13th International School of Biophysics

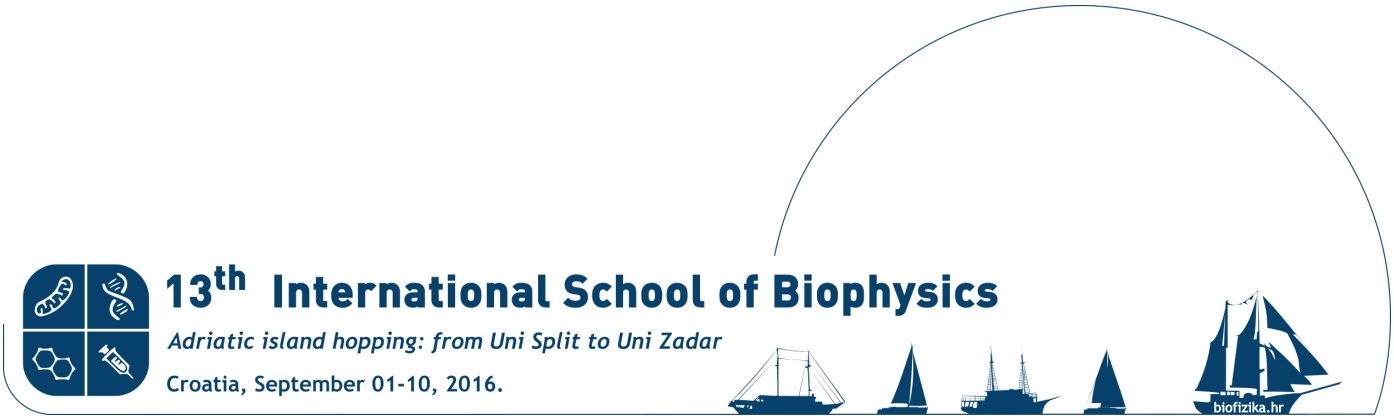
ABC of Physics of Life

BOOK OF ABSTRACTS

Croatia, September 01-10, 2016.

BOOK OF ABSTRACTS

ABC of Physics of Life



The 13th International "Greta Pifat Mrzljak" School of Biophysics

"ABC of Physics of Life: Book of abstracts"

Adriatic Coast, Croatia/ 1st– 10th September

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FOREWORD

The School

This session of the International School of Biophysics is the thirteenth in a series held for 35 years across Croatia: in Dubrovnik, Rovinj and Primošten. The previous sessions were held in 1981, 1984, 1987, 1990, 1994, 1997, 2000, 2003, 2006, 2009, 2012 and 2014. 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. Since then, we have had 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) networks CM1306 Molecular Machines (coordinated by dr. Fraser MacMillan), CM1207 GLISTEN (coordinated by dr. Peter Kolb) and BM1403 Native Mass Spectrometry (coordinated by prof. Frank Sobott). 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. Additional support is provided by the Faculty of Science of Split University and Zadar University. School is endorsed by IUPAB and sponsored by NanoTemper technologies, Beck's and Croatian Academy of Sciences and Arts.

Through the years, the School has been attended by more than one thousand Ph.D. students and postdocs interested in becoming acquainted with the state-of-the-art in biophysics. The Lecturers at the International School of Biophysics have always included top scientists in their respective disciplines and several Nobel laureates from this area of research, which spans across biology, chemistry and physics. Our intention is to keep the school as one of the focal events for European students and young scientists and to provide these young people with advanced training at the doctoral and postdoctoral levels in the field of biophysics and related fields like molecular and structural biology, physical chemistry, biochemistry, soft-matter physics... The boundaries of the traditional disciplines are not 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 interna-

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

The prospect for the enthusiastic young people to interact with top scientists in a relaxed manner is elementary in their development and a route to their 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. The 2016 School features a novel, itinerant concept in order to provide an intense experience, both for students and lecturers. The School starts at the new campus of University of Split with hands-on training in several experimental and computational biophysical techniques. From there on the School continues as an Adriatic island hopping cruise, towards University of Zadar. A comprehensive set of lectures in biophysics will be held throughout the cruise at different island-based venues.

Topics

An enormous amount of new knowledge on the molecular basis of various biological phenomena has emerged in the rapidly expanding field of bioscience. The principles and methods of biophysics provide the underpinning for all basic bioscience and a rational language for discussion among scientists of different disciplines. This was the general philosophy behind the organization of the summer school. The School is intended for young scientists (primarily Ph.D. students) at the beginning of their academic careers who are interested in the fundamental study of biomacromolecules: the structures of nucleic acid/protein complexes (ribosomes, viruses, chromatin), protein aggregation, conformational dynamics, folded and intrinsically disordered proteins, enzymatic activity—small molecule recognition, biomacromolecular interactions, bioenergetics and single molecule biophysics. The particular scope of the school has enabled the participants to become acquainted with state-of-art problem-oriented and/or methodology-oriented approaches to biological systems. Major topics are presented in a series of lectures and workshops, which can be roughly summarized as ABC of Physics of Life, that is: ASSEMBLING MOLECULAR MACHINES: viruses, ribosomes and other protein-RNA/DNA complexes, quaternary protein structures, DNA, proteins, polyelectrolytes with the focus on their structure, organization and function Interactions at BIOLOGICAL & BIOCOMPATIBLE interfaces: membranes, adhesion, extracellular matrix, protein-lipid/membrane interactions, biomimetic/hybrid surfaces CELLS: physical properties of biological and bio-inspired systems The outlook of this topics is done through the lenses 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, or even on the beach.

