# 12th Greta Pifat Mrzljak International School of Biophysics

## BIOMACROMOLECULAR COMPLEXES AND ASSEMBLIES

# BOOK OF ABSTRACTS

Primošten, Croatia, 27.9.-6.10.2014.

# BOOK OF ABSTRACTS

## BIOMOLECULAR COMPLEXES AND ASSEMBLIES

**12th Greta Pifat Mrzljak**
**International School of Biophysics**

**Primošten, Croatia** **27.9.-6.10.2014.**

**The 12th Greta Pifat Mrzljak International School of Biophysics**
**Biomolecular complexes and assemblies / Book of Abstracts**
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# FOREWORD

## The School

This session of the International School of Biophysics is the twelfth in a series held for more than 30 years across Croatia, in Dubrovnik, Rovinj and Primošten. The previous sessions were held in 1981, 1984, 1987, 1990, 1994, 1997, 2000, 2003, 2006, 2009 and 2012. 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. She was also awarded the American Biophysical Society's Emily M. Gray Award, primarily for her inspiring achievement with the School.

After passing of prof. Pifat Mrzljak in 2009, it was widely agreed that this 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. We had and still have in 2014 a strong support by EBSA (European Biophysical Societies' Association) and the Lecturers themselves, who commit their time and financial resources as a greatest sign of the need for the School to keep going. Here we would emphasize the role of professor Anthony Watts, Oxford University who is the greatest supporter of the School (and a lecturer), as well as the other members of the EBSA Executive Committee. This year, we coorganized the School with COST (European Cooperation in Science and Technology) network CM1306 Molecular Machines, coordinated by dr. Fraser MacMillan. School is also suppported by the Institute of physics, Zagreb and the Physics Department of Zagreb University where many of the organization team members work.

The Lecturers at the International School of Biophysics have always included highly prominent figures in 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 visible at this School. Indeed, this concept promoted at the previous sessions of the School has already benefitted 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 themselves. 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 select and enthusiastic young people to interact with top scientists in a relaxed manner is elementary in their development and a route to their ultimate succes in this quickly moving and challenging area. A thrilling experience is here also for the Lecturers, in teaching dozens of young and inquisitive minds. In the past, this interaction has been enhanced by the environment created by the local organizational team.

**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, which include inter- and intra-molecular interactions in biological systems; the structure, organization and function of biological macromolecules (proteins, nucleic acids, lipids, sugars) and supramolecular assemblies (membranes, viruses, ribosomes; quaternary protein structures; DNA, proteins and polyelectrolytes); major techniques in biophysics: spectroscopies (NMR, EPR, FTIR, Raman, mass spectrometry …), microscopies (AFM, fluorescence techniques, super resolution…), diffraction techniques (X-ray crystallography), computational solutions including modeling and simulations, and the evolutionary implications of molecular interactions, as well as molecular biology and biotechnology.

**Students and Lecturers**

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, in school discussion clubs or even on the beach. About 30 prominent lecturers give two to three lectures each per session.

Previous 11th School was attended by 70 Ph.D. students from 16 countries in Europe. 50 students presented the poster, of which 20 had a short poster talk. 11 students were supported by EBSA, with additional 4 supported from Greta Pifat Fund. There were 20 lecturers from Croatia, USA, UK, Germany, Austria, Italy, Spain, Switzerland and Slovenia. This year's school will feature similar numbers, over 70 students from all over Europe (16 European countries) as well as from Australia, Iran, Japan and Russia. EBSA supports 12 students, while Greta Pifat Fund supports additional 4 coming from outside Europe.

The School is chaired by Tomislav Vuletić, Institute of physics, Zagreb, programmed by our Programme Committee and prepared by the 5-person strong local team.

We wish the Students and Lecturers at the 12th 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 11th School:

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 11th Greta Pifat Mrzljak International School of Biophysics and read at the Gala dinner on 8th October 2012.

## ORGANIZING TEAM

**Dr. Tomislav Vuletić (Chair)**
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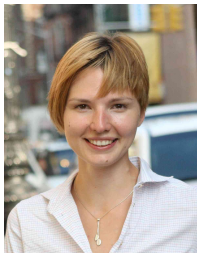
**Danijel Grgičin**, Institute for Physics, Zagreb, Croatia
**Jasmina Bilać**, ProConventa d.o.o., Zagreb, Croatia

## LECTURERS

**Prof. Dr. Nenad Ban**
ETH Zürich, Switzerland



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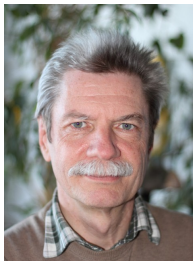

**Prof. Dr. Heinz-Jürgen Steinhoff**
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University of Melbourne, Australia

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**Prof. Anthony Watts**
University of Oxford, United Kingdom

**Dr. Bojan Žagrović**
University of Vienna, Max F. Perutz Laboratories, Vienna, Austria

## SPONSOR LECTURERS

**Dr. Aleksej Kedrov**, Gene Center LMU, München, Germany

**Dr. Primož Meh**, Instrumentalia d.o.o., Ljubljana, Slovenia

**Dr. Piotr Wardega**, NanoTemper GmBH, München, Germany

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## ORGANIZED BY

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COST CM1306 MOLECULAR MACHINES

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OF THE REPUBLIC OF CROATIA

RapidCell - IPA fund

# PROGRAM

## Saturday September 27 / SCHOOL DAY 1

15:30 - 17:30 REGISTRATION

**TOMISLAV VULETIĆ (Chair), Institute of physics, Zagreb, Croatia**
17:30 Welcome address

18:00 DINNER

**FRANCES SEPAROVIC, University of Melbourne, Australia**
19:30 Opening lecture

20:00 GET TOGETHER

## Sunday September 28 / SCHOOL DAY 2

**NENAD BAN, ETH Zürich, Institute for molecular biology and biophysics, Switzerland**
9:00 Ribosomes and their functional complexes involved in co-translational protein folding, processing and targeting to membranes

10:00 Beyond the prokaryotic ribosome: structural and functional insights into eukaryotic and mitochondrial ribosomes

11:00 COFFEE BREAK

**JASNA BRUJIC, New York University, USA**
11:30 Biomimetic emulsions probe lipid domain formation, cell-cell adhesion and hemifusion

13:00 LUNCH

**JASNA BRUJIC, New York University, USA**
15:30 Single molecule force spectroscopy reveals mechanisms of protein folding

**IVA TOLIĆ, MPI of molecular cell biology & genetics, Dresden, Germany & RBI, Zagreb, Croatia**
17:00 Unexpected actions in the mitotic spindle revealed by live-cell imaging and laser microsurgery

17:45 Total Internal Reflection Fluorescence (TIRF) microscopy *in vivo:* Single-molecule imaging of motor proteins

18:30 DINNER

**20-22 POSTERS TALKS, POSTERS & DRINKS**
20:00 **Ida Delač Marion:** Polyelectrolyte composite: Hyaluronic acid mixture with DNA

20:10 **Danijel Grgičin:** A novel approach to study deoxyribonucleic acid denaturation

20:20 **Sanjin Marion:** DNA with condensing proteins in spherical confinement

20:30 **Martina Lihter:** Solid-state nanopores

## Monday September 29 / SCHOOL DAY 3

**ANA - SUNČANA SMITH, Friedrich Alexander University Erlangen-Nürnberg, Germany & Ruđer Bošković Institute, Zagreb, Croatia**

9:00 Modeling protein organisation in adherent membranes (part I)

10:00 Modeling protein organisation in adherent membranes (part II)

11:00 COFFEE BREAK

**HOLGER STARK, MPI for Biophysical Chemistry, Goettingen, Germany**

11:30 Single Particle Cryo-EM as a tool to determine 3D structures of macromolecular complexes

12:30 Visualizing dynamic macromolecular complexes by cryo-EM at atomic resolution

13:30 LUNCH

**DAVID M. SMITH, Ruđer Bošković Institute, Zagreb, Croatia & Friedrich-Alexander-University, Erlangen- Nürnberg, Germany**

16:00 The integrated molecular orbital approach to accurate calculations of large molecular systems

17:00 Calculating CD spectra for flexible biomolecules

18:00 DINNER

**20-22 POSTERS TALKS, POSTERS & DRINKS**

20:00 **Olga Doroshenko:** The thermodynamics of dopamine / membrane interactions

20:10 **Sandor Szunyogh:** The disordered TPPP/P25, a prototype of the neomorphic moonlightning proteins

20:20 **Jelena Đorović:** Examination of antioxidant activity of gallic acid with different radicals

20:30 **Nevena Cvetešić:** Reassessment of LeuRS discriminatory power unveils norvaline as a prime quality control target

## Tuesday September 30 / SCHOOL DAY 4

**ANTHONY WATTS, University of Oxford, UK**

9:00 Principles of biological solid state NMR (part I)

10:00 Principles of biological solid state NMR (part II)

11:00 COFFEE BREAK

**ANTHONY WATTS, University of Oxford, UK**

11:30 NMR of membrane proteins - Drug targeting

**FRANCES SEPAROVIC, University of Melbourne, Australia**

12:30 Solid-state NMR of membrane-active peptides (part I)

13:30 LUNCH

**FRANCES SEPAROVIC, University of Melbourne, Australia**

16:00 Solid-state NMR of membrane-active peptides (part I)

**CHRIS OOSTENBRINK, University of natural resources and life sciences, Vienna, Austria**

17:00 Ensembles and sampling, leading to molecular dynamics simulations

18:00 DINNER

**20-22 POSTERS TALKS, POSTERS & DRINKS**

20:00 **Anna Wypijewska del Nogal:** Productive versus non-productive complex formation by DcpS enzyme and mRNA cap analogs

20:10 **Marine Bozdaganyan:** Computational study of interaction of C60-fullerene and tris-malonyl-C60-fullerene isomers with biomembranes

20:20 **Luca Ponzoni:** Dynamical domain decomposition of protein complexes based on pairwise distance fluctuations

20:30 **Joana Smirnovienė:** Comparison of enzymatic and biophysical assays using carbonic anhydrases as model proteins

## Wednesday October 1 / SCHOOL DAY 5

**CHRIS OOSTENBRINK, University of natural resources and life sciences, Vienna, Austria**

9:00 Structure refinement using molecular dynamics simulations (NMR observables)

10:00 Calculation of free energies from molecular simulation

11:00 COFFEE BREAK

**HEINZ-JÜRGEN STEINHOFF, University of Osnabrück, Germany**

11:30 Site-directed spin labeling and electron paramagnetic resonance spectroscopy: an introduction

12:30 LUNCH

14:00 EXCURSION to Krka National Park

20:00 DINNER (20-21h)

## Thursday October 2 / SCHOOL DAY 6

**YVES ENGELBORGHS, University of Leuven, Belgium**

9:00 Steady state and time resolved fluorescence applied to the study of molecular interactions

10:00 Fluorescence correlation spectroscopy: An elegant way to study molecular interactions in solution and in the living cell

11:00 COFFEE BREAK

**HELMUT GRUBMÜLLER, MPI for Biophysical Chemistry, Goettingen, Germany**

11:30 Forces and conformational dynamics in biomolecular nanomachines

12:30 Forces and conformational dynamics in biomolecular nanomachines

13:30 LUNCH

**HEINZ-JÜRGEN STEINHOFF, University of Osnabrück, Germany**

16:00 Inter- and intramolecular distance measurements using cw and pulse EPR spectroscopy

**BOJAN ŽAGROVIĆ, Max F. Perutz Laboratories & University of Vienna, Austria**

17:00 Protein-RNA interactions and the origin of the genetic code

18:00 DINNER

**20-22 POSTERS TALKS, POSTERS & DRINKS**

20:00 **Martin Calvelo Souto:** Study of a supramolecular assembly through molecular dynamics simulations: α,γ-peptide nanotubes

20:10 **Anna Komarova:** Key participants of mechanical stress response induced with cell wall microperforation in plant cells

20:20 **Katharina Rudi:** Conformational changes during GTP hydrolysis in wt and mutated orthologs of the human LRRK2 Parkinson kinase analyzed by DEER

20:30 **Daniel Klose:** Light-induced switching of HAMP domain conformation & dynamics revealed by time-resolved EPR spectroscopy

## Friday October 3 / SCHOOL DAY 7

**BOJAN ŽAGROVIĆ, Max F. Perutz Laboratories & University of Vienna, Austria**

9:00 More dynamic than we think? On conformational averaging in structural biology (part I)

10:00 More dynamic than we think? On conformational averaging in structural biology (part II)

11:00 COFFEE BREAK

**PIERRE-EMMANUEL MILHIET, Centre de Biochimie Structurale, Montpellier, France**

11:30 Near field microscopies in structural biology

12:30 AFM imaging in the field of biological membranes

13:30 LUNCH

**PIERRE-EMMANUEL MILHIET, Centre de Biochimie Structurale, Montpellier, France**

16:00 New developments in Bio AFM imaging

**PIOTR WARDEGA, NanoTemper GmBH, München, Germany**

17:00 Quantitative analysis of biomolecular interactions with Microscale Thermophoresis (MST)

18:00 DINNER

**20-22 POSTERS TALKS, POSTERS & DRINKS**

20:00 **Jaroslav Varchola:** Singlet oxygen production after photoexcitation of hypericin in DMSO/water mixture

20:10 **Leila Fotouhi**: Study of heme degradation and ROS production upon interaction of hemoglobin with n-alkyl sulfates

20:20 **Anna Mularski:** Interaction of a model antimicrobial peptide with bacterial cells

20:30 **Anna Katharina Weyrauch:** EPR- and IRRAS studies on Alpha-Synuclein

## Saturday October 4 / SCHOOL DAY 8

**ANTONIO ŠIBER, Institute of physics, Zagreb, Croatia**

9:00 Physics of viruses: electrostatics, elasticity and DNA condensation in viruses (part I)

10:00 Physics of viruses: electrostatics, elasticity and DNA condensation in viruses (part II)

11:00 COFFEE BREAK

**JANE KONDEV, Brandeis University, Waltham, USA**

11:30 DNA folding in bacteria

12:30 DNA folding in eukaryotes

13:30 LUNCH

**ANTONIO ŠIBER, Institute of physics, Zagreb, Croatia**

16:00 Physics of viruses: electrostatics, elasticity and DNA condensation in viruses (part III)

**LENNART NILSSON, Karolinska Institutet, Huddinge, Sweden**

17:00 Methodological aspects of nucleic acid simulations

18:00 DINNER

**20-22 POSTERS TALKS, POSTERS & DRINKS**

20:00 **Marta Kwiatkowska:** Glyphosate and its metabolites exhibit proapoptotic and prooxidative effects on human lymphocytes

20:10 **Kristina Perica:** Mass spectrometry-powerful tool for research of antibiotic resistance mechanisms in bacteria

20:20 **Edyta Pytel:** Effect of lipid-lowering therapy on homocysteine level and other plasma parameters in coronary artery disease (CAD)

20:30 **Yachong Guo:** How to make pores in lipid bilayers by tuning shape of embedded objects

## Sunday October 5 / SCHOOL DAY 9

**LENNART NILSSON, Karolinska Institutet, Huddinge, Sweden**

9:00 Molecular dynamics simulations of nucleic acids and protein-nucleic acid complexes (part I)

10:00 Molecular dynamics simulations of nucleic acids and protein-nucleic acid complexes (part II)

11:00 COFFEE BREAK

**JANE KONDEV, Brandeis University, Waltham, USA**
11:30 Functional organization of chromosomes in cells

**MARIO CINDRIĆ, Ruđer Bošković Institute, Zagreb, Croatia**
12:30 Mass spectrometry-back-to-basics

13:30 LUNCH

**PRIMOŽ MEH, Instrumentalia d.o.o., Ljubljana, Slovenia**
16:00 Protein stability and interactions by ITC and DSC

**ALEKSEJ KEDROV, Gene Center LMU, München, Germany**
17:00 Biophysical analysis on membrane protein targeting and assembly

19:30 GALA DINNER (19:30 - 23:30)

## Monday October 6 / SCHOOL DAY 10

**MARIO CINDRIĆ, Ruđer Bošković Institute, Zagreb, Croatia**
9:00 Protein sequencing- who, what, where, when, why and how

**FRASER MACMILLAN, School of Chemistry, Norwich, UK**
10:00 Understanding movement and mechanism in molecular machines: PsaBCA and manganese acquisition

11:00 COFFEE BREAK

11:30 CLOSING REMARKS

13:30 - 16:00 LUNCH

DEPARTURE

# LECTURES

**NENAD BAN**

ETH Zürich, Institute for molecular biology and biophysics, 8093 Zürich, Switzerland;
ban@mol.biol.ethz.ch

## Lecture 1: Ribosomes and their functional complexes involved in co-translational protein folding, processing and targeting to membranes

In all organisms mRNA directed protein synthesis is catalyzed by a ribonucleoprotein particle called the ribosome. Newly synthesized proteins leave the ribosome through the nascent polypeptide tunnel. Through the coordinated action of the ribosome associated chaperones, nascent chain processing enzymes, the signal recognition particle, and the protein insertion machinery newly synthesized proteins are brought into their native state and proper cellular localization. The interplay of these factors during ongoing synthesis requires spatial and temporal control of their interactions with the ribosome. We used electron microscopy in combination with crystallography and biochemical methods to study the structure of bacterial ribosomes and various nascent chain interacting factors.

[1] Ferbitz L, Maier T, Patzelt H, Bukau B, Deuerling E, Ban N (2004) Trigger factor in complex with the ribosome forms a molecular cradle for nascent proteins. *Nature* 431:590-6.

[2] Schaffitzel C, et al. and Ban N (2006) Structure of the E. coli signal recognition particle bound to a translating ribosome. *Nature* 444(7118):503-6.

[3] Bingel-Erlenmeyer R, et al. and Ban N (2008) A peptide deformylase-ribosome complex reveals mechanism of nascent chain processing. *Nature* 452(7183): 108-11.

[4] Ataide SF, Schmitz N, Shen K, Ke A, Shan SO, Doudna JA, Ban N (2011) The crystal structure of the signal recognition particle in complex with its receptor. *Science* 2011 Feb 18;331(6019):881-6.

