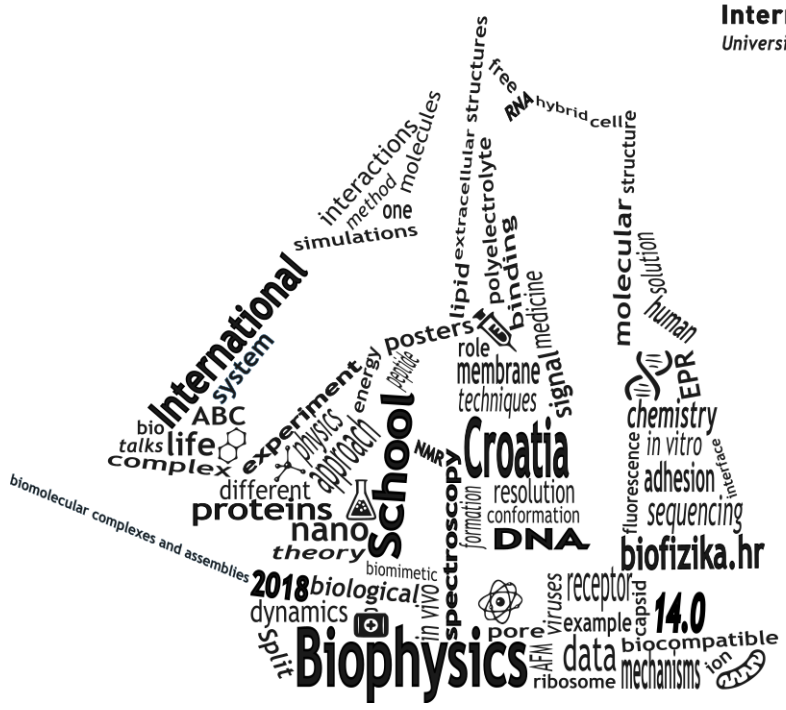
14th Greta Pifat Mrzljak
International School of Biophysics
University of Split, Croatia, Aug. 23 - Sept. 01, 2018



The 14th Greta Pifat Mrzljak International School of Biophysics

ABC of Physics of Life: Book of abstracts

University of Split, Croatia/ August 23rd – September 01st

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FOREWORD

FOREWORD

The School

This session of the International School of Biophysics is the fourteenth in a series held for 37 years across Croatia: in Dubrovnik, Rovinj, Primošten and along the Adriatic coast. The previous sessions were held in 1981, 1984, 1987, 1990, 1994, 1997, 2000, 2003, 2006, 2009, 2012, 2014 and 2016. In the period 1981-2009, the school was chaired by the late Prof. Greta Pifat-Mrzljak, an eminent Croatian biophysicist, president of Croatian Biophysical Society and a member of the International Union for Pure and Applied Biophysics (IUPAB) Council. Primarily for her inspiring achievement with the School, she was also awarded the American Biophysical Society's Emily M. Gray Award.

After passing of prof. Pifat Mrzljak in 2009, it was widely agreed that the school of biophysics for young scientists is still needed. In 2012 the Croatian Biophysical Society and Ruđer Bošković Institute have committed their resources in order to assure the future of the school and a new School was organized as a collective undertaking. Since then, we have had a strong support by EBSA (European Biophysical Societies' Association) and the lecturers themselves, who commit their time and effort preparing courses and lectures which are highly valued by the students of the School.

This year, we coorganized the School with COST (European Cooperation in Science and Technology) networks BM1403 Native Mass Spectrometry (coordinated by prof. Frank Sobott), CM1306 Molecular Machines (coordinated by dr. Fraser MacMillan) and CA15126 ARBRE-MOBIEU (coordinated by prof. Wouter Roos). School is also supported by the Institute of physics, Zagreb and Faculty of Science of Split University which is where many of the members of the organization team work. School is endorsed by Ruđer Bošković Institute and sponsored by NanoTemper technologies, Bruker, JPK Instruments, Oxford Instruments and Asolutic.

Through the years, the School has been attended by more than one thousand Ph.D. students and postdocs interested in becoming acquainted with the state-of-the-art in biophysics. The lecturers at the International School of Biophysics have always included top scientists in their respective disciplines and several Nobel laureates from this area of research, which spans across biology, chemistry and physics. Our intention is to keep the school as one of the focal events for European students and young scientists and to provide these young people with advanced training at the doctoral and postdoctoral levels in the field of biophysics and related fields like molecular and structural biology, physical chemistry, biochemistry, soft-matter physics... The boundaries of the traditional disciplines are not always visible at this School. Indeed, this concept promoted at the previous sessions of the School has already benefited hundreds of young scientists throughout Europe and other parts of the globe. With the passage of years, some of them have become lecturers at the School. With the legacy of Prof. Pifat-Mrzljak in mind, it is the intention of the organizers to position the School as a biennial event, complementary to, rather than competing with, relevant international

activities, e.g., the Regional Biophysics Conference or the EBSA Biophysics Courses.

The prospect for the enthusiastic young people to interact with top scientists in a relaxed manner is elementary in their development and a route to their success in this quickly moving and challenging area. The School is a thrilling experience also for the lecturers, as they get a chance to teach young and inquisitive minds from all over the world. In the past, this interaction has been enhanced by the environment created by the local organizational team. The lectures, workshops and training activities of the 2018 School will be held in the facilities of the new campus of University of Split.

Topics

An enormous amount of new knowledge on the molecular basis of various biological phenomena has emerged in the rapidly expanding field of bioscience. The principles and methods of biophysics provide the underpinning for all basic bioscience and a rational language for discussion among scientists of different disciplines. This was the general philosophy behind the organization of the summer school. The School is intended for young scientists (primarily Ph.D. students) at the beginning of their academic careers who are interested in the fundamental study of biomacromolecules: the structures of nucleic acid/protein complexes (ribosomes, viruses, chromatin), protein aggregation, conformational dynamics, folded and intrinsically disordered proteins, enzymatic activity - small molecule recognition, biomacromolecular interactions, bioenergetics and single molecule biophysics. The particular scope of the school has enabled the participants to become acquainted with state-of-art problem-oriented and/or methodology-oriented approaches to biological systems. Major topics are presented in a series of lectures and workshops, which can be roughly summarized as ABC of Physics of Life, that is:

ASSEMBLING MOLECULAR MACHINES: viruses, ribosomes and other protein-RNA/DNA complexes, quaternary protein structures, DNA, proteins, polyelectrolytes with the focus on their structure, organization and function

Interactions at BIOLOGICAL & BIOCOMPATIBLE interfaces: membranes, adhesion, extracellular matrix, protein-lipid/membrane interactions, biomimetic/hybrid surfaces

CELLS: physical properties of biological and bio-inspired systems

These topics are considered having always in mind the major experimental techniques in biophysics - spectroscopies (NMR, EPR, FTIR, Raman, mass spectrometry ...), microscopies (AFM, fluorescence techniques, super resolution...), diffraction techniques (X-ray crystallography) - computational and theoretical approaches which include modeling and simulations, and the evolutionary implications of molecular interactions, as well as molecular biology and biotechnology.

Students and Lecturers

Last three Schools were attended by 70-85 PhD students coming from all over Europe as well as from Australia, Iran, Armenia, Japan and Russia. EBSA supported 10-12 students each time, while different COST actions support more and more students each time. The structure of the School established from the very beginning, incorporating lectures, seminars and round tables, with emphasis on discussion, has shown to be successful and was later

accompanied by posters sessions and short poster talks held by participants. In addition to the School's inherent role in the transfer of knowledge and ideas, we emphasize its catalytic role in arranging future research collaborations, joint projects, visits and postdoc positions - and friendships. The scientific interaction of the participants among themselves or with the lecturers has always been extremely fruitful and active, often followed by future cooperation. Last but not least, the social contacts among the participants and the lecturers, as well as the contacts with the host Croatian culture, have proven that communication among scientists can be of mutual interest and of interest to Croatia. Students come from all over the world, participate very actively in discussions during or after the lectures, at poster sessions, or even on the beach.

For the fourth time in a row, the School is chaired by Tomislav Vuletić, Institute of physics, Zagreb, and organized by the local team. We wish the Students and Lecturers at the 14$^{\text{th}}$ School a warm welcome and hope that they will enjoy the extraordinary cool and friendly environment that we intend to create as we did at the previous three Schools.

Poem about the School

Biophysics summer school,
It was extraordinary cool.
Afternoons all on the beach,
Maybe once I missed a speech.

Monte Carlo random noise,
Stunning brilliant lecture choice.
Got us out of bed each day,
With some pain, but that's OK.

Short talks really rush your heart,
Yet the concept turned out smart.
Talking simply just went on,
Sometimes even all night long.

Sadly, we have to depart,
Can't wait for the next restart.
Coming years with you to bring,
Please keep up this brilliant thing.

And with that we want to conclude:
"Nosit ćemo vas u srcu svud."

Poem was written by students of the 11$^{\text{th}}$ Greta Pifat Mrzljak International School of Biophysics and read at the Gala dinner on 8$^{\text{th}}$ October 2012.

ORGANIZATION

ORGANIZING TEAM

- **Mario Cindrić**, Ruđer Bošković Institute, Zagreb, Croatia
- **Ida Delač Marion**, Institute of Physics, Zagreb, Croatia
- **Amela Hozić**, Ruđer Bošković Institute, Zagreb, Croatia
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- **Tea Mišić Radić**, Ruđer Bošković Institute, Zagreb, Croatia
- **Antonio Šiber**, Institute of Physics, Zagreb, Croatia
- **Tomislav Vuletić**, Institute of Physics, Zagreb, Croatia **(chair)**
- **Larisa Zoranić**, University of Split, Croatia

ORGANIZED BY

CROATIAN BIOPHYSICAL SOCIETY

CO-ORGANIZED BY

EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

BM1403 NATIVE MS
CM1306 MOLECULAR MACHINES
CA15126 ARBRE MOBIEU

SUPPORTED BY

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UNIVERSITY OF SPLIT

INSTITUTE OF PHYSICS, ZAGREB

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JPK INSTRUMENTS

OXFORD INSTRUMENTS

ASOLUTIC

LECTURERS

Nenad Ban
ETH, Zürich, Switzerland

Mario Cindrić
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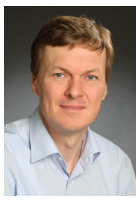
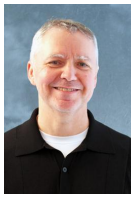


Helmut Grubmüller
Universität Göttingen, Germany



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Ruđer Bošković Institute, Croatia

Anthony Watts
University of Oxford, UK

Primož Ziherl
JSI, Ljubljana, Slovenia

Bojan Žagrović
MFPL, Vienna, Austria

WORKSHOPS

NanoTemper's MST and nanoDSF Workshop

The workshop will cover principles of biomolecular interaction analytics using Microscale Thermophoresis (MST) as well as protein stability and aggregation assessment with use of nanoDSF technology. We strongly encourage to bring your samples for analysis. Experts from Nanotemper will train the students.

Molecular dynamics simulations

This workshop will help you gain first experiences in performing molecular dynamics simulations. After a short theoretical introduction, you will find out about the basic ingredients for a molecular simulation. You will setup your own simulation of a small peptide and run some short simulations of it. Finally, you will analyse the resulting trajectories and find out what computational methods can offer in addition to experimental approaches. Held by by Prof. Zagrovic and Prof. Oostenbrink. Work on Linux PCs at the Faculty of Science, or you bring your own Linux laptop.

Atomic Force Microscopy (AFM)

AFM is a flexible tool to image and probe samples at the nanoscale. The workshop addresses Ph.D students and post-docs, as well as scientists, core facility technicians and engineers that are interested in the application of AFM to biology. A major emphasis will be on the preparation of biological samples for AFM imaging and spectroscopy and experimental activity will be the core of the workshop. Participants will be divided into groups: each group will focus on a specific scientific topic (biomolecules, cells) and each participant will be given opportunity to use AFM instruments provided by leading companies that are supporting the school. The presentation/lecture modules will be used only as an introductory component – as we strive to promote active learning experiences. Held by by Prof. Roos and dr. Ivosevic DeNardis. Trainers are also Dr. Kobiela and dr. Mišić-Radić, as well as the experts from AFM companies. Workshop is supported by COST action CA15126 ARBRE MOBIEU.

Proteome fingerprinting by MS

Mass spectrometry is routinely used to identify pathogens and malignant cell strains by directly fingerprinting their proteome. Prof. Mario Cindric has developed this method to the next level where it can be used for de-novo sequencing of peptides. The method is based on a proprietary chemical derivatization agent for preparation of the protein samples for MS analysis and heavily involves bioinformatics tools to analyze the MS spectra. Held by Prof. Mario Cindrić and Dr. Amela Hozić. Workshop is supported by COST action BM1403 NATIVE MS.

PROGRAM

August 23rd – September 01st

Thursday, August 23rd

08:00–13:00	**ARRIVALS & REGISTRATION**	
13:00-14:00	**Lunch**	
14:00-14:30	**Welcome address**	
14:30-16:15	**Chris Oostenbrink**	*BOKU, Vienna, Austria*
	Ensembles and sampling, leading to molecular dynamics simulations	
	Calculation of free energies from molecular simulation	
16:15	**Coffee break**	
16:45-18:30	**Tomasz Kobiela**	*Warsaw University of Technology, Poland*
	Introduction to bio-AFM	
18:30-20:10	**Short talks:**	
	Martina Petrencakova	*Conformational properties of dark and lit state of AsLOV2 domain*
	Anna B. Dobieżyńska	*Biophysical studies of protein interactions with mRNA transcripts containing different 5′ terminal structures*
	Oleksii Zdorevskyi	*Competitive interaction of water and hydrogen peroxide molecules with specific DNA recognition sites*
	Renata Biba	*Physiological and proteomic responses of tobacco seedlings exposed to silver nanoparticles*
	Dominik Saman	*Evolution of monodispersity in vertebrate HSP27*
	Anna Sobiepanek	*AFM/QCM-D methods in the diagnostics and prognostics of malignant melanoma*
	José Carlos Castillo Sánchez	*Looking for the roots of lung surfactant system: the tighter its package is, the better functionality it has*
20:15-21:30	**Poster session & snacks**	

Friday, August 24th

08:00–09:00	**Breakfast**	
09:00–10:45	**Helmut Grubmüller**	*MPI Göttingen, Germany*
	Forces and Conformational Dynamics in Biomolecular Nanomachines	
10:45–11:15	**Coffee break**	
11:15–13:00	**Frank Sobott**	*Universiteit Antwerpen, Belgium*
	Protein Mass Spectrometry: Defining proteoforms and Going native	
	Dynamic protein structure: From protein disorder to membrane pores	
13:00–14:00	**Lunch**	
14:00–16:00	**FREE TIME**	
16:00–17:45	**Intro for nanoTemper**	
	and/or AFM workshops	
17:45	**Coffee break**	
18:15–20:00	**Bojan Žagrović**	*MFPL, Vienna, Austria*
	On conformational averaging in structural biology	
20:15-21:15	**Dinner**	

Saturday, August 25th

08:00–09:00	**Breakfast**	
09:00	Parallel hands-on activity sessions	
10:45	**Coffee break**	
11:15	Parallel hands-on activity sessions	
13:00–14:00	**Lunch**	
14:00–14:45	**Nanotemper**	*TBA*
15:00	Parallel hands-on activity sessions	
18:30-20:10	**Short talks:**	
	Jelica Milošević	*The difference of amyloid fibril formation after reduction and denaturation of crude protein preparations*
	Ernesto Anoz-Carbonel	*Regulation of FMN biosynthesis in H. sapiens: Kinetics and thermodynamics of the riboflavin kinase activity*
	Elnaz Hosseini	*Conformational and functional studies of a novel thermostable xylanase from camel rumen metagenomic library*
	Guilherme G. Moreira	*Aggregation kinetics and bioimaging of monomeric tau*
	Shona Hepworth	*Studying conformational dynamics of the SLC6 gene family homologue LeuT using advanced EPR techniques*
	Martina Požar	*The structuring in the aqueous trifluoroethanol mixture*
	Andreas Weber	*Studying the effect of anticancer drugs breast cancer cell mechanics via AFM*
20:15-21:15	**Dinner**	

Sunday, August 26th

08:00–09:00	**Breakfast**	
09:00	Parallel hands-on activity sessions	
10:45	**Coffee break**	
11:15	Parallel hands-on activity sessions	
13:00–14:00	**Lunch**	
14:00–14:45	**David Goodlett**	*University of Maryland, Baltimore, USA & University of Gdansk, Gdansk, Poland, EU*
	The potential of Lipid A as a diagnostic and therapeutic	
15:00	Parallel hands-on activity sessions	
18:30-20:10	**Short talks:**	
	Veronika Alexandrova	*Protofilament length dispersion analysis in the context of microtubule catastrophes*
	Duygu Tarhan	*Characterization of intermittent hypoxia effects on bone structure by acoustic impedance*
	Marko Šterk	*The maximum entropy production principle and maximum Shannon information entropy in enzyme kinetics*
	Agathe Vanas	*Advancing Orthogonal Spin Labeling and pulsed EPR Distance Measurements*
	Charlotte Frankling	*Exploring the Binding Interfaces of the Type III Secretion System Chaperone-Translocator Complex*
	Yerko Escalona	*Exploring properties of soil organic matter at the microscopic level by using molecular dynamics and an upgraded Vienna Soil-Organic-Matter Modeler*
	Melanie Kampe	*Hydrophobins: the Microstructure of self-organized Films of amphiphilic fungal Proteins*
20:15-21:15	**Dinner**	

Monday, August 27th

08:00–09:00	**Breakfast**	
09:00	Parallel hands-on activity sessions	
10:45	**Coffee break**	
11:15	Parallel hands-on activity sessions	
13:00–14:00	**Lunch**	
14:00–14:45	**David Goodlett**	*University of Maryland, Baltimore, USA & University of Gdansk, Gdansk, Poland, EU*
	From a history of shotgun proteomics to the importance of longitudinal analysis for biomarker discovery in proteomics	
15:00	Parallel hands-on activity sessions	
18:30-20:10	**Short talks:**	
	Diana-Larisa Roman	*Computational assessment of biological effects of some xenobiotics*
	Catarina de Sousa Lopes	*Changes on elasticity and morphology of erythrocytes from amyotrophic lateral sclerosis patients*
	Giulia di Prima	*Casein-loaded proteoliposomes: novel delivery strategy to inhibit $A\beta_{1-40}$ fibrillogenesis in Alzheimer disease*
	Mohammad Behnam Rad	*Diagnostics applications of paper-based microfluidics*
	Emanuela Colucci	*Mg^{2+} transport in mitochondria*
	Borna Radatović	*Process for forming ready-to-use QCM sensors with atomically flat surface suitable for scanning probe microscopies*
	Tamas Gerecsei	*Single cell adhesion measurements using computer controlled micropipette and FluidFM BOT*
20:15-21:15	**Dinner**	

Tuesday, August 28th – Excursion

08:00-21:00	**EXCURSION by ferry to Island of Brač**

Wednesday, August 29th

08:00–09:00	**Breakfast**	
09:00–10:45	**Fraser MacMillan**	*University of East Anglia, Norwich, UK*
	EPR spectroscopy: The journey from a niche biophysical technique towards a universal structural biological tool	
	Magnets, Microwaves and Molecular Movement: An EPR Spectroscopist's View	
10:45	**Coffee break**	
11:15–13:00	**Heinz-Jürgen Steinhoff**	*University of Osnabrück, Germany*
	Multi-frequency EPR spectroscopy of membrane proteins	
13:00–14:00	**Lunch**	
14:00–16:00	**FREE TIME**	
16:00–17:45	**Nikolina Sekulic**	*University of Oslo, Norway*
	Hydrogen-deuterium exchange in studying protein dynamics	
17:45	**Coffee break**	
18:15–20:00	**Felix Rico**	*Aix-Marseille Université, France*
	Mechanics of single proteins probed with AFM	
20:00-21:30	**Poster session & snacks**	

Thursday, August 30th

08:00–09:00	**Breakfast**	
09:00–10:45	**Nenad Ban**	*ETH, Zürich, Switzerland*
	Protein synthesis: from ribosome assembly to targeting of membrane proteins	
10:45	**Coffee break**	
11:15–13:00	**Holger Stark**	*Max Planck Institute for Biophysical Chemistry, Göttingen, Germany*
	How to determine 3D structures of macromolecules by cryo-EM	
13:00–14:00	**Lunch**	
14:00–16:00	**FREE TIME**	
16:00–17:45	**Anthony Watts**	*Oxford University, Oxford, UK*
	Principles of biological solid state NMR	
	NMR of membrane proteins - Drug targeting	
17:45	**Coffee break**	
18:15–20:00	**Petr Novák**	*Institute of Microbiology, Prague, Czech Republic*
	Structural proteomics: from protein chemical modification to cross-linking and beyond	
20:00-21:30	**Poster session & snacks**	

Friday, August 31st

08:00–09:00	**Breakfast**	
09:00-10:45	**Ana-Sunčana Smith**	*Ruđer Bošković Institute, Zagreb, Croatia*
	Physics of cell adhesion: The role of the membrane in the protein recognition process	
10:45	**Coffee break**	
11:15-13:00	**Iva M. Tolić**	*Ruđer Bošković Institute, Zagreb, Croatia*
	Forces that divide the chromosomes	
13:00–14:00	**Lunch**	
14:00–16:00	**FREE TIME**	
16:00–17:45	**Antonio Šiber**	*Institute of physics, Zagreb, Croatia*
	Surface-tension-based models of tissues, Part I	
17:45	**Coffee break**	
18:15–20:00	**Primož Ziherl**	*JSI, Ljubljana, Slovenia*
	Surface-tension-based models of tissues, Part II	
20:30	**Dinner & Farewell Party**	

Saturday, September 01th

08:00–09:00	**Breakfast**
09:00–	**DEPARTURES**

LECTURE ABSTRACTS

NENAD BAN

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Protein synthesis: from ribosome assembly to targeting of membrane proteins

We are investigating bacterial and eukaryotic ribosomes and their functional complexes to obtain insights into the process of protein synthesis. Building on our studies of bacterial ribosomes we have increasingly shifted our attention to studying eukaryotic cytosolic and mitochondrial translation and were successful in obtaining first insights into the atomic structures of eukaryotic and mammalian mitochondrial ribosomes [1, 2, 3], which pose a significant challenge for structural studies as they are more complex and heterogeneous than their bacterial counterparts. The focus of our research has been to understand eukaryotic translation initiation, targeting of proteins to membranes, regulation of protein synthesis, and the assembly of eukaryotic ribosomes [4, 5, 6]. The complete molecular structure of the unusual mammalian mitochondrial ribosome specialized for synthesis of membrane proteins was one of the first examples of electron microscopic structure determinations that allowed de-novo building, refinement and validation of the structure. These results revealed the interactions between tRNA and mRNA in the decoding centre, the peptidyl transferase center, and the path of the nascent polypeptide through the idiosyncratic tunnel of the mammalian mitochondrial ribosome. Furthermore, the structure suggests a mechanism of how mitochondrial ribosomes, specialized for the synthesis of membrane proteins, are attached to membranes [3].