Previous 12th School was attended by 70 Ph.D. students from 16 countries in Europe as well as from Australia, Iran, Japan and Russia. EBSA supported 12 students, while Greta Pifat Fund supported additional 4 coming from outside Europe. There were 20 lecturers from Croatia, USA, UK, Australia, Belgium, France, Sweden, Germany, Austria, Italy, Spain, Switzerland and Slovenia. This year's School has gained additional international aspect by adding 8 more countries to the list, totaling in 28 countries from which are coming 86 participants and 22 lecturers. All participants are traveling on a fleet of six ships on the route from Split to Zadar while visiting and working at several locations along the Adriatic coast: Split, Sibenik, Dugi Otok, Zadar, Rogoznica and Stari Grad (Hvar). The School is further opening to the public by having evening activities outdoor, at the "waterfronts" (cro. *"riva"*) of the visiting towns. By abandoning the usual seclusion of a scientific meeting School is also getting additional attention of the public, where we would like to mention specifically that the School's fleet is participating in the Festival of the Sea, Maritime and Maritime Heritage "Days of Vali" 9-11.9.2016.", which is held in the Stari Grad on the island of Hvar under the auspices of the Croatian President Kolinda Grabar-Kitarovic who will during this events visit the ship and our young scientists.

For the third time in a row, the School is chaired by Tomislav Vuletić, Institute of physics, Zagreb, and prepared by the 5-person strong local team. We wish the Students and Lecturers at the 13th School a warm welcome and hope that they will enjoy the extraordinary cool and friendly environment that we intend to create as we did at the previous two Schools.

Tomislav Vuletić, Chair

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.

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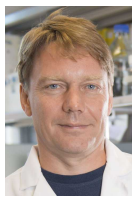

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ONBOARD A SHIP

General information

The captain of a ship, much like a captain of an airplane has an almost unlimited authority in order to ensure a safe and pleasant journey for everyone. In addition to the rules and suggestions listed below, the captain (or skipper) of any ship can impose additional rules in case of rough weather or other unpredictable circumstances.

All participants are encouraged to learn about sailing and navigation (both before and during the school) to better enjoy the trips between our venues. Passengers should take care when moving around. The floors can always be wet and slippery, (dis)embarking or crossing between the ships when in port should be done with care. Do not hesitate to ask a crew member for assistance when crossing. In case of rough weather move around with special care. The best place to stay is then in the salon. The portholes in cabins under deck should be closed during navigation, to avoid the cabins getting wet.

Because of the limited space and facilities on any ship, participants are encouraged to maintain their cabins and common areas in good order. Please take special attention not to throw anything into toilets except of toilet paper. Throwing garbage overboard (including cigarette butts) is strictly forbidden! Jumping ships underway or in port is dangerous and forbidden. There will be plenty of opportunity for swimming under safe circumstances! Swimmers are not easily noticed by ship's crews so they should never be in the sea close to moving or maneuvering ships.

All participants are responsible for any intentionally caused damage and for injuries caused by reckless and careless behaviour. We hope that you will not need your insurance policies, as we, our agent, or the ships crew and agent can not be held liable for your actions or actions of force majeure. Smoking is forbidden in closed spaces on the ships and on land (everywhere in Croatia). The two cruise ships feature bars and drinks are available at reasonable prices. It is against the house rules for passengers to bring food and drinks onboard. Space on a ship is especially valuable, so it is important to pack your luggage as efficiently as possible. The best way to achieve this is to pack everything into smaller, flexible, foldable bags or backpacks. The more space you save, the more time you'll have to enjoy our cruise Participants are responsible for looking after their personal belongings (cabins can be locked).

220V power is available on ships underway and in port and on sailboats in port. We have equipped the ships with mobile hot-spots that rely on cell phone, 3G network coverage. The bandwidth should be sufficient for email clients and browsers. We appeal to you to avoid video and cloud services to preserve capacity for others. Water is also a resource limited on a ship.

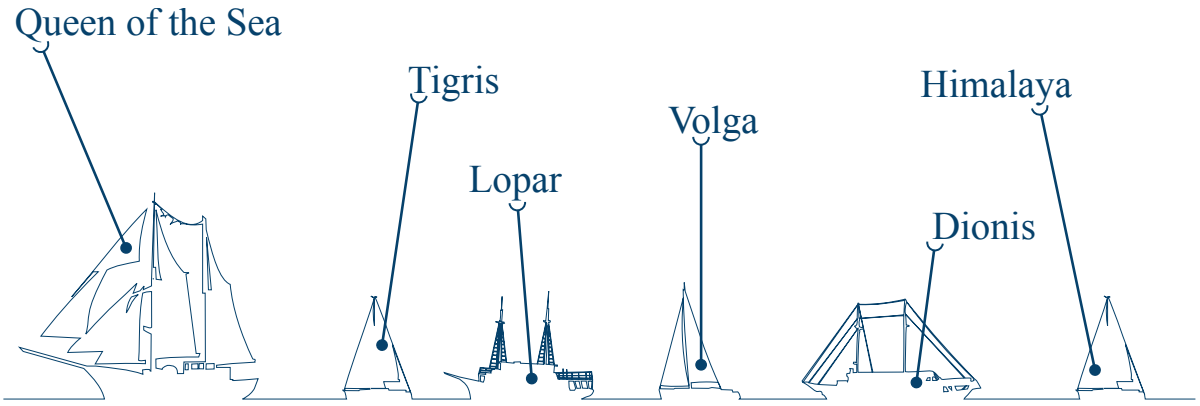
Passengers on "Queen of the Sea" are also members of the crew

The "Queen of the Sea" was purpose built as the national training ship for sailors and naval officers of the Republic of Croatia. Those sailing on it will have a unique opportunity to experience a recreational cruise, as well as the life of a cadet. You will observe operations of the professional crew, how order is maintained and how safety for ship, crew and passengers is ensured under all conditions. However, you will also participate in performing the daily

duties. At this ship smoking and alcohol are not allowed.

Notice to the sailboat crews

While these boats have cabins and amenities comparable to the bigger ones and provide comfortable accommodation, they provide an experience closer to recreational sailing (as the boat rolls under full sail, the deck can easily get flushed and spray is inevitable) which means special considerations are necessary. Still, in most cases it is more pleasant to spend time outside the cabin. Underway, especially with sails set, the utmost care has to be taken when moving around in cabin or in cockpit. Cockpit and deck of a sailboat are covered with different sailing hardware - watch your hands, feet and head. Also, be considerate to your fellow crew members - you'll have to share the same space for a week.