## Lecture 2: Beyond the prokaryotic ribosome: structural and functional insights into eukaryotic and mitochondrial ribosomes

Although basic aspects of protein synthesis are preserved in all kingdoms of life, eukaryotic ribosomesare much more complex than their bacterial counterparts, require a large number of assembly andmaturation factors during their biogenesis, use numerous initiation factors, and are subjected to extensiveregulation. In an effort to better understand the structure and the function of eukaryotic ribosomes wehave determined complete structures of both eukaryotic ribosomal subunits each in complex with aninitiation factor[1,2]. These results provide detailed structural information on the entire eukaryoticribosome, reveal novel architectural features of this ribonucleoprotein complex and offer insights in to the various eukaryotic-specific aspects of protein synthesis and ribosome evolution. Recently, using cryo electron microscopy we obtained first insights into the architecture of mammalian mitochondrialribosomes and revealed the mechanism of how mitochondrial

ribosomes, specialized for the synthesisof membrane proteins, are attached to the membrane[3].

[1] Rabl J, Leibundgut M, Ataide SF, Haag A, Ban N (2011) Crystal structure of the eukaryotic 40S ribosomal subunit in complex with initiation factor 1. *Science* 331(6018):730-6.

[2] Klinge S, Voigts-Hoffmann F, Leibundgut M, Arpagaus S, Ban N. (2011) Crystal Structure of the Eukaryotic 60S Ribosomal Subunit in Complex with Initiation Factor 6. *Science* 334(6058):941-948.

[3] Greber BJ, Boehringer D, Leitner A, Bieri P, Voigts-Hoffmann F, Erzberger JP, Leibundgut M, Aebersold R, Ban N (2014) Architecture of the large subunit of the mammalian mitochondrial ribosome. *Nature* 505(7484):515-9.

**JASNA BRUJIC**

New York University, Department of Physics, New York, NY 10003, USA; jb2929@nyu.edu

## Lecture 1: Biomimetic emulsions probe lipid domain formation, cell-cell adhesion and hemifusion

Emulsion droplets are a versatile system whose interactions can be tuned to mimic cellular functions, such as lipid domain formation, cell-cell adhesion or even hemifusion. For example, immiscible lipids on the surface of emulsion droplets create stable patterns of circular or stripy domains, reminiscent of lipid rafts in cell membranes. Functionalizing the lipids with biotins allows them to bind to each other either irreversibly through streptavidin or reversibly through cadherins or complementary DNA strands. We show that these mobile adhesion patches self-assemble linear chains of thermal droplets into well-defined compact structures. The size of adhesion is predicted by the balance between the binding energy and the energy of deformation of the droplets. Applying an external pressure to the system strengthens adhesions, which highlights the importance of homeostatic pressure on cell-cell adhesion and tissue integrity in vivo. Alternatively, functionalizing the lipids with E-cadherin proteins unexpectedly leads to droplets fusing together up to a given droplet size. Microscopically, we find that the lateral cis-interaction of cadherins clusters them into rings upon adhesion, which in turn ruptures the adjacent lipid monolayers to cause fusion. Emulsions are therefore not only a new class of liquid patchy particles for self-assembly, but also a model system for important problems in biophysics.

## Lecture 2: Single Molecule Force Spectroscopy reveals mechanisms of protein folding

Protein unfolding and refolding trajectories under a constant stretching force are manifestations of the underlying molecular processes in the end-to-end length. In the case of ubiquitin, I27 and NuG2 protein, the distribution of unfolding times at a given force is best fit with a stretched exponential function, while the collapse from a highly extended state to the folded length is well captured by simple diffusion along the free energy of the end-to-end length. Nevertheless, the estimated diffusion coefficient of $\sim 100 nm^2 s^{-1}$ is significantly slower than expected from viscous effects alone, possibly because of the internal degrees of freedom of the protein. The free energy profiles give validity to a physical model in which the multiple protein domains collapse all at once and the role of the force is approximately captured by the Bell model.

**MARIO CINDRIĆ**

Ruđer Bošković Institute, Bijenička 54, 10 000 Zagreb, Croatia; mcindric@irb.hr

## Lecture 1: Mass spectrometry-back-to-basics

The basics of mass spectrometry will be considered, starting with instrumentation, followed by different techniques of ionization and fragmentation necessary to understand development of mass spectrometry during the past two decades. Particularly, this tutorial lecture will be focused on technical aspects of mass spectrometry:

- ion sources
- ion optics
- ion separation
- cells for fragmentation
- ion detectors
- collecting MS data
- data processing

Techniques for molecules and macromolecules separation and fractionation hyphenated to mass spectrometers, particularly mass spectrometry techniques for the large scale identification of proteins will be reviewed. Also, different methods for protein quantification by mass spectrometry will be described and discussed. Finally, bioinformatics principles to convert mass spectrometry data into peptide/protein sequences and to quantify them will be introduced.

References:

- Hoffmann E and Stroobant V (2007) *Mass Spectrometry: Principles and Applications*, (3rd ed.) John Wiley & Sons: Chicester, England.
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## Lecture 2: Protein sequencing - who, what, where, when, why and how

Amino acid sequencing and more detailed structure elucidation analysis of peptides and small proteins is a very difficult task even if the state-of-the-art mass spectrometry is employed.

A novel method for peptide amino acid sequence determination upgraded with genome fingerprint scanning (GFS) was designed to investigate gene expression of the clinical organisms at the first instance but it can be used in further development as ultimate *de novo* sequencing method in “bottom up” proteomics. The method

links proteomics data, consisting of determined tryptic peptides amino acid sequences obtained by negative and positive ion modes. MALDI or ESI-MS/MS sequencing data readings are compared to sequenced genome of the observed organism and confirmed by overlapping of the sequence data from both modes (e.g. GAAGAK read in neg. and pos. ion modes; GAAGAK b ions neg. ion mode and KAGAAG y ions pos. ion mode). The idea that lies behind this new technology is enhanced *de novo* sequencing of unknown peptide amino acid sequences in negative MS/MS (enabled by derivatization that contains two negatively charged groups without observed side reactions or peptide degradation) and positive MS/MS of the same peptide used as quality control (MS/MS of derivatized ions in both cases). An overview of alternative techniques that are used for protein sequencing, *e.g*. Edman degradation or label-free mass spectrometry will be presented, as well.

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## Lecture 1: Steady state and time resolved fluorescence applied to the study of molecular interactions

The concepts and techniques to study time resolved fluorescence will be analyzed. Fluorescence is basically a kinetic technique. The kinetic equation for the formation (absorption) and disappearance of the excited state via radiative (emission) and non-radiative routes (internal conversion, intersystem crossing, quenching, energy transfer) will be discussed[1]. This equation can be solved (1) for continuous illumination leading to steady state fluorescence, (2) for a pulsed illumination, leading to photon timing, or for (3) a sinusoidally modulated illumination, resulting in phase fluorometry. The different processes will be discussed in detail and the molecular information that can be obtained in this way will be analyzed. Also the two types of techniques used to collect the data will be described and analyzed, and combined with a microscope for cellular applications. Using polarized light as excitation source a selection of molecules is made with their absorption dipole preferentially oriented parallel to the excitation plane. After excitation and before emission fluorescent labels can rotate, which results in a decrease of the anistropy of emission (time dependent or steady state). From the time dependence of the emission anisotropy the rotational correlation time can be obtained which is related to the volume of the molecule. Associations with other molecules lead to an alteration in the behavior of the anisotropy. Many protein have more than one tryptophan residue. The fluorescence of the whole protein can be explained on the basis of the contribution of each tryptophan residue by checking for additivity[2]. In the absence of this additivity, mutual interactions may be considered e.g. bidirectional energy transfer. Excited state processes of tryptophan will be considered, including reversible interconversions between rotamers. An attempt to correlate tryptophan rotamers by fluorescence and protein NMR will be discussed[3].

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## Lecture 2: **Fluorescence Correlation Spectroscopy: An elegant way to study molecular interactions in solution and in the living cell**

FCS is a single molecule technique with very high sensitivity. Since it measures concentration fluctuations, it needs small observation volumes and low concentrations of fluorescently labeledmolecules i.e. nanomolar as achieved in the observation volume of a confocal microscope. It is therefore able to study protein associations at very low concentrations, via the determination of the diffusion coefficient. But many proteins associate/dissociate in the micromolar or higher concentration regime. This challenge for the FCS technique can be overcome by using trace amounts of fluorescently labeled protein in the presence of a large excess of unlabeled protein. In this way the limitation to nanomolar concentrations can be overcome. An additional simplification thanks to this scheme is that every oligomer contains only one label. The application of this technique to the oligomerization of tubulin[1], alpha-synuclein[2], and tumor suppressor p53[3] will be presented. FCS also produces brightness information. This has been used extensively to study many proteinassociation systems. In brightness analysis typically, all proteins are labeled and the brightness should be proportional to the size of the oligomer. The method is powerful but does not always make use of the diffusion information and needs again nanomolar concentrations of fluorescent particles. In a recent study[4] a combination of the two techniques is presented by using mixtures of unlabeled protein with labeled one at concentrations much higher than just trace amounts. By doing FCS measurements as a function of increasing total protein concentration the decrease of the apparent diffusion coefficient as a function of total protein concentration can be obtained. This apparent diffusion coefficient is a kind of “average” diffusion coefficient of all species present, taking into account the weighting by the square of the brightness, the concentration and the relation between size and diffusion coefficient. In this way a quantitative analysis of a complex system is possible. If time allows the diffusion of HIV-integrase and LEDGF, in the cell, and their interaction with chromatin will be discussed[5].

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## Lectures 1 & 2: 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], mechanics of energy conversion in F-ATP synthase[3,4], the mechanical properties of viral capsids [5], and tRNA translocation within the ribosome. 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[6,7]. 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[8].

[1] Puchner EM, Alexandrovich A, Kho AL, Hensen U, Schafer LV, Brandmeier B, Grater F, Grubmuller H, Gaub HE, Gautel M. Mechanoenzymatics of titin kinase. PNAS 105: 13385-13390 (2008)

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[4] Czub J and Grubmüller H. Torsional elasticity and energetics of F1-ATPase. PNAS 108; 7408-7413 (2011)

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## DNA folding in cells

The length of DNA exceeds the size of the volume it occupies inside a cell by three or more orders of magnitude. Therefore, for DNA to fit inside a cell it must be folded up. Experimental techniques based on fluorescence imaging and DNA sequencing have begun to quantitatively characterize the folded state of DNA in cells, reveling mathematical rules that can be understood in the context of simple physics models. In this lecture series I will describe the emerging experimental and theoretical landscape of DNA folding in cell. I will also discuss how cells might control the folded state of DNA so as to regulate its biological functions such as recombination and transcription. These themes will be covered in the following three lectures:

Lecture 1. DNA folding in bacteria

Lecture 2. DNA folding in eukaryotes

Lecture 3. Functional organization of chromosomes in cells

**FRASER MACMILLAN**

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## Understanding movement and mechanism in molecular machines: PsaBCA and manganese acquisition

J H van Wonderen[1], A Mullen[1], S L Begg[2], R Campbell[2], B Kobe[3], J C Paton[2], M L O'Mara[3], C A McDevitt[2] and Fraser MacMillan[1]

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A fundamental aim of structural studies is to understand how structure relates to function. It is important to remember, however, that the majority of biological systems are not static. Only by visualising molecules in action it is anticipated to understand how structure and function are related. Without the ability to understand dynamic structural changes the design and creation of new materials, catalysts and therapeutics must rely on empirical rule-based approaches as well as a fair degree of serendipity. This is the basis of the new EU COST Network CM1306.

In this presentation I will briefly present the aims of this COST Action before I look specifically at Streptococcus pneumoniae, one of the world¹s foremost bacterial pathogens. Annually infections associated with *S. pneumoniae* cost the world¹s economy > several $billion and globally it is responsible for > 1 million deaths. *S. pneumoniae* infections are dependent on the acquisition of metals from the host environment. Manganese (Mn) is essential for pneumococcal virulence and is specifically acquired by the pneumococcal surface antigen protein A (PsaA), which is the substrate-binding protein component of an ATP-binding cassette (ABC) transport pathway (PsaBC). Although the role of PsaA in Mn acquisition has been definitively established in both in vitro and in vivo studies, the mechanism of metal binding remains poorly understood. Here I present new data on the molecular determinants of metal binding by PsaA and the potential implications for host-pathogen interaction.

This $Mn^{2+}$ substrate-binding protein, PsaA also reveals a strong $Zn^{2+}$ binding even though it is not transported. Metal competition is postulated to play a role in immune defence. We propose to design and create site-directed variants that will allow us to develop a site-directed spin-labelling (SDSL) approach to look at dynamic structural differences upon $Mn^{2+}$ and $Zn^{2+}$ binding in comparison to various crystal structures. The ultimate aim is to distinguish between two distinct metal binding mechanisms using a combination of biochemistry together with PELDOR spectroscopy and computational simulations. In addition, this work also directly reveals how the biological functions of proteins are ultimately beholden to the fundamental laws of chemistry.

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## Lecture 1: Near Field Microscopies in Structural Biology

**Synopsis**

1. INTRODUCTION TO NEAR-FIELD MICROSCOPIES IN BIOLOGY
2. ATOMIC FORCE MICROSCOPY IMAGING IN AIR AND IN LIQUID
   - Different modes and recorded signals
   - Tip-sample interaction
3. FORCE SPECTROSCOPY
   - Probing interaction and mechanics with the tip

## Lecture 2: AFM imaging in the field of biological membranes

**Synopsis**

1. FABRICATION OF MODEL LIPID MEMBRANES SUITABLE FOR AFM IMAGING
   - Monolayer and bilayer
   - How to mimic biological membranes with supported membranes?
   - Incorporation of membrane proteins
2. AFM IMAGING OF BIOLOGICAL MEMBRANES
   - Partition of membrane components within model membranes.
   - High resolution imaging of membrane proteins
   - Topology of intact biological membranes

## Lecture 3: New developments in BioAFM Imaging

Atomic Force Microscopy (AFM) is a very powerful tool to probe the topography of biological samples under physiological conditions with a vertical and lateral resolution in the nanometer range. However, with conventional microscope, two main drawbacks are encountered: i) it takes more than a minute to capture an image, while biomolecular processes generally occur on a millisecond timescale or less, and ii) it is difficult to identify the structures delineated by the tip, especially for complex systems such as eukaryotic cells. During this talk, I will review the main advances achieved for solving theses two problems, focusing on recent developments and imaging of biological samples performed in my group.

**LENNART NILSSON**

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## Lecture 1: Methodological aspects of nucleic acid simulations

Molecular dynamics simulations of nucleic acids are performed using the same methods and software as for other biomolecules. The differences are mainly in the need of for a force field that describes the building blocks, nucleotides, of DNA and RNA, and the high surface charge density of a nucleic acid compared to a protein. The latter initially caused severe problems when the first attempts were made at running MD simulations of DNA *in vacuo*; this had worked well for proteins (BPTI, lysozyme, myoglobin), but was a catastrophe for DNA where the strong electrostatic forces between backbone phosphate groups led to severe distortions and unfolding of the DNA.

Three central aspects of a force field and model physics are:

- The size of the basic unit – atom, united atom, or groups of atoms
- The charge model – additive force fields with fixed partial charges, polarizable force fields
- Solvent representation – vacuum, explicit solvent, implicit solvent

For nucleic acids most of the development, as well as applications, have been with all atom additive force fields and explicit solvent, but there are coarse-grained force fields, implicit solvent models and polarizable force fields of various flavors available. In the AMBER family of force fields there are several variants of the parmff99 that have been optimized to remedy some issues that had been uncovered when longer (10+ ns) simulations were attempted, and the nucleic acid part of the CHARMM36 force field contains updates of backbone torsion angles to correct the 2'OH orientation in RNA and the BI/BII backbone conformational equilibrium in DNA. The charged phosphate groups in DNA/RNA necessitate a proper treatment of the solvent and of the handling of long-ranged Coulombic interactions, for example by using proper spherical cutoffs, or methods that account explicitly for the longe range part, such as Particle Mesh Ewald summation or reaction-field methods.

## Lectures 2 & 3: **Molecular dynamics simulations of nucleic acids and protein-nucleic acid complexes**

Some examples of simulations of RNA, DNA and their complexes with proteins will be presented.

### tRNA-mRNA recognition on the ribosome

We have carried out molecular dynamics simulations of the tRNA anticodon and mRNA codon, inside the ribosome, to study the effect of the common tRNA modifications cmo5U34 and m6A37. In tRNAVal, these modifications allow all four nucleotides to be successfully read at the wobble position in a codon. Previous data suggest that entropic effects are mainly responsible for the extended reading capabilities, but detailed mechanisms have remained unknown. We have performed a wide range of simulations to elucidate the details of these mechanisms at the atomic level and quantify their effects: extensive free energy perturbation coupled with umbrella sampling, entropy calculations of tRNA (free and bound to the ribosome), and thorough structural analysis of the ribosomal decoding center.

### Loop-loop interactions in a riboswitch

Riboswitches are mRNA-based molecules capable of controlling the expression of genes. They undergo conformational changes upon ligand binding, and as a result, they inhibit or promote the expression of the associated gene. The close connection between structural rearrangement and function makes a detailed knowledge of the molecular interactions an important step to understand the riboswitch mechanism and efficiency. We have performed all-atom molecular dynamics simulations of the adenine-sensing add A-riboswitch to study the breaking of the kissing loop, one key tertiary element in the aptamer structure. We investigated the aptamer domain of the add A-riboswitch in complex with its cognate ligand and in the absence of the ligand. The opening of the hairpins was simulated using umbrella sampling using the distance between two loops as the reaction coordinate. A two-step process was observed in all the simulated systems.

### p53-DNA

Zinc ions are frequently found in DNA-binding proteins. p53 is a cancer-related transcriptional factor, and its DNA-binding domain (DBD) contains a $Zn^{2+}$, which has been shown to be important for aggregation and sequence-specific DNA binding. We have carried out molecular dynamics simulations to investigate the influence of $Zn^{2+}$ on the p53 DNA recognition and the stability of the DBD. In the simulation with $Zn^{2+}$ present, the protein attracted to the DNA phosphate backbone, allowing for Arg248 on loop L3 to be inserted into the minor groove for specific contact with the DNA base. The insertion of Arg248 between the backbone phosphate groups in the minor groove caused a narrowing of the minor groove, which is not seen in the simulation without $Zn^{2+}$. Structurally, the zinc ion coordinated the motions among the different protein structural elements, which could also be important for optimal binding and core packing.