[1] Rabl J, Leibundgut M, Sandro F. Ataide SF, Haag A and Ban N. (2010) Nature 331(6018):730-6

[2] Klinge S, Voigts-Hoffmann F, Leibundgut M, Arpagaus S, Ban N. (2011) Science, 334, 941-8

[3] Greber BJ, Bieri P, et al. (2015) Science. 348(6232):303-8

[4] Erzberger JP et al. (2014) Cell 158(5):1123-35

[5] Greber BJ, et al. (2016) Cell 164(1-2):91-102

[6] Kobayashi K, Jomaa A, et al. (2018) Science. 360(6386):323-327

MARIO CINDRIĆ

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Sequencing of peptides/proteins and organisms ID

The organism identification tests are used to characterize microorganisms in human body, environmental samples (microorganisms and organisms) or simply to identify organisms of interest (*e.g.* what we have had for lunch?). Currently used techniques for species identification are mostly relied on genome database matching (limited to 20 000 species), commercially available protein database matching (limited to 3000 species) or alternatively on immunological procedures (limited to a couple of hundreds species). Although widely used, such an approach does not always provide satisfactory matching, sequence coverage or specific antigen-antibody reaction to unambiguously identify DNA, RNA, lipid, and sugar or peptide/protein of selected species. Mass spectrometry as a tool for species determination was introduced about ten years ago. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identifies microbes using either intact cells or cell extracts. The most abounded proteins from the cell detected in the instrument are matched against commercially available databases that cover relatively limited number of microbes. On the other hand, high resolution mass spectrometers in contrast to MALDI-TOF MS could provide more accurate and precise results after *de novo* sequencing of analyzed proteins. A brief historical retrospective, as well as state-of-the art methodology of species identification will be covered in the lecture.

DAVID R. GOODLETT

University of Maryland, Baltimore, MD USA &
University of Gdansk, Gdansk, Poland, EU
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The potential of Lipid A as a diagnostic and therapeutic

Lipid A is the membrane anchor for Gram-negative bacteria that holds the much larger lipopolysaccharide (LPS) molecule in place in the outer membrane. Importantly in mammals, Toll receptor 4 (TLR4) recognizes lipid A the result of which is activation of a cytokine cascade that can aid the host in clearing the infection or if unchecked lead to a deadly cytokine storm. There are a range of activities from agnostic to antagonistic that are directly related to structure (e.g. [1]). To exploit this we are working to better define the lipid A structure activity relationship for use as a vaccine adjuvants and antisepsis therapeutics (e.g. [2]). We are also using lipid A and related Gram-positive molecules to identify bacteria direct from source in under an hour [3]. We are also interested in investigating the classic use of bacterial extracts as an immunotherapy (i.e. Coley's toxins late 1800s NYC) that have been recently revived [4].

[1] Li Y, Yoon SH, Wang X, Ernst RK, Goodlett DR. (2016) Structural derivation of lipid A from Cronobacter sakazakii using tandem mass spectrometry. Rapid Commun Mass Spectrom. 2016 Oct 30;30(20):2265-70

[2] Scott AJ, Oyler BL, Goodlett DR, Ernst RK. Lipid A structural modifications in extreme conditions and identification of unique modifying enzymes to define the Toll-like receptor 4 structure-activity relationship. Biochim Biophys Acta. 2017 Nov;1862(11):1439-1450

[3] Leung LM, Fondrie WE, Doi Y, Johnson JK, Strickland DK, Ernst RK, Goodlett DR. (2017) Identification of the ESKAPE pathogens by mass spectrometric analysis of microbial membrane glycolipids. Sci Rep. Jul 25;7(1):6403

[4] Kim OY, Park HT, Dinh NTH, Choi SJ, Lee J, Kim JH, Lee SW, Gho YS. (2017) Bacterial outer membrane vesicles suppress tumor by interferon-γ-mediated antitumor response. Nat Commun. 2017 Sep 20;8(1):626

From a history of shotgun proteomics to the importance of longitudinal analysis for biomarker discovery in proteomics

This presentation will be in part a history lesson of the development of mass spectrometry based proteomics. Specifically, we will review the work of Prof Don Hunt who during the 1980s led efforts to sequence MHC peptides [1] for use as cancer vaccines. His work led to the development of what we know today as shotgun proteomics [2]. The story begins with the development of the triple quadupole mass spectrometer by Prof Enke in 1978, moves to development of nanoHPLC by Prof Jorgenson and ESI by Prof Fenn both in the mid 1980s. While none of these three developments came directly from Prof Hunt's laboratory, he quickly and cleverly combined them to identify and sequence active MHC peptides present in complex mixtures at attomole levels on column by the early 1990s [1]. From there we will look at how automation drove the field to be more competitive with genomics, how quantitative analysis in proteomics began and how recently longitudinal samples has emerged as an important tool in biomarker discovery. Among others, examples will be given from our work in pediatric diabetes [3, 4] and more recently sepsis the latter of which is unpublished.

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Forces and Conformational Dynamics in Biomolecular Nanomachines

Proteins are biological nanomachines. Virtually every function in the cell is carried out by proteins ranging from protein synthesis, ATP synthesis, molecular binding and recognition, selective transport, sensor functions, mechanical stability, and many more. The combined interdisciplinary efforts of the past years have revealed how many of these functions are effected on the molecular level [1]. Computer simulations of the atomistic dynamics play a pivotal role in this enterprise, as they offer both unparalleled temporal and spatial resolution. With state of the art examples, this talk will illustrate the type of questions that can (and cannot) be addressed, and its (current) limitations. The examples include aquaporin selectivity [2], high affinity ligand unbinding [3, 4], mechanics of energy conversion in F-ATP synthase [5, 6], and the mechanism of antibiotics within the ribosome [7, 8, 9]. We will further demonstrate how atomistic simulations enable one to mimic, one-to-one, single molecule experiments such as FRET distance measurements, and thereby to enhance their accuracy [10]. We will, finally, take a more global view on the 'universe' of protein dynamics motion patterns and demonstrate that a systematic coverage of this 'dynasome' allows to predict protein function more reliably than purely structure- based methods [11].

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EPR spectroscopy: The journey from a niche biophysical technique towards a universal structural biological tool

Electron Paramagnetic Resonance (EPR) or Electron Spin Resonance (ESR) spectroscopy is the only technique that detects unpaired electrons unambiguously. It is typically used to identify the nature of the molecule hosting the unpaired electron; since EPR is very sensitive to local environments it can provide information both on the molecular structure of that molecule (structure, dynamics, binding) and through application of further advanced studies it can also investigate its molecular environment ($<$ 0.8 nm for nuclear spins and up to 5.0 nm for other electron spins).

Sometimes, the EPR spectra exhibit dramatic line shape changes, providing insight into dynamic processes such as molecular motions or fluidity. The EPR spin-trapping technique, which detects short-lived, reactive free radicals, nicely illustrates how EPR detection and identification of radicals can be exploited. This technique has been vital in the biomedical field for elucidating the role of free radicals in many pathologies and toxicities. EPR spin-labelling is a technique used by biochemists whereby a paramagnetic molecule (a spin label) is used to "tag" macromolecules in specific regions. From the EPR spectra reported by the spin label, they can determine the type of environment (hydrophobicity, pH, fluidity, etc.) in which the spin label is located.

ESEEM and ENDOR are two examples of advanced EPR methods that measure the interactions of the electron with nuclei from the immediate surrounding. They are extremely powerful techniques for probing the structure of "active sites" in metalloproteins. Another important application for quantitative EPR is radiation dosimetry. Among its uses are dose measurements for sterilization of medical goods and foods, detection of irradiated foods, and the dating of early human artefacts.

One of the most popular pulsed EPR experiments currently is double electron-electron resonance (DEER), which is also known as pulsed electron-electron double resonance (PELDOR). This uses two different frequencies to control different spins in order to detect the strength of their coupling. The distance between the spins can then be inferred from their coupling strength (in the range of 2 – 12 nm). A typical experimental approach focuses on the use of various EPR techniques in combination with molecular biological, biochemical and other biophysical methods including theoretical MD approaches which studies the structure and dynamics of large bio-molecules through determination of such coarse-grained structural constraints.

Magnets, Microwaves and Molecular Movement: An EPR Spectroscopist's View

Research in the Henry Wellcome Unit for Biological EPR at UEA focuses on the architecture and functional dynamics of membrane proteins, many medically relevant with a special interest on membrane transport systems and their interaction with intra-cellular signalling pathways. There is increasing evidence that membrane proteins do not act alone, but that they are organised as nano-machineries which function through the concerted action of its individual components with high precision and specificity observed in both time and space. We seek to unravel the principles underlying the architecture and dynamics of these protein nano-machineries as well as their function and regulation. Our experimental approach focuses on the use of Electron Paramagnetic Resonance (EPR) techniques in combination with molecular biological, biochemical and other biophysical methods including theoretical MD approaches. Our expertise lies in the development and application of EPR techniques to address these key questions.

I will review the methods before focusing on recent method developments, which are allowing a shift of focus of this technique away from being considered purely a niche technique towards a more universal structural biological tool. I will use examples from our recent work on membrane and metallo-proteins (myoglobin, terminal oxidases & nitrate reductase), membrane transport proteins including ABC and secondary active transports to demonstrate the range and power of this technique to deliver key mechanistic insight into e.g. how to resolve multiple distances in complex macromolecular complexes, how to observe conformational change within membrane proteins at a molecular level and finally to identify the molecular determinants of ligand binding.

This research is funded by The Royal Society and the Wellcome Trust as well as being embedded within the current EU COST Action CM1306 "Understanding Movement and Mechanism in Molecular Machines".

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Structural proteomics: from protein chemical modification to cross-linking and beyond

Even the first protein structural models were built using X-ray crystallography and NMR spectroscopy sixty and thirty years ago, respectively. There are still many protein sequences and protein complexes with unknown 3-D structure. The tremendous progress in mass spectrometry in last decades opened the space for studying protein folding, protein/ligand interactions and protein dynamics in solution.

A common feature of protein chemical labeling and cross-linking in conjunction with mass spectrometry (MS) is the identification of a novel covalent bond formed upon treatment of the protein by a modifying agent. Labeling experiments assume that the degree of exposure to the solvent correlates with the reactivity of the target sites on the protein landscape. Sterically protected or heavily H-bonded parts of protein are modified to a lesser extent than those that are freely accessible to solvent. Several different types of chemical probes are currently in use targeting polypeptide backbone (hydrogen/deuterium exchange) or amino acid side chains (chemical probes, hydroxyl radicals). The output of the MS analysis of modified proteins is the knowledge of surface accessible and/or reactive area of the protein molecule which enables to monitor protein dynamics in solution or formation of non-covalent protein-ligand complexes. In contrast to protein labeling, the cross-linking experiment introduces covalent bonds between specific amino acids side chains via bi-functional chemical probes that serve as molecular rulers. The subsequent MS analysis unambiguously identifies connected amino acids side chains and obtained distance constrains are utilized as a starting point for the design of 3D-structural model of proteins and protein complexes when combined with computational modeling approaches. Thus the combination of the covalent labeling, cross-linking and mass spectrometry significantly contributes to the understanding of protein conformations and their dynamics.

The potential of structural proteomics techniques such as covalent labeling, chemical cross-linking and hydrogen/deuterium exchange for structural biology will be discussed.

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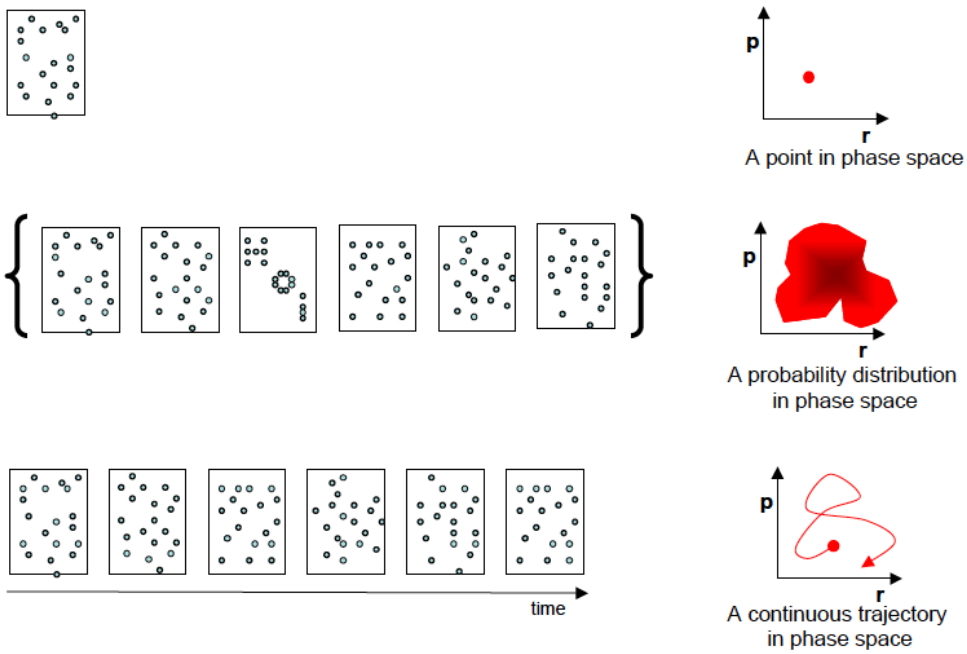
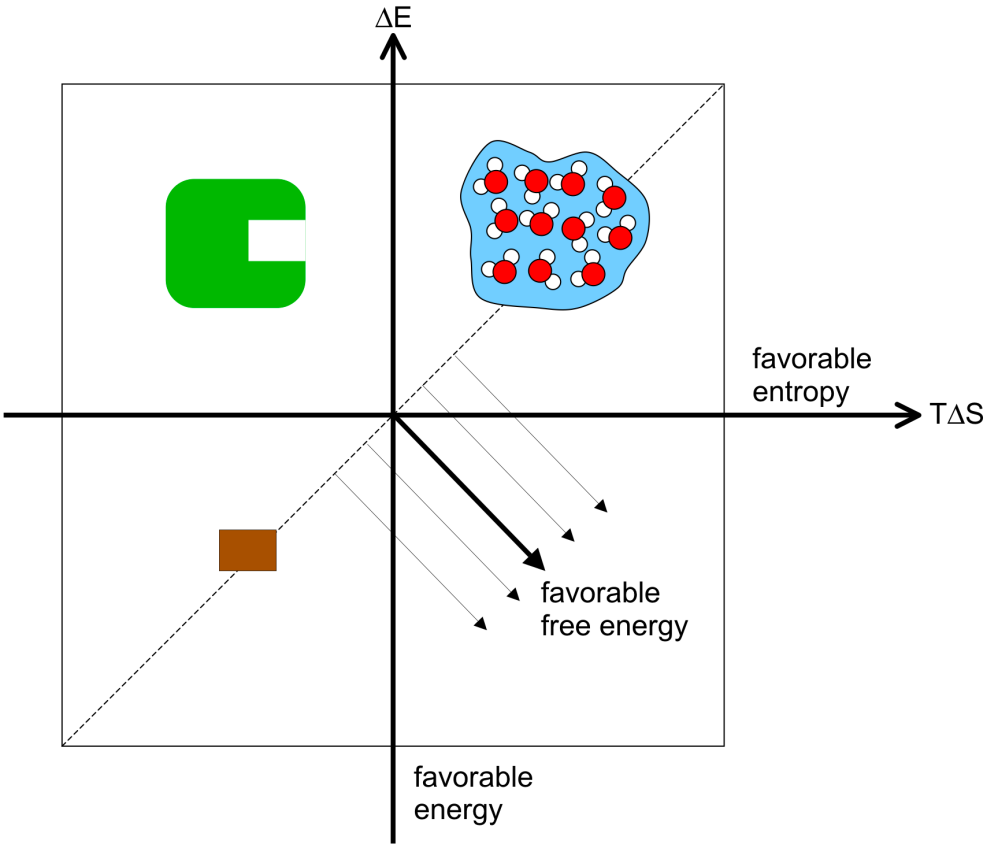
Ensembles and sampling, leading to molecular dynamics simulations

In this lecture, we will discuss the basics of molecular dynamics simulations. Starting from the link between a molecular structure and the (potential) energy of a system, we will discuss various algorithm to modify the structure in physically meaningful ways. This will lead to the definition of ensembles and the computational tools to generate ensembles of complex biomolecular systems. We can subsequently use the statistical mechanical ensembles to calculate averages of molecular properties which may be compared to experimental data directly. Next, we will mention methods to define ensembles at different thermodynamic state points. Finally, we will discuss possibilities to use experimental observations as boundary conditions for molecular simulations, in order to e.g. refine molecular structures. Using NMR parameters like NOE distance restraints or ^{3}J-coupling constants as examples, the various ways of restraining the molecular structure will be discussed.



Calculation of free energies from molecular simulation

The free energy forms the driving force of any molecular process. Intrinsically containing both enthalpic and entropic contributions, the accurate estimation of free energies is possible from statistical mechanical principles. This lecture will use the free energy of ligand binding as a representative example for which such calculations may be performed. We will first introduce the various actors and their enthalpic or entropic contribution to the binding affinity and subsequently focus on the alchemical free energy methods that can be used to calculate the free energies. Real case examples from our own work will be used to demonstrate the use of the methods.



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Mechanics of single proteins probed with AFM

The mechanical properties of individual proteins play a crucial role during essential cellular processes such as migration, muscle contraction and adhesion. For example, cytoskeleton proteins are stretched and may unfold during cell contraction, while adhesion complexes link the cell cytoskeleton with the extracellular space and support mechanical force. Thus, knowing the mechanical response at the single molecule level is important to understand biological function. While the number of crystal structures of proteins has been growing over the last decades, the number of tools that directly measure protein mechanics is still limited. Atomic force microscopy (AFM) [1] is a unique technology that combines nanometric-imaging capabilities with piconewton force resolution. Here, I will review some of the available approaches to quantify single protein mechanics using AFM [2, 3]. I will describe the basic principles of the technique, the various force spectroscopy modes and related theoretical developments for data interpretation. Finally, I will introduce recent advances, such as high-speed force spectroscopy [4, 5], and its combination with molecular dynamics simulations.