PROGRAM

1^{st}– 10^{th} September

NOTICE: The schedule may change due to weather and other unforeseen events. Please follow the official announcements, on the premises and on the web.

Thursday, September 1st – Split

9:00–12:00	**Arrivals and Registration**	
13:00-14:00	**Lunch**	
14:00-14:30	**Welcome address**	
14:30-15:30	**Ana-Sunčana Smith**	*Institute for Theoretical Physics I, FAU, Erlangen & IRB, Zagreb*
	Effects of the noisy environment on ligand-receptor binding	
15:30-16:15	**Marek Zurawski**	*NanoTemper GmbH, München, Germany*
16:15	**Coffee break**	
16:45	Parallel hands-on activity sessions	
19:00	**Dinner**	
20:10-21:30	**Short talks: Protein Machines**	
	Shiran Barber Zucker	*Hypermanganesemia as a Result of a Cation Diffusion Facilitator Cytoplasmic Domain Loss of Structure*
	Jelena Dragojević	*Functional characterization of organic anion transporters in zebrafish (Danio rerio): Oat2a-d*
	Franziska Heydenreich	*A GPCR as a Michalis-Menten enzyme: Understanding biased signalling of vasopressin V2 receptor*
	Faezeh Mofidi Najjar	*Mechanistic study on catalase activation by curcumin*
	Stephan Rempel	*The new vitamin B12 degradation product transporter BtuM*
	Tomas Šneideris	*Looking for a generic inhibitor of amyloid-like fibril formation among flavone derivatives*
	Olga Tkachenko	*Chaperone Mechanism of αB-Crysrallin*
	Oleksii Zdorevskyi	*Understanding the mechanism of DNA deactivation in ion therapy of cancer cells: hydrogen peroxide action*
21:30-23:59	**Poster Session**	

Friday, September 2nd – Split

09:00	Parallel hands-on activity sessions	
10:45	**Coffee break**	
11:15	Parallel hands-on activity sessions	
13:00	**Lunch**	
14:00-14:45	**Marek Zurawski**	*NanoTemper GmbH, München, Germany*
14:45	Parallel hands-on activity sessions	
16:45	**Coffee break**	
17:15	Parallel hands-on activity sessions	
19:00	**Dinner**	
20:20-21:30	**Short talks: MS, EPR, X-ray, NMR**	
	Miranda Collier	*Dynamic small heat-shock protein interactions with non-aggregating clients*
	Christoph Gmeiner	*Pulse EPR with Gd(III)-spin labels to determine distance distributions in a large protein/RNA complex*
	Daniel Mayer	*The structural basis for recognition of the „Phosphorylation Barcode“ of G protein-coupled receptors by arrestin*
	Anna Mullen	*Understanding structures & functional dynamics of membrane proteins using EPR spectroscopy*
	Barbora Kalouskova	*Preparation and Structural Characterization of Human NK Cell Activating Immunocomplex NKp80:ACIL*
	Agnieszka Sztyler	*Analysis of intrinsically disordered region of human topoisomerase I using hydrogen/deuterium exchange mass spectrometry*
	Lara Štajner	*Investigation of selected amino acids influence on calcium carbonate precipitation as simple models of organic matrix*
21:30-23:59	**Poster Session**	

Saturday, September 3rd– Split/Šibenik

9:00-10:45	**Iva M. Tolić**	*Ruđer Bošković Institute, Zagreb, Croatia*
	Forces that divide the chromosomes	
	Live-cell fluorescence microscopy and laser-cutting inside the cell	
10:45	**Coffee break**	
11:15	**Nuška Tschammer,**	*NanoTemper Technologies GmbH, Münich, Germany*
	Microscale thermophoresis based biomolecular interaction analysis	
12:15	**Transfer**	
13:00	**Sailing: Split-Šibenik**	
20:00-23:00	**Posters (Riva Šibenik)**	

Sunday, September 4th – Šibenik/Dugi otok

06:00	**Sailing: Šibenik-Sali**	
11:15	**Holger Stark**	*Max Planck Institute for Biophysical Chemistry, Göttingen, Germany*
	Single Particle Cryo-EM as a tool to determine 3D structures of macromolecular complexes	
	Visualizing dynamic macromolecular complexes by cryo-EM at atomic resolution	
13:00	**Lunch & free time**	
16:00	**Zvonimir Dogic**	*Brandeis University, Waltham, USA*
	Colloidal membranes	
17:45	**Antonio Šiber**	*Institute of physics, Zagreb, Croatia*
	Conformations of DNA ropes condensed in cylindrical confinement	
19:30	**Dinner**	

20:40-21:30 **Short Talks: MD/simulations/models**

Andrea Cesari *Using the maximum entropy principle to enforce NMR data on RNA simulations*

Yossa Dwi Hartono *Investigation of tautomerisation and protonation equilibria of nucleic acid with lambda dynamics*

Willem Jespers *Binding mode prediction using free energy perturbations*

Jakub Kollár *Computational design of selective histone deacetylase inhibitors as epigenetic agents targeting cancer*

Negar Nahali *Density effects in entangled solutions of ring polymers*

21:30-23:59 **Poster Session**

Monday, September 5th – Dugi otok

9:00 **Dmitry B. Veprintsev** *Paul Scherrer Institute, Villigen, Switzerland & Department of Biology, Swiss Federal Institute of Technology Zurich, Zurich, Switzerland*

Allostery in GPCR signaling

Fluorescence based methods to study protein stability and conformation

10:45 **Coffee break**

11:15 **Fraser MacMillan** *University of East Anglia, Norwich, UK*

Understanding Movement and Mechanism in Membrane Proteins: An EPR Spectroscopist's View

EPR spectroscopy: Up close and from afar, recent developments of a niche biophysical technique working towards a universal structural biological tool

13:00 **Lunch & free time**

16:00 **Arwen Pearson** *Universität Hamburg, Germany*

Time-resolved structural biology

17:45 **Nenad Ban** *ETH, Zürich, Switzerland*

Ribosomes and their functional complexes involved in cotranslational protein folding, processing and targeting to membranes