**CHRIS OOSTENBRINK**

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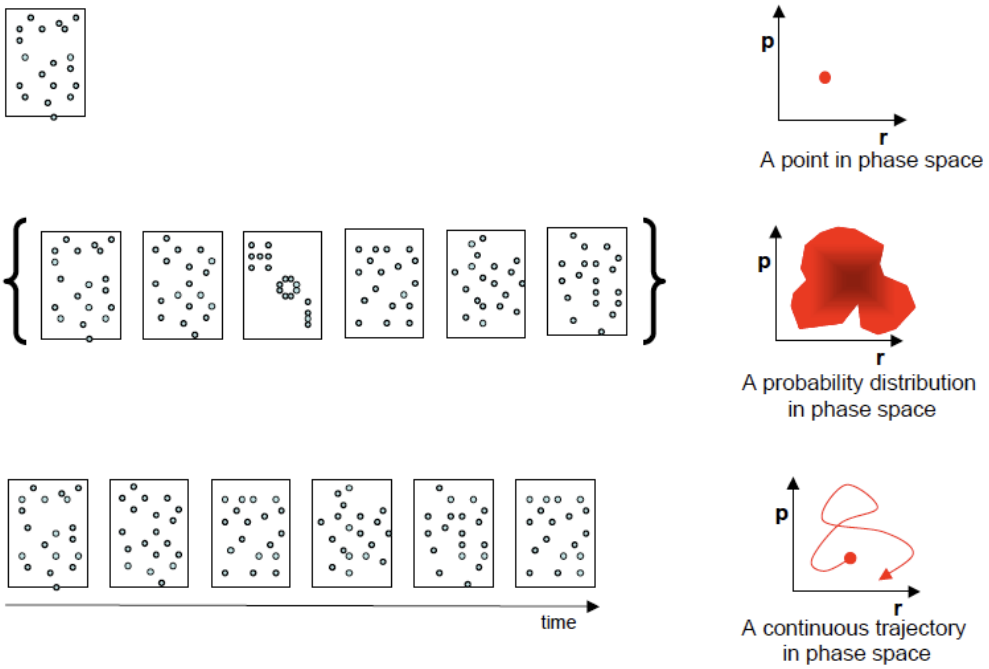
## Lecture 1: Ensembles and sampling, leading to molecular dynamics simulations

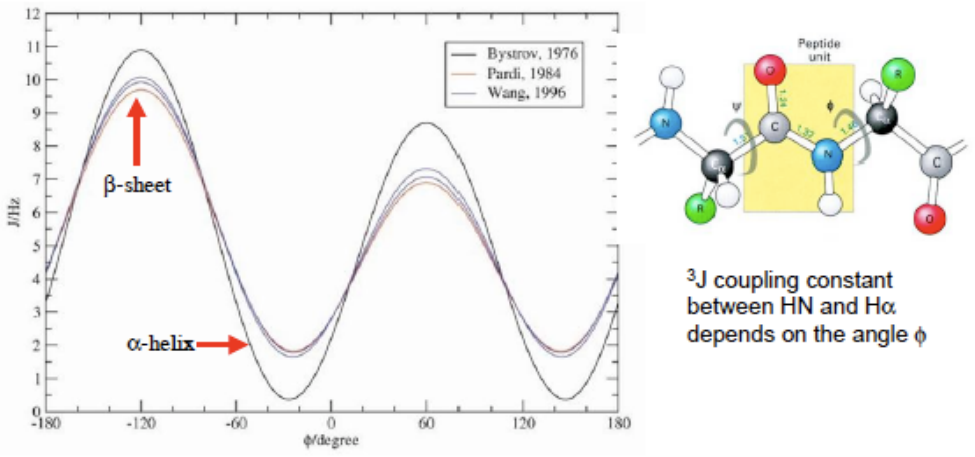
In this lecture, we will discuss the basics of molecular dynamics simulations. Starting from the link between a molecular structure and the (potential) energy of a system, we will discuss various algorithms to modify the structure in physically meaningful ways. This will lead to the definition of ensembles 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. Finally, we will hint at the methods to define ensembles at different thermodynamic state points.




## Lecture 2: Structure refinement using molecular dynamics simulations (NMR observables)

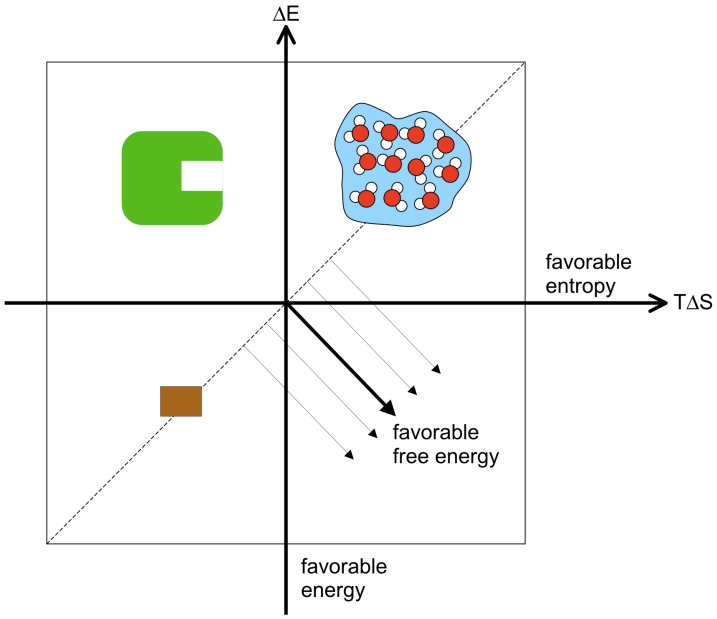
In the previous lecture, we have seen how molecular simulations can be used to generate conformational ensembles and how averages over such ensembles may be correlated to experimental properties. In the current lecture, we will discuss possibilities to do the inverse: use experimental observations as 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.




## Lecture 3: **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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## Lectures 1 & 2: Solid-state NMR of membrane-active peptides

### Lecture synopsis

A. BIOLOGICAL SOLID-STATE NMR (SS-NMR): INTRODUCTION

1. Main interactions for ss-NMR
2. Magic angle spinning (MAS) vs. oriented systems
3. Phospholipid membranes: $^{31}P$ and $^{2}H$ NMR
4. Antimicrobial peptides in membranes
5. Structural studies of melittin in bilayers
6. 2D ss-NMR of peptides in bilayers

B. MEMBRANE INTERACTIONS OF ANTIMICROBIAL AND AMYLOID PEPTIDES

1. Antimicrobial peptides from Australian frogs
2. Membrane interactions by ss-NMR
3. Conformation in phospholipid bilayers
3. Amyloid peptide Ab42
4. Effect on phospholipid bilayers
5. Structural studies in model membranes

### Abstract

Membrane-active peptides and proteins present a challenge for structural biology, which can often be resolved using solid-state NMR spectroscopy (ss-NMR). An introduction to ss-NMR will be given and illustrative examples taken from studies of the interaction of antimicrobial peptides (AMP) and amyloid peptides with phospholipid membranes. The interactions are contingent on the nature of the constituent lipids and, hence, we have studied these membrane-active peptides with membranes of different lipid types. We focused on AMP secreted from the skin of Australian tree frogs and Aβ42, a 42-residue peptide from amyloid plaques found in the brains of Alzheimer's disease patients. The dynamic and structural changes of lipid vesicles mimicking *E. coli*, *S. aureus* or eukaryotic plasma membranes were monitored using solid-state NMR in order to understand the selectivity and mechanism of action and the role of lipids. The AMP activity showed a strong dependence on lipid membrane composition whereas the Aβ42 peptide had

superficial interactions with model membranes. The results demonstrate the importance of choosing appropriate model membranes to study membrane-active peptides.

Selected References:

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See also website: http://www.chemistry.unimelb.edu.au/professor-frances-separovic

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## Lectures 1 & 2: Modeling protein organisation in adherent membranes

In embryogenesis, vertebrate cells assemble into organized tissues. In cancer, metastasis tumor cells spreading in the circulatory system use mechanisms of adhesion 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 a material substrate or scaffold. 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-substrate 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 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 control the binding affinity to the cell-adhesion molecules. In my talk, over the two lectures, I will show how these mechanisms can be studied in mimetic models both experimentally and theoretically, the result of which can be discussed in the cellular context.

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## Lecture 1: The integrated molecular orbital approach to accurate calculations of large molecular systems

The ubiquitous difficulty facing researchers interested in studying the mechanism of chemical reactions in large systems is that the computational expense of techniques that are satisfactorily accurate is too large to apply to macromolecular systems. Of the many suggestions put forward to address this difficulty, one of the simplest yet most general is the integrated molecular orbital approach.[1] This technique employs a relatively simple additivity approximation in order to couple a more accurate method (for chemical effects) to a less accurate one (for steric and electrostatic effects).

The present contribution will outline the basis for the integrated molecular orbital approach and demonstrate, by example, its applicability in studying large molecular systems. Particular emphasis will be placed upon incorporating the effect of the protein environment in enzyme-catalyzed reactions, while striving to maintain "chemical accuracy" for chemical transformations and spectroscopic properties.[2]

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## Lecture 2: Calculating CD spectra for flexible biomolecules

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Circular dichroism (CD) spectroscopy is one of the key experimental methods employed in the structural characterization of optically active chiral molecules. It is widely used in studies of biologically important systems, such as proteins with well-defined 3D-structures. The application of CD spectroscopy to small, flexible molecules, such as short peptide chains, is a more complex endeavor. This apparent paradox is due to the large number of widely varying conformers present in the room-temperature ensemble of such species.

The successful interpretation of the CD spectra for flexible molecules, therefore, requires a complementary theoretical treatment. In this contribution, we present our efforts towards establishing a reliable methodology capable of calculating the CD spectra for flexible systems. The inherently multi-scale approach involves

successive averaging over the solvent and solute degrees of freedom using a combination of classical (MM), quantum (QM) and hybrid (QM/MM) treatments.[1]

The construction of such a multi-scale approach requires careful calibration of the appropriate means in which to treat the solvent (explicit or implicit), the conformational space of the solute (force field and sampling), and the requisite electronic transitions (quantum chemical approach).[2] In addition to addressing the methodological issues, the presentation will demonstrate efforts to connect the structural ensemble to the observed spectra, for flexible signaling peptides, as well as the applicability in determining the absolute configurations of novel natural products.

[1] Brkljača Z, Čondić-Jurkić K, Smith A-S, Smith DM (2012) *J Chem Theory Comput* 8:1694-1705.

[2] Brkljača Z, Mališ M Smith DM, Smith A-S (2014) *J Chem Theory Comput* 10:3270-3279.

**HOLGER STARK**

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## Lecture 1: Single Particle Cryo-EM as a tool to determine 3D structures of macromolecular complexes

In single particle electron cryo-microscopy (cryo-EM) macromolecular complexes are embedded in a thin film of vitrified water and imaged in an electron microscope at low (liquid nitrogen) temperature. The imaged molecules can adopt random orientations leading to different “views” of the molecules which can be exploited to calculate the 3D structure of the imaged object. Electron microscopic images can be considered as projection images and are notoriously noisy. Therefore computational image processing of large image datasets is required to recover the signal from noise and to compute a 3D structure of the macromolecular complex at the highest possible resolution.

The method can be applied to very large macromolecules but has a lower size limit of several hundred kDa. Large macromolecules such as viruses and ribosomes were recently determined at very high resolution due to significant technical improvements in electron microscopic hardware as well as software. The latest state of the art technical equipment thus allows structures to be determined at near-atomic resolution and becomes comparable to resolutions obtained by X-ray crystallography.

## Lecture 2: Visualizing dynamic macromolecular complexes by cryo-EM at atomic resolution

Recently numerous high-resolution structures were obtained for macromolecular complexes by single particle cryo-EM techniques. The importance of the development of new generation pixel detectors and the possibility to correct for motion by the alignment of image frames has been particularly stressed and is considered to be one of the main important recent hardware developments leading to high resolution structures of macromolecules. We studied a biochemically well-defined macromolecular complex (70S ribosome-EF-Tu-kirromycin complex) at the highest possible resolution using a direct pixel detector (DDD) and a high-brightness gun (XFEG) in a Cs corrected electron microscope (Titan Krios).

Using state of the art equipment and image processing, we obtained the structure of the ribosome at 2.9 Å resolution which is identical to the resolution obtained by X-ray crystallography for the same complex from a different organism. The local resolution obtained for this cryo-EM structure is even higher (up to 2.5 Å resolution) which is sufficient to build the entire atomic structure of the ribosome making use of

crystallographic model building software. At this level of resolution we were even able to directly visualize all 30 chemical modifications in the E. coli ribosomal RNA. This has long been tried by crystallographers but without success so far. This is therefore the first time that we have obtained better resolution than X-ray crystallography. Simultaneously, cryo-EM can be used to sort the molecules and calculate 3D reconstructions of the ribosome in different conformations from the same image dataset. The level of computational sorting has reached a level by which it becomes possible to identify the motion at atomic resolution within small flexible parts of the ribosome representing functionally important dynamic regions. With cryo-EM it is thus possible to determine high-resolution structures and the dynamics of macromolecular complexes simultaneously.

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## Lecture 1: Site-directed spin labeling and electron paramagnetic resonance spectroscopy: an introduction

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. The lecture presents the basics of this method and covers the following paragraphs:

1. PRINCIPLES OF EPR SPECTROSCOPY AND SITE DIRECTED SPIN LABELING
   - 1.1. Site directed spin labeling
   - 1.2. The Zeeman effect
   - 1.3. Hyperfine interaction
   - 1.4. The EPR powder spectrum of a nitroxide spin label
   - 1.5. Motional narrowing: isotropic and anisotropic reorientational motion of a nitroxide spin label

2. SITE DIRECTED SPIN LABELING AND PROTEIN STRUCTURE
   - 2.1. Spin label side chain dynamics and protein structure
   - 2.2. Accessibility for paramagnetic quenchers: saturation and protein topography
   - 2.3. Spin-spin interaction: inter-residue distance measurements
   - 2.4. Determination of membrane protein structures using site directed spin labeling

Suggested reading:

[1] Bordignon E, and Steinhoff H-J (2007) Membrane protein structure and dynamics studied by site-directed spin labeling ESR. In: Hemminga MA, and Berliner LJ (eds) *ESR Spectroscopy in Membrane Biophysics.* (Springer Science and Business Media, New York) pp 129-164.

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## Lecture 2: Inter- and intramolecular distance measurements using cw and pulse EPR spectroscopy

The second lecture of the series is dedicated to the application of site directed spin labeling to determine inter- and intramolecular distances[1, 2]. The basics of cw EPR and pulse electron-electron double resonance (DEER) methods to determine interspin distances are presented and illustrated with studies of the structure and dynamics of soluble and membrane bound proteins: the Rpo4/7 stalk module of RNA polymerase[3], Mnme, the (ABC) maltose importer $MalFGK_2$, and the proline

transporter PutP. Molecular modeling approaches will be introduced which relate inter-spin distances measured between NO groups of nitroxide spin labels to the protein structure (MMM 2011 package[4]). We will then focus on DEER studies on the spin labeled halobacterial phototaxis receptor sensory rhodopsin (pSRII) in complex with the receptor specific transducer (pHtrII). This complex is considered as a general model system for transmembrane signal transduction. Inter-spin distances determined from pairs of interacting nitroxide spin labels lead to a unique structural model of the dimeric complex. Time resolved detection of inter-spin distance changes after light activation reveals conformational changes of pSRII and uncovers the mechanism of the signal transfer from pSRII to the associated transducer pHtrII. Conformational changes in the first HAMP domain of pHtrII are shown to play an essential role in the signal transfer[5].

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## Lectures 1, 2 & 3: Physics of viruses: electrostatics, elasticity and DNA condensation in viruses

As particularly simple complexes of proteins and DNA or RNA, viruses present an opportunity to study the interaction between the two types of molecules characteristic of all life. However, although viruses appear simple when compared to bacteria and cells, from the physical viewpoint they are still quite involved. This is due to complexity and versatility of the molecules they are comprised of. In particular, the molecular versatility enables them to self-assemble from proteins and RNA, to densely pack DNA in small internal space of the shell formed by proteins (capsid), to disassemble upon entry to the cell, to deliver DNA by injecting it through the bacterial membrane (as bacteriophages do), to somehow discriminate between different RNA pieces available in the cell and to pack those required for further infection. The physics behind these and other functions that the viruses perform during their "life-cycle" is still not understood in detail, yet, it is known that it must involve combination of different classical disciplines, including elasticity, electrostatics, chemical kinetics, and possibly even quantum mechanics.

The application of the different physical approaches to viruses will be illustrated and explained in the lectures which should provide introduction to the, already established field of physical virology.

### 1. Geometry of viruses: "buckyballs", "nanotubes", and how to self-assemble them

Viruses are highly symmetric nano-particles. The two dominant shapes of the virus particles are sphere- (e.g. hepatitis B virus) and rod-like (e.g. tobacco mosaic virus). Spherical particles can be thought of as triangulations of a nearly spherical surfaces with icosadeltahedral geometry, formed by a precise assembly of (often identical) virus proteins. Rod-like particles are "tubes" formed by spiral assembly of (identical) proteins. Different ways to form these particles can be clearly mathematically defined. The physical and chemical processes leading to such precise assembly of virus proteins will be discussed with a particular emphasis on protein "design" required.

### 2. Physical interactions in viruses: elasticity and electrostatics

The virus capsid can be thought of as an interwoven, two-dimensional (2D) medium of interacting proteins. In the continuum representation, the capsid shape depends only on the elastic properties of a 2D protein sheet. I will show how the classical theory of elasticity (of shells) can be applied to predict the exact shape of the capsid, including its deviations from the perfect sphere (perfect icosahedron).

The electrostatic interactions are known to be important for the assembly and structure of viruses which come in two essential classes – one containing the, usually, single-stranded RNA molecule, and the other containing the, usually, double-stranded DNA molecule. Both types of genomes are strongly negatively charged in the solution, and their encapsulation in the viral protein coating (capsid) may sometimes require specific form of electrostatic “screening”, such as positive protein tails on the capsid interior, or the encapsulation of the histone-like, positively charged proteins, together with the genome. These effects will be discussed in the framework of the mean-field electrostatics (Poisson-Boltzmann).