Keywords: protein flexibility, protein mechanics, protein unfolding, compression, elasticity, atomic force microscopy, force, binding strength, high-speed AFM

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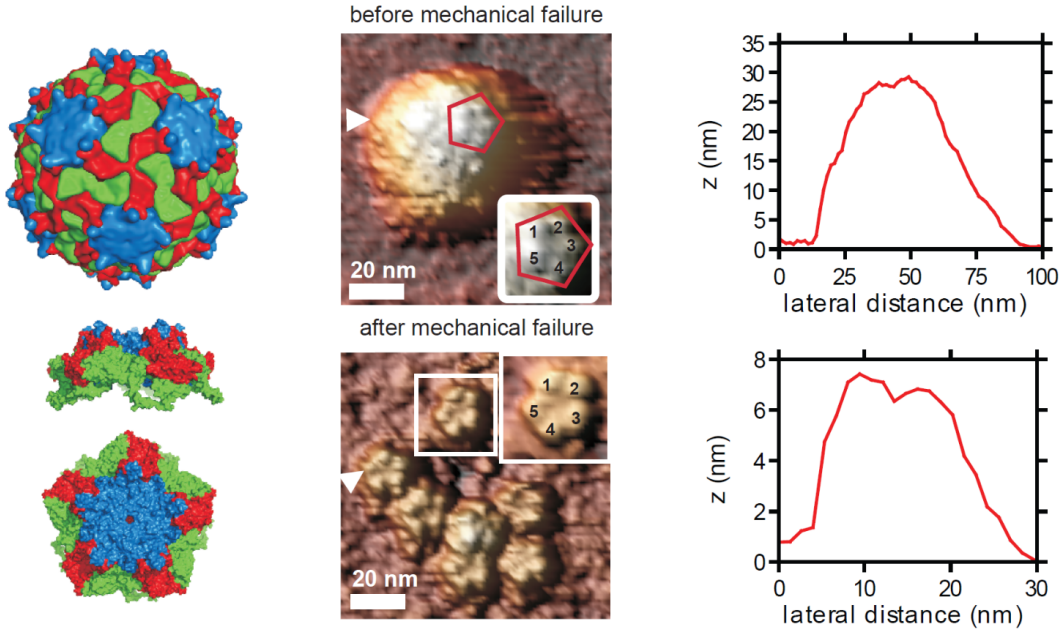
Introduction to bio-AFM

Cellular life harbours a fascinating variety of complex processes and we are still at the beginning of our understanding of how the cell manages all these processes. Using Atomic Force Microscopy imaging and force spectroscopy we are now making big steps towards the elucidation of the mechanisms behind (supra)-molecular cellular processes. Here I will provide an introduction to the principles of AFM imaging and sample manipulation [1]. Building onto this background knowledge, I will show how we are studying the physics of the fascinating sub-cellular dynamics. This will be illustrated by discussing the mechanics and material properties of viruses and cellular protein nanocages; In particular by revealing the existence of pre-stress in nanoshells, by scrutinizing the interactions between viral RNA and its capsid and by showing how viral infectivity is in essence a mechanical process [2, 3, 4]. Furthermore, recent studies on viral assembly and disassembly will be discussed. Finally, extracellular vesicle dynamics is presented, currently a hot topic in nanomedicine as these particles are expected to have great potential in diagnosis and treatment [5, 6].



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Hydrogen-deuterium exchange in studying protein dynamics

The pattern of hydrogen bonds between the amino and carbonyl group in the main chain of proteins is critical for the stability of protein secondary structural elements, such as α-helices and β-strands. The formation of secondary structures governs protein folding and eventually yields to a specific protein tertiary structure. However, rather than having only one static conformation, even when completely folded, proteins are in equilibrium between several different conformations. Ligand binding, posttranslational modifications or different cellular environments can shift this equilibrium, favoring some conformation(s) over other(s). The main-chain hydrogen-bonding pattern often differs between different conformations. By monitoring the rate at which main-chain hydrogen bonds are formed and broken, we can gain insights into the existence and stability of different protein conformations. The technique of hydrogen-deuterium exchange (HDX) is based on the fact that, when proteins are incubated in buffer with D_2O, they incorporate deuterium in their main-chain amide group, if they are able to form a hydrogen bond with the solvent. This happens more quickly for amide groups on the surface of the protein than for those that are buried in a protein interior. In addition to providing useful structural and dynamic data for different aspects of protein analysis, hydrogen-exchange is extremely powerful in providing a dynamic component to high-resolution structural approaches like X-ray crystallography and NMR. In my lecture I will explain basic principles behind HDX, possible detection methods and instrumental set-up, together with advantages and challenges in using this approach. I will also give examples (from literature and from my own lab) for application of the technique in addressing different biological problems.

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Physics of cell adhesion: The role of the membrane in the protein recognition process

In embryogenesis, vertebrate cells assemble into organized tissues. In metastatic cancer, cells spreading in the circulatory system build cell-cell contacts with the surrounding tissue to establish new tumors. At the root of these life-forming or life-threatening biological phenomena is cell adhesion, the binding of a biological cell to other cells or to extracellular matrix. The most obvious fundamental question to ask is then as follows: What factors control or govern cell adhesion? For a long time, the paradigmatic answer to this question was that specific protein molecules embedded in the cell wall (or membrane) were responsible for cell adhesion, in either a key-lock fashion (in cell-cell adhesion) or a suction-cup fashion (in cell-matrix adhesion). But, a new realization has emerged during the past two decades that physical mechanisms, promoted by the cell membrane, play an unavoidable, yet not fully understood role. Although these physical elements, namely membrane fluctuations and ability to change shape, do not at all depend on any specific proteins, they can have a major impact on the protein-mediated adhesion, and can be viewed as mechanism that controls the binding affinity to the cell-adhesion molecules. In my talk I will show how these mechanisms can be studied in mimetic models both experimentally and theoretically, the result of which will be discussed in the cellular context.

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Protein Mass Spectrometry: Defining proteoforms and Going native

In classic, "bottom-up" proteomics approaches the samples (e.g. cellular extracts) are often very complex with up to 10 000 different proteins present. "Bottom-up" means that after a – typically tryptic – digest, the peptide mixtures are analysed for the presence of proteins, their (relative) amounts, and sometimes also for post-translational modifications. Often data from a few unique peptides per protein is sufficient for this type of analysis, as it typically delivers a little bit of information about a lot of proteins. Finding out, on the other hand, a lot about one protein or complex however requires a different approach. It is often underestimated how important sequence variations (e.g. truncations, mutations, frame shifts) and post-translational modifications are for protein function. Such detailed, molecular characterization typically works with intact, undigested proteins which can be denatured (so-called "top-down" MS) or even remain under non-denaturing conditions ("native MS") which allows to study higher-order structure such as folding, conformational ensembles and formation of complexes. In this first lecture, we apply the knowledge of electrospray ionization, tandem MS and ion mobility to study various aspects of protein primary to quaternary structure, illustrating the experimental strategy with a number of examples.

Dynamic protein structure: From protein disorder to membrane pores

This lecture focuses on aspects of dynamic and heterogeneous protein conformations and assemblies, using an integrated structural approach based on "native" mass spectrometry, ion mobility, and other biophysical methods. We will briefly introduce the different mass spectrometry-based Structural Proteomics approaches, and highlight the type of data which they can generate, and how they can be integrated with other structural information and with computational models. Specifically, we are going to show recent results on the detection and characterization of intrinsic disorder in proteins, including alpha-synuclein and the apoptosis-related BAX protein. A range of folding states, from disordered to compact, are characterized and interpreted using molecular dynamics approaches. These data link the conformational state of the protein with their association into larger oligomers, which are believed to be able to form membrane pores. We use detergent micelles, lipid bilayers (bicelles) and nanodiscs for both native MS and covalent labelling of exposed parts of the protein, and apply these techniques to various different ion channels including the mechanosensitive channel of large conductance (MscL). Using covalently attached, charged ligands inside the MscL channel, we can mimic the effect of mechanical pressure on the surrounding membrane and characterize various opening states using ion mobility-MS, electron microscopy, EPR spectroscopy and other biochemical and computational methods, in the absence of lipids.

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How to determine 3D structures of macromolecules by cryo-EM – Methodological aspects

Single particle cryo electron microscopy (cryo-EM) has developed into a powerful technique to determine 3D structures of large macromolecular complexes. Due to improvements in instrumentation and computational image analysis, the number of high-resolution structures is steadily increasing. The method can be used to determine high-resolution structures but also to study the dynamic behavior of macromolecular complexes and thus represents a very complementary method to X-ray crystallography. It requires macromolecular complexes purified to homogeneity and imaged in a cryo-electron microscope at low temperatures. 3D structures can then be computed from the 2D projection images using advanced computational image processing tools. The basic idea of the single particle cryo-EM method will be described in the lecture as well as the requirements on samples and the current limitations of the technology.

Visualizing the dynamic behavior of macromolecules by cryo-EM

The number of high-resolution structures obtained by cryo-EM is still exponentially growing. Even though the number of recently solved high-resolution structures is impressive, the resolution peak in the EMDB database is still rather in the 5-6 Angstrom regime which does not allow building of atomic models. A typical observation is also that large parts of a macromolecular complex can be observed at low resolution only whereas high resolution can be obtained for the core of the complex. A certain amount of motion represents the typical limitation in cryo-EM structure determination which is related to the intrinsic dynamic behavior of macromolecules and normally results in a resolution loss. However, using advanced image processing tools it is now also possible to study this dynamic behavior and extract valuable information about the conformational dynamics of macromolecules. This information can be very useful to correlate internal motion with function and visualize effects such as the allosteric regulation of a complex by drug binding.

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Multi-frequency EPR spectroscopy of membrane proteins

Electron paramagnetic resonance (EPR) spectroscopy in combination with site-directed spin labeling (SDSL) has emerged as a powerful method to study the structure and conformational dynamics of membrane proteins. EPR spectroscopy of spin label side chains introduced into a protein provides structural and dynamic information for restraint modeling of protein domains, protein-protein or protein-lipid interaction and for following conformational changes with high temporal and spatial resolution. Double electron-electron resonance (DEER) spectroscopy not only reveals average interspin distances but also provides distance distributions making this approach very valuable for determining heterogeneity of complex systems, e.g., of membrane protein complexes in their native environment or for characterizing thermodynamic equilibria of protein conformations. The first part of the lecture will cover the basics of SDSL EPR and new developments in the area of SDSL [1, 2]. The second lecture will present applications with examples on the conformational dynamics of the halobacterial phototaxis sensory rhodopsin-transducer complex and transmembrane substrate transporters. Chemotaxis and phototaxis of bacteria and archaea regulate cell motility through two-component signal transduction pathways. The lecture will provide an overview about the findings of the last decade, which served to understand the basic principles microorganisms use to adapt to their environment. We document the time course of a signal being perceived at the membrane, transferred across the membrane [3] and show, how this signal modulates the dynamic properties of a HAMP domain [4], an ubiquitous signal transduction module found in various protein classes.

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Surface-tension-based models of tissues

Many mechanical aspects of epithelial tissues can be interpreted in terms of models where cells are viewed as drops of incompressible liquid carrying a surface tension. We review the basic theoretical concepts related to this model and the key experiments supporting it. We discuss the differential adhesion hypothesis as an early yet still fresh incarnation of the surface-tension-based model, which can explain tissue aggregation, mixing, engulfment, and dispersal. After sketching the general aspects of the in-plane structure of epithelia, we turn to the tension-related elements of physical models of these tissues. Then we describe the tension-based models of non-trivial epithelial shapes such as folds, which highlight the role of apico-basal internal stresses in the formation of these shapes. We also discuss the theoretical models of gastrulation in *Drosophila*, the differential-adhesion-hypothesis interpretation of convergent extension, the two-drop model of epiboly in zebrafish embryo, and selected other applications of the model including the recent results obtained within the dynamical 3D vertex-model implementation.

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Forces that divide the chromosomes

Our bodies are built from around 100 trillion individual cells, each of which contains 46 chromosomes that carry our genetic material. This enormous number of cells originates from a single cell that is the product of fertilization of an egg with a sperm. Thus, cell division is one of the most fundamental processes in the living world. At the onset of division the cell assembles the mitotic spindle, a fascinating and complex micro-machine made of microtubules and the accompanying proteins. The microtubules move the chromosomes around and finally line them up in the middle of the spindle. When all chromosomes are ready, sister chromatids are moved apart towards the opposite spindle poles. The central question in this field of research is how the mitotic spindle manages to divide chromosomes into two equal sets without errors. Understanding how proper chromosome segregation is achieved in cells and how it is controlled will help to identify new strategies for prevention and treatment of diseases such as cancer and genetic disorders caused by chromosome segregation errors. By taking an interdisciplinary approach, in which we combine cell and molecular biology, cutting-edge microscopy, optogenetics, laser microsurgery, computer science and theoretical physics, my group explores how the mitotic spindle self-assembles and how forces that move the chromosomes are generated.

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Principles of biological solid state NMR

Lecture Synopsis:

- NUCLEAR MAGNETIC RESONANCE: FUNDAMENTALS
    1. Sample form for solid state NMR
    2. Why solid state NMR – comparison with solution state NMR
    3. Concept of anisotropy, membrane examples
    4. The magic angle
    5. Magic angle spinning, cross polarization, and recoupling
    6. Distance measurements through dipolar recoupling
    7. Isotopic substitutions
    8. Instrumental requirements
- STRUCTURAL BIOLOGY
    1. Silk fibres
    2. Amyloids
    3. SH3 domain
    4. Antibiotic confirmation at site of action
    5. Oriented peptides in membranes
    6. Retinals in photoreceptors

Solid state NMR can be applied to a wide range of sample morphologies and hydration states, and for large (MWt $\gg$ kDa) systems, is ideally placed to complement other structural methods[1]. In particular, fibrous proteins, membrane systems and crystalline systems have been studied, with very high resolution atomistic details being resolved through the use of recoupling and magetization transfer approaches[1, 2]. NMR is a short-range method, and so nuclear detail over short distances can be resolved. In addition, since anisotropy can be exploited in oriented systems, the vectorial arrangements of secondary structure elements can be resolved, something that is lost in isotropic approaches. Here, the principles of solid state NMR will be explained in a practical way, and then some illustrative examples presented[3, 4, 5, 6].

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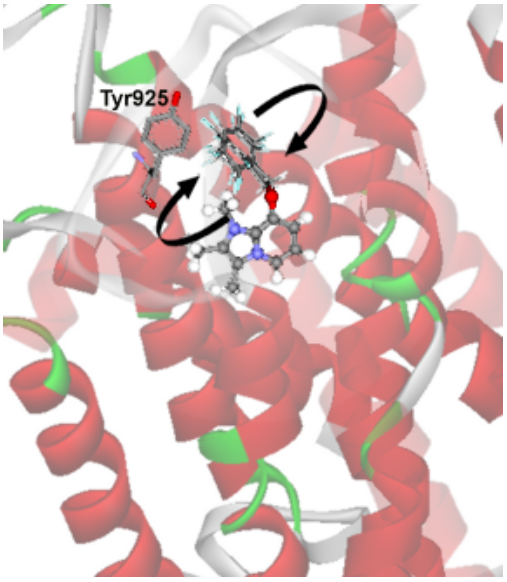
NMR of membrane proteins - Drug targeting

Systems studied



1. H/K-ATPase and peptic ulcer inhibitors
2. Mapping inhibitor binding site
3. Importance of dynamics in drug design
4. Na/K-ATPase and oubain conformation
5. Ligand gated ion channel, nAChR
6. Cation-p interactions, ligand conformation
7. Understanding the gating mechanism
8. GPCRs

The interactions between macromolecules and small molecules take place on a wide range of timescales. Probing their structure and dynamics is a major challenge, especially for membrane targets, and such information is required to supplement rigid atom detail and functional description, where available. It is now possible to resolve local dynamics within a membrane bound protein at near physiological conditions in natural membrane fragments or in reconstituted complexes, using solid state NMR approaches[1, 2]. This information is obtained by isotopically (^{2}H, ^{1}3C, ^{1}9F, ^{1}5N, ^{1}7O) labeling selective parts of either a ligand, or the protein understudy, and observing the nucleus in non-crystalline, macromolecular complexes[3, 4, 5].

Ligands with complex structure have differential mobility at their binding sites. Substituted imidazole pyridines, for example, which inhibit the H^+/K^+-ATPase and have therapeutic use, are constrained in the imidazole moiety, but shows significant flexibility at the pyridine group[6] (see figure). It is this group which has a direct interaction with an aromatic (phe198) residue, with the potential for p-electron sharing[7]. Similarly, the steroid moiety of ouabain undergoes motions which are similar to those of the protein, but the rhamnose undergoes a high degree of flexibility at fast rates of motions whilst interacting with Tyr198[8]. The quaternary ammonium group of acetyl choline, undergoes both kinds of interaction which are driven by thermal fluctuations and may be functionally significant[9, 10]. More recently, challenging GPCR-ligand interactions have been examined, ahead of crystal studies[11].

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Lecture 1: More dynamic than we think? On conformational averaging in structural biology

The majority of experimental methods in structural biology provide time- and ensemble-averaged signals and, as a consequence, molecular structures based on such signals often exhibit idealized, average features. Moreover, most experimental signals are only indirectly related to real, molecular geometries, and solving a structure typically involves a complex procedure, which may not always result in a unique solution. To what extent do such conformationally-averaged, non-linear experimental signals and structural models derived from them accurately represent the underlying microscopic reality? Are there certain structural motifs that are actually artificially more likely to be "seen" in an experiment simply due to the averaging artifact? Finally, what are the consequences of ignoring the averaging effects when it comes to functional and mechanistic implications of experimentally-based structural models? Here, I will discuss these questions with a particular focus on nuclear magnetic resonance, X-ray scattering methods and different types of spectroscopy and address their individual susceptibility to conformational (motional) averaging.

Lecture 2: RNA-protein interactions and the origin of the genetic code

The relationship between mRNA and protein sequences as embodied in the genetic code is a cornerstone of modern-day molecular biology. However, a potential connection between physico-chemical properties of mRNAs and cognate proteins, with implications concerning both code's origin and mRNA-protein interactions, remains largely unexplored. In this talk, I will present some recent evidence which both supports as well as markedly redefines the stereo-chemical hypothesis concerning the origin of the genetic code i.e. that the code evolved as a consequence of direct interactions preferences between nucleobases and amino acids. Importantly, I will explore the possibility that the physicochemical rationales, which led to the development of code's structure, may still be relevant in present-day cells.

TRAINERS CONTRIBUTIONS

Accurate identification of microorganisms

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Majority of currently used LC-MS/MS techniques for protein identification is based on database matching of non-derivatized peptide signals recorded in positive ion mode. Although widely used, such an approach does not always provide satisfactory sequence coverage to unambiguously identify a protein sample.

Protein de novo sequencing concept using CAF-/CAF+ reagent (chemically activated fragmentation negative/chemically activated fragmentation positive) enables fast, highly accurate, reliable and easy to use identification of microorganisms down to the species and subspecies level. CAF-/CAF+ (5-formylbenzene-1,3-disulfonic acid*) is a chemical reagent for the derivatization of peptide samples prior to analysis by tandem mass spectrometry (MS/MS) that enables gathering of both positive and negative peptide ions datasets. Peptide sequences are read from both positive and negative MS/MS spectra and matched against the NCBInr database by developed software named ProteinReader.

We present results that show identification of microorganisms down to the subspecies level using either MALDI- or ESI-TOF technique.

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Nanomechanical characterization of marine microalgal cells in buffer solution

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Marine microalgae as the globally important primary producers, drivers of essential biogeochemical cycles as well as the basis of the aquatic food web. Many stressors (e.g. salinity, temperature, pH, light, nutrients, pollutants, toxicants) in the aqueous environment affect algal cell growth, adaptation, physiological activity, and complex biochemical processes in the cell, which may have consequences in the food web structure. In addition to their ecological roles as oxygen producers and as the food base for almost all aquatic life, algae are economically important for biofuel production and as sources of food and pharmaceutical and industrial products. The unicellular green flagellate *Dunaliella tertiolecta (D. tertiolecta)* is widespread in aquatic ecosystems due to their tolerance to a wide range of salinities, light intensities, and temperature. *Dunaliella* lacks a rigid polysaccharide cell wall and is enclosed by a thin elastic plasma membrane covered by a mucous surface coat and possesses pronounced motility. Aim is to determine and compare the mechanical properties of algal cells before and after exposure of culture to high concentration of heavy metal which may help us to understand better algal cell response and adaptation to stress condition. Students will be introduced with immobilization procedure of motile algal cells to a modified substrate. High resolution AFM imaging will be used to determine structural details. AFM coupled to fluorescence microscopy will be used to determine nanomechanical properties of the cells and to correlate mechanical properties with certain cellular structure.

Application of AFM technique for monitoring the treatment of selected skin diseases

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Skin diseases are a problem affecting a growing percentage of the population. One of the most common dermatological diseases is atopic dermatitis (AD) (*dermatitis atopica / eczema atopicum*). Lack of diagnostic tests which would enable an easy and precise identification of this disease and its variable clinical features often cause a problem in diagnosis. AD is a condition caused by a large number of endogenous and exogenous factors that interact with various processes of the immune system. Atopic dermatitis is characterized by a wide spectrum of clinical features which differ accordingly to patients' age. Symptoms include strong pruritus, exudative changes, scabs, abnormal dryness of the skin or changes in its pigmentation. Due to differences in the course of the disease, the type of treatment is selected individually. Treatment programs include both daily care in the form of skin moisturizing as well as a number of local and oral medicines, phototherapy or systemic therapies.