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

19:30 **Dinner**

20:30 **Short talks: Lipid Membrane/ Membrane proteins**

Barbara Eicher *Joint analysis of SAXS and SANS data of asymmetric lipid vesicles*

Dietmar Hammerschmid *Characterization of the globin coupled sensor from Geobacter sulfurreducens using native mass spectrometry*

Steven Lavington *Exploring NTS1 helix 8 conformation and dynamics in lipid environments*

Aliaksandra Skarabahatava *In vitro and in vivo changes in lipids erythrocyte membrane affected aluminum ions*

Lilit Tonoyan *The Influence of Sodium Dodecyl Sulfate on Stability of Bilayer Lipid Membranes*

Maria Tsemperoulli *Optical and Electrical Characterization of Voltage Sensitive Dyes in Lipid Bilayers*

21:30-23:59 **Poster Session**

Tuesday, September 6th – Dugi otok/Zadar

09:00 **Chris Oostenbrink** *UNRLF, Vienna, Austria*

Ensembles and sampling, leading to molecular dynamics simulations

Structure refinement using molecular dynamics simulations (NMR observables)

Calculation of free energies from molecular simulation

10:45 **Coffee break**

11:15	**Bojan Žagrović**	*Max F. Perutz Laboratories & University of Vienna, Austria*
	Protein-RNA interactions and the origin of the genetic code	
	More dynamic than we think? On conformational averaging in structural biology	
13:00	**Lunch**	
14:00	**Peter Kolb**	*Pharmaceutical Chemistry, Marburg, Germany*
	Structure-based ligand discovery & chemoinformatics	
15:45	**Sailing: Sali-Zadar**	
20:00	**Welcome Address, University of Zadar**	
20:20	**Short talks: Optics & imaging based approaches**	
	Alexandra Falamas	*Raman microspectroscopy of stem cells: approach to monitor the CBCT radiation effect*
	Marija Nišavić	*Positive/negative ion mode nano-electrospray ionization mass spectrometry of metallated peptides*
	Ana Milas	*Sliding of microtubules in the bridging fiber segregates chromosomes by creating poleward flux of the attached k-fibers*
	Rita Nagypál	*Fluorescence kinetics of flavin adenine dinucleotide in different microenvironments*
	Aiswaria Prakash	*Position dependent fluorescent properties of coupled fluorescent dyes in RNAs and proteins*
	Ioana Maria Simon	*Study of the biological effects of oral diagnostic irradiation with Raman spectroscopy*
	Dora Sviben	*Stability of proteins and viruses determined by differential scanning fluorimetry*
21:30-23:59	**Poster Session**	

Wednesday, September 7th – Zadar/Rogoznica

9:00 **Anthony Watts** *Oxford University, Oxford, UK*

Principles of biological solid state NMR

NMR of membrane proteins - Drug targeting

10:45 **Coffee break**

11:15 **Eurico J Cabrita** *Universidade Nova de Lisboa, Portugal*

Molecular Structure and Interactions by Nuclear Magnetic Resonance

Nuclear Magnetic Resonance and the study of Protein Ligand Interactions

13:00 **Sailing: Zadar-Rogoznica**

20:30-23:00 **Posters in the Marina**

Thursday, September 8th – Stari grad, Hvar

06:00 **Sailing: Rogoznica-Stari Grad, Hvar**

13:00 **Lunch**

14:00 **Edwin De Pauw** *Université de Liège, Belgium*

Biological mass spectrometry

15:45 **Mario Cindrić** *Ruđer Bošković Institute, Zagreb, Croatia*

A Novel Positive and Negative Mass Spectrometry Chemically Activated Fragmentation for Genome Fingerprinting Scanning

17:30 **Coffee break**

18:00 **Frank Sobott** *Universiteit Antwerpen, Belgium*

Protein Mass Spectrometry: Defining proteoforms and Going native

Dynamic protein structure: From protein disorder to membrane pores

19:45 **Dinner**

20:40 **Short talks: Nanobio/soft matter physics**

Jiandong Feng *Coulomb blockade in ion transport*

Sanjin Marion *Nucleic acids and condensing proteins in viral confinement*

Martina Požar *The microscopic structure of hot and cold alcohol mixtures*

Biljana Stojković *Micro and macro-rheology study of DNA-levan mixtures*

Pradeep Waduge *Angstrom-Sensitivity Protein Sizing and Conformation Detection using Solid-State Nanopores*

21:30-23:59 **Poster Session**

Friday, September 9th – Stari grad, Hvar

9:00 **Marija Drndić** *University of Pennsylvania, USA*

Review of solid-state nanopores and their applications

10:45 **Coffee break**

11:15 **Sabrina Leslie** *McGill University, Montreal, Canada*

Squeezing New Information out of DNA Using Adjustable Nanoconfinement

How DNA do the twist? Visualizing supercoil-induced site-unwinding and site-invasion in DNA loops

13:00 **Lunch & free time**

16:00 **Meni Wanunu** *Northeastern University, Boston, USA*

Biomolecular Capture and Transport Through Synthetic Nanopores

17:45 **Helena Danielson** *Uppsala University, Sweden, and Beactica AB, Uppsala, Sweden*