**3. DNA condensation in viruses**

The DNA molecule packed in viruses is often condensed. In such a configuration, the DNA strands form locally a lattice of parallel strands, i.e. the DNA is bundled. In free space, this leads to toroid-like DNA condensates. The condensation of DNA in geometrical constraint of the virus capsid is obviously more complicated, thus the structure of the DNA in viruses is still not resolved. The intricacies of the DNA condensation in viruses will be discussed in a phenomenological (Ubbink-Odijk) model representing the formation of the DNA bundle as an interplay of the elastic energy of the DNA bending and the surface energy of the bundle.

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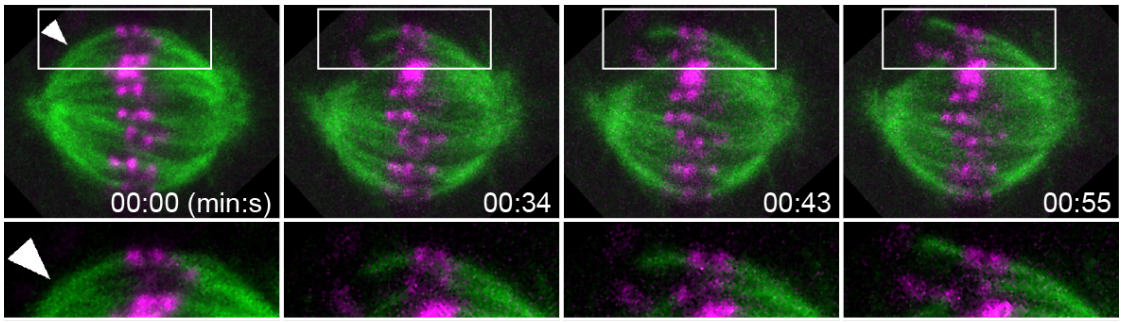
## Lecture 1: Unexpected actions in the mitotic spindle revealed by live-cell imaging and laser microsurgery

At the onset of division, the cell forms a spindle, a micro-machine based on microtubules and the associated proteins, which divides the chromosomes between the two nascent daughter cells. The attachment of microtubules to chromosomes is mediated by kinetochores, protein complexes on the chromosome. Spindle microtubules can be divided into two major classes: k-fibers, which are bundles of parallel microtubules connecting the kinetochore with the spindle pole, and interpolar microtubules, which extend from the spindle poles and interact with each other in the center of the spindle, forming antiparallel bundles. During metaphase, microtubules and the associated motor proteins move the kinetochores to position them at the spindle equator. Sister kinetochores then experience pulling forces, which regulate the spindle assembly checkpoint and segregate the kinetochores in anaphase. Thus, it is crucial to understand the forces that position and move the kinetochores.

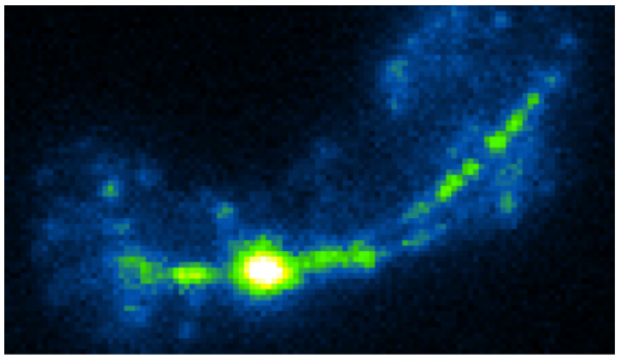



According to the current paradigm, sister kinetochores in metaphase are connected with the spindle poles by k-fibers and with each other only by the chromatin. Interestingly, we observed in living HeLa cells expressing tubulin-GFP and mRFP-CENP-B (a kinetochore protein) a weak microtubule signal in a line bridging the sister kinetochores, in addition to the strong signal of the k-fibers. Nevertheless, imaging alone cannot reveal whether the microtubules bridging the sister kinetochores are linked with the k-fibers and kinetochores. To test whether such links exist, we designed a laser microsurgery assay in which we cut the outmost k-fiber. We found that after cutting, sister kinetochores together with the k-fibers and the microtubules that bridge them moved outwards, away from the central spindle. These results show that the microtubules bridging sister kinetochores are connected to k-fibers and the kinetochores into a single object, and we term this new class of microtubules "bridging microtubules". To examine whether the forces that position the kinetochores can be explained by taking into account the bridging microtubules

as a link between sister k-fibers, we introduce a theoretical model. Predictions of the model were tested experimentally by combining live-cell imaging and genetics with our laser microsurgery assay. We conclude that the forces acting on kinetochores can be explained by considering bridging microtubules. Understanding the role of these microtubules in chromosome movements and tension generation will shed light on the establishment of proper kinetochore-microtubule attachments and the force balance in the entire spindle.

### Lecture 2: **Total Internal Reflection Fluorescence (TIRF) microscopy *in vivo:* Single-molecule imaging of motor proteins**

To exert forces, motor proteins bind with one end to cytoskeletal filaments, such as microtubules and actin, and with the other end to the cell cortex, a vesicle, or another motor. A general question is how motors self-organize to generate large-scale movements in the cell. An example of a system where a motor binds to a microtubule and to the cell cortex is provided by dynein, which during meiotic prophase in fission yeast drives oscillations of the spindle pole body and of the nucleus. These oscillations are crucial for proper chromosome pairing and recombination. Quantitative live-cell imaging and laser ablation experiments together with a theoretical description show that the mechanism of these oscillations relies on the asymmetric distribution of dyneins, with more dyneins bound to the leading than to the trailing microtubule. The observed asymmetry is a consequence of preferred unbinding of dynein from the trailing microtubule. Thus, spatio-temporal pattern formation within a cell can occur as a result of mechanical cues, which differs from conventional molecular signaling, as well as from self-organization based on a combination of biochemical reactions and diffusion.



To generate force for the movement of large organelles, dynein needs to be anchored, with the anchoring sites being typically located at the cell cortex. However, the mechanism by which dyneins target sites where they can generate large collective forces was unknown until recently. By using Total Internal Reflection Fluorescence (TIRF) microscopy *in vivo*, we have directly observed single dynein motors during nuclear oscillations, and identified the steps of the dynein binding process: from the cytoplasm to the microtubule, and from the microtubule to cortical anchors. We uncovered that dyneins on the microtubule move either in a diffusive or directed manner, with the switch from diffusion to directed movement occurring upon binding of dynein to cortical anchors. This dual behavior of dynein on the microtubule, together with the two steps of binding, enables dyneins to self-organize into a spatial pattern needed for them to generate large collective forces.

**ANTHONY WATTS**

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## Lectures 1 & 2: Principles of biological solid state NMR

### Lecture Synopsis

A. 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

B. 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

### Abstract

Solid state NMR can be applied to a wide range of sample morphologies and hydration states, and for large (MWt >> 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].

Selected References

[1] Watts A, Straus SK, Grage S, Kamihira M, Lam Y-H, Xhao Z (2003) Membrane protein

structure determination using solid state NMR. In: *Methods in Molecular Biology – Techniques in Protein NMR* Vol. 278 (ed. Downing K), Humana Press, New Jersey, pp. 403-474.

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See also: www.bioch.ox.ac.uk/~awatts/

## Lecture 3: **NMR of membrane proteins - Drug targeting**

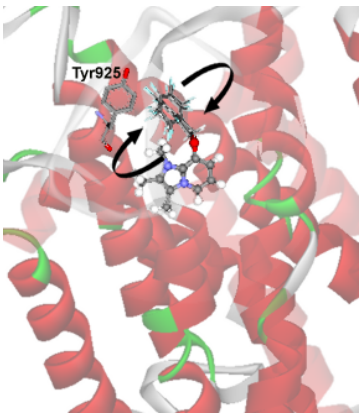
### Lecture Synopsis

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

### Abstract

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 ($^{2}H$, $^{13}C$, $^{19}F$, $^{15}N$, $^{17}O$) 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[11,12].

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[2] Watts A, Straus SK, Grage S, Kamihira M, Lam Y-H, Xhao Z (2003) Membrane protein structure determination using solid state NMR. In: *Methods in Molecular Biology – Techniques in Protein NMR Vol. 278* (ed. K. Downing), Humana Press, New Jersey, pp. 403-474.

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### Lecture 1: Protein-RNA 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 between amino acids and cognate codons. Importantly, I will explore the possibility that the physico-chemical rationales, which led to the development of code's structure, may still be relevant in present-day cells.

### Lectures 2 & 3: 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 complicated 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? In the first part of this lecture, 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.

Biomolecules exhibit rich dynamics on different time- and length scales, and this dynamics directly affects the properties of the molecular structures derived in typical structural experiments. In the second part of this lecture, I will focus on different theoretical approaches that are being increasingly used to aid experimentalists in interpreting structural biology experiments, most notably molecular dynamics simulations. In particular, I will illustrate how computer simulations can be used to not only study molecular features that are typically inaccessible to experiment, but also to probe the limitations of different experimental structural biology techniques when it comes to the problem of conformational averaging.

# SPONSOR LECTURES

**ALEXEJ KEDROV**

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## Biophysical analysis on membrane protein targeting and assembly

Membrane proteins form a large part of a cellular proteome and represent the major target for pharmacological therapy. In spite of a recent progress in structural analysis, mechanisms of membrane protein folding and assembly within lipid bilayers remain unresolved. We implement a comprehensive biophysical analysis for studying membrane protein biogenesis – from selective targeting translating ribosomes towards the lipid surface to membrane protein insertion, folding and release via the Sec translocon. High-sensitivity fluorescence-based studies allow probing a range of protein:protein interactions along this pathway, both in solution and at lipid membrane interfaces. During the talk I will overview our recent applications of fluorescence correlation spectroscopy and the microscale thermophoresis to analyze ribosome:ligand and protein:protein interactions, and demonstrate how nanodisc-incorporated membrane proteins can be implemented in biophysical and structural analysis.

**PIOTR WARDEGA**

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## Quantitative analysis of biomolecular interactions with Microscale Thermophoresis (MST)

Microscale Thermophoresis (MST) allows for quantitative analysis of protein interactions in free solutions and with low sample consumption. The technique is based on thermophoresis, the directed motion of molecules in temperature gradients. Thermophoresis is highly sensitive to all types of binding-induced changes of molecular properties, be it in size, charge, hydration shell or conformation. In an all optical approach, thermophoresis is induced using an infrared laser for local heating, and molecule mobility in the temperature gradient is analyzed via fluorescence. In addition to fluorescence by labels or fusion proteins attached to one of the binding partners, intrinsic protein fluorescence can be utilized for MST thus allowing for label-free MST analysis.

Its flexibility in assay design qualifies MST for biomolecular interaction analysis in complex experimental settings, which we herein demonstrate by addressing typically challenging types of binding events from various fields of life science. The interaction of small molecules and peptides with proteins is, despite the high molecular weight ratio, readily accessible via MST. Furthermore, MST assays are highly adaptable to fit to the diverse requirements of different biomolecules, e.g. membrane proteins to be stabilized in solution. The type of buffer and additives can be chosen freely. Measuring is even possible in complex bioliquids like cell lysate and thus under close to in vivo conditions and without sample purification. Binding modes that are quantifiable via MST include dimerization, cooperativity and competition.

**PRIMOŽ MEH**

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## Protein stability and interactions by ITC and DSC

Studying a protein in isolation (for example, obtaining the crystal or NMR structure of a purified protein) is critical to establish a structural basis for the protein's function(s). However, true functional characterization of the protein generally requires manipulation of multi-component systems under stringently-controlled conditions, evaluating the roles of the various components in regulating and tuning the physical characteristics of the protein.

Isothermal titration calorimetry (ITC) is a universally-applicable technique for determining the thermal effects arising from molecular interactions. For life scientists, ITC is a particularly powerful approach for quantifying molecular interactions between two or more proteins or other macromolecules, or a protein and small molecule ligands such as drugs or enzyme inhibitors. Since nearly every reaction is accompanied by the absorption or evolution of heat, most reactions, often requiring only nanomoles of material, can be studied using calorimetry. ITC has two major life science applications: first, for studying binding interactions between, for example, proteins and drugs, or ligands and receptors, and second, for quantifying rates of enzymatic catalysis and product or drug inhibition. Experiments are rapid, sample derivatization is not required, impurities are tolerated, and often only nanomoles of the target macromolecule are required.

Differential Scanning Calorimetry (DSC) measures the change in energy in a sample as the temperature is raised or lowered, and thus can determine absolute thermodynamic data for thermally-induced transitions. All macromolecules will denature when heated, and many proteins denature when cooled. Ultra-sensitive calorimeters quickly quantify the thermodynamic parameters (and hence stability) of proteins and nucleic acids in their native state, following mutation, or when bound to a drug candidate. Calorimetric measurements are direct: the intrinsic thermal properties of the sample are measured without the need for extensive purification, chemical derivatization, immobilization or spectroscopic probes, and if the unfolding process is reversible, the technique is non-destructive. Depending on the complexity of the unfolding profile, only nanomoles of material may be required to provide a complete analysis of thermally-induced transitions. Importantly, DSC data provide fundamental thermodynamic insights into the stabilizing and binding interactions crucial for rational protein and drug design. DSC is a straightforward approach towards understanding the enthalpic and entropic changes that occur as a biopolymer is thermally denatured. Since the biopolymer can be free in solution (such as a protein or nucleic acid), associated with another molecule (as in a

DNA/drug complex) or part of an assembly of molecules (such as lipids in a membrane), DSC can be used to study the stability of essentially any biological sample, including whole cells. Experiments are rapid, sample derivatization is not required, impurities are tolerated, and often only nanomoles of the target macromolecule are required.

# POSTERS & POSTER TALKS

# Thermal stability of human serum albumin – DSC study

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Human serum albumin (HSA) is the most abundant protein in human plasma (concentration about 45 mg/ml) with ascribed ligand binding and transport properties, antioxidant functions, enzymatic and antioxidant activities[1,2]. This protein also contributes significantly to colloid-osmotic blood pressure. HSA is a non-glycosylated 65 kDa protein consisting of 585 amino acids having three homologous a-helical domains. Design of this molecule provides a variety of binding sites for many physiological important ligands, fatty acids, hemes, amino acids, bilirubin, steroids and metal ions, including a wide variety of drugs, helping thus in the transport, distribution and metabolism of these compounds. HSA has two primary binding sites named Sudlow site I and II located in IIA or IIIA subdomain, respectively, which serve as binding cavities for a variety of ligands[1,3].

Understanding the relationship between the structure of proteins and the energetics of their stability and binding with other biomolecules is very important in biotechnology and is essential to the engineering of stable proteins and to the structure-based design of pharmaceutical ligands[3].

In the previous years, the thermal stability of HSA has been widely investigated mainly by differential scanning calorimetry (DSC)[4]. This technique enables direct determination of the thermodynamic parameters (calorimetric and van't Hoff enthalpies ($\Delta H_{cal}$ and $\Delta H_{vH}$) and temperature ($T_m$)) of the denaturation as well as evaluation of entropy and Gibbs energy of the transitions between folded and unfolded states of the studied biopolymer.

In this work we study by means of DSC the dependence of the thermal denaturation of HSA on the concentration, scan rate and presence of fatty acids. Deconvolution analysis suggests that DSC endotherms are well approximated as the sum of two independent two state transitions in both types of albumin, defatted as well as containing fatty acids. Our results show that the presence of fatty acids in HSA significantly stabilizes the structure of this protein. Further, DSC curves exhibit a marked dependence on protein concentration in both fatty acid containing and fatty acids free HSA. The increase of HSA stability with increasing of the concentration enables to determine oligomeric state of albumin at denaturation process. From the

linear dependence of $1/T_m$ on albumin concentration follows that HSA tends to form dimers.

Our data show only a slight increase of $T_m$ and $\Delta H_{cal}$ with a scan rate increase. This finding is in accordance with the previous observations[5]. The reversibility of the transition of HSA molecule depends on the temperature at which the protein was heated. Reversibility of the thermal transition after heating of the sample to the temperature ≈90°C and cooling up to ≈20°C is negligible, however heating only up to the temperature of the thermal denaturation (≈67°C in the case of fatty acids containing HSA) leads to 60% reversibility.

This work contains also preliminary results about the influence of several drugs (ibuprofen, warfarin, emodin) on the thermal stability of HSA. Interaction of these molecules with HSA leads to a significant stabilization of the protein (higher temperature and enthalpy of the denaturation). This type of measurements may be beneficial for the easy determination of the binding site(s) of ligands to HSA.

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# The role of ATP binding in the structure of the presynaptic filament of human Rad51

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Rad51 is a key protein component of homologous recombination, the error free repair process of double strand DNA breaks. The human Rad51 is a 37 kDa protein of two domains which forms a helical filament around single stranded overhangs at the double strand breaks in the repair process. The formation of the presynaptic filament is a prerequisite of the next steps in the repair, and thus its structure is of vital significance. The Rad51 recombinase possesses DNA dependent ATPase activity in the presence of both single and double stranded DNA, however, it has not been clearly shown, whether this enzyme activity played important role in the formation and structure of the presynaptic filament. In this study our goal was to provide evidence for the possible structural role of ATP excluding its hydrolyzis by adding Ca to the solutions instead of Mg. We applied pressure perturbation fluorescence spectroscopy to characterize the strength of interaction at protomeric interfaces in the Rad51 filaments. We also used transmission electron microscopy to unravel the topology and structural parameters of the filamentous structures under the studied experimental conditions. Four conditions of filament formation were compared in 40 mM Hepes buffer at pH 7.5 containing 200 mM KCl. The protein concentration was 1-2 mM. It was shown that human Rad51 forms filamentous structures by self-association without ATP/Ca and ssDNA being present. It also forms filaments when only ssDNA is present. These filaments, however, do not have ordered, helical structure. The formation of the ordered structure clearly requires the presence of ATP/Ca. The results of pressure perturbation measurements show that the interface binding strength is the highest in the presynaptic filament of helical structure. The study provides evidence for the significant structural role of ATP in the studied step of homologous recombination.