The aim of the study was to conduct a research of corneocytes of people affected by atopic dermatitis. We present a process for imaging and analyzing topographical and mechanical properties of corneocytes in their most native conditions, combining a novel method for tape stripping, AFM characterization and statistical analysis. Both cells from pathological areas, as well as from those showing no pathological changes for comparative purposes, were used for the study. Our results indicate a probable relationship between the Young's modulus and the health state of the cells.

Role of EPR spectroscopy in syntehesis and sterilization of dental bone graft materials based on calcium sulfate

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Calcium sulfate (CaS) based materials are frequently used as dental implants. CaS is higly biocompatible material and does not interfere with healing process [1]. In the use, there are two modifications, calcium sulfate dihydrate (CSDH) and calcium sulfate hemihydrate (CSHH). Before the implementation all implants, does not matter of the origin, need to be sterilized and it is usually done with X or gamma irradiation. Unfortunately, the research of irradiation on the implants based on phosphates are scarce [2], but for sulfates are not to be find. Briefly it should provoke the formation and stabilization of radicals. That is why the structures of CSDH and CSHH as well as commercial material Bond bone, which is the mixture of both, are investigated before and after the gamma irradiation with electron paramagnetic resonance (EPR) spectroscopy, thermogravimetry (TG) and Fourier transform infrared spectroscopy (FTIR). The results have shown that EPR spectroscopy is a method which can be used to improve the quality control of bone graft materials after syntering, processing and sterilization procedure.

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Supramolecular structure of marine gel studied by atomic force microscopy

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Self-assembly of marine biopolymers into gel phase has emerged as a dominant concept of abiotically formed organic particles in seawater. It is suggested that about 10% of surface seawater dissolved organic matter (DOM) can self-assemble as polymer gels. A broad polydispersity of marine gels ranges from microscopic to macroscopic dimensions and the massive appearance of gelatinous macroaggregates known as 'mucilage have been reported for a few coastal seas including northern Adriatic Sea. The phenomenon manifests itself in a massive appearance of large amounts of gelatinous aggregates up to several metres in diameter in the water column ('clouds') and on the sea surface covering up to tens square kilometres, as documented by satellite images.

Determination of the supramolecular structure of marine gel networks emerges as a key step towards understanding mechanism of marine gel formation and understanding the role of the gel phase in the ocean. Here, we introduced atomic force microscopy (AFM) as a direct imaging technique to reach this goal.

PARTICIPANT CONTRIBUTIONS

Protofilament length dispersion analysis in the context of microtubule catastrophes

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A property called dynamic instability enables microtubules (MT), biological molecular motors assembled from tubulin polymers, to abruptly switch between elongation and shortening phases, promoting an ability of effective cellular space investigation when searching and capturing chromosomes in mitosis. Rapid disassembly of microtubules, termed catastrophes, occurs many times during the microtubule life. Still, the mechanism of catastrophe transition remains unclear. One of the well-documented fact is that catastrophe events are more likely for microtubules with a longer lifetime, a phenomenon termed aging. A hypothesis of gradual tapering tip was suggested [1] to explain the uneven catastrophe distribution through the MT lifetime. To confirm the hypothesis, a mathematical model of microtubule dynamics was used, as well as the results of total internal fluorescence microscopy. However, we have demonstrated that the established model found no tapering in the usual sense of the word. Our simulation of non-hydrolysable tubulin dimers in terms of kinetic approach revealed two cases when morphological changes at the MT tip could have long-term effect depending on parameter sets. In the first case an increase of catastrophe frequency is turned out to be mostly a consequence of artificially implemented limitations, which eventually give rise to implausible forms of the microtubule tip that would be detectable with cryo-electron microscopy, if any. Meanwhile the second mechanism provide more "natural" tip configuration by gaining more autonomy over interprotofilament interaction. In addition, our theoretical calculations were supplemented with experimental underpinnings as we propose an explanation of the observed tapering in TIRF microscopy basing on peculiarities of the method. This analysis expands and illuminates existing ideas and what's more, implies to review some widely held assumptions about catastrophe triggers.

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Regulation of FMN biosynthesis in *H. sapiens*: Kinetics and thermodynamics of the riboflavin kinase activity

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The human riboflavin kinase (HsRFK) exhibits ATP:riboflavin kinase activity (RFK) and, therefore, catalyzes the biosynthesis of the essential cofactor flavin mononucleotide (FMN) [1, 2]. This flavin cofactor, together with flavin adenine dinucleotide (FAD), is key essential not only in energetic metabolism but also as cofactor of a plethora of flavoproteins and flavoenzymes [3]. Hence, its production should be thoroughly regulated to maintain the cellular and flavoproteome homeostasis. In our group, we have previously characterized the catalytic activity of the C-terminal RFK modules of different prokaryotic bifunctional FAD synthetases (FADS) [4], enzymes exhibiting sequence and structural similarity with the eukaryotic monofunctional counterparts. Herein, we focus on the regulation of the FMN synthesis in Homo sapiens through the kinetic and thermodynamic characterization of the interactions of HsRFK with their substrates and products, in the context of the available structural information.

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3D modeling of BmpA, BmpB, BmpC and BmpD from *Borrelia burgdorferi*

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B. burgdorferi is one of the main Borrelia species causing Lyme disease in humans. The pathogens are transmitted by the *Ixodes* ticks, and there are 60 000 – 200 000 Lyme disease infections in Europe annually. The BmpA, BmpB, BmpC and BmpD proteins are expressed by *B. burgdorferi* in infected patients, but the exact role of the proteins is still unknown. The Bmp proteins are reported to be homologous to *T. pallidum* PnrA (Purine nucleoside receptor A), which has been characterized as a substrate-binding lipoprotein of the ATP-binding cassette (ABC) transporter family, preferentially binding purine nucleosides. Based on our 3D homology models, the Bmp proteins share the typical fold of the substrate-binding protein family. Moreover, the residues involved in binding the ribose moiety of the nucleoside are highly conserved in the Bmp models, whereas the residues in the purine binding site are less conserved. In particular, the BmpC model has differences in the residues binding the base moiety of the nucleoside. In conclusion, the revealed differences indicate that the Bmp proteins could prefer different nucleosides and, thus, might have distinct biological functions.

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NMR study on interaction of silver nanoparticles with biothiols

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Introduction of nanoparticles (NPs) to biological environments results in the formation of the so-called "biomolecular corona" – a layer of adsorbed biomolecules on the surface of NPs. The composition and nature of corona and its impact on pharmacodynamics and pharmacokinetics of thusly-modified NPs is a major question in nanomedicine. In this study, we employed nuclear magnetic resonance (NMR) to evaluate interaction of silver NPs with glutathione (GSH), an important biothiol. The selected silver NPs were functionalized with three distinct coatings - non-ionic polyvinylpyrrolidone (PVP), positively charged poly-L-lysine (PLL), and negatively charged sodium bis(2-ethylhexyl)sulfosuccinate (AOT). The experiments involved exposing different silver NPs to GSH, analysis of size and surface charge by light scattering technique and tracking the change in NMR spectra of GSH.

Diagnostics applications of paper-based microfluidics

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SHORT TALK

Diagnosis is a critical first step in effectively managing infectious diseases spread. On the other had early detection is very crucial in the treatment of cancer disease. But prevalent and precise diagnostic technologies such as enzyme-linked immunosorbent assay and polymerase chain reaction are not accessible in resource-limited regions. Microfluidics is the technology of engineered manipulation of fluids on sub micrometers scales and through its application, we can have precise control of fluids and rapid processing of samples beside using inexpensive materials. Paper-based microfluidic devices have lots of applications and had been used for blood typing, detection of molecular biomarkers and pathogenic microorganisms. These systems are used as analytical systems for diagnostics with several advantages such as low cost, high availability in undeveloped regions, user-friendly, rapid and precise detection. Microfluidic paper-based analytical devices (μPADs) are easily scalable for large-scale production and because of their cheap raw material could be used as the disposable point of care testing systems.

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Thermodynamics of interactions within ternary complexes of eIF4E, 4E-BP and mRNA 5' cap

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The eukaryotic translation initiation 4E factor (eIF4E) is a highly conserved small globular protein, which is responsible for recognition and selective binding of an mRNA 5' terminal regulatory structure called "cap". The interaction of eIF4E with the cap is particulary important, since this is a rate-limiting step of initiation of protein biosynthesis and therefore plays a crucial role in cell development, growth and survival [1]. The elevated level of eIF4E leads to the efficient translation of oncoproteins and is closely related to the progression of cancer. eIF4E is thus thought to be a promissing target of anticancer therapy [2]. A thermodynamic approach to the search for small molecule inhibitors of eIF4E is still an open idea [3].

The goal of the studies was to determine thermodynamic parameters of the binary interactions of eIF4E with chemical mRNA 5' cap analogues, and within ternary complexes including 4E-BP1. The results obtained reveal the relationship between the cap analogues structure and thermodynamic parameters of the binding (ΔH^0, ΔS^0, ΔG^0, ΔC_p^0). This approach provided us with a deeper insight into the association process of mRNA 5' cap analogues with eIF4E.

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SHORT TALK

Physiological and proteomic responses of tobacco seedlings exposed to silver nanoparticles

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Since silver nanoparticles (AgNPs) are the dominating nanomaterial in consumer products, there is a growing concern about their impact on the environment. Although numerous studies on the effects of AgNPs on living organisms have been conducted, interaction of AgNPs with plants is still not fully clarified. To reveal the plant mechanisms activated after exposure to AgNPs and to differentiate between effects specific for nanoparticle and ionic silver, we investigated physiological and proteomic changes in seedlings of tobacco (*Nicotiana tabacum*) after 30 day-exposure to commercial AgNPs and $AgNO_3$. The higher Ag content was measured in seedlings exposed to AgNPs than to $AgNO_3$ of the same concentration. However, obtained results on oxidative stress parameters revealed that in general higher toxicity was recorded in $AgNO_3$-treated seedlings compared to those exposed to nanosilver. The presence of silver in the form of nanoparticles was confirmed in the root cells, which may explain the lower toxicity of AgNPs. Proteomic study showed that both AgNPs and $AgNO_3$ can affect photosynthesis. Moreover, majority of the proteins involved in the primary metabolism were up-regulated after both types of treatments, indicating that enhanced energy production, which can be used to reinforce defensive mechanisms, enables plants to cope with silver-induced toxicity.

Quantitative analysis of overlapped peaks

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One of the main goals in chromatography is to achieve optimum resolution in the minimum time. Often this is simply not possible, or it presents a timeconsuming obstacle. Resolution values less than 1.5 between two partially separated peaks do not ensure accurate and precise quantitative analysis of components separated to a degree at which the area or height of each peak may be accurately measured. For that reason, we have developed a mathematical tool that can predict peak shape and area of potentially several unseparated peaks in such complex signals. In our research we have used the Exponential Gaussian Hybrid (EGH) function to model the single peak [1].

We have also tested our method on the following peak types that are frequently mentioned in the literature, the partial cases of the universal peak shape: Gaussian, Cauchy, Pseudo - Voigt (additive mixture of Gaussian and Cauchy), Fraser-Suzuki (asymmetric Gaussian), Laplace, asymmetric Laplace and asymmetric Cauchy [2].

Because the measured signal is impaired by noise, some preprocessing is required. Denoised signal is then, using the Levenberg-Marquardt (LM) method, decomposed to a sum of EGH functions such that their superposition yields the processed signal with relative L^2 error ranging from 0.2% to 2%.

We have developed a universal and robust tool that can reconstruct complex chromatographic signal consisting of several unseparated chromatographic peaks.

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Rapid intact DNA/RNA analysis via ESI-MS

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The use of nucleic acids within research for treatment of genetic diseases and cancer has become increasingly more prevalent. Specifically, research into small RNA and nucleic acids associated with CRISPR is of particular value within this field. Therefore, rapid analysis of nucleic acids with high accuracy is crucial for quality control and sequence confirmation.

This research outlines a method for rapid analysis of DNA and RNA oligonucleotides via HPLC-QTOF-ESI-MS. This was achieved by trapping the oligonucleotide on a C18 guard column whilst removing buffers and salts, before flowing through the column in the opposite direction to remove the nucleic acid and direct it to the Mass Spectrometer.

The method successfully ionised 14 out of the 16 samples analysed. Oligonucleotide length appears to be the primary factor determining the ability of the method to retain the nucleic acid, and it is proposed this is due to the varying polarity of the molecules. Further, a poly C-10mer was not retained. Further investigation is required to determine the cause of this lack of retention.

This method provides a suitable method for the desalting and analysis of small oligonucleotides, with a specific focus on oligonucleotides with mixed base composition. Further work is required to optimise the method for a broader range of oligonucleotides, and to determine the upper limit of analysis.

Encapsulation of a fibrinolytic agent in lipid vesicles and its clot degradation activity

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There is increasing evidence for a consistent association of denser fibrin clot structure and higher resistance to degradation (fibrinolysis) with cardiovascular diseases (CVDs). CVDs account for nearly one-third of deaths worldwide and there is an urgent need to overcome this scenario. Fibrin polymerization starts by thrombin-mediated cleavage of fibrinopeptides A and B from the N-terminal of Aα- and Bβ-chains of fibrinogen, respectively. This exposes small residue sequences, knobs A and B, that interact with their respective binding pockets on the C-terminal region of the Aα- and γ-chains of another fibrinogen molecule, leading to the formation and growth of protofibrils, which culminates in fibrin fibers. The aim of the work is to develop an encapsulated fibrinolytic nanoparticle strategy with lower bleeding risk, to be incorporated in the clot structure. We studied the impact of the empty liposome nanoparticle on blood clot formation and lysis and demonstrated that it does not affect haemostasis properties, by recording clot polymerization and lysis kinetics. Using dynamic light scattering and zeta potential assays, we concluded that the nanoparticle is stable over time, without any measurable aggregation or change in its surface charge. Turbidimetry studies showed that the presence of the nanoparticles reflected a non-significant increase in fibrin fiber radius, protofibril packing and protein content with increasing lipid concentrations. The fibrinolytic agent tissue plasminogen activator (tPA) was added as liposome cargo, using two different methods of encapsulation, with one of them achieving 90% encapsulation efficiency. Ultracentrifugation was used to separate non-encapsulated material without triggering liposome nanoparticle aggregation. Preliminary results demonstrated a controlled release of tPA in a solid emulation of a clot, without activity loss. The work is now focused on optimizing the liposome nanocarrier by surface decoration with a targeting element toward fibrin clots.

Looking for the roots of lung surfactant system: the tighter its package is, the better functionality it has

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SHORT TALK

The alveolar epithelium is a large structure where gas exchange takes place along respiratory compression-expansion cycles. To enable such an indispensable physiological process, alveolar type II cells (ATII) synthesize and secrete the so- called lung surfactant (LS). LS is a complex composed of lipids and proteins and its mainly function is to reduce the surface tension in the alveolar air-liquid interface. Historically speaking, pulmonary surfactant has been exhaustively studied using surface-active materials purified from bronchoalveolar lavages of animal lungs. To deepen in the biophysical properties of LS, we ought to go back to the drawing board. The main reason is that LS is packed into particularly active structures called lamellar bodies (LB) by ATII. Due to respiratory mechanics, LBs form a multilamellar membrane film into the alveolar air-liquid interface. Additionally, LS is recycled by ATII and alveolar macrophages closing its metabolic cycle. Therefore, several LS structures coexist in the alveolar aqueous subphase.

In this context, understanding how lipids and proteins are packed into LBs forming a freshly synthesized LS is increasingly relevant in biomedicine and biophysics. On the one hand, their functional properties have been proposed as an alternative to treat respiratory pathologies where LS functionality is impaired, because surfactant administration therapies have not been effective. On the other hand, LBs have been described as intriguingly dehydrated membrane structures where surfactant proteins have extremely important roles and lipid protein interactions are unknown and engaging to understand the molecular mechanisms underlaying the synthesis and activity of LS system.

Here, we propose a ground-breaking approach to obtain a human LS structured as LBs: from human amniotic fluid. Experimentally speaking, we have compared its structure and surface-active properties with those of a standard LS purified from bronchoalveolar lavages of porcine lungs. To address this objective, we have employed several biophysical techniques and we confirmed that the surface-active material obtained from human amniotic fluid is assembled into LB like structures.

Mg^{2+} transport in mitochondria

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Mg^{2+} is one of a few absolutely essential ions for every living organism. It is the most abundant divalent cation and its biological importance is highlighted by its critical role in cellular energy metabolism, DNA transcription and replication, protein translation and many signal transduction systems, membrane stability, and hormonal regulation. I investigate the mammalian Mg^{2+} transporter Mrs2, located in the mitochondria, which is distantly related to the bacterial CorA family of proteins. Currently only the structure of Mrs2 soluble domain is known [1], which is not sufficient to deduct the transport mechanism of this important molecular machinery. In my work I am using a combination of macromolecular X-ray crystallography and cryo-electron microscopy. In this contribution I will present my preliminary results on the expression screening and purification of this important biological target.

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Characterization and functionalization of transferred synthesized MoS_2 monolayer

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Significant scientific and technological interest in two-dimensional (2D) materials is closely related to the fact that today's microelectronic devices are based on 2D architectures. Additionally, properties of 2D materials can be easily tuned *e.g.* by chemical functionalization or mechanical modulation. Along with the most famous and widely researched 2D material, graphene, attention is increasingly turning to other 2D materials, especially those with intrinsic semiconductor properties. One such material is molybdenum disulphide (MoS_2) monolayer, a prominent member of the transition metal dichalcogenide (TMD) family. We have explored MoS_2 monolayers synthesized on a SiO_2/Si wafer and transferred to Ir(111), which enabled detailed characterization, in particular scanning tunneling microscopy and spectroscopy (STM/STS). The established transfer procedure is applicable to a diversity of substrates, thus opening a way for different applications. The samples were extensively characterized during every step of the transfer process, and MoS_2 on the final substrate examined down to the atomic level [1]. Conducted procedures yielded high quality monolayer MoS_2 of millimeter-scale size with an average defect density in the same range as samples obtained by exfoliation from the bulk MoS_2. The lift-off from the growth substrate was followed by a release of the tensile strain as measured by photoluminescence (PL). Additional functionalization with tetrahedron shaped DNA origami constructs with thiol-groups in three of the vertexes resulted in 16 fold increase in PL signal, while the overall spectral shape remains unchanged. This research advances interdisciplinary research between DNA nanotechnology and 2D TMD based device technology. The strong and stable PL from defects sites of MoS_2 may have promising applications in optoelectronic devices.

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Casein-loaded proteoliposomes: novel delivery strategy to inhibit Aβ_{1-40} fibrillogenesis in Alzheimer disease

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Background: Alzheimer's disease (AD) is a chronic and progressive syndrome, which represents the most common cause of dementia worldwide. A pathological and characteristic AD hallmark is the deposition of amyloid plaques, composed by well-ordered amyloid β-peptide (Aβ) fibers, in brain tissue. The Aβ aggregation process follows typical nucleation-polymerization kinetics, characterized by structural intermediates with specific dimensions, morphologies and cytotoxic activity. Some evidences shifted researchers' attention to smaller soluble Aβ prefibrillar oligomers as they result the most toxic species. Therefore, novel therapeutic strategies target oligomers or prefibrillar aggregates rather than mature fibers. In particular, α_{s1}-Casein, a natural bovine milk protein, resulted able to slow down the entire fibrillogenesis process, increase the lag-time of the nucleation phase and sequester the Aβ peptide on its surface [1].

Future perspectives and scope: to benefit from the remarkable therapeutic option represented by α_{s1}-Casein in AD treatment, it is crucial to define a way to efficiently protect proteins and deliver them to the brain in appropriate amounts. Liposomes are spherical phospholipids-based vesicles characterized by excellent biocompatibility and biodegradability, low toxicity, ability to incorporate and protect both hydrophilic and hydrophobic drugs as well as ability to cross the Blood-Brain Barrier (BBB) in order to access the CNS. Based on these considerations, novel proteoliposomes composed by phospholipids, cholesterol and α_{s1}-Casein were prepared in order to exploit the potentiality of liposomes in brain delivery together with the fibrillogenesis inhibition activity of α_{s1}-Casein. The proteoliposome preparation protocol was optimized in order to obtain the best results in terms of protein-loading and stability. Nanosystems have been characterized by different biophysics techniques, such as light scattering, zeta-potential, circular dichroism, fluorescence and AFM imaging.

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Optimising protein backbone parameters combining Hamiltonian reweighting and elaborate search schemes

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Proteins play a very important role in everyday life of living organisms from bacteria to humans. They are very versatile macromolecules and their function often depends upon their shape and structure. Therefore it is crucial to describe the properties and dynamics in the protein backbone as accurate as possible in molecular dynamics simulations.