Biophysics at the interface between academia and pharmaceutical industry

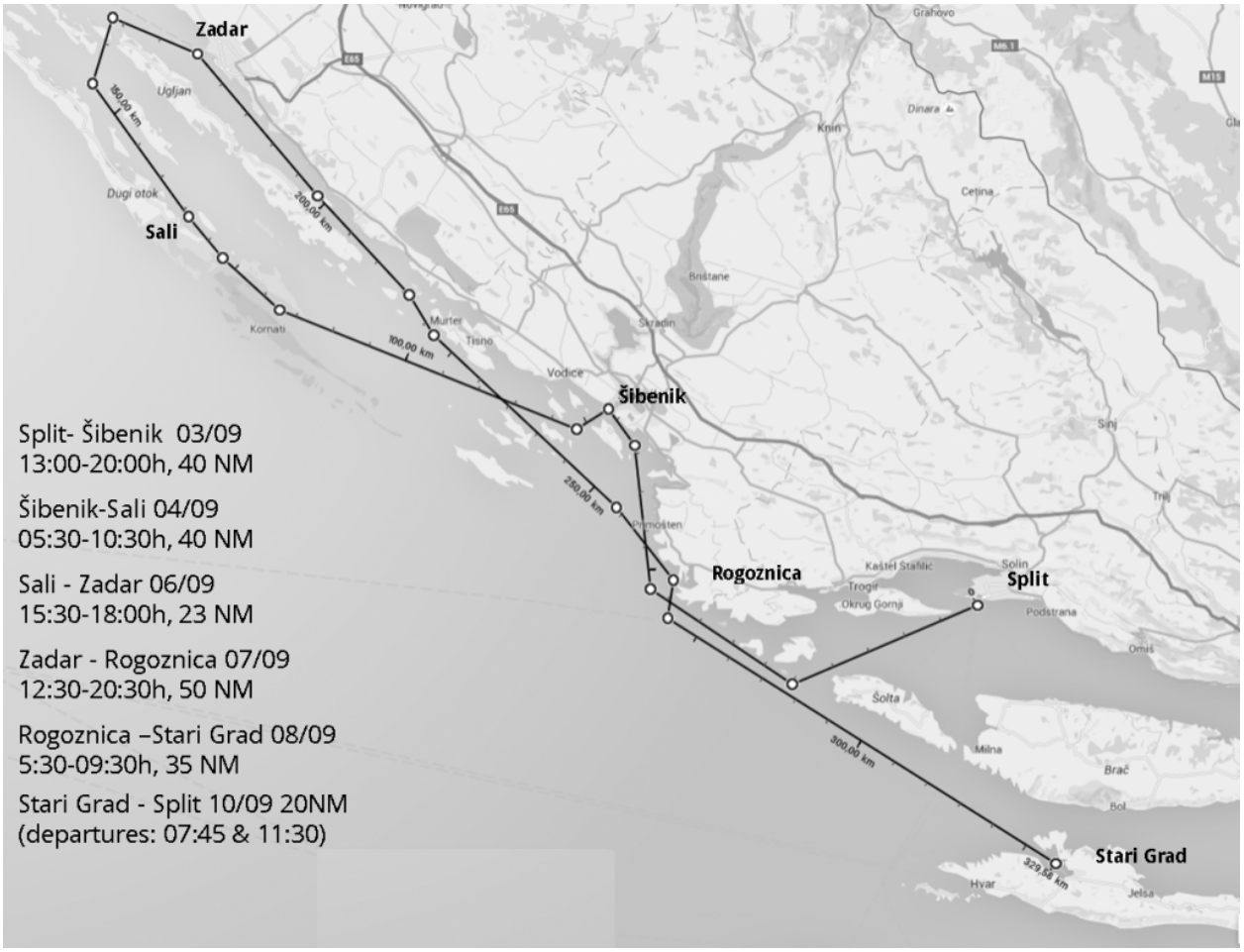
19:30 **Dinner**

20:30 **Farewell Party**

Saturday, September 10th – Stari grad, Hvar

6:00– **DEPARTURES: Sailing & Ferry: Stari Grad - Split**

12:00 **Visit by the President of Croatia**



LECTURE ABSTRACTS

ANA-SUNČANA SMITH

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Effects of the noisy environment on ligand-receptor binding

The existence of living matter is based on the defiance of equilibrium while maintaining order at the expense of energy consumption. This makes the cell a prime example of an active system that plays a pivotal role in the development of non-equilibrium physics. Due to their softness, cells exhibits strong fluctuations that are typically comprised from a thermal and an active component. While it is becoming conceptually clear that these fluctuations are coupled to the driving of biological processes, their physiological role remains widely unknown. One reason for this lack of clarity is that both the measurement of fluctuations, and the determination of their relation to a particular molecular recognition process, remains a significant challenge. Using the example of cell adhesion, I will show how recent experimental advances in measuring cell-surface undulations can be combined with experiments in reconstituted systems and multiscale modelling to demonstrate that active fluctuations of cells can be harnessed for the control of the formation of adhesion clusters and mechanosensing.

IVA M. TOLIĆ

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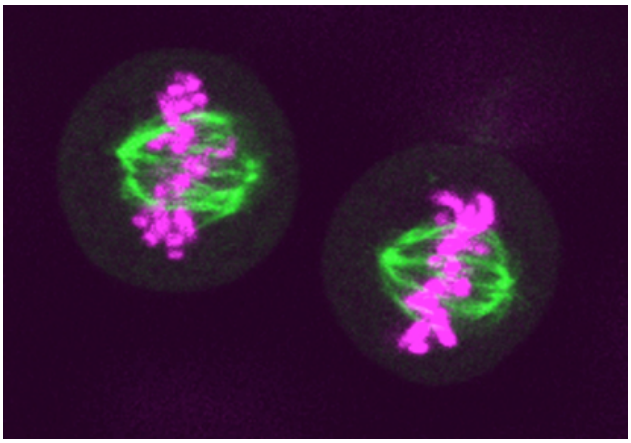
Forces that divide the chromosomes

Cell division is the most fundamental process without which there would be no life, but we still do not really understand how it works. At the onset of division, the cell forms a spindle, a precise self-constructed micromachine composed of microtubules and the associated proteins, which divides the chromosomes between the daughter cells[1]. The main big question is how cells succeed in dividing chromosomes into two equal sets without errors. Today we know the identity of hundreds of molecules involved in mitosis, and the challenge is to understand how these molecules self-organize to create intricate structures such as the spindle, and to generate precise movements of chromosomes. Because biology is incredibly complex, theoretical approaches help us to isolate key mechanisms, which are difficult to uncover from complicated experimental data.