# IleRS eliminates norvaline from *Escherichia coli* proteome via pre- and post-transfer editing pathways

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Isoleucyl-tRNA synthetase (IleRS) catalyzes ATP-dependent covalent pairing of cognate isoleucine with cognate $tRNA^{Ile}$, thus providing accurate substrates for ribosomal protein synthesis. IleRS may also efficiently substitute isoleucine with structurally similar valine in the synthetic reaction. To prevent erroneous aminoacylation, IleRS developed extensive hydrolytic proofreading. This enzyme employs both the tRNA-dependent pre-transfer (hydrolysis of Val-AMP intermediate) and the post-transfer (hydrolysis of $Val\text{-}tRNA^{Ile}$) editing reactions within the synthetic and editing site, respectively[1]. Norvaline is a natural non-proteinogenic amino acid, structurally similar to isoleucine. It may accumulate in *Escherichia coli* under hypoxic conditions to milimolar concentrations. We have recently shown that norvaline is the major threat for error-free $Leu\text{-}tRNA^{Leu}$ synthesis in *E. coli*[2]. To establish the mechanism of norvaline discrimination by IleRS, we utilized steady state and single-turnover kinetic analyses of the synthetic and editing pathways. We show that norvaline, similarly as valine, is not discriminated well in the synthetic reaction, and is efficiently activated and transferred to $tRNA^{Ile}$. However, the rapid post-transfer editing reaction precludes accumulation of $Nva\text{-}tRNA^{Ile}$, acting as the main defense against misincorporation of norvaline in place of isoleucine into cellular proteome. We further demonstrate that tRNA-dependent pre-transfer editing, that efficiently operates against Val-AMP, is also employed by IleRS to hydrolyze Nva-AMP. Our data thus establish that the tRNA-dependent pre-transfer editing activity, an idiosyncratic feature of IleRS proofreading, is characteristic of the IleRS:tRNAIle complex and is independent on the identity of non-cognate amino acid substrate.

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# Computational study of interaction of $C_{60}$-fullerene and tris-malonyl-$C_{60}$-fullerene isomers with biomembranes

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Oxidative stress induced by excessive production of reactive oxygen species (ROS) has been implicated in the etiology of many human diseases. It has been reported that fullerenes and some of their derivatives – carboxyfullerenes – exhibits a strong free radical scavenging capacity.

The permeation of $C_{60}$-fullerene and its amphiphilic derivatives – $C_3$-tris-malonic-$C_{60}$-fullerene ($C_3$) and $D_3$-tris-malonyl-$C_{60}$-fullerene ($D_3$) – through a lipid bilayer mimicking the eukaryotic cell membrane was studied using molecular dynamics (MD) simulations. The free energy profiles along the normal to the bilayer composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) for $C_{60}$, $C_3$ and $D_3$ were calculated. We found that $C_{60}$ molecules alone or in clusters spontaneously translocate to the hydrophobic core of the membrane and stay inside the bilayer during the whole period of simulation time.

The incorporation of cluster of fullerenes inside the bilayer changes properties of the bilayer and leads to its deformation. In simulations of the tris-malonic fullerenes we discovered that both isomers, $C_3$ and $D_3$, adsorb at the surface of the bilayer but only $C_3$ tends to be buried in the area of the lipid headgroups forming hydrophobic contacts with the lipid tails. We hypothesize that such position has implications for ROS scavenging mechanism in the specific cell compartments.

# Peptide *de novo* sequencing - mutation detection

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With the ongoing development of high throughput DNA sequencing technologies and bioinformatics tools, the number of sequenced genomes is exponentially growing. The vast amounts of acquired data are currently available and organized in computer databases[1]. Peptide *de novo* sequencing presents the analytical process for the reading of amino acid sequences directly from tandem mass spectra (MS/MS). This process is in opposite to "database search" process that matches experimental data against annotated genomes. *De novo* sequencing is independent from assistance of a sequence database, so it is powerful tool in single mutation determination in organisms with annotated genomes or even in organisms of unknown genomes[2].

In this study we searched for the strain specific mutations by peptide *de novo* sequencing. We identified particular mutations between *Lactobacillus brevis* L62 isolated from sourdough and *L. brevis* ATCC 367 with known genome sequence (National Center for Biotechnology Information, NCBI Accession number NC_008497.1)[3]. Furthermore, we used *de novo* sequencing to determinate mutations between the AmpC enzymes in clinical isolates of *Pseudomonas aeruginosa* confirming β-lactams antibiotic resistance. For peptide *de novo* sequencing we used CAF-/CAF+ technique (chemically activated fragmentation negative/chemically activated fragmentation positive)[4]. Acquired peptide sequences are additionally confirmed by Sanger direct DNA sequencing.

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# Study of a supramolecular assembly through molecular dynamics simulations: α,γ-peptide nanotubes

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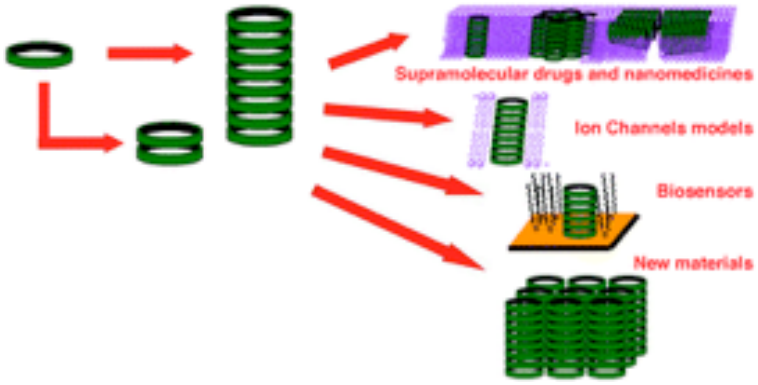
Supercomputing applied to biology is giving us the ability to understand how life works at the molecular level and it is revolutionizing our vision of living beings. Molecular Dynamics (MD) simulations have become a more and more useful and necessary partner in experimental techniques in the field of supramolecular chemistry, as they are able to capture the behavior of biological systems at atomic level through a range of time scales with a very high spatial resolution, where the experimental characterization is not able to reach.

Using MD simulations we have obtained interesting results about the dynamical behavior of self-assembling α,γ-peptide nanotubes (α,γ-SPNs),[1] a supramolecular assembly with very promising properties. We have simulated them in several solvents (water, chloroform and methanol) and also inserted into a lipid bilayer (acting as transmembrane transporters), demonstrating the power of theoretical calculations when acting as an atomic microscope to analyze the fine details of a complex supramolecular structure.

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# Reassessment of LeuRS discriminatory power unveils norvaline as a prime quality control target

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Leucyl-tRNA synthetases (LeuRS) covalently couple $tRNA^{Leu}$ with leucine, and thereby provide the pool of Leu-$tRNA^{Leu}$ for ribosomal protein synthesis. LeuRS may also activate and transfer to $tRNA^{Leu}$ structurally and chemically similar norvaline, a non–canonical amino acid that accumulates in *Escherichia coli* under micro-aerobic conditions. However, incorporation of norvaline into proteins is prevented by efficient intrinsic LeuRS hydrolytic activity toward norvalyl-$tRNA^{Leu}$ within a dedicated post-transfer editing domain. In spite of the prevailing opinion that noncognate isoleucine mimics leucine well in the LeuRS synthetic reactions and thus requires editing to prevent errors in leucyl-$tRNA^{Leu}$ synthesis, we now demonstrate that isoleucine is discriminated with high specificity within the synthetic site. Thermodynamic, structural and kinetic approaches establish that both very weak ground state binding and the decreased rate of the chemical step contribute to isoleucine discrimination. These results were complemented by *in vivo* experiments, where we show that while *E. coli* strain with editing deficient LeuRS grows normally in the presence of high isoleucine concentration, it displays growth defects under micro-aerobic conditions where norvaline accumulates. Our results reveal that LeuRS-mediated translational quality control represents the essential part of the major *E. coli* adaptive response necessary for survival in environments with low oxygen levels.

# Polyelectrolyte composite: Hyaluronic acid mixture with DNA

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We studied salt-free studied salt-free, highly concentrated (5-200 g/L) mixtures of unfragmented (μm contour length) DNA and hyaluronic acid (HA) as a border-line example of rigid-rod/flexible-chain composite, across a broad range of concentration ratios cHA/cDN = 0.05-50. By polarizing microscopy (PM) we established that the DNA and HA form clearly separated thread-like domains defined and oriented by solution shear. Within its domains DNA shows birefringent banded patterns, routinely observed for long chain mesogens. We applied small angle x-ray scattering (SAXS) to the mixtures and observed a PE correlation peak. This peak was ascribed to DNA subphase and was used as a measure of effective DNA concentration in the subphase, according to deGennes scaling relationship between the DNA mesh size $x = 2\pi/q^* \approx c^{-1/2}$ and monomer concentration c[1]. From cDNA we inferred effective cHA of HA subphase, and found a proportionality cHA ≈ 0.8 cDNA. As DNA and HA subphases are in the osmotic pressure equilibrium, HA osmotic pressure ΠHA = ΠDNA is inferred, since DNA equation of state is known. That is, ΠHA(c) scales as for the other PEs (DNA and polystyrene sulfonate, PSS), $\Pi \sim c^{9/8}$, up to about c = 1M. The osmotic pressure of PEs is regulated by Manning uncondensed counterion concetrations, ci /c = ϕ < 1. Since HA, a weak PE due to a low linear charge, does not feature condensation, i.e. ci = c, it may be used as a measure of counterion concetrations for strong PEs. Eventually, we corroborate the work by Raspaud et al.[2] who found that the concentration of counterions controlling the osmotic pressure is double the Manning-condensation theory defined value for DNA or PSS.

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# A toolkit and benchmark study for FRET-restrained high-precision

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A comprehensive toolkit for FRET-restrained modeling of biomolecules and their complexes is presented for quantitative analysis in structural biochemistry. A dramatic improvement in precision of FRET-derived structures is achieved through explicitly considering spatial distributions of dye positions and motions[1], which greatly reduce uncertainties due to flexible dye linkers. The precision and confidence levels of the models are calculated by rigorous error estimation. Furthermore, we introduce a new approach of FRET-guided “screening” of a large structural ensemble created in silico via all-atom molecular dynamics or coarse grained simulations. An experiment planning tool is introduced which allows one to determine efficient labeling positions and distance pairs for FRET measurements based on known structural information or simulation results. Topology space analysis can also be performed. Per- distance information content contributions can be evaluated. Using this approach, formerly unknown conformations of various biomolecules were determined[2]. The software package is available from authors[3].

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# Examination of antioxidant activity of gallic acid with different radicals

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Gallic acid (GA) is broadly distributed in plants all around the world, both as free compound and as part of hydrolysable tannins [1]. GA may be found in gallnuts, witch hazel, tea leaves, oak bark, etc [2]. It has great importance and application in medicine acting as an antioxidant and helping to protect human cells against oxidative damage. Due to its free radical scavenging activity, investigation of gallic acid has great practical and theoretical importance. In its structure, GA has phenolic groups which, as a source of readily available hydrogen atoms, enable the subsequently produced radicals to be delocalized over the whole phenolic structure. The free radical scavenging activity of GA has been studied in non-polar and aqueous solutions, using the Density Functional Theory (DFT). Antioxidant mechanism depends directly on nature of solvent in which the reaction occurs. Those mechanisms are investigated by analyzing the thermodynamic properties of the base molecule, the corresponding radical cation, radicals and anions using DFT method M05-2X implemented in Gaussian 09 software package [3]. According to the investigation of the BDE, PA and PDE values of GA, it is clear that the 4-OH group is the most reactive OH group of GA. In order to confirm this result, the reaction of GA with peroxyl radicals (methylperoxyl (MPR), ethylperoxyl (EPR), *izo*-propylperoxyl (*i*-PPR) and *terc*-butylperoxyl radicals (*t*-BPR)) has been performed. All transition states for reactions between GA and the corresponding peroxyl radicals have been found. Two mechanisms of antioxidative action – Hydrogen atom transfer (HAT) and Proton coupled electron transfer (PCET) have been investigated in two solvents – water and pentyl ethanoate (PE). Our results show that the HAT mechanism seems to be the most probable reaction path in water and in PE, while the reaction via PCET mechanism may takes place only in PE.

On the basis of activation energies for both reaction mechanisms, we can conclude that the HAT mechanism is more probable than PCET mechanism in PE. On the other hand, it is the only possible mechanistic pathway in water (Table 1). The obtained results are in prefect agreement with the thermodynamic approach; i. e. they confirm that the 4-OH group is most responsible for the good antioxidant properties of GA.

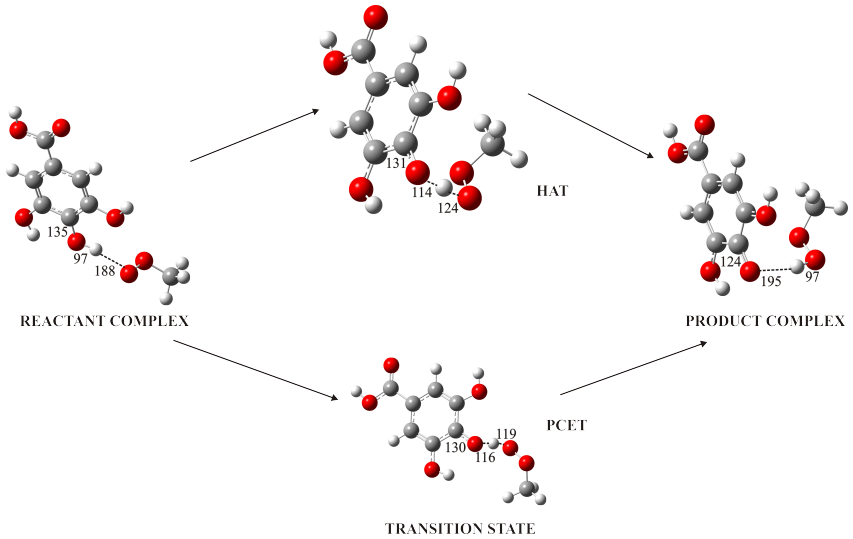


Figure 1. Possible reaction paths for H atom transfer from 4-OH-group of gallic acid to methylperoxyl radical in pentyl ethanoate. Selected bond distances are given in pm.

Table1. Activation energies ($\Delta G^{\ddagger}_{a}$) in water and pentyl ethanoate, for reaction under investigation

| | M05-2X/6-311++G(d,p) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | HAT | | | | PCET | | | |
| | $\Delta G_a$ (kJ/mol) | | | | $\Delta G_a$ (kJ/mol) | | | |
| Gallic acid | MPR | EPR | *i*-PPR | *t*-BPR | MPR | EPR | *i*-PPR | *t*-BPR |
| Water | | | | | | | | |
| OH-3 | 54.4 | 63.3 | 65.8 | 64.1 | | | | |
| OH-4 | 54.6 | 54.6 | 42.3 | 64.2 | | | | |
| Pentyl ethanoate | | | | | | | | |
| OH-3 | 60.7 | 64.3 | 67.3 | 65.1 | 77.8 | 79.4 | 70.8 | 76.6 |
| OH-4 | 56.6 | 57.2 | 56.2 | 55.9 | 69.5 | 73.5 | 74.4 | 72.4 |

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# RNA junctions structure and distance determination via accurate single-molecule high-precision FRET measurements

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Förster-Resonance-Energy-Transfer (FRET) restrained high-precision structural modeling is a powerful tool for determining the structure of the RNA three- and four-way-junctions (4WJs and 3WJs). Multi-parameter fluorescence detection (MFD) and fluorescence correlation spectroscopy are applied to perform super resolution FRET studies with a high level of precision in distance measurements better than 1% of the Förster radius[1]. To extend the database with already existing information of the RNA 3WJs, we introduced new molecule with different sequence to understand the influence of this factor on the structural behavior. Overall 203 Donor-Acceptor pairs were measured with single-molecule MFD. This allowed us to collect simultaneously all fluorescence information such as intensity, lifetime and anisotropy in wide spectral range from picoseconds to seconds. With the analysis toolkit[2] it is possible to observe the structural changes of the molecule.

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# Study of heme degradation and ROS production upon interaction of hemoglobin with n-alkyl sulfates

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According to our previous study[1], it was confirmed that the interaction between human adult hemoglobin (Hb) and sodium dodecyl sulfate leads to heme degradation. Generation of hydrogen peroxide during this process was responsible for heme degradation in Hb. In current study the question is how the tail lengths of alkyl (hydrophobicity) in n-alkyl sulfates homologues and reactive oxygen species (ROS) production and so heme degradation pattern of hemoglobin correlate.

Spectroscopic techniques including UV-visible, circular dichroism and fluorescence spectroscopy were applied. The intrinsic fluorescence, circular dichroism and UV-Vis spectrums showed third and second structural changes by lengthening the tail and increasing the concentration in each homologue. It was found that during Hb reaction with different concentrations of n-alkyl sulfates some fluorescent degradation products were formed. The existence of these fluorescence bands are a clear indication that the heme is being degraded. The detection of the fluorescent products suggested that the heme degradation may involve the reaction of ROS with Hb. To address this point a chemiluminescence study was performed and then to understand the type of ROS, catalase was applied. It was shown that the hydrogen peroxide, produced during this interaction, is responsible for heme degradation. To have a quantitative insight into the amounts of hydrogen peroxide produced, a chemiluminescence calibration curve was obtained. It was concluded that the increase of hydrophobic chain length correlates with second and third structural changes, ROS production and heme degradation.

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# Atom transfer radical polymerization catalyzed by naturally occurring catalysts

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Atom transfer radical polymerization (ATRP) is one of the most important polymerization methods in modern polymer chemistry. Well defined polymers can be obtained with control over molecular weight and polydispersity.[1] Naturally occurring catalysts derived from plants are promising catalysts for ATRP. Recently Bruns and coworkers as well as di Lena and coworkers discovered that enzymes are capable to catalyze ATRP.[2,3] Moreover, Matyjaszewski and coworkers showed that hemin, which is an iron porphyrin, is an effective ATRP catalyst.[4] Here, we present a naturally occurring catalyst for ATRP that is abundantly available, cheap and environmentally friendly. It mediates the polymerization of poly (ethylene glycol methyl ether acrylate) in water with a good control over the molecular weight of the polymer, which is proven by first order kinetic plot and GPC elugrams.