The parameterisation of force fields relies on the comparing of properties obtained from wet-lab experiments to quantities observed in simulations. Using single amino acids with an extended backbone as model system, we simulated all 20 canonical amino acids and parameterised to fit the experimental values on J-values and secondary structure propensities obtained by Avbelj, Grdadolnik and co-workers [1, 2]. In order to achieve sufficient sampling in the parameter space Hamiltonian reweighting was used in combination with heuristic seach- and sampling schemes [3, 4]. First the best fitting parameters were obtained by Monte Carlo search scheme and the most promising candidates were then further optimised using steepest descent minimisation on the shifts and force constants. Finally the best sets obtained, are simulated and validated on an set of 40 proteins.

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Biophysical studies of protein interactions with mRNA transcripts containing different 5′ terminal structures

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In the eukaryotic cell, the mRNA 5′ cap [1] is an essential structure that allows efficient mRNA translation, limits mRNA degradation, directs pre-mRNA splicing and mRNA export from the nucleus, as well as allows recognition of viral RNAs as "non-self". In eukaryotes, mRNA is protected by a 5′ cap structure consisting of an N7-methylguanosine moiety linked by a triphosphate bridge to the first transcribed nucleoside. In higher eukaryotes, the mRNA cap can be further modified by methylation of ribose 2′-O. Viruses have also evolved mechanisms to protect their RNA's end against degradation and to be translated by the host machinery [2]. Viral mRNAs that carry only a triphosphate group at its 5′ terminus (pppN) are recognised by human IFIT proteins [2], directly or indirectly. IFITs are expressed in cells infected by viruses and affect the innate immune system. They block viral proteins biosynthesis leading to inhibition of virus multiplication.

The aim of the work was to rank the affinities between the IFIT proteins and mRNA transcripts containing different 5′ terminal structures. Firstly, we estimated qualitatively the interactions of IFIT1 and IFIT5 proteins with mRNA fragments by a competitive assay, involving Bis-ANS as a fluorescent probe. Secondly, we quantitatively measured the direct protein-mRNA binding kinetics by biolayer interferometry. Both approaches yielded concordant results, that the preferential ligand of both IFIT1 and IFIT5 is the pppG-capped transcript, and the binding is more efficient for the former protein. These results provided us with a deeper insight into how IFIT proteins recognized the mRNA 5′ terminal cap structures.

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Protein self-association

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Monomeric protein structure can form dimers, trimers and other aggregates induced by different types of stressors. During protein association process different types of bonding may occur, such as covalent, especially disulfide bonds, and non-covalent bonds: hydrogen bonds, electrostatic interactions, Van der Waals interactions and hydrophobic bonds. Protein structure complexity makes mechanism of aggregates emergence entirely unrevealed or poorly described.

Affected by these stressors, covalent and non-covalent bondage may occur and produce irreversible or reversible protein aggregates. Irreversible aggregates can be produced through heating, freezing-thawing, over-concentrating, isomerization, oxidation etc. On the other hand, reversible aggregates or self-associates might be formed by the aforementioned processes but most likely by agitation [1].

We examined formation of dimers, trimers and tetramers on rHuG-CSG, also known as Granulocyte Colony Stimulating Factor, induced by agitation through time period of 60 - 600 s. Analysis was performed immediately after agitation by liquid chromatography (gel permeation) at pH = 7.0 (50 mM NH4HCO3 mobile phase). Due to increased pressure caused by centripetal acceleration during agitation monomeric structures merges and makes dimers, trimers, tetramers and other aggregates. Increased agitation time results in significant increase of reversible self-associate quantity.

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Effect of liposome composition on calcium phosphate precipitation

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Biomineralization is formation of hard tissues in organisms [1]. From physical - chemical point of view it can be considered as a precipitation/crystallization process of inorganic minerals within the organic matrix and/or confined environments. The liposomes, closed lipid bilayers, are typical experimental model of confined environment in which biomineralization can occur [2]. The influence of lipid molecules' structure or their chemical composition and charge (anionic, cationic, zwitterion), on biomineralization is still not fully understand. The aim of this study is to investigate the influence of three lipids (dimyristoylphosphatidylcholine, DMPC, dimyristoylphosphatidylserine, DMPS and phosphatidylcholine, EPC) with different charge of their polar heads, on precipitation of calcium phosphates (CaP). Two precipitation strategies were investigated: fusion of liposomes loaded with constitutive ions (Ca^{2+} and HPO_4^{2-}) and spontaneous precipitation of CaP in the presence of liposomes. DMPS exhibited the strongest influence on the precipitation and transformation of the CaP, in both precipitation systems. The obtained results may contribute to the understanding of the general mechanism of inorganic - organic interactions underlying the biomineralization processes, as well as for materials science.

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Exploring properties of soil organic matter at the microscopic level by using molecular dynamics and an upgraded Vienna Soil-Organic-Matter Modeler

SHORT TALK

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The Vienna Soil-Organic-Matter Modeler (http://somm.boku.ac.at/; VSOMM) is an online tool dedicated to generating condensed phase models of humic substances (HS), one of the most important constituents of Soil-Organic-Matter (SOM). This tool has permitted us to investigate the structure and dynamics of different SOM systems, including the Standard Leonardite Humic Acid, as well as, their interaction with small organic compounds.

One of the challenges associated with the development of these systems, is to create a representative environment of the humic substances, at a nanoscale, taking into account that HS are composed of complex and heterogeneous organic compounds produced by biological degradation. For that reason, the modeler use a database of organic fragments called building blocks (BBs). Each BB has a different chemical structure, which, in combination, are able to reproduce the carbon distribution estimated by 13C NMR. Here, we have constructed a number of new BBs with the current parameter set 54A7 of the GROMOS force field, including BBs with Methoxyl-C and Alkyl-C groups that improves the agreement with the NMR data. Additionally, an improved core algorithm of VSOMM was developed and written using PYTHON, which allows us to automatically generate and equilibrate multiple models of greater chemical and geometric diversity.

Using the new version of VSOMM, we extend our investigation from the Standard Leonardite Humic Acid to several other experimentally available HA samples, including the Suwannee River II, Elliott Soil, Elliott Soil IV and Pahokee Peat Humic Acids. We characterized these systems by doing a plethora of analyses in order to observe differences in their structure and dynamics. The binding of pollutants and pesticides affects the erodibility, moisture retention and soil structure of SOM impacting possibly the carbon cycle, reducing the Carbon storage on soil. We also investigated the sorption of these systems.

Keywords: Humic substances (HS); Soil-Organic-Matter (SOM); Molecular models; Molecular Dynamics (MD).
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Studies of the disordered regions of FUS and SRSF1 with EPR

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Electron paramagnetic resonance (EPR) in combination with site-directed spin labelling is a powerful technique that can provide information on side chain dynamics and on the site-to-site distance distributions in biomacromolecules [1]. The mobility of the spin label can be estimated from the lineshape of the continuous wave EPR spectrum. The double electron-electron resonance experiment provides information on the mean distance between two spin labels as well as on the shape of the spin-spin distance distributions.

We will discuss how EPR can be used to gain information on the disordered regions of the RNA-binding proteins FUS and SRSF1, in particular the N-terminal low-complexity domain of FUS and the flexible linker between the two RNA recognition motifs of SRSF1.

The N-terminal low-complexity domain of FUS phase separates in vitro into liquid droplets, which resemble RNP granules [2]. Singly spin labeled FUS is used to infer information of mobility changes during phase separation and to obtain inter-protein distance distributions.

SRSF1 is a member of the SR protein family, which are crucial regulators of gene expression [3]. The flexible linker between RRM1 and RRM2 of SRSF1 has been shown to be involved in the binding of RRM2 to RNA [4]. The role of the linker in binding to RNA is analyzed by measuring the distance distributions between RRM1 and RRM2 in a free state of spin labeled SRSF1 and in its complex with RNA.

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Exploring the Binding Interfaces of the Type III Secretion System Chaperone-Translocator Complex

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SHORT TALK

The type III secretion system (T3SS) is adopted by many gram-negative bacteria for infection via the direct passage of effector proteins into host cells. This occurs through the translocon pore, formed from two large membrane proteins called the major and minor translocators. For pore formation, both translocators depend on binding to and being transported by small specialised chaperones after synthesis in the bacterial cytoplasm. Therefore, the chaperone-translocator complex is vital for effective secretion of toxins and infection of the host.

Here we investigate the specificity of the binding interfaces from the translocator-chaperone complex found in *Pseudomonas aeruginosa*. In particular we probed the two major interaction sites of the translocator-chaperone complex - "N-terminal arm" versus "N-terminal anchor" - via the selection of directed peptide libraries using ribosome display and next generation sequencing. At the N-terminal arm interface, we show that peptide libraries based on the chaperone sequence (PcrH) produce heavy enrichment for wildtype from selections against the major translocator (PopB) but less significant enrichment from the minor translocator (PopD). In contrast, at the N-terminal anchor interface, we recover little enrichment, outside 2 of the 3 consensus positions, for libraries based on the PopB/D sequence against the chaperone PcrH. Interestingly, the difference in binding between the two translocators at the N-terminal arm interface would seem to be compensated at the N-terminal anchor interface where ITC shows that PopD has a 2-fold increase in KD versus PopB. Importantly, our results show that there is no evolution of peptides that bind stronger than wild-type in either interface therefore suggesting the need for an unnatural mimic of the most important "N-terminal arm" peptide to abrogate the complex.

Evolution of α-synuclein fibril morphologies from the E46K variant associated with familial Parkinson's disease

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Parkinson's disease is the second most-prevalent neurodegenerative disorder, estimated to affect seven to ten million people around the world. The protein α-synuclein, involved in synaptic vesicle trafficking in neurons and glia, has been shown to convert from a monomeric form into fibrils that manifest themselves as protein deposits called Lewy Bodies in the brains of patients. Recent evidence has suggested that α-synuclein aggregates can seed aggregation of native protein within neighbouring cells and propagate cell-to-cell spreading. Research has revealed that fibrils formed from the disease-related E46K variant have three distinct *in vitro* morphologies: flat, tight pitch, and long pitch. This project seeks to determine the morphological evolution of the E46K fibril structure through cross-seeding. Fibril formation for each generation was monitored using Thioflavin T fluorescence assays while the protein structure was analysed using circular dichroism spectroscopy. Microscopy techniques, including atomic force microscopy and transmission electron microscopy, were used to image the fibril morphologies. This is the first step toward investigating a potential preferential type of E46K fibril morphology and how these structures may relate to disease pathology.

SHORT TALK

Single cell adhesion measurements using computer controlled micropipette and FluidFM BOT

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Measuring the interaction of cells with different surfaces is one of the great challenges of the emerging science of single cell biology. Extracting trustworthy, quantitative information has proved to be a difficult task using traditional methods such as AFM tip functioanliaztion. In our laboratory we have been actively working on applying and developing novel methods to measure single cell adhesion [1] . Using a powerful micropipette based method we were able to selectively measure the adhesion of HeLa cells in different cell cycles in the same cell culture. We were able to differentiate between cell cycles based on the Fucci genetic system that compels the cells to express different fluorescent dyes in their G1 (directly after division) and G2 (preceding mitosis) states [2, 3]. By measuring both sub-populations at practically the same time, we can avoid any secondary effects coming from chemical cell cycle manipulation and purely measure the different adhesion strength caused by the differing cell stages. The average time needed per cell is half a minute, demonstrating the exceptional high-throughput capacity of our system.

Another approach, focusing on the extraction of accurate force curves is based on the FluidFM BOT device, and automated AFM based system with a nanofluidic channel incorporated in the cantilever. By applying negative vacuum in this channel, it becomes possible to lift cells up from the surface while measuring their adhesion curve.

Within this presentation we intend to show that combining these methods present a powerful new tool in the investigation of single cell adhesion in a high-throughput, reproducible manner.

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Interplay of intracellular regulation and population dynamics in bacterial restriction-modification systems

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In-vivo dynamics of protein expression in bacterial cells depends not only on intracellular regulation, but also on the rates of cell and plasmid division that can change with time. That additionally couples the dynamics of proteins, which results in increased dimensionality of parameter space from which is computationally very hard to infer parameters. We consider this problem on a relatively simple case of bacterial restriction-modification systems, where we exploit one of the first available measurements of in-vivo expression of the restriction enzyme and the methyltransferase in these cells, which are under control of a specialized transcription factor – control protein.

We use a biophysical model of the system gene expression regulation that we previously developed to model the system dynamics. Now, we include complete cell population dynamics effects, due to both dividing cell and plasmid population. We resolve the parameter inference problem iteratively, through a "mean-field like" procedure that we here developed. Including the dynamics effects into the model clearly improves its agreement with experimental data, while neglecting such effects can lead to falsely identifying (otherwise non-existent) regulatory mechanisms. Next, we also systematically perturb the population dynamics. Such perturbations significantly change kinetics of the protein synthesis. However, an early peak in methyltransferase protein expression remains robust in these perturbations, which is clearly related with the main functional constraint to protect the host cell from cutting by the restriction enzyme. This clearly aligns with the idea of robustness of intracellular networks, where we now also propose that the regulatory interactions may be hardwired to preserve the main system dynamical constraints with respect to changes in the population dynamics – these changes might be expected in the natural environment, due to changes in the external conditions.

Magnetic Resonance Spectroscopy of Molecular Transport Machines

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This abstract describes the work of two ongoing collaborative projects, centred around the use of advanced magnetic resonance techniques for protein studies:

1. GltPh, an archael homolog of the Excitatory Amino Acid Transporters (EAATs), was the first of the Solute Carrier 1 (SLC1) transporter family to be crystallised. Various human diseases, including Amyotrophic lateral schlerosis, Huntington's disease and stroke have been linked to dysfunction of the EAATs and GltPh serves as an excellent model system for dynamic studies of these membrane proteins [1]. Membrane proteins, however, continue to prove challenging for structural and mechanistic studies, extending to the determination of substrate and inhibitor binding affinities, essential for drug screening. Here we present a novel approach for the determination of ligand K_D values in both detergent and reconstituted proteoliposomes using Saturation Transfer Difference (STD) NMR spectroscopy.

2. Metal ion efflux transporters known as Cation Diffusion Facilitators (CDF's) are responsible for maintenance of divalent cation homeostasis in all domains of life. Accumulation of divalent metal cations can be toxic, and CDF's have been implicated in Type-II diabetes and neurodegenerative diseases. MamM, a magnetosome-associated CDF protein of magnetotactic bacteria, is currently used as a model system for further understanding of CDF's [2]. Here we report pulsed EPR studies of the cytoplasmic domain of MamM revealing dynamic structural changes upon metal binding.

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Studying conformational dynamics of the SLC6 gene family homologue LeuT using advanced EPR techniques

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LeuT is a small amino-acid transporter from *Aquifex aeolicus*. It is used as a model for the SLC6 class NSS neurotransmitters: sodium symporters. Such transporters are implicated in several disease states including anxiety and depression. Large scale conformational changes and several inhibitor complexes have been suggested previously. Wild-type LeuT and selected cys-variants have been spin labelled at sites of interest and studied using advanced EPR techniques, such as distance determination methods (e.g. PELDOR) to probe for conformational changes not previously observed in crystal structure models. Further, novel detergent free approaches using SMALPs are being employed and results obtained will be compared to both proteoliposomes and detergent micelles of wild type and the selected variant LeuT.

SHORT TALK

Ovalbumin thermal stability dependence on pH

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Parameter that highly affects stability of the protein structure is pH since it affects charges of amino acid residues and, therefore, affects intra- and intermolecular noncovalent interactions responsible for protein stability. Protein thermal shift assay (TSA) revealed greater stability of ovalbumin (PDB code: 1OVA) in alkaline conditions (pH= 8) than in acidic conditions (pH= 4). In order to understand molecular basis of ovalbumin's thermal stability dependence on pH, molecular dynamics simulations at various temperatures and various pH were applied. Two different procedures were used, one for the protein that was simulated at acidic conditions where aspartate and glutamate amino acid residues were protonated, and the other one for the protein that was simulated at alkaline conditions with aspartate and glutamate amino acids deprotonated. Each system, acidic and alkaline, was simulated at five temperatures: 37 °C, 42 °C, 57 °C, 74 °C, and 85 °C. Results of simulations were in agreement with experimental results, it was observed that the instability of the acidic system is much more pronounced than the instability of the alkaline system. The most noticeable difference between these two systems were the H-bond interactions of arginine 50 and arginine 58 with their surrounding residues. In case of the alkaline system, both, Arg50 and Arg58, made higher average number of stronger and more persistent H-bond interactions. The same differences in H-bonding between alkaline and acidic systems were noticed, both, during the simulation at lover and higher temperatures. Different behaviour of Arg50 and Arg58 in acidic and alkaline systems is also supported by the analysis of fluctuations (RMSF).

Characterization of azide modified lipid monolayers for lipid-peptide photocrosslinking

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Photocrosslinking of lipids with membrane peptides represents a promising technique for studying peptide insertion into lipid layers. With this technique it is possible to analyse the insertion depth of membrane proteins or peptides within their native environment.

Photocrosslinking makes use of lipids with UV activated reactive sites at different positions within their hydrophobic tails. The key for future photocrosslinking studies is an accurate knowledge of physical and chemical properties of the used crosslinking lipids. Therefore, we have chosen lipid monolayers at the air-water interface as a model system for investigating some properties of a photocrosslinkable lipid to create the base for crosslinking experiments with synthetic peptides incorporated into the interfacial film.

We present a stable monolayer forming azido lipid and its characteristics at the air-water interface determined by thermodynamic and spectroscopic measurements. The temperature dependence of the surface pressure-area isotherm of pure azido lipid and its miscibility with DPPC were investigated by film balance measurements. Additionally, angle and polarization dependent infrared reflection-absorption spectroscopy (IRRAS) was used to study lipid chain conformation and order in the lipid monolayer.

The combination of the film balance technique and FTIR spectroscopic measurements of interfacial films in reflection-absorption provides deep insight in physical chemical properties of the studied films and our results set up the base for future photocrosslinking experiments.

SHORT TALK – EBSA

Conformational and functional studies of a novel thermostable xylanase from camel rumen metagenomic library

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Thermostable enzymes are very important because their considerable potential in many industrial applications. The metagenomic data obtained from camel rumen was considered as the potential source of microbial xylanase enzymes with proper activity in extreme conditions. The metagenomic data was assembled and contigs were used for in silico identification of microbial enzyme-encoding genes and their corresponding amino acid sequences. The most similar predicted enzymes to those reported in literature as thermostable xylanase were extracted and their active sites approved. Using the DNA template extracted from camel rumen metagenomic samples, the candidate enzyme genes were cloned, and expressed using proper E. coli strains. Structural and functional studies were performed on purified enzymes to determine their thermal stability characteristics and their activity respectively. In this study, a novel thermostable xylanase enzyme with 1146-bp full-length gene which encodes a 381-amino acid peptide was identified and the candidate xylanase gene was isolated from the camel rumen metagenomic DNA. Conformational studies of enzyme provided considerable information about its stability. Interaction between identified enzyme with n-dodecyl trimethylammonium bromide (DTAB; as a cationic surfactant) confirmed high thermostability. Thermal unfolding experiment confirmed the high thermostability of enzyme and its Tm was 71.5 °C. The maximum activity of enzyme was observed at pH 8.0 and temperature 40 °C. By increasing the temperature up to 90 °C, fifty percent of the enzyme activity was retained at pH 8.0. These superior properties make this enzyme ideal candidate for application in the industrial applications.

Does the shape of titanium dioxide nanoparticles modulate their biological effects in human keratinocytes?

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Skin protection is important element in reducing the level of sunburns (erythema), and cancer risk from the ultraviolet radiation exposure. Titanium dioxide nanoparticles (TiO_2NPs) are nowadays commonly used in sunlight protective products in spite of knowledge gaps on the safety issues concerning their use in medicine and cosmetics. This study aimed to examine the effects of differently shaped TiO_2NPs on immortalized human keratinocytes (HaCaT cell line) before and after UVB exposure. The UVB radiation was applied at dose equivalent to the UV component observed in one medial erythemal dose with 2 kJ/m^2 UVB (lamp maximal output 312 nm). Stability evaluation of fully characterized TiO_2NPs was performed by the light scattering technique and transmission electron microscopy (TEM). Biological effects on HaCaT cells were observed by means of cell viability assays (MTT assay, neutral red assay, Live/Dead assay) and the oxidative stress response (level of reactive oxidative species, extent of membrane damage and intracellular glutathione - GSH and activity of glutathione peroxidase - GPx).