The current paradigm says that the chromosomes get pulled apart only by those microtubules that end at the chromosome, forming k-fibers. In my lab, we recently found out that other microtubules, which we called 'bridging microtubules', also exert forces on chromosomes[2, 3]. These microtubules act like a bridge between sister kinetochores and their k-fibers. By cutting a k-fiber with a laser, we have shown that the bridging fiber moves together with sister k-fibers, revealing that these three fibers are strongly linked. Our theoretical model and experiments show larger relaxation of the interkinetochore distance for cuts closer to kinetochores[2, 4], which challenges the paradigm that the tension on kinetochores is generated by molecular events occurring only at the ends of the k-fibers. We conclude that the bridging fiber, which links sister k-fibers, is required to balance the interkinetochore tension. We are now exploring the role of bridging microtubules in the process of chromosome capture by spindle microtubules[5] and in chromosome segregation.



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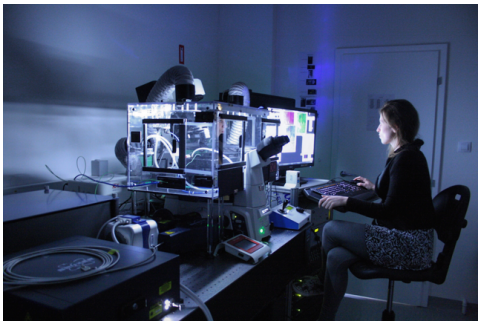
Live-cell fluorescence microscopy and laser-cutting inside the cell

A cell can be viewed as a dynamic puzzle, where pieces shuffle in space, bind different partners, and new pieces are generated while old ones are destroyed. Live-cell microscopy has become capable of directly observing the pieces of the puzzle in action. Moreover, microscopy can be combined with mechanical perturbations to investigate the complex system of mechanical interactions in a living cell. The idea underlying all perturbation techniques is to perform controlled modifications of a selected structure and to observe the reaction of the cell to those alterations. In last decades, optical manipulations became the main tool for introducing perturbations on a very small scale. This is due to many advantages of optical over non-optical manipulation techniques, including easy integration with standard microscopy systems, high spatial and temporal resolution, and minimal interaction with the sample. As a result, numerous studies have been performed using optical techniques such as laser ablation in diverse model systems, e.g. [1, 2, 3, 4]. Laser ablation exploits the confined deposition of energy induced by a highly focused laser beam to locally modify the sample. This method can be used to cut individual microtubule bundles in the mitotic spindle without affecting the function of the whole spindle [2, 3]. The resulting movements of the severed bundle can be used to deduce the forces that are present in an unperturbed system, helping us to resolve the puzzle of mechanical interactions in the cell.



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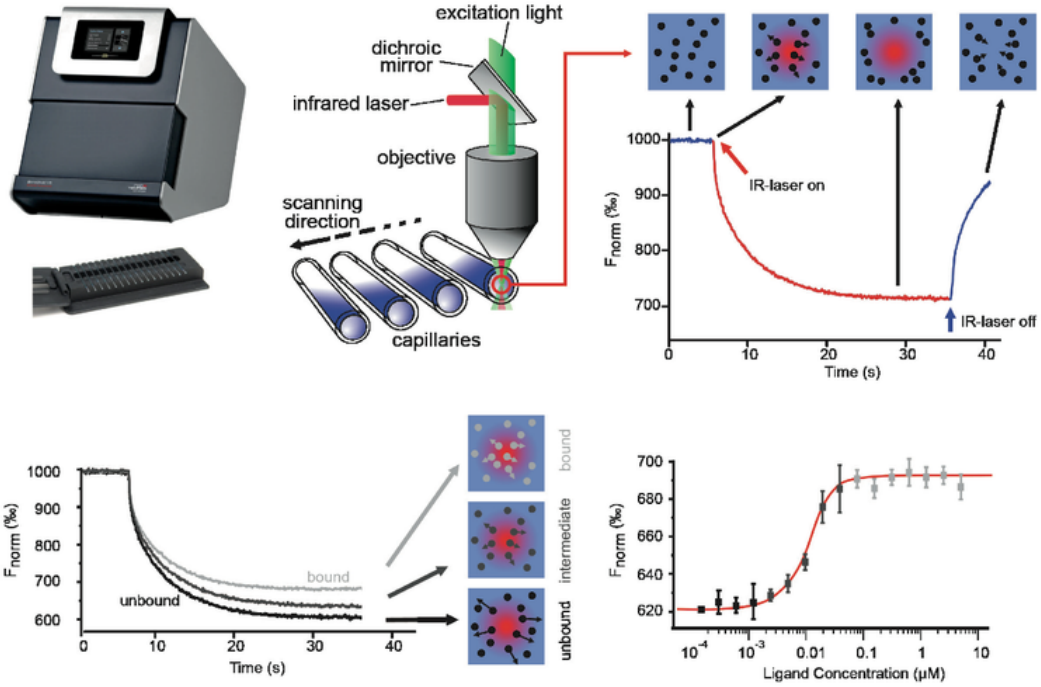
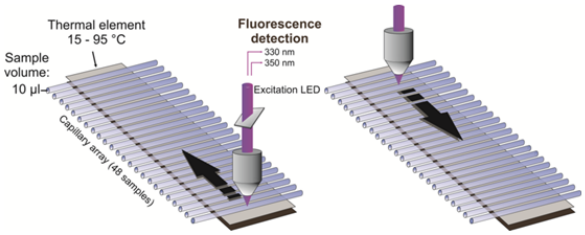
Microscale thermophoresis based biomolecular interaction analysis

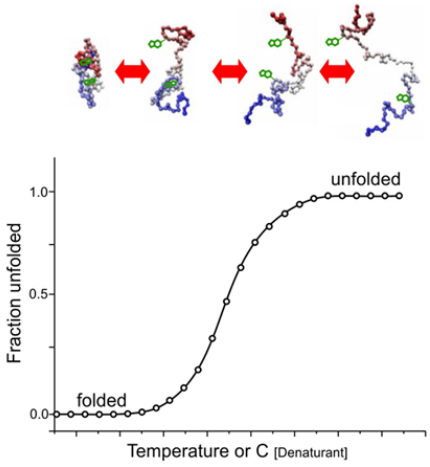
MicroScale Thermophoresis (MST) is a powerful technique to quantify biomolecular interactions in free solution and with low sample consumption. It is based on thermophoresis, the directed movement of molecules in a temperature gradient, which strongly depends on a variety of molecular properties such as size, charge, hydration shell or conformation. In an all-optical approach, an infrared laser is used for local heating, and molecule mobility in the temperature gradient is analysed by fluorescence. For this purpose, one binding partner is fluorescently labelled, however, MST can also be performed label-free by exploiting intrinsic protein UV-fluorescence. By combining the precision of fluorescence detection with the variability and sensitivity of thermophoresis, MST provides a flexible, robust and fast way to dissect molecular interaction.