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# A novel approach to study deoxyribonucleic acid denaturation

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In living organisms deoxyribonucleic acid (DNA) is stored in double strand (ds) conformation. When tasks such as replication or transcription are performed DNA must be at least locally in single strand (ss) conformation. Base stacking and hydrogen bonds between bases keeps DNA in ds conformation while repulsion of phosphate groups destabilize ds conformation. When later prevails DNA denatures into ss conformation. DNA denaturation as a function of the temperature was studied since 1970's with UV absorbance[1]. The novel approach uses dielectric spectroscopy coupled with conductometry[2,3]. We will compare results with the one obtained from UV absorbance and comment on benefits of the novel approach.

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# How to make pores in lipid bilayers by tuning shape of embedded objects

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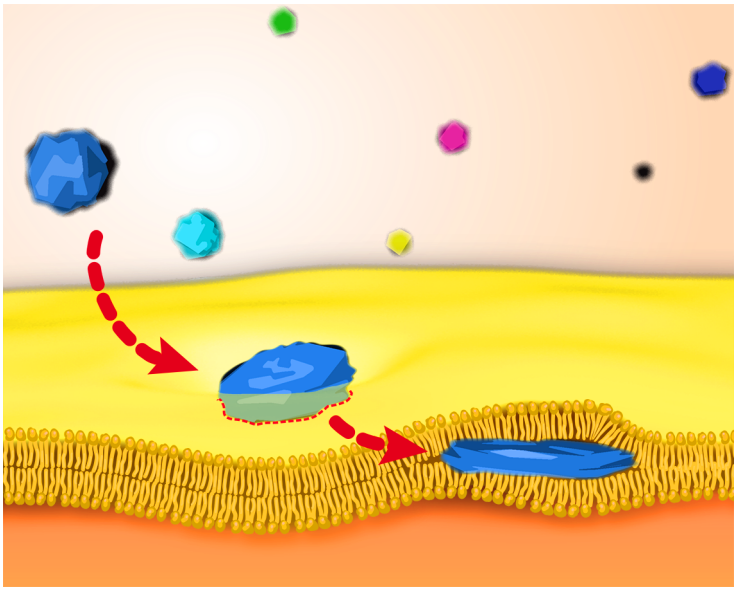
Membrane active peptides and proteins often undergo conformational changes while in contact with lipid membranes.[1] Shape and size variation can be a key ingredient for membrane activity of certain peptides and proteins. Using Single Chain Mean Field (SCMF) theory we demonstrate that the pores in lipid bilayers can be opened and closed by tuning shape and size of the embedded hydrophobic object: (1) Changing size and hydrophobicity of globular proteins may mechanically induce two kinds of pores around ion-channel in the equilibrium bilayer. (2) By changing slightly the conformation of the protein the pore can be opened or closed with almost no energy cost. We also find under certain conformation, protein can open and close the pore in the membrane only by thermal fluctuations.



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# New pharmaceutical derivates and their binding properties to serum albumin studied through EPR spectroscopy

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Besides globulins and other sparingly occurring plasma proteins, in the term of percentage, serum albumin is the major transport protein in human blood plasma. It thus regulates many endogenous processes of the organism and can serve as a drug carrier of many pharmaceutical ligands. Our aim is to understand this complex transport process, specifically the transport connection from ligand to protein by the selective elimination or exchange of specific functional groups in a fixed position of chemical related pharmaceuticals[1]. E.g., by comparison with unmodified pharmaceuticals or by the exchange of specifically selected functional groups at one and the same pharmaceutical, we are able to make more precise statements as to which functional groups contribute to protein binding. To this end, some selected (chemically related) pharmaceutical ligands were labeled with EPR active spin probes via Steglich synthesis. In a screening approach with several spin-labeled pharmaceuticals we utilize continuous wave (CW) electron paramagnetic resonance (EPR) methods with human serum albumin (HSA) at physiological conditions and varying ratios of ligand to protein. With aid of appropriate simulations of the recorded CW EPR spectra we can extract association constants ($K_A$) and the maximum number (n) of binding sites per protein of the modified pharmaceuticals. As indicated above with the comparison of the binding affinities from literature to our system or with the comparison of the binding affinities of similar modified pharmaceuticals to each other, e.g. through the exchange of different functional groups or elimination of atoms in one position, we aim at understanding the mechanisms of ligand-protein association. Furthermore, we hope that, based on our results, new drugs can be designed by a suitable choice of functional groups with predictable protein binding tendency.

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# Light-induced switching of HAMP domain conformation & dynamics revealed by time-resolved EPR spectroscopy

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Achaea and bacteria can “see” and “sniffle”, they have photo- and chemosensors that measure the environment. On the cell poles, these sensor proteins form large arrays built of several thousands of different receptors.[1] The interactions between proteins in such arrays are responsible for the remarkable properties of this large signal transduction machinery: its high sensitivity, its ability to integrate the signals from diverse receptors, and its capability to adapt to constant stimuli, preserving the ability to react to signal changes.[1] However, the molecular mechanism of signal transduction remains obscure.[1]

For the HAMP domain, a widely abundant signaling module in these arrays, several mechanisms were suggested, all comprising two distinct conformational states. These can be observed by two-component cw-EPR spectra at ambient temperatures existing in a thermodynamic equilibrium in the dark state. [2]

To trace the conformational signal and it’s propagation throughout the elongated transducer, we applied cw- and pulse-EPR spectroscopy in conjunction with nitroxide spin labeling. We follow transient changes by time-resolved cw-EPR spectroscopy and compare the resulting spectral changes to difference spectra corresponding to the above shifts in the thermodynamic equilibrium. The light-driven conformational changes are in agreement with a shift towards a more compact state of the HAMP domain.

Following this signal beyond the HAMP domain requires a mechanism compatible with the formation of trimers of SRII/HtrII dimers which activate CheA. An activation scheme within the framework of hexagonal arrays formed by the trimers of SRII/HtrII will be the key step to understanding the enormous cooperativity leading to signal amplification in networks formed by clusters of interacting receptors.

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# Key participants of mechanical stress response induced with cell wall microperforation in plant cells

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Dynamic physical forces and mechanical stress are common in everyday life of plants. Unlike animals, plants could not actively avoid stress factors because of their sessile life habit. Perception of environmental mechanical stimuli is crucial for adaption to variable surroundings and survival of plants. To investigate primary processes of mechanoperception, arising from invasion of pathogens or feeding of herbivorous insects, we performed the cell wall (CW) incision with a glass micropipette of *Chara corallina* internodes and recorded changes in $H^+$ concentration in the apoplast with antimony pH-microelectrodes. We discovered the localized increase in apoplastic pH up to 3 units as an early event in mechanoperception. The pH response was suppressed by lowering cell turgor. We suppose that the key role in alkalinization belongs to the tensile stress produced upon membrane impression into a microscopic cavity or a pore left in the CW after micropipette withdrawal, not to the compression produced by the micropipette during its impact on CW. The local weakening of CW disrupted the balance between turgor pressure and the elastic force, which led to local deformation of CW, the plasmalemma, and underlying cytoskeleton. The involvement of cytoskeleton in the origin of alkaline patch was documented by observations that the incision-induced pH transients were suppressed by the inhibitors of microtubules (oryzalin and taxol) and, to a lesser extent, by the actin inhibitor (cytochalasin B). The next step in mechanoperception is the plasma membrane impression into the CW defect under high hydrostatic internal pressure. Stretching of the plasma membrane during its impression into the cell wall defect is likely to activate the $Ca^{2+}$ channels, as evidenced from sensitivity of the incision-induced alkalinization to the external calcium concentration and to the addition of $Ca^{2+}$-channel blockers, such as $La^{3+}$, $Gd^{3+}$, and $Zn^{2+}$. Thus, early events of mechanoperception include the initial cytoskeleton deformation and changes of intracellular calcium, which promote the increase in plasmalemmal $H^+$ conductance, the apoplast alkalinization, and the eventual CW hardening.

# Laser synthesis and characterization of colloidal silver nanoparticles

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Nanoparticles of various materials are today implemented in wide variety of industrial, scientific and medical applications. Biocompatible nanoparticles are used for cell treatment; as nano-biomarkers, for therapy and diagnostics. Nanoparticles can be synthesized conventionally either using wet chemistry methods or gas phase processes. Such nanoparticles are often characterized by impurities which are reaction products of additives and precursors. Laser ablation in liquids appeared to be a solution for that drawback. It is recognised as simple and versatile technique and there are almost no limitations in a selection of materials for the nanoparticle synthesis. The remarkable advantages of this technique over other techniques are absence of impurities in the final product (free of contaminating processes, chemical precursors not required), possibility of preparation of multicomponent nanoparticles, weak agglomeration, narrow size distribution of nanoparticles, etc. In our work we studied laser synthesized silver nanoparticles produced in water by ns Nd:YAG laser operating at 1064 nm with 100 mJ of output energy and 5 Hz of repetition rate. From AFM figures size-distribution of nanoparticles is obtained. Laser ablation is monitored by optical emission spectroscopy while colloidal solution is characterized by photospectrometry and photoluminescence. Our results show that average diameter of laser synthesized silver nanoparticles is around 10 nm and size distribution is relatively narrow. Our colloidal solution is very stable in time (few months) implying that nanoparticles are well dispersed and/or charged.

# Glyphosate and its metabolites exhibit proapoptotic and prooxidative effects on human lymphocytes

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Glyphosate (*N*-phosphonomethylglycine) is a non-selected broad spectrum ingredient of herbicide formulations used worldwide to protect agricultural and horticultural crops. Glyphosate was believed to be environmentally friendly but recently, a large body of evidence has revealed that glyphosate can negatively affect on environment and humans. It has been found that glyphosate is present in the soil and groundwater. It can also enter human body which results in its occurrence in blood in low concentrations of 73.6 ± 28.2 ng/ml. Poisonings still pose a challenge and problems for toxicological investigations.

That is why we have investigated apoptotic and oxidative effects of the most commonly used pesticide: glyphosate and its metabolites: amino-methylphosphonic acid (AMPA) and methylphosphonic acid on human peripheral blood mononuclear cells (PBMCs), mostly lymphocytes. Due to the Regulation of the European Parliament and Council Regulation 1107/2009/EC on 21$^{st}$ of October 2009, it is necessary to identify metabolites present in the technical pesticide and undertake toxicological researches concerning these substances. Apoptosis, was detected by alterations in membrane permeability (by flow cytometry using YO-PRO-1 and propidum iodide), activation of caspase-3 (by fluorimetric assay) and changes in transmembrane mitochondrial potential. Moreover, oxidative damage was estimated through detection of reactive oxygen species (ROS) formation including hydroxyl radical by measuring the level of fluorescent labels – 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate ($DCFH_2$-DA) and 3'-(p-hydroxyphenyl) fluorescein (HPF). The PBMCs were incubated with xenobiotics at concentrations from 0.01 to 5 mM for 4 hours.

It was found that all the compounds studied caused statistically significant increase in the number of apoptotic cells, increased caspase-3 activity, decreased transmembrane mitochondrial potential and induced ROS formation in human peripheral blood mononuclear cells. Statistically significant changes in transmembrane mitochondrial potential were observed from 0.05 mM of glyphosate and from 0.1 mM of methylphosphonic acid and AMPA. For other parameters, changes were observed for higher concentrations.

# A metadynamics way to Hamiltonian replica exchange

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The computational study of RNA dynamics and de novo prediction of its native state is a hot topic in molecular biophysics. Simulations based on molecular dynamics technique has been successfully used to this purpose, from folding RNA tetraloops[1–2] to explore the conformational space of oligonucleoties[3]. These calculations often employ replica exchange methods such as parallel tempering[4] or Hamiltonian replica exchange[5]. However, the former is expensive and the latter is usually difficult to use. We propose a protocol where well-tempered metadynamics[6] is used with Hamiltonian replica exchange to enhance conformational sampling of an RNA tetranucleotide. Using this method, free-energy barriers of individual collective variables are significantly reduced compared with simple force-field terms scaling at the same effective temperature. The introduced methodology is very flexible and allows adaptive bias potentials to be simultaneously constructed for a large number of simple local collective variables, such as distances, angles, and dihedral angles.

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# Solid-state nanopores

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Solid-state nanopores have become a new single-molecule tool in biophysics. In comparison to biological nanopores, they offer many advantages due to their robustness, high stability, tunable pore size and potential for integration into devices. A precursor in the preparation of a nanopore is a thin, solid supported membrane of e.g. SiN (thinner than 20 nm) or ultrathin 2D materials like graphene or MoS2. Translocation of a macromolecule, e.g. DNA through a nm sized pore in such a thin membrane influences the ionic current of the surrounding electrolyte through the pore and electrical properties of the membrane itself. Both these transduction mechanisms provide single-molecule sensing capability and are being tested for an even finer role: DNA sequence readout - next generation DNA sequencing. Conventionally, nanopores are drilled in these membranes within a transmission electron microscope which is a tedious and resource intensive procedure. A latest development in this research field is a simple method of pore formation by the controlled dielectric breakdown of a membrane immersed in an electrolyte solution[1]. We constructed a setup for nanopore characterisation and translocation measurements based on a commercial current preamplifier and some analog devices built in-house. Our setup performs with the same noise levels as an order of magnitude more expensive setups developed elsewhere. It is also capable of performing the dielectric breakdown. We present the initial results on lambda DNA translocation events through a nanopore made in 20 nm SiN membrane.

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# The effect of PKCδ on hypericin photo-induced cell death

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In U87 MG cells (human glioma cell line), Hyp is predominantly localised in endoplasmic reticulum, mitochondria, lysosomes and Golgi apparatus[1]. Cell death induced by Hypericin photoactivation is triggered via mitochondrial apoptotic pathway.

A member of lipid–regulated serine/threonine protein kinase C (PKC) family -PKCδ, functions as a proapoptotic protein during apoptosis. It also has a critical role on the regulation of various cellular processes including cell proliferation and tumor promotion[2].

We used small interfering RNA to find out how would post–transcriptional silencing of *pkcδ* gene influence the cell death pathway. Flow cytometry was used to determine cell survival, type of cell death and production of reactive oxygen species (ROS). By fluorescence microscopy sub–cellular distribution of Bcl-2 family protein members (Bax and Bak) and PKCδ were monitored.

We examined that gene silencing did not influence the type of cell death (apoptosis or necrosis), however amount of death cells (%) where PKCδ was not blocked was higher compared to siRNA transfected cells. This can be due to the fact, that production of ROS was notably decreased in cell treated with PKCδ siRNA. It was shown that inhibition of PKCδ leads to translocation of Bcl2 family members (Bax, Bak) from cytosol to plasma membrane which slowly disappears 24 hours after Hyp activation. This translocation could be explained as effort of cells to target possible PKC after reduction of cytosolic PKCδin cells.

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# FRET triangulation of OmpF in polymersomes

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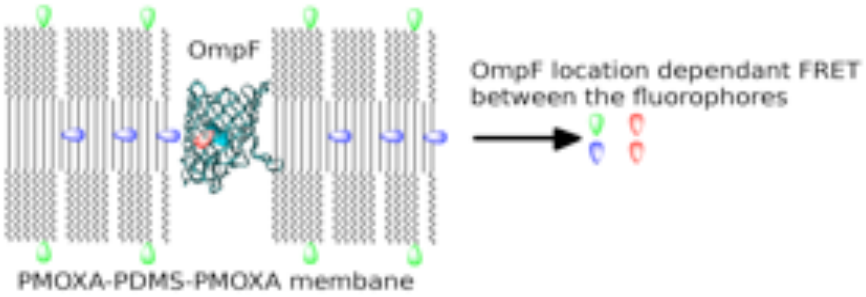
In nature, membrane proteins represent key elements necessary for energy production, selective uptake and secretion, motion and interactions between the compartments like organelles, cells or organisms and their environment. Lipid based membranes are nature's choice of compartmentalisation with the advantage of high fluidity but the drawback of low stability. The aim of molecular systems engineering is the adaptation of the functionality offered by nature's membrane proteins by their reconstitution in more stable and tuneable synthetic polymeric membranes.

A library of triblock-co-polymers PMOXA-PDMS-PMOXA was synthesized via cationic ring opening polymerization with slight modifications.[1] The polymers were shown to self-assemble into vesicular structures (polymersomes) with membrane thickness ranging from about 6.0 to 16 nm. To study the reconstitution mechanism of membrane proteins into these polymeric membranes, the outer membrane protein F from E. coli (OmpF) was expressed as a model protein in its wild type form and two cysteine mutant forms (K89C and R270C). Polymer and OmpF mutant forms allow site specific attachment of fluorescent probes and thereby FRET between them.

FRET experiments allow the determination of the OmpF's location within the polymeric membrane and enable a deep insight in reconstitution mechanism of membrane proteins into synthetic membranes.

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# DNA with condensing proteins in spherical confinement

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Nature has found various ways to pack DNA into small spaces. A well known example is the packing of DNA in chromosomes where highly basic histone proteins wrap the DNA, forming thus protein-DNA complexes called nucleosomes which can be more easily fitted in small volume[1]. Sim- ilar packing mechanisms have been found in adenoviruses where DNA-condensing proteins encoded in the viral genome condense the DNA in the capsid[2]. This is an unusual device in the world of DNA viruses[3]. Packing of DNA in adenoviruses has long evaded precise description since the viral DNA molecule condensed by proteins (core) lacks icosahedral order characteristic of the virus protein coating (capsid)[4,5]. Still, the dominant view is that the core has an ordered structure.

We show that useful insights regarding the organization of the core can be inferred from the analysis of spatial distributions of the condensing proteins. These were obtained from the inspection of contrast in cryo-EM cross-sections of mature and immature adenoviruses. Our analysis shows that the core lacks symmetry and strict order, yet the distribution of the condensing proteins is not entirely random. Comparisons between mature and immature virions showed no visible differences.

The features of the distribution can be explained by modelling[6] the condensing proteins and the part of the DNA each of them binds as very soft spheres, interacting repulsively with each other and with the capsid. Results show that a backbone of DNA linking the condensing proteins is not needed to explain the experimental results. Although these condensing proteins are connected by DNA in disrupted virion cores, the in vivo capsid is a crowded environment which changes the effective interactions involved in the packing of the DNA material[7].