How the N-terminal saposin of the surfactant protein B precursor can be produced using yeast: a deeper structural and functional characterization

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Saposin-like family (SAPLIP) is a set of proteins characterized by containing a highly conserved hydrophobic amino acid distribution that folds into 4 or 5 amphipathic α-helices, which facilitates transient or permanent interaction with membranes. Moreover, this structure contains a polypeptide motif characterized by the strictly conserved position of six cysteines involved in forming three intra-molecular disulphide bridges. The most relevant protein in lung homeostasis is surfactant protein B (SP-B) that belongs to SAPLIP. SP-B is characterized by hydrophobic amino acid sequence that folds in α-helices and interacts with lung surfactant lipids promoting their adsorption to the alveolar air-liquid interface. Two additional saposins are contained within the sequence of SP-B proprotein; the N-terminal one (SP-B^N) has recently been purified. Interestingly, SP-B^N exhibited certain antimicrobial properties at low pH, suggesting a potential but yet unknown role in innate host defense. Nevertheless, purifying the recombinant human SP-B^N from *E. coli* has some limitations including its necessary reconstitution from inclusion bodies, a potential contamination with endotoxin and absence of glycosylation. To address those constraints, we have characterized the human version of SP-B^N by its recombinant expression in *Pichia pastoris*, with and without glycosylation. Adequate yields of soluble SP-B^N are obtained by this procedure and both forms can be independently purified, as confirmed by amino acid composition, N-terminal sequencing and mass spectrometry. High thermostability together with saposin-fold acquisition and dimerization ability is revealed by far-UV CD. SP-B^N does not perform a stable interaction with lipid vesicles although it promotes the leakage of phosphatidylglycerol vesicles at low pH, specially the glycosylated form. Regarding its role in host defense, this improved version of the recombinant SP-B^N does not show remarkable antimicrobial properties. Instead, antiviral activity against several viral pseudotypes is suggested.

Hydrophobins: the Microstructure of self-organized Films of amphiphilic fungal Proteins

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Hydrophobins are small cysteine rich proteins, which are only produced by filamentous fungi. Because of the separation of the hydrophilic from the hydrophobic regions in the molecule, they show a strong amphiphilicity and as a result a strong surface activity [1]. The proteins are used in industrial application because of their mentioned properties, in the form of emulsifying agents, as coatings or as transport material for pharmaceutical substances [2]. Furthermore, there are amyloid fibrils formed by some proteins of special species of fungi. These are structurally equal to formations, which were discovered in relation to some diseases, for example Alzheimer's or Parkinson's disease [3].

Little is known about the structural arrangement of these proteins. Here, the self-organization of two hydrophobins were investigated under external constraint in the form of compression at the water-air-interface. These proteins differ in their physical properties; hence they are divided into two different classes. Here, SC3 from Schizophyllum commune was chosen as a member of class I, and HFBII from Trichoderma reesei as a member of class II hydrophobins. The organization of the proteins over multiple compression and expansion cycles on a film balance were investigated by atomic force microscopy after Langmuir-Schaefer transfer.

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The structural study of leucine rich repeat synaptic adhesion molecules

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Synaptic adhesion molecules play a crucial role in the regulation of synapse development and maintenance. Recently, several families of leucine rich repeat domain containing synaptic adhesion molecules have been characterized, including netrin G-ligands, leucine rich repeat transmembrane (LRRTM) neuronal protein family, and the synaptic adhesion-like molecules (SALM) protein family. Most of these are expressed at the excitatory glutamatergic synapses in post-synaptic cells, and dysfunctions of these genes are genetically linked with cognitive disorders, such as autism spectrum disorders and schizophrenia. My PhD study is focused in the structural and functional characterization of proteins under SALM and LRRTM family. The SALM family contains SALM1-5 proteins. SALM2, SALM3 and SALM5 have been shown to bind to the presynaptic receptor protein tyrosine phosphatase (RPTP) family ligands. We have solved the 3 Å crystal structure of the SALM5 LRR-Ig domain construct and we have done biophysical studies that verify the crystallographic results and binding of the ligand. The work is under process for publication [1]. Similarly, LRRTM family contains LRRTM1-4 proteins. The identified ligand of LRRTM1-2 is neurixin in the presynaptic cells. We have solved the engineered structure of LRRTM2 [2] and currently, I am working to solve the structure of LRRTM2-neurixin complex using crystallization or cryo-EM method, map the binding site of the complex using MS or NMR method.

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Fine structure and dynamics of EB3 comets in fibroblast cells

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Microtubules (MT) - molecular structures consisting of a tubulin protein bound to GTP or GDF molecules, are necessary in cells to construct a cytoskeleton, provide cellular mobility and form a fission spindle. Microtubules grow by attaching GTP-tubulins to their end. Subsequently, the GTP molecules inside the MT are hydrolyzed, as a result of which tubulins appear in the body of the MT in combination with GDF, the bonds between which are less stable. If a sufficient amount of stabilizing GTP-tubulins - the so-called "GTP caps" - is removed from the end of the MT, the tube can pass from growth to depolymerization [1]. Important proteins that regulate the dynamics of MT are the proteins of the EB family (end binding), which bind to the plus-ends of MT [2], forming structures resembling comets. It is believed that EB proteins recognize tubulins in combination with GTP.

In this study, we investigated the binding profiles of EB3 protein from the EB protein family at the ends of MT in living mouse fibroblast cells. We showed that the average distribution of EB3 proteins is fairly well approximated by a piecewise function that combines the exponential and Gaussian parts. However, individual EB3 profiles in some cases have a more complex distribution, not described by this function. As a rule, this is due to the presence on MT of additional regions of EB3 protein binding in the form of one or several immobile "islands". We decided to check whether the "islands" data can be the result of the stochastic nature of GTP hydrolysis. To do this, we analyzed the theoretical profiles of EB3 protein distribution generated by the previously published VanBuren et al., 2002. It turned out that under no assumptions of EB3 binding to tubulins, the model did not predict the presence of EB3 islets. Moreover, we found that the classical model of the dynamics of the MT considered by us also does not describe the distribution of the EB3 amplitudes and the velocities of the cometary EB3 motion observed in the experiment. Our detailed analysis of the velocity spectra in the experiment and theory has shown that MTs are likely to polymerize to the brink of stability and quite often experience microcatastrophes during polymerization.

Thus, our analysis made it possible to detect inconsistencies between the classical ideas about the dynamics of MT and the observed experimental data in living cells.

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A novel approach in bacterial growth modeling: *E. coli* exposed to silver nanoparticles

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There are many growth and inactivation models in the literature which are purely empirical and do not offer a mechanistic insight [1, 2]. Here, we present a novel, intuitive and mathematically relatively simple model for bacterial growth and inactivation. The proposed model well describes *E. coli* batch culture growth curves obtained for different inoculum sizes and different initial nutrients concentration. The model is, furthermore, expanded in order to fit and explain growth curves obtained for *E. coli* treated with different concentrations of laser produced silver nanoparticles. The model is described by three nonlinear mutually dependent differential equations that give time evolution of bacterial, nutrients and nanoparticle concentrations. These equations are of general nature and can be used to predict growth of different bacteria in batch culture since the equations do not include a parameter that is characteristic for *E. coli*. As our model can be linked to the logistic model, it is possible that the equations could be applicable to any single species growth.

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Changes on elasticity and morphology of erythrocytes from amyotrophic lateral sclerosis patients

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Patients' complications, such as venous thromboembolism (VTE), promote changes in haemodynamic properties and abnormalities in red blood cells (RBC) membrane and on its lipid content. Our main goal was to evaluate changes in the elastic and morphological properties of RBCs in ALS and compare them with the erythrocytes from healthy donors. By atomic force microscopy (AFM), RBC membrane roughness, elasticity and morphological parameters were analysed for both groups. Patients' RBCs are stiffer, have higher penetration depth and are more capable to deform, presenting an increased membrane roughness. Morphological changes on RBCs from ALS patients were also assessed by AFM, showing lower thickness and higher cell area. Zeta-potential analysis showed that the surface of patients' RBCs is less negatively charged, which may be due to a lower density of sialic acid residues. Fluorescence spectroscopy showed that RBC membranes from ALS patients are more fluid. This may be associated with changes on membrane lipid composition and packing. We conclude that ALS disease leads to significant electrostatic and morphologic changes in RBC membranes. These findings may contribute to understand the complex interplay between ALS disease progression rate and RBC lipid profile.

LRM calculation of the vibrational frequencies and amplitudes of fluoroethen izotopomers

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A new method, named the "Low Rank Modification" (LRM) method [1], is applied to calculate the out-of-plane frequencies of some fluoroethen isotopomers, together with corresponding amplitudes of vibrations of pure fluoroethen. The main advantage of the LRM compared with other methods is that the LRM method does not require force field constants to be determined, whichs significantly simplifies calculations.

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Temperature-dependent structural changes in Cas3 protein in *Escherichia coli*

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The CRSISPR-Cas system is a significant mechanism of bacteria and archea that provide adaptive immunity against viruses and plasmids. It consists of DNA repeats separated by spacers of foreign origin (CRISPR locus), and cas genes responsible for various stages of defense. In *E. coli*, Cas3 protein is involved in a degradation of invader DNA as a last stage of defense. Recent studies showed that Cas3 could be the limiting factor for regulation of the CRISPR-Cas immunity due to its unusual property – loss of activity at 37°C, unless the protein is present in abundance. In this work we wanted to investigate if the loss of Cas3 activity is caused by structural change of protein which is temperature-dependent. We monitored structural changes in the purified protein by measuring a change of ellipticity using circular dichroism and by measuring intrinsic tryptophan fluorescence using fluorescence spectrometry. Both methods gave the same result, a subtle conformational change in helical region at 35°C which is in agreement with the protein activity change *in vivo*. This is probably the first experimental evidence that Cas3 activity from *E. coli* is temperature-dependent due to the change in protein conformation. Also, similar structural change was observed in archaeal Cas3 suggesting that this trait is preserved in other species as well. The results of this research will contribute to better understanding of regulation of Cas3 activity as well as to the progress of the CRISPR-Cas field.

The difference of amyloid fibril formation after reduction and denaturation of crude protein preparations

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SHORT TALK – EBSA

Formation of highly stable amyloid fibrils is considered as a generic property of polypeptides. This process got a lot of interest of structural biochemists as one of the causes of many severe human diseases. Although many medically important proteins are extensively investigated in the means of amyloid fibrillation, it is assumed that all proteins can undergo amyloid fibrillation under specific conditions. Some easily accessible proteins such as ovalbumin and lysozyme are reported to occupy amyloid forms in destabilizing conditions. In the case of lysozyme, the importance of 4 disulfide bonds in folding, as well as fibrillation processes, is well described.

Our goal was to investigate if crude extracts of various widely available proteins show the difference in fibrillation processes if previously reduced and denatured in the presence of chaotropic agents.

The differences in denaturation and fibrillation were monitored by Fourier Transform Infrared Spectroscopy, Attenuated Total Reflectance mode, and Thioflavin T binding. Significant differences in fibrillation with and without reduction steps were observed in all proteins investigated. Pretreatment of proteins with reducing and chaotropic agents resulted in diversification of the conditions and pathways that lead to fibrillation. The greatest differences in fibrillation were observed in lysozyme and in papain which has never been reported to adopt amyloid forms before.

Our results lead to a conclusion that reversible denaturing and reducing pretreatment of various proteins have an important role in amyloid-like fibrillation. As shown with papain, and lysozyme, this approach can lead to the development of new fibrillation procedures.

Aggregation kinetics and bioimaging of monomeric tau

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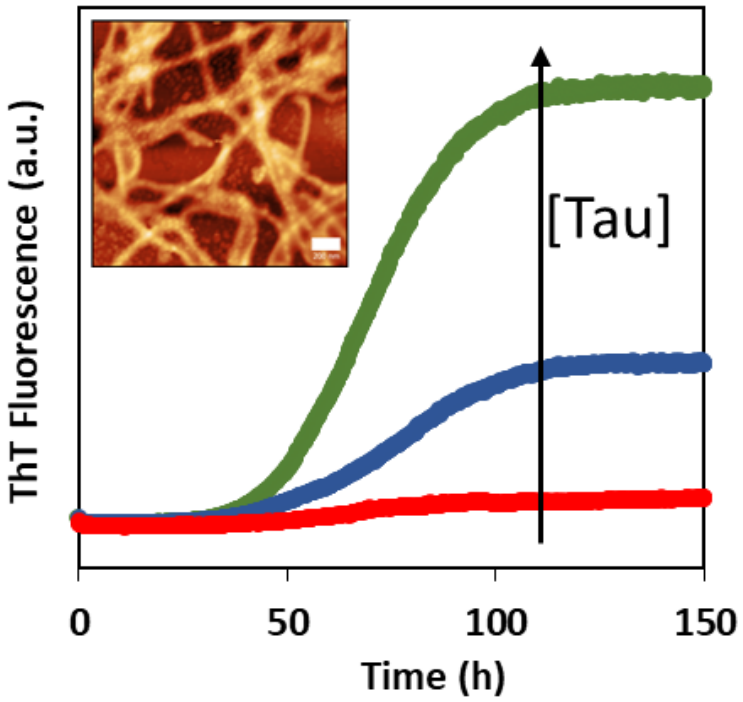
Alzheimer's disease (AD) is the most common cause of dementia (60-70%), affecting millions of people worldwide. The extracellular accumulation of amyloid-beta (in senile plaques) and the intracellular aggregation of hyperphosphorylated tau (in neurofibrillary tangles) are two well-known disease hallmarks. Tau hyperphosphorylation also leads to microtubule disintegration resulting in neuronal death [1].

Tau is an intrinsically disordered protein with a high aggregation propensity which in solution is known to forms oligomers that evolve into amyloid fibrils [2]. The exact sequence of molecular events describing this process starting from monomeric tau remains to be fully understood, and this is critical to develop anti-aggregation therapies.

Towards this goal we here report a series of investigations using biophysical techniques including thioflavin-T fluorescence monitored kinetic assays, circular dichroism (CD), and atomic force microscopy (AFM) that are allowing us to gain insights into tau pathomechanisms from in vitro approaches. We here describe an improvement of an established protocol for the expression and purification of recombinant full length human tau (hTau441) [3] that results in a fraction enriched in monomeric tau. Using this purified monomeric hTau441 we have performed systematic heparin-induced aggregation experiments monitored by thioflavin-T (ThT) fluorescence in 96-well plates. The reaction aggregation rates are then determined from curve fitting (see figure). A number of distinct conditions were tested in these kinetic assays (different tau concentrations, in the presence of NaCl and reductants) whose effects on tau structure were also investigated using far-UV CD spectroscopy. We then used AFM bioimaging to characterise the morphology of the fibrillar species formed at the reaction end-points.

Overall, with this improved protocol for production of monomeric hTau441 and the ability to carry out detailed aggregation kinetic assays coupled to AFM imaging we expect to soon achieve a more complete understanding of tau aggregation phenomena.



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Net Charge Changes in the Calculation of Protein-Ligand Binding Free Energies via Classical Atomistic MD Simulation

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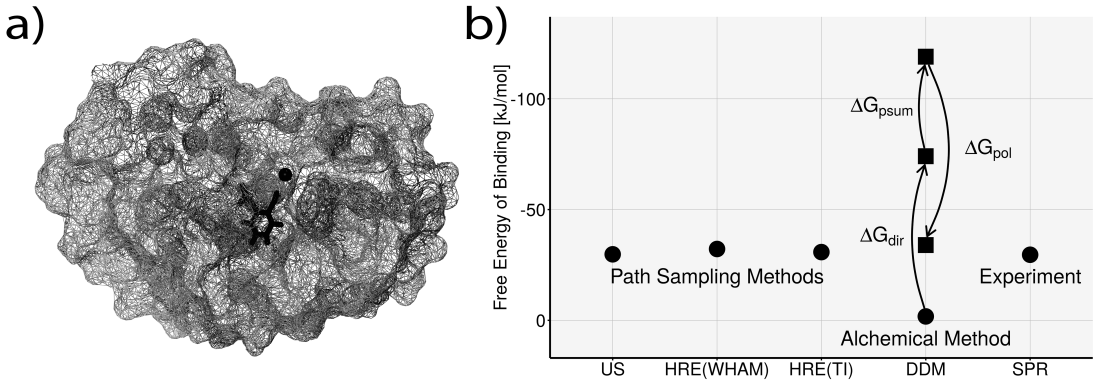
The calculation of binding free energies of a charged ligand to a target protein is an often encountered problem in molecular dynamics (MD) studies. Absolute free energies as well as their relative changes (compared to a ligand with a different net charge) are both affected by artifacts caused by the representation of electrostatic interactions in a non-macroscopic simulation box using periodic boundary conditions[1]. In particular, electrostatic interactions are calculated either using a lattice-summation scheme or a cutoff-truncation scheme with Barker-Watts reaction-field correction rather than summing over individual potentials. The application of correction terms had already been proposed but so far only been applied for simple systems, including polyatomic molecules binding to a functionalized C_{60}-buckyball [2] or the binding of a ligand to a cytochrome c peroxidase where the MD simulation was performed with fixed solute atom positions [3]. Here, we present a study where the estimated binding free energy of acetylsalicylic acid coupling to a phospholipase A2 [4] (see Figure a) was succesfully derived by applying the correction terms to the alchemically computed free energy estimate. The corrections split up into individual terms for the direct ligand-protein interactions (ΔG_{dir}, see Figure b), for the P-summation over the individual solvent molecules (ΔG_{psum}) and for the wrong solvent polarization around the charged ligand (ΔG_{pol}). The corrected estimate was found to be in a very good agreement with experiments.



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Conformational properties of dark and lit state of AsLOV2 domain

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SHORT TALK – EBSA

Plants, algae, bacteria and fungi contain Light-Oxygen-Voltage (LOV) domains that function as blue light sensors and control cellular responses to light. Blue light activation occurs when the non-covalently bound FMN chromophore absorbs a photon and forms covalent bond between its C4a atom and nearby cysteine residue within the protein. After covalent bond formation protein undergoes a conformational change that results in the unfolding of the terminal Jα helix. The photoreaction process is fully reversible in the dark [1, 2]. Thanks to its light induced conformational change LOV domains have potential widepread use in optogenetics, i.e. as molecular switch in cell signaling and can also be used as a fluorescent protein.

For the purpose of this study we used 2 forms of Avena sativa LOV2 domain (AsLOV2): native form, so-called lit form, and the variant, so-called dark state form. In dark state form, photoreactive cysteine is replaced by alanine and it does not undergo a photoreaction upon blue light illumination.

Here, we present our findings regarding the influence of pH and temperature on the structural response in secondary and tertiary structure by CD, fluorescence spectroscopy and differential scanning calorimetry. In addition, we investigated the effect of pH, temperature and protein concentration on dark reversion kinetics of native AsLOV2.

Our results indicate that despite an ability to undergo significant ligh-induced conformational transition, AsLOV2 is resilient against changing solvent conditions.

This work was supported by Slovak grant agency VEGA1/0423/16 and APVV-15-0069.

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Structural and functional studies of the interplay between three virulence factors from *Listeria monocytogenes*

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Listeria monocytogenes is a pathogenic intracellular bacterium, which causes listeriosis, a relatively rare disease, which can however result in death, especially in the case of immunocompromised patients. The ability to escape primary and secondary vacuoles of the host cell is an important factor in pathogenicity of this intracellular pathogen. The key virulence factors recognized in facilitation of vacuolar escape are a cholesterol dependent cytolysin Listeriolysin O (LLO), a broad-range phospholipase C (PC-PLC) and a phosphatidylinositol-specific phospholipase C (PI-PLC). All three proteins were prepared recombinantly from bacteria. Methods were introduced to characterize the enzymatic activity of both phospholipases: colorimetric test of enzymatic activity on multilamellar vesicles with malachite green dye for PC-PLC and activity test with synthetic substrate X-IP for PI-PLC. The effect of PI/PC-PLC on vesicle leakage induced by LLO has been observed by measuring calcein release from model lipid membrane systems in the presence of both proteins and various membrane cholesterol concentrations. Our preliminary data suggest that PC-PLC facilitates LLO pore formation in membranes with cholesterol content lower than required by LLO alone (i.e. min 40 mol %). Our studies are now oriented towards imaging of these protein-protein and protein-lipid interactions with various biophysical and structural approaches.

SHORT TALK

The structuring in the aqueous trifluoroethanol mixture

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Aqueous mixtures of amphiphiles display a rich variety of molecular self-assembly, from defined objects such as micelles in micro-emulsions to micro-heterogeneity in molecular liquids. 2,2,2-trifluoroethanol (TFE), a flourinated mono-ol, is an example of a such an amphiphile. Its aqueous mixture is relevant in the biological context, as TFE-water mixtures are known for promoting or inducing the regular secondary structuring in peptides and proteins [1] and are often used in CD and liquid NMR experiments. On the molecular scale, aqueous TFE mixtures are micro-heterogenous, as witnessed by scattering experiments [2]. This particular type of microheterogenous organization may also contribute in promoting the regular structuring in the solvation process of a peptide. For example, at around 40v/v concentration of TFE - where the microheterogeneity shows the strongest signals - the transition from unfolded to folded protein states is also observed [3].