Analysing thermal and chemical unfolding of proteins: nanoDSF

A detailed analysis of proteins stability is a prerequisite for both, the basic understanding of protein folding mechanisms, ligand-induced protein stabilization or destabilization as well as for the successful development of biologicals in the pharmaceutical industry. The nanoDSF (miniaturized differential scanning fluorimetry) is a cutting-edge method to determine the thermal and chemical stability of proteins by following the changes in fluorescence. The basis of this method lies in the properties of the fluorescent amino acid tryptophan. Since tryptophan is a hydrophobic amino acid, it is mostly located in the hydrophobic core of proteins where it is shielded from the surrounding aqueous solvent. Upon unfolding tryptophan is exposed and its photophysical properties. By detecting changes in tryptophan fluorescence intensity and its emission peak shift, the transition of a protein from the folded to the unfolded state can be precisely recapitulated. In this way the melting temperature (Tm) and thermodynamic parameters can be determined. Applications include membrane protein research, ligand screening, antibody engineering, formulation and quality control.





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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.

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 Åresolution) 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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Colloidal membranes

Daily experience demonstrates that upon mixing, oil and water quickly phase separate. While rare in pure substances, such liquid-liquid phase separation is ubiquitous in molecular mixtures as well as suspensions of nanoparticles, proteins and colloids. With few notable exceptions, surface-tension minimizing spherical droplets continuously coalesce, increasing in size with any bound, before achieving bulk phase separation. In comparison, the phase behaviour of nanoparticle or proteins dissolved in 2D fluids membrane is significantly more complex. Inclusions distort local membrane structure leading to membrane-mediated interactions that are fundamentally different from well-studied bulk interactions. We investigated liquid-liquid phase separation in a highly simplified system of colloidal membranes. The bulk phase separation of dissimilar rods is inherently unstable giving way to formation of finite-sized, highly monodisperse colloidal rafts. Using single molecular techniques we quantify kinetics by which thousands of rods assemble into an isolated raft. Subsequently, we quantify raft-raft repulsive interactions and correlate them to raft-induced membrane distortions; demonstrating that particle chirality is an essential requirement for formation of colloidal rafts. At high densities rafts assemble into cluster crystals which constantly exchange rods with the membrane background to robustly maintain a self-limited size. Finally, we demonstrate raft polymorphism by form supra-rafts, 2D liquid droplets with complex highly non-spherical shapes such as a beads-on-a string polymer.

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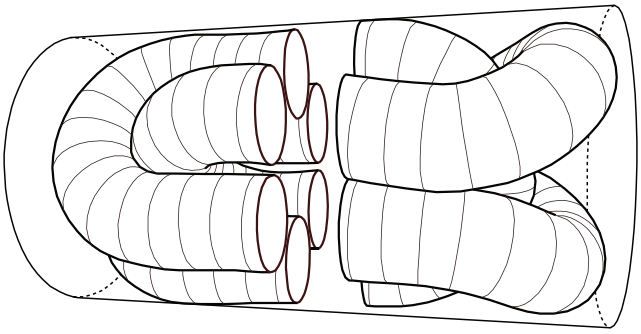
Conformations of DNA ropes condensed in cylindrical confinement

Some sort of DNA "compression" is required to pack the long DNA strand in a small space, such as the cell nucleus or bacteria. Viruses have, in particular, developed several strategies to physically compress the DNA genome molecule. In presence of basic proteins[1] or multivalent counterions, DNA can be "condensed", i.e. brought to a state where it self-attracts. When condensed in free space, long DNA typically assumes a shape of a toroid. The toroidal shape arises from the interplay between the (unfavorable) surface energy of the condensate and the (unfavorable) bending energy of the DNA strands in it – the only favorable contribution to the free energy is the volume term, requiring that the DNA strands be next to each other[2]. When condensed in confinement, e.g. in virus protein coatings (capsids), it is known that the, sufficiently short, DNA also assumes toroidal conformations, but the free energy balance is in that case additionally complicated by the adsorption energy (DNA-capsid interaction) and by the capsid confinement[3]. It has been proposed in the literature that the, sufficiently long DNA, may condense in conformations which are non-toroidal, i.e. which do not have the cylindrical axis of symmetry[4]. Nevertheless, such propositions were never tested in a suitable model, explaining the free energies of all the conformations that can be envisioned. Furthermore, it is not known how the conformations depend on the geometry of a virus, in particular whether its capsid is icosahedral or elongated, as is often the case for bacteriophage viruses. I will show a generalization of the previously proposed models[2] to account for non-toroidal conformations of DNA condensed in cylindrical confinement. The model that I will present reproduces conformations that were previously predicted[4, 5], but also several intriguing conformations that were never predicted in the context of viruses[6].



One of the conformations of the DNA "rope" condensed in a cylinder. A slab, parallel with the cylinder bases, has been cut out from the shape in order to illustrate better its 3D nature.