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# spFRET experiments reveal conformational dynamics of nucleosomes and binding of GR to DNA

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Chromatin, the structure in which DNA is compacted in eukaryotic cells, plays a key role in DNA regulation by governing DNA accessibility. Förster Resonance Energy Transfer (FRET) experiments on single nucleosomes, the basic units in chromatin, have revealed a dynamic nucleosome where spontaneous unwrapping from the ends provides access to the nucleosomal DNA[1]. Here, we investigate how the dynamics of individual nucleosomes couples to the higher order structure of chromatin by studying DNA dynamics in nucleosomes flanked by linker DNA or a second nucleosome. We also started investigating the binding of a nuclear receptor to DNA.

A combination of single-pair (sp) FRET, Alternating Laser EXcitation (ALEX) and Fluorescence Correlation Spectroscopy (FCS) on nucleosomes with increasing linker DNA length reveals that the fraction of partially unwrapped nucleosomes increases when the exiting DNA tails come close to each other. Electrostatic repulsion between the entering and exiting DNA tails drives nucleosomes to a more open conformation.

In dinucleosomes, an interaction mediated by histone tails is expected to bring nucleosomes face to face in order to form a folded chromatin fiber. spFRET experiments show that the fraction of partially unwrapped nucleosomes increases for dinucleosomes with 55 bp linker DNA as compared to nucleosomes with 20 and 50 bp of linker DNA and mononucleosomes. This additional unwrapping might help alleviate bending of the linker DNA in stacked nucleosomes[2].

Binding of the nuclear receptor GR (glucocorticoid receptor) to DNA constructs containing or lacking a glucocorticoid response element was investigated using ALEX and FCS. Examination of the stoichiometry revealed a concentration-dependent increase in the GR-DNA subpopulation for both DNA constructs.

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# Relative binding free energies of OppA ligands from MD simulations

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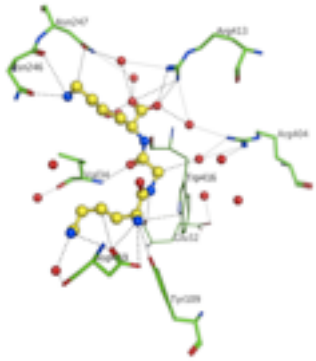
The oligopeptide binding protein A (OppA) is a vital transporter protein in the periplasm of gram-negative bacteria.1 It represents a well-known example of water-mediated protein-ligand binding. OppA relies on water molecules to accommodate a broad range of ligands with different physico-chemical properties. Different water configurations have been observed for different oligopeptides, binding to the active site. This observation, combined with the highly flexible peptidic character of the ligands, make the OppA system an excellent test case for advanced computational approaches.



Here we investigate the free energy of binding of three tripeptide ligands crystallized with OppA,2 using thermodynamic integration (TI) approaches. Thermodynamic cycles were constructed for the three different peptides, once bound to OppA, and once freely solvated in water.

For the free tripeptides in water, it was observed that the difference between an alanine and a glycine amino acid residue, though chemically small, leads to a large physical difference in flexibility, and thus to a surprisingly slow convergence of the calculations. This can be overcome by either extending the simulations, or by slightly modifying the Hamiltonian of the tripeptides, followed by appropriate corrections to obtain unbiased free energy differences. Furthermore, substantial improvements in cycle closure accuracy could be achieved by thoroughly investigating the high curvature areas of TI transformation curves, which represent (unphysical) transitional states close to the polar peptide species.

However, for the tripeptides in complex with OppA, significant discrepancies between independent simulations still remained. These could be traced to a slow relaxation of the functional water molecule network within the active site. Here a brute force approach has been employed to reach fair agreement of calculated free energies of binding with experimental affinities. It might however still be necessary to combine brute force with the more elegant Hamiltonian modification solution to finally close the thermodynamic cycles.

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# Interaction of a model antimicrobial peptide with bacterial cells

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Growing antimicrobial resistance necessitates the development of new, effective therapeutics for the continued treatment of infection and disease, for which antimicrobial peptides are promising candidates. Many antimicrobial peptides are membrane-active but their mode of action remains elusive. Our work provides insight into the action of antimicrobial peptides by measuring the effect of a model antimicrobial peptide on living planktonic *Klebsiella pneumoniae* cells. This has been achieved using atomic force microscopy to probe the mechanobiology of live bacteria to obtain the turgor pressure, cell wall elasticity, and bacterial capsule thickness and level of organisation.

The addition of the peptide had a significant effect on the turgor pressure and Young's modulus of the cell wall. The turgor pressure increased upon peptide addition and the subsequent decrease suggests that cell lysis occurred and that cytoplasm leaked through a compromised membrane. The Young's modulus increased after exposure to the peptide, indicating that interaction with the peptide increased the stiffness of the cell wall. A surprising result is that the bacterial capsule does not prevent cell lysis by the lytic peptide. Nor does the capsule appear to be affected by exposure to the peptide, as the thickness and organisation varied no more than expected within a population of bacteria. This result is ratified by mechanical measurements.

# The signal transduction in the archeal photosensoric complex *Np*SRII:*Np*HtrII as studied by molecular dynamics simulations

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Bacterial and archeal signaling systems share similar two-component architecture and are highly homologous. The central role in these systems belongs to the histidine kinase CheA, which is bound along with the CheW protein to the tips of the transmembrane receptors. Transmembrane receptors organized in trimers-of-dimers recieve various signals and convey them to the kinases: a single conformational disturbance arising in the receptor upon its activation appears to be transduced along the receptor molecule of 20-30 nm in length to the tip region, where it alters kinase activity of the bound CheA and eventually changes cell mobility.

Adjusting of the TCSs to the current level of input signal is furnished by a reversible methylation process of specific Glu residues in the cytoplasmic domain of the receptors acomplished by CheB/CheR proteins. Since CheB is activated by CheA kinase, this grounds a robust feedback mechanism called adaptation.

We performed coarse-grained MD simulations of the trimer of *Np*HtrII:*Np*SRII in methylated and demethylated states to reveal molecular mechanisms of signal trunsduction and to envision kinase activation explointing strong analogy between activation and adaptation processes.

Upon the methylation/activation of the complex the interacting with CheA tip region does not undergo significant structural rearrangement but rather its dymanics changes. We suggest a universal model of signaling in bacterial and archeal receptors: different conformational input signals cause distinct pattern of dynamical changes along the trimer modulating CheA activity via dynamical allostery.

# Mass spectrometry-powerful tool for research of antibiotic resistance mechanisms in bacteria

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Infections induced by bacteria that have become resistant to commonly used antibiotics have become a global healthcare problem in the 21st century [1]. According to report by Centers for Disease Control and Prevention each year at least 2 million people in USA have been infected with bacteria that are resistant to antibiotics and at least 23,000 people die from these infections. Therefore, it is important to understand mechanisms of bacteria self-defense against antibiotics, in order to develop new drugs.

The development of soft ionization techniques such as matrix assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI) in mass spectrometry that were in-line with development of human genome project positioned mass spectrometry as an invaluable tool for proteome research and elucidation of protein function[2] in living organisms.

In this work we studied the effect of antibiotic Penicillin G on proteome of *Escherichia coli* MFBF 10519. For the identification of proteins we were used *de novo* sequence analysis of peptide (MALDI-TOF/TOF mass spectrometer). Results of the effect of Penicillin G on *Escherichia coli* MFBF 10519 showed 8 different protein expressions compared with strain of *Escherichia coli* MFBF 10519 that do not grow in the presence of Penicillin G.

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# Hemoglobin catalyzed ATRPase of acrylamide

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Recent advances in polymer chemistry allow scientist to perform controlled/living radical polymerization which yield polymers with predetermined molecular weights and a narrow distributions of molecular weight. Among the various techniques, atom transfer radical polymerization (ATRP) is the most popular one since it is versatile, tolerant toward functional groups and yields halogen-terminated polymer chains[1]. The main drawback of this method consists in the transition metal complexes that it requires as catalysts, because they can be toxic and are present in the final product. A “green” approach to ATRP is the use of enzymes, so called ATRPase, instead of conventional catalysts[2].

Controlled polymerization of acrylamide by ATRP is still a challenge due to its fast propagating rate and its tendency toward chain transfer. Recent work by Broekhuis’ group presents homopolymerization of acrylamide with a PDI as low as 1.39[3]. We aim to obtain better results in acrylamide polymerization by using hemoglobin, already established as an efficient catalyst for controlled polymerization of water soluble monomers[4]. Latest results in the kinetic study of acrylamide ATRPase will be presented.

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# Dynamical domain decomposition of protein complexes based on pairwise distance fluctuations

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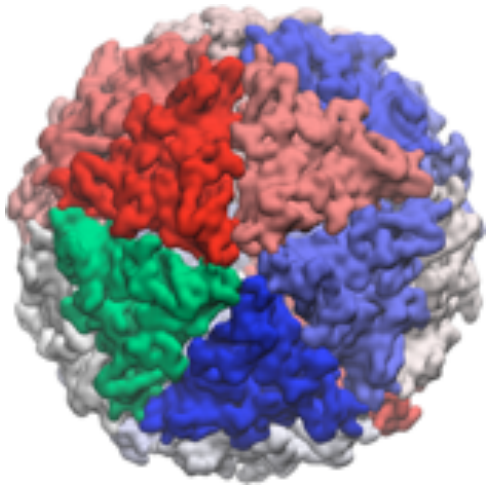

The internal dynamics of proteins and enzymes often impacts their biological functionality, e.g. by favouring the binding of substrates or their processing. Functionally-oriented movements typically entail the concerted displacement of several amino acids. For this reason, it is both convenient and physically appealing to characterize the large-scale internal dynamics of proteins as resulting from the relative motion of few quasi-rigid domains. Here we want to present a novel and general domain-subdivision technique, which is based on the analysis of distance fluctuations between pairs of amino acids from a limited ensemble of alternative conformations, either obtained from experimental measurements (for instance, NMR structures), extensive molecular dynamics simulations or elastic network models. The method employs advanced clustering techniques to identify the innate number of quasi-rigid domains and their identity in terms of groups of amino acids (in figure, the resulting dynamical domains, in different colors, of the Satellite Tobacco Mosaic Virus capsid). It has been validated in previously well-characterized contexts (for example, on systems already analyzed with a different technique[1]) and next applied to more challenging systems such as voltage-gated ion channels and entire viral capsids, for which the domain subdivision strategy is shown to provide valuable biological insight.

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# Molecular order in benzene mixtures

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The complexity of the organization in liquid mixtures is due to the competing hydrophobic and hydrophilic contributions. These are two key physical principles that define the forming of structures on both the molecular and the cellular scale. Structuring in aqueous mixtures is defined by the difference in the structuring in mixtures of simpler liquids such as benzene and acetone in the sense that they convey a new scale of disorder that is fundamentally different from the simple disorder found in mixtures of the latter liquids[1]. This type of organization differs profoundly from the bigger scale organization, which is characterized by associations such as micelles and bilayers.

We are examining the structuring in liquid mixtures, using molecular dynamics simulations, in order to span different molecular types of organization from simple liquid mixtures to more complex ones. Three systems were chosen: benzene-pentane, benzene-ethanol and benzene-acetone. By keeping one component constant, we're able to determine the influence of the chaging component on the structure of the mixture as a whole. The key idea is to qualitatively describe the differences in the molecular organization between the mixtures, using radial distribution functions (to describe the structure of the mixtures and indicate the type of interactions between the molecules) and running Kirkwod-Buff integrals (as a means of quantifying concentration fluctuations).

A combination of experimental results found in literature and our simulation results will present a global picture of the molecular scale ordering in liquids.

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# Effect of lipid-lowering therapy on homocysteine level and other plasma parameters in coronary artery disease (CAD)

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Increase concentration of homocysteine is one of the risk of cardiovascular diseases. Coronary artery disease (CAD) accompanied the increase of LDL cholesterol level and in treatment are used the hipolipemic drugs. These drugs have also pleiotropic effects that are not very good known. The aim of that study was to compare the effect of two different hipolipemic therapies (atorvastatin and atorvastaton + ezetymibe) on concentration of homocysteine and lipid peroxidation in plasma of CAD patients.

The study involved 18 patients aged from 53 to 77 with angiographically confirmed coronary artery disease who despite at least 6 months hypolipidemic treatment did not achieve LDL C<70mg/dl and 20 healthy subjects in appropriate age. Participants were randomized to 2 groups: 12 patients received atorvastatin 40mg (A40) and 6 patients combination therapy: atorvastatin 10 mg with ezetimibe 10 mg (A+E). The treatment duration was 6 months. The following parameters were studied: homocysteine level, lipid peroxidation in plasma and lipids parameters.

Our results show, increased level of homocysteine (46%), lipid peroxidation (22%), LDL-C concentration (34%) and total cholesterol level (19%) in patients with CAD in comparison to the healthy subject. After six months therapy was observed the following changes in comparison to the values before therapy: decrease of homocysteine level in plasma in group A40 about 26% and in group A+E about 28%; decrease of lipid peroxidation in plasma in group A40 about 27% and in group A+E about 32%, decrease of LDL-C cholesterol level in group A40 about 17% and in group A+E about 33% and in total cholesterol level in group A40 about 15% and in group A+E about 17%.

Our results suggest that intensive lipid-lowering therapy has a beneficial effect on certain parameters of the blood of CAD patients.

# Conformational changes during GTP hydrolysis in *wt* and mutated orthologs of the human LRRK2 Parkinson kinase analyzed by DEER

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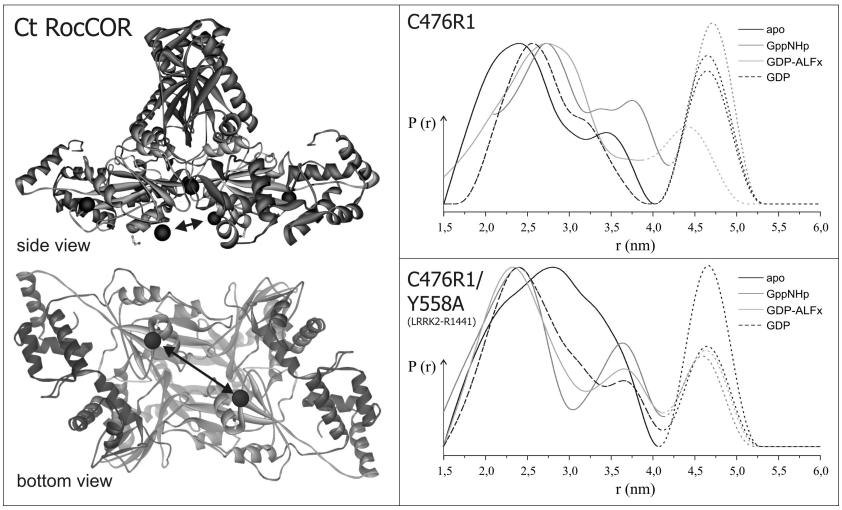
Leucine-rich repeat kinase 2 (LRRK2) is a dimeric multidomain protein containing a kinase domain, a GTPase domain (Ras of complex proteins, Roc) appearing in tandem with the COR (C-terminal of Roc) domain, and numerous protein–protein interaction domains. Mutations linked to autosomal dominant forms of Parkinson's disease result in alterations in both its enzymatic properties and interactions. For example, the best characterized mutations to date, G2019S in the kinase domain and R1441C and R1441G in the GTPase (G) domain have been reported to influence kinase as well as GTPase activity.

We applied site directed spin labeling and distance measurements by Double electron electron resonance (DEER) spectroscopy to study the G domain motions in course of the GTPase cycle and a possible influence of mutations in the Roc–COR domain tandem of *Chlorobium tepidum* (*Ct*), a prokaryotic homologue of the human LRRK2 Parkinson kinase. Our results reveal conformational changes of the G




domains that are not in line with current models. Furthermore, we observed a strong influence of the mutations on the relative motion of the G domains in the Roc-COR dimer.

# Multiple pH regime Molecular Dynamics simulation for pK calculations

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The main goal of this work is the theoretical prediction of the ionization equilibrium constants of the titratable groups of Staphylococcal Nuclease Δ+PHS (SNase Δ+PHS)[1]. The approach applied in the study is based on generation of conformational sets, corresponding to different protonation states of the ionizable groups in the molecule[2].

The X-ray structure of the protein of interest (PDBID: 3bdc) suggests disordered N- and C-termini of the molecule. Two models of the enzyme were built- a short model with acetylated and amidated N- and C-termini, and a model with all the missing residues added to the molecule (computational work in progress). Two sets of structures for the short model of SNase were prepared, with protonated and deprotonated states of the GLU and ASP residues.

The current stage of the project includes molecular dynamics simulations using CHARMM package[3]. The simulations with the truncated X-ray structure were completed and the pKa values of the glutamates and aspartates were calculated with the multiple hydrogen location approach[4].

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# Hofmeister salts regulate the population distribution of the conformers of FAD molecule: a fluorescence kinetics study

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The flavin adenine dinucleotide (FAD) molecule in water prefers a stacked conformation, where the adenine group quenches the fluorescence of the flavin one. In the presence of alcohol and other organic solvents an open structure is also populated with the lack of quenching. Consequently, the population of the stacked and the open forms can be characterized by short- and long-lived fluorescence, respectively[1]. As we observed earlier[2], even in pure water a considerable amount of molecules are in open conformation, and the fluorescence kinetics distinguishes three different closed states.

The Hofmeister series represents the relative effectiveness of different salts on the aggregation and conformation of proteins and other colloids. The aggregated states are facilitated by the kosmotropic anions ($SO_4^{2-}$, $F^-$), while the open structures by the chaotropic ones ($ClO_4^-$, $SCN^-$)[3]. We have found that the conformation states of the small FAD molecule also follow this rule, unexpectedly well. We measured the time-resolved fluorescence of FAD in aqueous solution in the presence of high concentration of characteristic kosmotropic and chaotropic salts (NaF and $NaClO_4$, respectively) in the 100 fs – 10 ns region, applying fluorescence upconversion and time resolved single photon counting (TCSPC) techniques.

The kinetics was analyzed by a basis pursuit denoising (BPDN) method optimized for multicomponent exponencel decay[1], making possible to sensitively monitor the population of the different conformational states of FAD. In the presence of the Hofmeister salts, the populations of these states are markedly changed, in the directions according to the Hofmeister's rule.