In this work, we aim at characterizing the structuring in TFE-water mixtures using computer simulations. Since recent studies have shown the issues in achieving correct mixing of the TFE and water [4], a lot of consideration was put into the choice of the force field models, the system sizes and simulation times. By calculating static site-site properties, we track the way the mixture evolves with the change in the molar fraction of the co-solvents. The self-association, induced by self H-bonding, and cross-species associations due to the effect of solvent are particularly distinguished. Oftentimes in the literature, the term "clusters" is used for both cases, which hides the fact that these structures are due to different mechanisms of associations. We also present thermodynamic properties, such as enthalpies and entropies of mixing and concentration fluctuations which again highlight the complex nature of the TFE-water mixture.

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Local fluctuations in ethanol mixtures

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We study different ethanol mixtures using classical molecular dynamics computer simulations. The theory of fluctuations, developed by Kirkwood and Buff in the 50's [1], allows us to connect integrals of radial distribution functions, obtained through simulations, with a variety of thermodynamic quantites, such as partial molar volumes, compressibility and most importantly, the thermodynamic factor. The value of thermodynamic factor is twofold. Firstly, it is an indicator of the stability of a mixture, with respect to mixing or demixing, since it can be written as a second derivative of the Gibbs free energy with respect to concentration. Secondly, it is particularly useful in comparing experimental and simulation results for diffusion coefficients.

Here, we look for the most precise and accurate ways of calculating the thermodynamic factor from finite size systems and see what we can learn about hydrogen bonding associative mixtures and their deviation from ideality. Understanding the structural characteristics on molecular level is important for the understanding of more complex biological structures and processes.

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Binding of PI(4,5)P_2 to class A G-Protein Coupled Receptors

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Cell surface receptors allow cells to exchange information between different cells and thereby to react to external stimulants. One of the main classes of cell surface receptors in the human body are G-Protein Coupled Receptors (GPCR). GPCRs fulfil a wide range of physiological functions despite sharing a very similar architecture. One explanation for this phenomenon is allosteric modulation by the membrane environment. Recent studies showed the key role of lipids in regulating the membrane protein function [1, 2]. In this project we compare binding of Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) in a Phosphatidylcholine membrane to class A GPCRs through coarse-grained molecular dynamics simulations following the MemProtMD pipeline [3].

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Process for forming ready-to-use QCM sensors with atomically flat surface suitable for scanning probe microscopies

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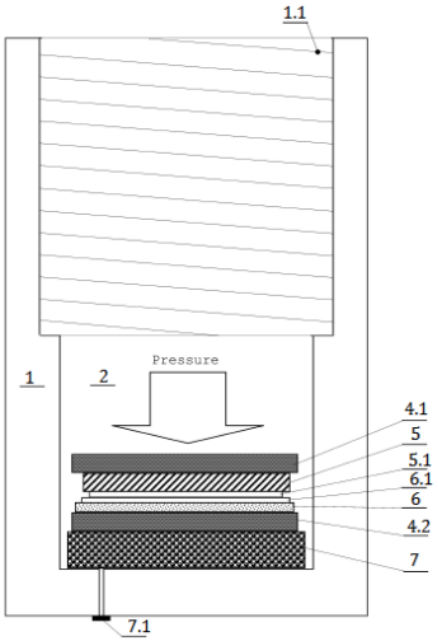
SHORT TALK

QCM (quartz micro balance) is a routinely used method in studies of biomacromolecular interactions with various substrates. It's application goes from biophysics, drug discovery, cell adhesion to new biomaterials and material science itself. As with any research tool, QCM is constantly upgraded and optimized in order to open new applications and new combinations with other techniques. [1]

One of the most attractive upgrades is combining scanning probe techniques like AFM (atomic force microscope) and STM (scanning tunneling microscope) in parallel with QCM, which would give for example not only information of total adsorption on surface, but also the information of exact placement of adsorption. To accomplish that, main challenge is producing ultra flat, functionalized surface of quartz chip that is used for QCM sensing - as the flatness is the key requirement for AFM and STM. [2]

Here we present our process for forming such a surface, essentially a modification of a regular, commercially available chip into a chip with atomically flat gold surface. We use a proprietary press showed on image bellow. The press provides another advantage, as it protects this gold surface from adsorption of contaminants after preparation. We will present some AFM and STM studies that may utilize the flatness of this surface for imaging of biomacromolecules.



1 casing
1.1 casing thread
2 interior
3 closure
3.1 closure thread
3.2 closure tip
3.3 closure head
4.1 upper buffer
4.2 lower buffer
5 Si wafer
5.1 deposited gold surface
6 QCM sensor
6.1 gold surface of QCM sensor
7 load cell/pressure sensor
7.1 load cell wirings

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Thermodynamic and structural studies of homocyclopeptide-anion complexes

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Within the field of supramolecular chemistry an intense focus has recently been given to research of anion receptors due to the important role of anion recognition in the life processes and its numerous medicinal and environmental applications [1]. Neutral ion receptors containing amide, urea/thiourea or pyrrole groups have been developed with the aim of creating receptors with specfic affinities for a given molecule or ion through the formation of N-H–anion hydrogen bonds. Amongst them, cyclic peptides present promising receptor properties due to their bioavailability as well as the hydrogen-donor properties of amide group, macrocyclic ring flexibility and the variability of the subunits [2, 3].

In this work, two cyclopeptide receptors were studied, **L1** that contains five lysine subunits with amino groups of the side chains protected by a BOC group (Lys-BOC) and **L2** that is comprised of six Lys-BOC subunits. Complexation affinities of **L1** and **L2** ligands toward halides and structural anions (HSO_4^-, $H_2PO_4^-$, NO_3^-, NO_2^-, SCN^-) in acetonitrile were determined by means of mass spectrometry, ^{1}H NMR spectroscopy and isothermal microcalorimetric titrations. More information on the structural characteristics of the cyclopeptide binding sites was obtained by performing molecular dynamics simulations with explicit solvent molecules. The Gibbs energies of complexation reactions were also calculated by means of molecular dynamic simulations. Combining the results of computational studies with the experimental ones provided a detailed insight into the microscopic image of the anion-binding processes.

This work has been fully supported by Croatian Science Foundation under the project IP-2014-09-7309 (SupraCAR).

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Biophysical modeling of CRISPR-Cas regulation

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In the advanced bacterial immune system CRISPR-Cas, CRISPR array, which consists of foreign DNA sequences sampled during past infections, is transcribed into a long pre-crRNA. One of the Cas proteins specifically processes (i.e. cuts) pre-crRNA into a large number of crRNAs. A crRNA complementary to invasive DNA guides Cas proteins to destroy this DNA. Experimentally established transcriptional regulatory feature of CRISRP-Cas is highly cooperative repression of both CRISPR and *cas* promoters, which can be abolished by some transcription activators. However, dynamics of the native system activation in *E. coli* have not been observed as it stays silent under standard conditions even during phage infection. We previously showed that fast non-specific pre-crRNA degradation significantly contributes to the fast system OFF-ON transition by dynamically modeling pre-crRNA processing upon Cas overexpression [1]. To also investigate the role of the observed high cooperativity in transcription regulation, we propose and biophysically model a synthetic circuit for CRISPR-Cas activation in which cas genes are put under transcriptional control of a restriction-modification system exhibiting qualitatively similar regulatory features and performing a common immune function [2]. Using this approach, we found that CRISPR-Cas regulation is optimized to achieve fast switching on and generating very large amounts of crRNAs, which is under some conditions close to physical limit of infinitely abrupt induction. Additionally, fast pre-crRNA degradation provides a delay in crRNA generation, which may be related with evading autoimmunity, through favoring primed adaptation (sampling foreign, rather than host, DNA in CRISPR array).

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Computational assessment of biological effects of some xenobiotics

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The aim of this study is to use computational approaches to predict pharmacokinetics, biological activity spectra and side/toxic effects of some synthetic steroids and commonly used parabens in humans. The following computational tools are used: (i) SwissADME for predicting pharmacokinetics;(ii) PASS online for predicting biological activities; (iii) PASS online, admetSAR and Endocrine Disruptome for envisaging the specific toxicities; (iv) SwissDock to assess the interactions of investigated xenobiotics with cytochromes involved in drugs metabolism.

Synthetic steroids usually reveal a high gastrointestinal absorption and a good oral bioavailability, may inhibit some of the human cytochromes involved in the metabolism of drugs (CYP2C9 being the most affected) and reflect a good capacity for skin penetration. There are predicted numerous side effects of investigated steroids in humans: genotoxic carcinogenicity, hepatotoxicity, cardiovascular, hematotoxic and genitourinary effects, dermal irritations, endocrine disruption and reproductive dysfunction. Investigated parabens reveal no carcinogenicity and mutagenicity, a weak potential to inhibit the hERG channel and skin irritation/sensitization, apnea, ulcer and muscle weakness as side effects. The parabens under investigation are able to inhibit human cytochromes that are involved in metabolization of numerous endogenous and exogenous compounds, butylparaben having the higher inhibitory potential These results are important to be known as an occupational exposure to steroids and/or parabens at workplaces may occur.

Membrane-active antimicrobial peptide identified in *Rana arvalis* by targeted DNA sequencing

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Skin secretions of many tested anuran species have been shown to contain a variety of antimicrobial peptides (AMPs) acting directly towards pathogens, including multidrug resistant isolates, and also showing immunomodulatory properties [1]. We have selectively amplified transcripts likely to encode for AMPs, thus providing their sequences. After RNA extraction from frog skin tissue samples, cDNA synthesis followed by PCR amplification was performed. For this purpose, forward degenerate primers were designed based on highly conserved signal peptide regions [2], together with a reverse primer designed on the poly-A tail of mRNA. Signal peptide regions were derived from sequences deposited in DADP database [3] and transcriptome data in SRA database [4]. Resulting amplicons were size-selected and processed by ion semiconductor sequencing, obtaining several thousand sequencing reads. Those were then assembled into contigs representing nearly full-length AMP-encoding transcripts. Analysis of the assembled sequencing output allowed to identify more than a hundred full-length mature peptides from 5 different specimens belonging to 5 different frog species, mostly from Ranidae species. Based on appropriate biophysical properties (e.g. charge, hydrophobicity, amphipathicity) six of the most promising candidates were chosen for chemical synthesis and extensive characterization.

All peptides were tested against a panel of Gram-negative and Gram-positive bacteria, as well on tumour cell lines. One peptide, identified in *Rana arvalis*, proved to be active against both reference ATCC strains and epithelial cancer cell lines, while less toxic for circulating MEC-1 cells in a range of concentrations several fold higher then minimal inhibitory concentration (MIC) and IC_{50} values. Furthermore, the peptide was found to disrupt the bacterial membrane even at sub-MIC concentrations, as observed by flow cytometry and visualised by atomic force microscopy (AFM).

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Investigation of oligoamine-based systems for the formation of colloid-like clusters in solution

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Ionic self-assembly is responsible for constructing colloid-like ionic clusters in solution, named ionoids. Mostly, these "soft" yet durable globular structures present a highly defined size and shape [1]. Through utilizing various molecular building units we can build up such systems with ionic components of different size and charge ratios to study their influence on to the dynamic self-assembly process. Due to acting electrostatic interactions as well as weaker noncovalent interactions (hydrogen bonding, van der Waals, solvation) we receive tunable structures with sensitivity to environmental changes, implying possible future technological applications as "material by design" [1, 2, 3].

Here, we examine the size and long-term stability of the oligoamine-based system containing spermine and methanedisulfonic acid dipotassium salt in our established solvent mixture DMSO:glycerol:water 50:43:7 (v/v/v) [1] with dynamic light scattering (DLS). Additionally, to these systems we use electron paramagnetic resonance (EPR) spectroscopy to understand the local structure and environmental constitution mainly around the anionic component. We utilize the dianion of Fremy's salt as observer component. Intend to highlight expected changes of the solvation shell around Fremy's salt while adding the oligoamine-based compound. Therefore, we want to partially deuterate our established solvent mixture and measure the spin-spin as well as spin-lattice relaxation time through pulsed EPR spectroscopy.

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Enhanced protection of biological membrane using flavonoid embedded nanoparticles

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Flavonoids, polyphenolic biomolecules with antioxidative activity, have recently emerged as potential novel therapeutics for neurodegenerative diseases[1]. In addition to the fact that the mechanisms of their antioxidant effects have not yet been fully elucidated, their applicability is rendered by poor water solubility and chemical instability under physiological conditions encountered during pharmaceutical product consumption[2]. Flavonoid incorporation in nanoparticles (NPs) as carriers has been proposed as possible solution to surpass these obstacles. The aim of the research is to overcome the problem of poor water solubility and chemical instability of flavonoids by delivering them loaded in biodegradable mesoporous NPs to model membranes and neurons whereby their protective effects should be enhanced. The incorporation of flavonoids from the subgroups of flavonols (quercetin[3], myricetin[4] and myricitrin[5]), anthocyanins (cyanidin 3-0-glucopyranoside[6]) and flavons (luteolin and apigenin[7]) was investigated. Such choice of flavonoids will enable determination of a relationship between flavonoid structure and protective activity towards oxidative stress. All selected flavonoids contain planar moiety but differ in the degree of monosaccharide unit substitution. In addition, cyanidin 3-0-glucopyranoside is the only one bearing charge. One kind of biodegradable mesoporous NPs, Fe_3O_4 will be investigated as flavonoid nanocarriers due to their superior drug-loading and controlled release properties. Mesoporous NPs were selected with the goal to increase the flavonoid loading and entrapment efficiency, as compared to so far used organic or inorganic NPs, and to enable protection of flavonoids in physiological conditions. This research will ultimately generate detailed knowledge about the effects of the size, shape, charge, hydrophobicity and applied magnetic field of NPs with flavonoids and their release.

Results from the studies proposed within research will pave the way towards development of innovative and improved therapies for oxidative stress-associated neurological disorders. In addition, the knowledge obtained within this research could be extended to designing effective delivery systems for the incorporation, protection and release of other unstable bioactive molecules with an aim to improve human health or to increase the shelf life of pharmaceutical or food products.

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Evolution of monodispersity in vertebrate HSP27

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SHORT TALK

The small heat shock proteins (sHSPs) play an important role as a part of cellular protein quality control and are present in all kingdoms of life. sHSPs exist as monomers and dimers, but many also form larger oligomers. Even though some sHSPs, like wheat HSP16.9, only form specific large oligomers, all characterized vertebrate sHSPs so far that oligomerize beyond a dimer are polydisperse in nature.

Here, we present a vertebrate sHSP whose oligomeric state is monodisperse – HSP27 from *Callorhinchus milii*. Using native mass spectrometry and interferometric scattering mass spectrometry, we show that in addition to monomers and dimers, *Callorhinchus milii* HSP27 only forms octamers to appreciable extent. Despite this deviation from vertebrate sHSP canon, *Callorhinchus milii* HSP27 still shows chaperoneactivity in assays that monitor ability to suppress aggregation. Since the N-terminal region of sHSPs is known to affect their oligomeric state, we hypothesize that this reduction of dispersity might be caused by an approximately 15-residue-long insert in the N-terminal domain, specific to *Callorhinchus milii* HSP27. Since monodispersity seems to be a quite unique trait among vertebrate sHSPs, we use phylogenetics to trace their evolution through the vertebrate subphylum. We then use native mass spectrometry to study HSP27 from *Leucoraja erinacea* to determine whether this reduction in dispersity only developed in *Callorhinchus milii*, or is present in the whole class of cartilaginous fish (sharks, rays and skates).

Future studies will examine the structure of the octamer and the exact role of the insert region in the N-terminal domain. Using computational approaches, we can also obtain protein sequence for HSP27 of the last universal common ancestor of all vertebrates and find whether it was originally polydisperse, and monodispersity developed later, or vice versa. The results will improve our understanding of the functions of different modes of oligomerisation in biology.

FTIR spectroscopy of thin films made of human DNA

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Investigations of DNA vibrational properties using Fourier transform infrared spectroscopy can be traced back to 1960s. There is a high interest among researchers for this method given the fact that it's nondestructive and suitable for investigation of DNAs vibrational properties which are related to the DNAs structural properties. In most cases, fragments are short sequences of DNA which are extracted from Salomon testes or calves gland timus. In this type of research, there is a little investigation of DNA from biological tissues [1] and even less on human DNA [2]. FTIR spectrum of DNA with the range of wave numbers from 4000 to 400 cm^{-1} contains about 40 spectral lines which are the result of the DNAs double stranded structure. Range from 1800 to 800 cm^{-1} is of special interest because in this range we can find the most vibrations that can be divided into two groups: vibrations of bases and backbone of DNA [3].

In this research, we have used DNA that is extracted from the human placenta of regular pregnancies (archive samples of CERRM). Measurements using infrared spectroscopy were performed on thin films that were made from DNA solutions. Normalization and baseline correction was performed using program Kinetics which is part of the MatLab software and in program eFTIR. This processed spectra will enable us to assign the certain characteristic vibrational bands so we can compare them with relevant information and finally with the pathological human placenta.

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Biophysical characterization of lipid-tagged peptides as fusion inhibitors for respiratory viruses

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Human parainfluenza viruses (HPIV) and respiratory syncytial virus (RSV) are paramyxoviruses and are among the most common respiratory pathogens affecting infants and children worldwide. Nowadays, acute respiratory infections are the leading cause of mortality in children, accounting for nearly 20% of childhood deaths worldwide (nearly 3 million children each year). There are no effective treatments available. Consequently, there is an urgent demand for efficient antiviral therapies. Infection of healthy cells by these respiratory viruses requires fusion of the viral membrane with the target cell membrane, a process mediated by a trimeric viral fusion protein, the F protein. Inhibitory peptides inhibit viral fusion by binding to F's transient intermediate, preventing it from advancing to the next step in membrane fusion. We assessed variants of lipid-tagged F-derived peptides to search for properties that may associate with efficacy and broad-spectrum activity. Fluorescence spectroscopy was used to study the interaction of the peptides with biomembrane model systems, using partition assays. Using acrylamide, a quencher of tryptophan fluorescence, it was possible to understand the preferential localization of the peptides in lipid bilayers. The interaction of the peptides with human blood cells was also evaluated, using the dipole potential probe di-8-ANEPPS. Understanding the membrane biophysical processes involved in enveloped viruses entry may enable the development of new therapeutic strategies.

AFM/QCM-D methods in the diagnostics and prognostics of malignant melanoma

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Malignant melanoma is one of the most common type of skin cancer originating from melanocytes [1]. Its diagnosis is difficult and generally relies on subjective assessments. In particular, there is a lack of quantitative methods allowing melanoma diagnosis as well as monitoring melanoma therapies.

Changes in the glycosylation pattern of cells are often associated with various diseases including cancer [2]. Lectins are proteins recognizing various carbohydrate structures with specific binding affinities [3]. Lectin-carbohydrate interactions can be found in a wide variety of regular biological processes (adhesion and migration) or can be applied for detecting glycans present on cancerous cells [4].

Label-free methods like the quartz crystal microbalance with the dissipation monitoring (QCM-D) or the atomic force microscopy (AFM) in the spectroscopy mode may be applied for the differentiation of cancer cells in different progression stages based on lectin-glycan interaction as well as viscoelastic properties of cells [5, 6].

On the other hand, the only hope for patients with advanced melanoma is the inhibition of metastasis. Anandamide is an endocannabinoid, that has a potential anti-tumor effect. As for now, its influence on the cancer cells' proliferation, adhesion, apoptosis and cell cycle was evaluated [7].

In our studies, the real-time binding between lectin Concanavalin A and glycans present on the surface of melanoma cells from different stages of cancer progression was examined by label-free methods and compared with that observed on the melanocyte surface. Two procedures have been developed to detect the differences in the cellular glycosylation profile using cell-based sensors. The observed changes in lectin-glycan interactions among the studied cell types (melanoma cells and melanocytes) enabled an early cancer detection as well as the distinction of melanoma cell types. The treatment of metastatic melanoma cells with anandamide results in a possible change in the glycosylation profile towards the less aggressive forms of melanoma.

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SHORT TALK

Structural studies of Gorab – a novel component of Drosophila centrioles

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Centrioles are barrel-shaped organelles essential for the formation of cilia, flagella, and centrosomes. As such, they provide cell motility and mitotic spindle organization in almost every cell type. A universal 9-fold radial symmetry is observed in cross-sections of most centrioles across the evolutionary tree, and it is present in both the inner "cartwheel" structure and the outer microtubule wall of the centriole. The inner cartwheel acts as the first step in centriole assembly and is thought to determine the stability and the 9-fold symmetry of centrioles. Although essential components of the cartwheel have been identified, the exact mechanism of the cartwheel assembly remains unclear. Glover lab has identified a novel cartwheel component in Drosophila centrioles – a Golgi-associated protein Gorab. In collaboration with them, we are investigating the role and function of this new centriolar protein by employing various structural and biophysical techniques. Our results indicate that Gorab binds directly to the main cartwheel protein Sas6 in such a way that suggests a crucial role of Gorab in establishing a functional centriole with the specific 9-fold symmetry. Ongoing structural studies will further help us elucidate the role of Gorab in Drosophila centriole assembly.