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# Comparison of enzymatic and biophysical assays using carbonic anhydrases as model proteins

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Protein-ligand interactions are important in drug discovery. A wide range of methods is used for determination of binding affinity and all of them have pros and cons. For this reason, a careful look is needed to interpret the data correctly.

In this study, a class of nanomolar range inhibitors of carbonic anhydrases (CA) is reported and the limits of some enzymatic and biophysical assays are discussed. The carbonic anhydrases are considered as potential therapeutic targets. At least 30 CA sulfonamide inhibitors have been used as drugs to treat glaucoma, epileptic seizures, altitude sickness, and as diuretics. The carbonic anhydrase is a highly efficient catalyst of reversible interconversion of carbon dioxide and bicarbonate ion. The stopped-flow $CO_2$ hydration assay was used to monitor CA activity and inhibitor potency. Determination of inhibition constant $K_i$ in traditional enzyme inhibition methods is limited by the enzyme activity and concentration of the enzyme in a solution. Moreover, CA stability and ligand binding was tested by the fluorescent thermal shift assay, which is not limited in the range of affinities. However, dissociation constant $K_d$ does not reveal enough information about inhibitor binding to the protein. Isothermal titration calorimetry was used to confirm the $K_d$ and determine other thermodynamic parameters, such as Gibbs free energy, enthalpy, and entropy. The combined use of these methods provides a greater understanding of protein-ligand interactions. Despite different principles of the methods, the observed $K_d$ determined by biophysical methods correlates with $IC_{50}$ obtained by an enzymatic method.

# 3D model structure of thermostable pernisine

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Pernisine is a calcium mediated serine extracellular protease originated from *Aeropyrum pernix* K1. *Aeropyrum pernix* is hyperthermophilic archaea living in coastal sea water at temperatures above 90°C. Nowadays is increasing interest in thermostable enzymes appropriate for the biotechnological industries.

Pernisine was overexpressed in *E.coli* expression system.[1] Purification was done using affinity and size-exclusion chromatography. Active pernisine is produced after self-activation process. Proform of pernisine band appears at molecular weight 50 kDa on SDS-PAGE and is activated by heat treatment for 30 min at 90°C in the presence of 1 mM $CaCl_2$. Activated pernisine revealed at 36 kDa and was biochemically characterized using azocasein assay under broad range of pH and temperatures. Optimal activity was determined around pH 7.0 and temperature 105°C (Fig.1). Further on, we designed 3D model of a proform as well as mature form of pernisine. Model was calculated with Geno3D2 software using published 3D structure of Tk-subtilisin. Amino acid alignment showed us that Tk-subtilisin and pernisine share most of the amino acid residues involved in $Ca^{2+}$ binding sites (Fig.1). Model of pernisine and azocasein assays implies that $Ca^{2+}$ ions are crucial for pernisine activation and stability at temperatures above 80°C under standard assay conditions.

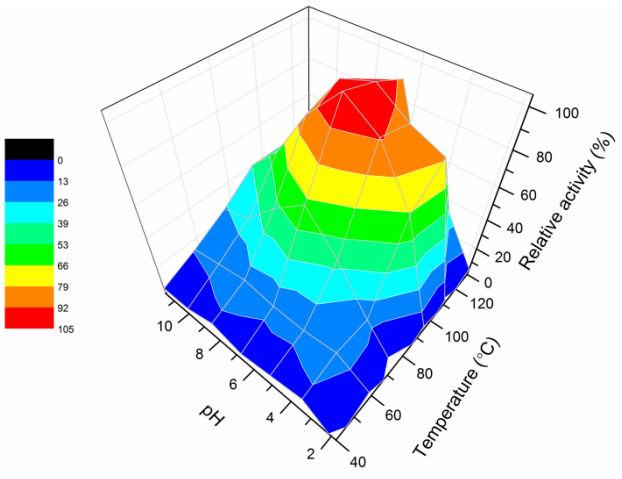


Figure 1: Recombinant pernisine activity dependence of temperature and pH. Relative activity of recombinant pernisine was incubated at different temperatures from 40-120°C and under six different pH ranged from pH 2-12.

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# On the role of Rac1 GTPases in the regulation of cell polarity in *Dictyostelium*

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*Dictyostelium* cells use the crawling migration to explore the space as they search for food. Typically, they travel in one direction shorter than 1 minute, and then reorient. These reorientations are a consequence of spontaneous cell re-polarizations in which small Rac1 GTPase proteins play a prominent role. A mechanism was recently unraveled in which the spatiotemporal dynamics of Rac1 activity appears to be regulated by the opposing interactions of Rac1-GTP with two different effector proteins[1]. The active Rac1 is simultaneously present at the leading edge, where it participates in the Scar/WAVE-mediated actin polymerization, and at the trailing edge, where it binds to the IQGAP-related protein DGAP1 and induces its interaction with the actin-bundling proteins cortexillin I and II. We hypothesize that a competition between DGAP1/cortexillins and Scar/WAVE effectors for the common Rac1-GTP activator could govern the oscillatory re-polarization of *Dictyostelium* cells[2]. Using a highly sensitive configuration for confocal microscopy of live cells, we monitored with improved temporal resolution the anticorrelated dynamics of fluorescent probes that report localization of the two complexes. Based on the experimental data, we propose a preliminary mathematical model that has the ability to reproduce certain characteristics of the observed dynamics, such as the fluctuating relocation of the probes with a correct characteristic repetition rate. Further development of the modelling approach will enable us to predict the values of the kinetic rate constants for the formation and decomposition of the effector-activator complexes *in cellulo*.

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# Curcumin impacts the EGFR lateral diffusion *via* its interaction on plasma membrane

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The Epidermal Growth Factor Receptor (EGFR) is a transmembrane protein with an intracellular tyrosine kinase activity, which is stimulated by the receptor dimerization. As the over-activation of EGFR is involved the development of many tumors, this receptor has become a target in cancer therapy. In this context, the curcumin action on EGFR was investigated, but its mechanism of action is not fully elucidated. There are numerous works reporting the effect of curcumin on the kinase activity, autophosphorylation yield [1], expression level and processes related to EGFR function like cells proliferation. Although many different modes of action were examined, to our knowledge the plasma membrane alterations leading to limitation of the receptor diffusion were not considered. This work highlights the curcumin driven rigidification of the membrane as revealed by fluorescence anisotropy and FRAP studies in biomimetic membrane models. Since the level of another membrane rigidifying constituent, the cholesterol, is shown to impact the EGFR phosphorylation level by modulating its ability to diffuse and dimerize [2], we expected that curcumin might act in the same way. Single particle tracking analyses confirmed that the diffusion coefficient of EGFR in the cell membrane significantly decreased in the presence of the curcumin, which might influence the receptor dimerization and in turns its activation. In conclusion, we have demonstrated that the curcumin action mode is dual: its direct inhibitory action on the tyrosine kinase domain is reinforced by its indirect action on the receptor dimerization *via* the membrane.

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# The disordered TPPP/P25, a prototype of the neomorphic moonlightning proteins

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Proteins without well-defined 3D structures represent a significant part of the human gene products. These intrinsically unstructured proteins play critical role in the etiology of conformational diseases such as Parkinson's disease (PD). The involvement of the hallmark proteins of these diseases such as $\alpha$-synuclein has been revealed, however, the pathomechanism remains inconclusive. The evaluation of the role of novel players could largely contribute to the understanding of the molecular mechanisms of these conformational diseases. In this respect the discovery of the Tubulin Polymerization Promoting Protein (TPPP/p25) has great potency since its enrichment in brain inclusions from PD patients has recently been demonstrated by the Ovádi's lab[1].

The disordered TPPP/p25, a prototype of neomorphic moonlighting proteins, displays distinct physiological and pathological functions by interacting with distinct partners. Experiments with human recombinant protein and in living cells revealed that TPPP/p25 modulates the dynamics and stability of the microtubule system due to its bundling and tubulin acetylation promoting activities detected by different biophysical methods and immunofluorescence microscopy. Analysis of atomic structure of human TPPP/p25 by multinuclear NMR revealed that extended disordered segments are localized at the N- and C-terminals (44-45 amino acid) straddling a flexible region (130 amino acid) which extensively fluctuates among multiple conformations. This middle flexible segment of TPPP/p25 is responsible for binding of $Zn^{2+}$ and GTP[2]. TPPP/p25 occurs both in monomeric and homo-dimeric forms and this could be controlled by its local concentration as well as the intracellular GTP level. The binding of GTP promotes the dimerization coupled with conformational changes, which favors its tubulin polymerization promoting activity[3].

In normal brain TPPP/p25 occurs in oligodendrocytes; it is indispensible for the differentiation of the progenitor cells via its role in the rearrangement of the microtubule network in the course of elongation of projections leading to oligodendrocyte maturation necessary to the axon ensheathment [3]. The non-physiological expression level of TPPP/p25 is coupled with different central nervous system diseases, such as multiple sclerosis (destruction of the myelinization resulting in altered TPPP/p25 level in patients), oligodendroglioma (lack of TPPP/p25 expression), or conformational diseases (co-enrichment of TPPP/p25

and alpha-synuclein in neuronal or glial inclusion bodies characteristic for PD and multiple system atrophy, respectively). Therefore the detailed characterization of the interactions of TPPP/p25 with its physiological (tubulin) and pathological (alpha-synuclein) partners is of great importance to understand the molecular mechanisms of these conformational diseases and to selectively prevent/arrest the pathological interactions.

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# Singlet oxygen production after photoexcitation of hypericin in DMSO/water mixture

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Photodynamic therapy (PDT) is a very promising modern medical method of cancer treatment. Elementary mechanism for PDT is based on formation of singlet oxygen, which is generated by energy transfer from excited photosensitizer (in our case Hypericin) triplet state to molecular oxygen. Singlet oxygen is highly reactive oxygen species used to cause oxidative stress in cancer cells. In the present work we built experimental apparatus to detect very low phosphorescence of singlet oxygen which has its maximum in near infrared region around 1270 nm. There are still only few apparatuses in the world which are able to directly measure singlet oxygen through its luminescence. In this project we studied production of singlet oxygen after photoexcitation of hypericin in different aggregation degree. Hyp is in pure DMSO in its monomeric form. But Hyp is not water soluble so when the water content in DMSO is increased the molecules of Hyp generate nonfluorescent aggregates. We measured lifetime of singlet oxygen in different DMSO/water mixtures by time resolved detection of phosphorescence singlet oxygen after photoexcitation of Hyp by laser pulse. We used the method of quasi-continuous excitation with relatively long laser pulses (20 μs); this method decrease of bleaching of sample and enabled us to see saturation of signal in better signal to noise ratio. The measured lifetime is in correlation with viscosity of DMSO/water mixture. Singlet oxygen luminescence lifetime exhibits its maximum mixture exhibits its maximum at 30% water content in DMSO/water mixture (around 11 μs); in pure water 3 μs and in pure DMSO 5 μs.

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# Higher order periodicity in *Tribolium castaneum* identified with computational method Global Repeat Map

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There are a vast number of tools that are used for identification of tandem repeats, elements in DNK that are arrays of sample of nucleotides that are arranged in head to tail fashion. Researchers showed that they have an increasing importance in gene expression and in their regulation. Higher order repeats, which are secondary repeating unit of tandem repeats, shows an important role in centromere formation and structure, as is shown in case of alpha satellites ~171 bp in human chromosomes in region of centromere, as well as an indicator for evolutionary jump from primates to humans in brain development[1]. With Global Repeat Map - GRM computational method [2], we have analyzed insect *Tribolium castaneum* genome, in order to identify higher order repeats (HORs). Along the other algorithms for detection of tandem repeats, who works on principles of dynamical matrix alignments, data compression, mapping sequence in numerical one and Fast Fourier Transform, our method is effective tool for analysis of a DNA sequence. Advantage of GRM method is in direct mapping of symbolic DNA sequence in frequency domain making global map and with the complete assemble of “key words”, with no input parameters, it can detect repeats of all lengths in spite of copy deviations of perfect sample. With this method we have identified a great number of higher order repeats in insect *T.castaneum*, which are usually characteristic for mammals. Those HORs are assembled from monomers of length ~360 bp, as well as HOR-s based on other monomer lengths. Identification of HOR’s is relevant for further investigations of regulatory roles ie. gene expression as well as mechanisms of development.

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# EPR- and IRRAS studies on Alpha-Synuclein

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α-Synuclein is a protein composed of 140 amino acids that forms a major constituent of the Lewy bodies characteristic of Parkinson's disease. Although no precise function has yet been determined, binding to membranes seems to be important for its physiological role. α-Synuclein lacks a well-defined secondary structure in free solution and can thus be counted to the class of intrinsically disordered proteins (IDPs). These proteins show an unusual dynamic flexibility and are thus not crystallisable by means of X-ray crystallography as a central method in structural biology.

Upon binding to negatively charged membranes α-Synuclein adopts a more ordered structure, i.e. folding-upon binding. α-Synuclein exhibits two coexisting binding modes, an extended α-helical form and a helix-loop-helix form (horsehoe model).[1]

In order to study the membrane-bound state of α-Synuclein, single cysteine mutants labelled with the MTS spinlabel [1-oxyl-(2,2,5,5-tetramethylpyrroline-3-methyl)methanethiosulfonate] are examined by studying the interaction between α-Synuclein and liposomes composed of the negatively charged lipid POPG.

CW-EPR spectroscopy is used to obtain information about the dynamics and polarity of the environment of the incorporated spinlabel.

In order to obtain information about the self-assembly of α-Synuclein on membranes distances between the spin labels are measured by using pulsed EPR-methods, like the double-electron-electron-resonance (DEER) experiment, where distances between 1.5 nm and 8 nm can be obtained.

Further examinations of the α-Synuclein wild-type will include the usage of the Infrared-Reflection-Absorption-Spectroscopy (IRRAS). This is an ideal technique to study the interaction of proteins with lipid monolayers (at the air/water interface) and in addition to study changes of secondary structure upon self-assembly of proteins[2].

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# Characterization of the hysteretic behavior of thermoresponsive polymers by spin probing EPR

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Biological[1] and synthetic[2] thermoresponsive polymers are of particular importance due to their increasing use in drug delivery or tissue engineering applications.

The inverse phase transition of thermoresponsive polymers is a complex process. Continuous wave (CW) electron paramagnetic resonance (EPR) spectroscopy on reporter molecules (spin probes) as a non-invasive intrinsically local technique can provide a holistic understanding of the temperature dependent phase transition of thermoresponsive polymers.

To understand the molecular details of the phase transition of - in the present case - hysteretic polymers as a function of the key molecular parameters chain length and composition, different thermoresponsive polymers are investigated by CW EPR. To overcome the disadvantage, that the diamagnetic polymers show no EPR signal, nitroxide radicals as spin probes are added to the samples as reporter molecules that self-assemble into the polymer-rich and water-rich regions of the phase-separated systems.

The major aim of this study is to examine and compare the EPR spectra recorded at different temperatures during heating and cooling of the polymers to analyze how the dynamics of the hysteretic polymers behave upon increase and decrease of temperature.

The measured EPR spectra are analyzed by simulating the spectra with regard to in particular two parameters: the $^{14}$N-hyperfine coupling of the spin probe NO-group, which reports on the hydrophobicity/hydrophilicity and polarity of the molecular environment, and the rotational diffusion tensor to calculate the rotational correlation times of the spin probe molecules.

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# Productive versus non-productive complex formation by DcpS enzyme and mRNA cap analogs

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Dinucleotides containing the cap structure, i.e. 7-methylguanosine linked to the second nucleoside by a triphosphate chain ($m^7$GpppN), are the main substrates of DcpS (Decapping Scavenger) enzyme. The enzyme catalyzes $m^7$GpppN hydrolysis, yielding $m^7$Gp and ppN, to preclude inhibition of other proteins by $m^7$GpppN species. This is important for proper gene expression. DcpS inhibition, in turn, was proposed as a therapeutic approach to spinal muscular atrophy (SMA).

We identified a cellular DcpS inhibitor – 7-methylguanosine diphosphate ($m^7$Gpp). This interesting finding allowed us to analyze DcpS complexes with several cap analogs modified within $m^7$G, ppp or N. Using fluorescence spectroscopy and HPLC chromatography we determined kinetic parameters ($K_M$, $v_{MAX}$) for cap analogs retaining substrate properties. By means of fluorescence titration we determined association constants ($K_{AS}$) for cap analogs which appeared non-hydrolysable. These quantitative experimental data correlated with computational docking analysis, which let us pinpoint the specific molecular determinants for formation of productive (enzyme-substrate) versus non-productive (enzyme-inhibitor) DcpS-cap analog complexes.

Since the role of DcpS inhibition in SMA is poorly understood, our characterization of DcpS complexes might be useful for solving the molecular basis of this devastating neuromuscular disorder.

# Formation of oligomers, fibrils, and other aggregates of Amyloid beta protein

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Several lines of evidence suggest that the amyloid-β-peptide (Aβ) fibrils play a central role in the pathogenesis of Alzheimer's disease (AD). Not only Aβ fibrils, but also small soluble Aβ oligomers in particular are suspected to be the major toxic species responsible for disease development and progression. Despite remarkable efforts, the exact mechanism and process of Aβ oligomer and fibril formation out of Aβ monomers is poorly understood. The wealth of postulated on- and off-pathway intermediates of Aβ oligomer and fibril formation is irritating. The aim of the work is to gain insight into growing process of Amyloid beta from monomers to fibrils though time resolved experiment at different temperature as well as with various concentrations.

Kinetics of different concentrations Aß(1-42) has been observed by time resolved DLS at temperatures between 7°C and 37°C in Napi buffer. DLS measurements showed that the aggregation at low temperature (7°C) is generally slower than it at normal body temperature, 37°C. But even at 7°C immediately after the addition of buffer in protein large aggregates were formed. SEC and AUC indicated the presence of monomers in solutions and the lower the concentration the higher the percentage of monomers, which are not visible in DLS. In addition, time resolved SANS experiment exhibited the change in shape of aggregates during the kinetic, from flat oligomers to rod like protofibril, by the change in the slop of scattering curve (power-law). At the beginning of the kinetics all kinds of aggregates grow by addition of monomer, while in the later stage small aggregates start to dissociate.

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## PARTICIPANTS