The maximum entropy production principle and maximum Shannon information entropy in enzyme kinetics

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Enzymes in living cells are involved in biochemical reactions that represent open non-equilibrium thermodynamic systems. Such reaction systems are often shifted far from thermodynamic equilibrium. While classical thermodynamics offers elaborated theoretical instruments to study behavior of systems that are in or close to equilibrium, the processes in biological organisms are mostly far from equilibrium, and therefore the search for principia governing nonequilibrium systems is of great interest. The Maximum Entropy Production Principle (MEP) is a type of thermodynamic optimization which states that open non-equilibrium systems spontaneously approach toward steady states where they dissipate energy and produce entropy with maximal possible rate. It is claimed that among all possible steady states the steady state of MEP is selected because it is statistically the most probable one.

In this work the MEP principle is applied for analyzing the kinetics of enzymatic reaction catalyzed by Triosephosphate-isomerase (TPI), which is an important enzyme in glycolysis. It is investigated whether the most probable steady state of TPI enzyme is characterized by the maximum in the entropy production. The entropy production and Shannon information entropy are derived as functions of six independent enzyme rate constants. Mass conservation of the reaction system and the fixed free Gibbs energy of the reaction are taken as optimization constraints. The co-existence of well-defined maxima in the entropy production and Shannon information entropy for an arbitrary chosen enzyme rate constant is demonstrated. The calculated optimal enzyme rate constants are in good agreement with experimental data[1]. Moreover, by using the local stability analysis, we found that co-existence of the maxima in entropy production and Shannon information entropy might be a consequence of flexible enzymatic structure, which is necessary for effective enzymatic catalysis. In conclusion, our results suggest that the enzyme TPI operates close to the maximum in entropy production and requires the most flexible enzymatic structure for its optimal thermodynamic performance in the steady state.

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Biophysical characterisation of patient-derived immunoglobulin light chains

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Patients with a B-Cell proliferative disorder produce abnormally high amounts of monoclonal plasma free immunoglobulin light chains. Each patient presents a monoclonal light chain with a different amino acid sequence due to the variable domain. Because of this high sequence diversity, patient-derived light chains could serve as a model to correlate the differences in the amino acid sequence with biophysical behavior, in particular with respect to the tendency to aggregate to highly ordered fibrillar structures. In this study, monoclonal light chains isolated from the urine of patients were examined with different biophysical methods.

Development of new functional supramolecular systems based on β-cyclodextrin and liposomes for gene delivery

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The aim of the present study was preparation and characterization of supramolecular systems with adamantyl guanidine (AG) and probing of their interaction with DNA using fluorescence correlation spectroscopy (FCS). In the continuation of our study dealing with adamantyl guanidines and investigations of lipid-based nanovesicles as drug delivery systems [1, 2] we prepared and characterized functionalized supramolecular systems of AG 1-5 with phosphatidylcholine liposomes, liposomes incorporating amphiphilic β-cyclodextrin (β-CD) derivative and cyclodextrin vesicles composed only from the amphiphilic β-CD derivative. Efficiency of incorporation of AG 1-5 into prepared supramolecular systems was measured spectrophotometrically, and surface charge were determined by dynamic light scattering method. FCS was applied to examine the interactions between functionalized supramolecular systems and the Cy5 fluorescently labelled, double-stranded, 120 bp DNA (DNA120*). The obtained results have clearly demonstrated that the supramolecular systems with entrapped AG 1-4 present guanidinium groups on the surface of vesicles, which leads to interaction with DNA120* via guanidine-phosphate interaction. Preliminary results have shown that prepared functional supramolecular systems strongly bind DNA and as such could be used in gene delivery.

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The new role of Maf1 protein in the cytoplasm under glucose-rich conditions

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Maf1 has been identified as a repressor of RNA Polymerase III (RNAP III), the enzyme responsible for transcription of non-coding RNAs, among others tRNAs synthesis in yeast, invertebrates and vertebrates [1]. Under unfavorable growth conditions, when cells grow on glycerol, Maf1 is dephosphorylated by PP2A and PP4 [2] phosphatases and moves from cytoplasm to the nucleus [3]. In the nucleus, Maf1 physically interacts with RNAP III. This is the only RNAP III repression mechanism, so far revealed in *Saccharomyces cerevisiae*. Maf1 has several interaction partners in the nucleus, the kinases, such as: PKA, TORC, Sch9 [4] or CK2 [5]. Due to this interaction, Maf1 is phosphorylated and changes its cellular localization back to the cytoplasm. The biological role of Maf1, when localized in the cytoplasm, under optimal growth conditions is not yet well understood.

Here, we investigate the protein-protein interactions of Maf1 and its cytoplasmic partners using Bimolecular Fluorescence Complementation (BiFC) *in vivo* approach. We provide evidence that the interactions are essential for transduction of glucose signaling within the Snf1 pathway [6]. The novel protein-protein interactions require further studies and will be investigated using biophysical methodologies *in vitro*.

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Characterization of intermittent hypoxia effects on bone structure by acoustic impedance

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Intermittent hypobaric hypoxia is defined as to be below the normal values of partial oxygen pressure at tissue due to hypoxia periods [1]. It can lead to increased reactive oxygen species which causes oxidative damage to bone tissue [2].The amount of oxygen in the organism affects the production of erythrocytes. Since erythrocytes are produced in the bone marrow, the bone tissue is also affected by hypoxia. It can be characterized by measurement of acoustic velocity in soft and hard tissues with acoustic microscope or by measurement of acoustic impedance values [3]. The reason for using zinc (Zn), copper (Cu) and bicarbonate (HCO3) in our work is due to their role in erythrocyte, oxygen transport and cellular structure. The aim of our study was to investigate the effects of intermittent hypobaric hypoxia on bone structure by using acoustic impedance microscopy. Forty adult male Sprague Dawley rats randomly divided into five groups (Control, Hypoxia, Hypoxia+Zn, Hypoxia+Cu and Hypoxia+HCO3). Rats were exposured hypoxia a daily 8 hours for 5 days/week until completing 3 weeks in hypoxia cabine at a simulated pressure of 400-500 mmHg. The animals of Hypoxia+Zn, Hypoxia+Cu and Hypoxia+HCO3 experimental groups received through drinking water 30 mg/kg Zn, 7 mg/kg Cu and 3 mmol/kg NaHCO3, respectively. At the samples of bone taken from rats at the end of experiment were measured acoustic impedance. Our study is a preliminary study and we believe that we will be able to shed light on more molecular level studies considering statistical analyzes.

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Dipeptidyl peptidase III from a mesophilic and thermophilic bacterium – similarities and differences

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Dipeptidyl peptidase III (DPP III) is a two-domain protein, a metallopeptidase from the M49 family which contains a zinc ion within its active site and cleaves dipeptides from N-termini of its substrates [1]. Structure and flexibility of DPP III from the mesophilic bacterium *Bacteroides thetaiotaomicron (Bt)* and the thermophile *Caldithrix abyssi (Ca)* as well as their complexes with synthetic substrates and selected inhibitors were studied using computational methods in order to elucidate the differences between DPPs III from a thermophilic and a mesophilic organism. Several force fields have been tested to determine the most suitable one for studying long-range conformational motions within the enzyme. Comparison of results obtained for a termophile with those of a mesophile revealed the structural reasons behind thermal stability of *Ca*DPP III. The biologically relevant binding modes of substrates and inhibitors were identified and correlated with structural characteristics of the respective complexes, as well as with the enzyme flexibility [2]. The identified binding modes qualitatively agree with the kinetic data [3, 4]. These results were used to build a model system of *Bt*DPP III which was used in a quantum mechanics study in order to propose the reaction mechanism behind the peptide bond hydrolysis.

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Effects of I(h) ablation in principal neurons on excitatory and inhibitory synapses in the mouse hippocampus

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Spontaneous electrical activity guides developmental processes such as synapse maturation and refinement of neuronal circuits. The hyperpolarization activated current I(h), which is conducted by the hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN), has been suggested to contribute to development-dependent network activity and maturation of the CNS. By expressing a dominant negative HCN construct under the control of the CaMKII alpha promoter and Tet-off doxycycline system, we ablated I(h) in principal neurons during different developmental windows. We found that lifelong loss of I(h) starting around birth affects behavior and cortico-basal ganglia circuits in that I(h)-deficient mice developed antipsychotic-sensitive locomotor hyperactivity with stereotyped circling. Notably, restricting I(h) loss to an early postnatal period, i.e., to the first three weeks of life, had more severe consequences than lifelong ablation of I(h), and mutant mice, in addition to hyperactivity, also exhibited cognitive deficits. Our goal was to further investigate the effects of I(h) ablation on the synaptic coverage of the hippocampus. Immunofluorescently labeled sections for vesicular glutamate transporters 1 and 2 (VGLUT1 and VGLUT2, respectively) and vesicular inhibitory transmitter transporter (VGAT) were used to assess excitatory and inhibitory synaptic coverage of principal neurons in hippocampus subdivisions CA1, CA3 and dentate gyrus (DG). Results indicated that lifelong loss of I(h) causes an increase of VGLUT1 expression levels in stratum oriens and stratum radiatum of the CA1, indicating increased commissural and CA3 input, respectively, into this region. Furthermore, we found increased VGLUT2 expression in the hilus of the DG, probably originating from the mossy cells in this region. There were no significant differences in the number of perisomatic VGAT-positive terminals in any hippocampal area, indicating undisturbed inhibitory transmission. Thus, lifelong postnatal ablation of I(h) in the excitatory projection neurons causes increased excitatory synaptic transmission in the hippocampus and does not change inhibitory synapses. This increase in the excitation/inhibition ratio may be a potential mechanism to compensate for altered network activity upon I(h) loss during postnatal development.

Advancing Orthogonal Spin Labeling and pulsed EPR Distance Measurements

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SHORT TALK

Electron paramagnetic resonance (EPR) has become a versatile tool in the study of biomolecular structures, particularly providing access to distance information in the low nanometer range [1], typically in conjunction with site-directed spin labeling (SDSL) or by exploiting suitable metal cofactors.

Beyond the canonical distance determination between two identical spin labels, using two orthogonal labels holds the advantage of spectroscopic separability of the labeling sites. This particularly enables the detection of distances between subunits of low-affinity complexes, which are challenging to probe otherwise [2]. Currently, we use the well-characterized heterodimeric protein Rpo4/Rpo7 [3] as a model system, which we genetically engineered to contain one cysteine residue and one unnatural amino acid per subunit, both of which can be specifically targeted by SDSL [4]. The resulting system opens up the possibility of specific incorporation of up to four different spin labels arranged in a well-defined protein complex. Spectroscopically this poses the interesting possibility of selective excitation and detection of the different paramagnetic centers. From a structural biology point of view, it enables the selective detection of specific distances as well as the correlation of multiple distances within one sample, thus potentially reducing the required number of samples for structural characterization.

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Alpha satellites and Higher Order Repeats in human, Neanderthal and chimpanzee genomes - new database

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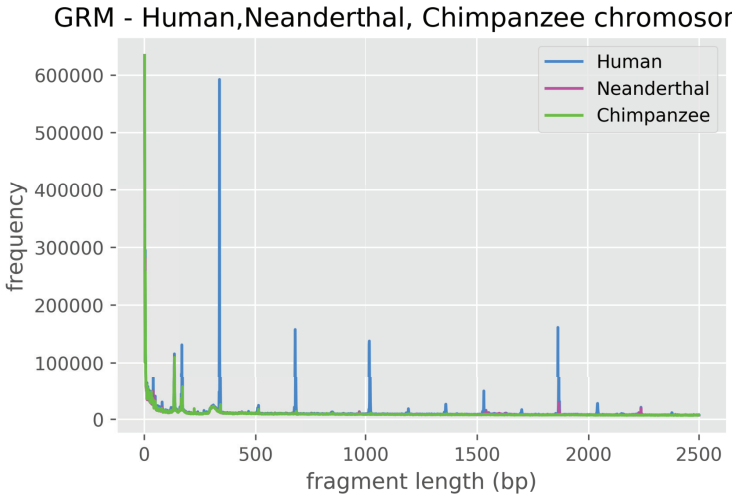
Recent DNA technologies enabled researchers around the world to sequence DNA of different species, even the extinct ones such as Neanderthals, human close relatives. Those DNA sequences, publicly available, enabled us to investigate part of unjustly called "junk DNA" - repeats of all kinds using computational tools. Our group developed special algorithm for repeat identification called Global Repeat Map (download at http://genom.hazu.hr/tools.html [1]). Using this algorithm, we identified many tandem repeats of which some appear in form of higher order -HORs (tandem within tandem arrays of DNA sequence). Those HORs are mostly found in centromere with repeat basic unit of 171 bp, but some of them can be found in genes (an example is NBPF family gene in humans responsible for brain development and occurrence of diseases like macrocephaly, microcephaly and autism). We build a database (http://genom.hazu.hr/) where we store those repeats and their higher order structures along with sequence analysis for human, Neanderthal and chimpanzee DNA. This database can serve to other researchers for experiment designs to reveal their roles, and especially roles of higher order repeats, as is known form other research that some of them might have regulatory roles [1, 2]. With this poster we present our database and some large HORs based on alpha satellite sequence (see figure), as one of the main result of our project.



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Compartmentalization of dynamic monolayers

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Morphogenesis and wound healing both require migration of large number of constituent cells. We address these complex problems by using MDCK II model epithelium grown on collagen I coated glass substrates. Usually, to study such a system, a part of an expanding monolayer is carefully analyzed. Here we take the complementary approach and look at the global development of an, initially droplet seeded, system of cells which is allowed to expand freely over time. In contrast to most studies majority of our experiments performed have very long time windows of at least 10 days. On the basis of experimental findings the known model of exponential growth of small (< 0.1 mm^2) cell clusters is expanded with an additional parameter which accounts for the slowing down of area growth. Thus, with the use of a simple differential equation, and easily interpreted parameters - initial colony area (A_0), colony doubling time (tau) and effective slowing down of growth (b) - one can successfully predict the area expansion of clusters in the range of four orders of magnitude. Further data analysis shows a stunning picture of a perpetually accelerating monolayer border, in stark opposition to the concept of constant speed limits supposedly already reached by macroscopic (> 10mm^2) monolayers.

Quantitatively obtained parameters from 3 distinct experimental observations are combined together and fed to simulations that approximate cells with 2D Voronoi objects [1]. Results of modelling show that even such a small set of parameters can be complete and accurately predict the global colony morphology after as much as 10 days of growth.

Looking in, analysis of the flow fields down to microscopic scales shows the distinct behaviour not seen in simple diffusion processes. Distributions of velocities discern two different cell populations. A "static" and an "active" one. Non intuitively, monolayers show coexistence of these two populations even deep inside the jammed colony bulk. To our knowledge this aspect of "active jamming" has not been observed previously [2].

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Studying the effect of anticancer drugs breast cancer cell mechanics via AFM

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The study of mechanical properties of eukaryotic cells has gained an increased interest in recent years. An important finding in this field is that different cellular states (e.g. disease, cancer, age ...) lead to significant alterations in those properties [1]. By studying cell mechanics, one can therefore derive important knowledge to further understand the complex mechanisms of e.g. cancer formation. In addition, the quantification of those changes can be used as novel diagnostics tool [2].

In this study, atomic force microscopy (AFM) in force spectroscopy mode was used to determine, at the nanoscale, the mechanical properties of MCF-7 (Michigan Cancer Foundation) human breast cancer cells. The time and concentration dependent effect on cell mechanics by different agents (tamoxifen, resveratrol) was studied [3, 4]. Thus, mechanics-related factors such as cell elasticity, adhesion, as well as cell rheology were obtained (and their dependence upon incubation with the aforementioned substances). Batch data processing was performed by means of self-developed R language protocols (afmToolkit) [5]. Complementarily, fluorescence microscopy experiments were performed to study cytoskeletal rearrangements. By this combined methodology new insights on drug treatment influence (or resistance) on cancer cells could be achieved.

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Hydroxyl Radical Foot Printing of Ubiquitin

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Hydroxyl radical (OH), the most reactive form of reactive oxygen species, can react with any amino acid side chains that are solvent accessible. Therefore, the reaction of reactive oxygen with proteins has been used to probe the structure of proteins and protein complexes. To introduce hydroxyl radicals to biological systems, several methods such as radiolysis [1], photolysis [2] and Fenton chemistry [3] can be utilized.

In this study, Top Down mass spectrometry (FT-ICR) analysis has been applied to the structural characterization of oxidized ubiquitin induced by metal-mediated hydroxyl radicals. Oxidation of ubiquitin was achieved using the Fenton oxidant consisting of $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$, H_2O_2 and EDTA. Two parameters in the Fenton process were optimized in order to obtain higher yield of oxidized products - concentration of Fe(II) and H_2O_2. When reasonable oxidation of ubiquitin was observed, Top Down analysis - collision induced dissociation and electron transfer dissociation - of intact and oxidized ubiquitin was performed in order to find the modification sites. Detailed interpretation of the MS/MS spectra from isolation of the +11, +10 and +9 charge states was used to identify the amino acid side chains on the protein surface that are the most susceptible to oxidation. Our results show that the Fenton reaction is a simple and an inexpensive way of radical generation for protein structural foot-printing and Top Down strategy is the most promising technology for such a comprehensive analysis.

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Competitive interaction of water and hydrogen peroxide molecules with specific DNA recognition sites

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Water environment is an important factor in DNA secondary structure stabilization. Thus, changes in the medium will affect significantly on DNA stability. It was shown [1] that hydrogen peroxide is highly accumulated in the water medium during ion beam cancer treatment. In the work [2] we studied interaction of hydrogen peroxide molecules with non-specific DNA recognition sites.

In the present work the competitive interaction of water and hydrogen peroxide molecules with specific nucleic recognition sites — DNA bases, are studied. Complexes of DNA bases (Adenine, Thymine, Guanine or Cytosine) with solvent molecules (water or hydrogen peroxide) are under investigation. Interaction energies are calculated using atom-atom potential functions method taking into account van der Waals, Coulomb interactions and hydrogen bonds. As a result, the sites of DNA bases where hydrogen peroxide molecules can create more stable complexes with nucleic base compared with the same complex with water molecules are determined. Our study shows that recognition of Cytosine can be blocked by hydrogen peroxide from major groove, and recognition of Guanine – from minor groove. Adenine and Thymine can be blocked on the stage of DNA transcription when complementary pair is already opened. Therefore, the presence of hydrogen peroxide molecules in DNA major and minor grooves can block the processes of genetic information transfer.

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Bacterial-phytoplankton interface in natural lake ecosystems

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Cryptophytes, chrysophytes and dinoflagellates are groups of phytoplankton largely consisting of species with well documented mixotrophy. These groups also comprise a major part of phytoplankton biomass in karstic lakes with good to high ecological status. The aim of the study was to establish the link between bacterial community and potential mixotrophs in the planktonic community of a karstic mesotrophic Visovac Lake (Krka National Park, Croatia), in order to provide a precise assessment of the ecological status of lake ecosystem. Integrated vertical samples for phytoplankton, samples for physical-chemical parameters, water chemistry analyses and bacteriology were taken simultaneously from the deepest point of the lake, once a month in the period between April and September 2016. Phytoplankton composition and biomass were determined by standard methods, while fluorescence *in situ* hybridization with signal amplification and catalyzed reporter deposition (CARD-FISH) was applied for bacterial microscopic quantification. Additionally, filtered water was used for DNA extraction and 16S rRNA gene sequencing for bacterial identification. Total phytoplankton biomass varied from 0.24 mgL^{-1} (June) to 0.75 mgL^{-1} (July). Potentially mixotrophic species characterizing the community were: *Dinobryon divergens, Dinobryon bavaricum, Dinobryon crenulatum, Chromulina* sp., *Ochromonas danica, Plagioselmis nannoplanctica, Cryptomonas* sp., *Parvodinium inconspicuum* and *Ceratium hirundinella.* The lowest total bacterial abundance was determined in April (2.61 x 10^6 cell mL^{-1}), whilst the highest was in July (11.39 x 10^6 cell mL^{-1}). Sequencing of 6 integrated samples resulted in a total of

132 429 nucleotide sequences, with 5 bacterial groups identified: *Betaproteobacteria, Actinobacteria, Bacterioidetes, Chloroflexi* and *Veruccomicrobia. Actinobacteria* was the most abundant class in all investigated months (avg. 34%), while *Betaproteobacteria* was subdominant (avg. 22%). Multivariate analysis provided a statistically significant corelation between the selected phytoplankton species and determined bacterial groups. The results will be used to determine relation between mixotrophic species and bacterial groups in lakes with variable ecological status.

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