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Allostery in GPCR signaling

The signaling events in G protein coupled receptors (GPCRs) propagate through protein by concerted local conformational changes, forming allosteric pathways. Using a combination of NMR and mutagenesis as well as structural bioinformatics, we identified several independent allosteric activation pathways of the β1-adrenergic receptor that connect the ligand binding pocket with the activity of the receptor. In order to identify the allosteric networks in the G protein, we generated comprehensive single amino acid resolution maps of the residues stabilising the human Gα_{i1} subunit in nucleotide- and receptor-bound states. We generated these maps by measuring the effects of alanine mutations on the stability of Gα_{i1} and of the rhodopsin-Gα_{i1} complex. We identified stabilization clusters in the GTPase and helical domains responsible for structural integrity of the protein, the conformational changes associated with activation, as well as changes in the dynamics of individual amino acids. The proposed methods can be readily applied to identify and study allosteric pathways in other proteins.

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Fluorescence based methods to study protein stability and conformation.

Fluorescence spectroscopy is one of the most widely applied techniques in protein biophysics to study protein stability, conformational changes, protein-ligand and protein-protein interactions. In this lecture I will briefly revisit the principles of fluorescence, as well as provide several examples of how it can be applied to study conformational changes in proteins.

[1] Principles of Fluorescence Spectroscopy. Joseph Lacowicz, Springer, 2006, ISBN 978-0-387-46312-4

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Understanding Movement and Mechanism in Membrane Proteins: An EPR Spectroscopist's View

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

I will focus on recent method developments, which may allow a shifting of the focus of this technique away from being considered purely a niche technique towards a more universal structural biological tool. I will use examples from our recent work on membrane and metalloproteins (cytochrome c oxidase & nitrate reductase), membrane transport proteins including multidrug efflux pumps (P-glycoprotein) and bacterial pathogens (PsaA in Streptococcus pneumonia) to demonstrate the power of this technique to deliver key mechanistic insight into e.g. how to resolve multiple distances in complex macromolecular complexes, how to observe conformational change within membrane proteins at a molecular level and finally to identify the molecular determinants of metal binding by PsaA and the potential implications for host-pathogen interactions.

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

EPR spectroscopy: Up close and from afar, recent developments of a niche biophysical technique working towards a universal structural biological tool

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

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

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

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

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Time-resolved structural biology 1

In this lecture we will discuss the time-scales of interest in structural biology and explore a series of complementary crystallographic and spectroscopic approaches that can be used to trap and characterize reaction intermediates, as well as tools to probe global dynamic behaviour, with several case study examples. We will also look at the problem of radiation damage and how this affects crystallographic data collection.

Time-resolved structural biology 2

In this lecture we will look at at time-resolved structural experiments using both diffraction and scattering. We will discuss the general principles of pump-probe experiments and then examine the challenges associated with a time-resolved structural experiment. We will explore different reaction initiation ("pump") strategies as well as different probe methods. We will also touch on the different X-ray sources now available and the importance of matching source and initiation strategy to the specific system that is being studied. Finally we will briefly discuss how to analyse the data from a time-resolved experiment.

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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, 2, 3, 4].

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[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 *331(6019):881-6.*

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

Eukaryotic ribosomes are much more complex than their bacterial counterparts, require a large number of assembly and maturation factors during their biogenesis, use numerous initiation factors, and are subjected to extensive regulation. We have investigated the structures of eukaryotic ribosomes and their complexes involved in initiation and maturation and complexes involved in regulation of protein synthesis[1, 2, 3, 4]. These results provide insights into the architecture of the eukaryotic ribosome and into various eukaryotic-specific aspects of protein synthesis. Recently, using electron microscopy, we determined the complete molecular structure of the 55S mammalian mitoribosome. The maps that we calculated between 3.4 and 3.6 Åresolution allowed de-novo tracing of a large number of mitochondrial specific ribosomal proteins and visualization of interactions between tRNA and mRNA in the decoding centre, the peptidyl transferase center, and the path of the nascent polypeptide through the idiosyncratic tunnel of the mammalian mitoribosome. Furthermore, the structure suggested a mechanism of how mitochondrial ribosomes, specialized for the synthesis of membrane proteins, are attached to membranes [5, 6].

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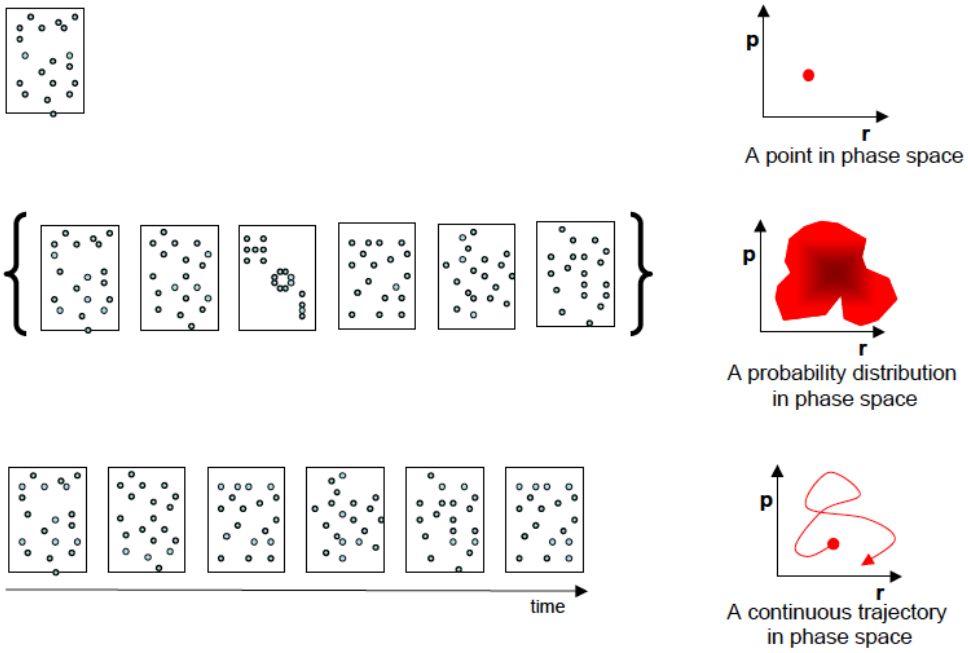
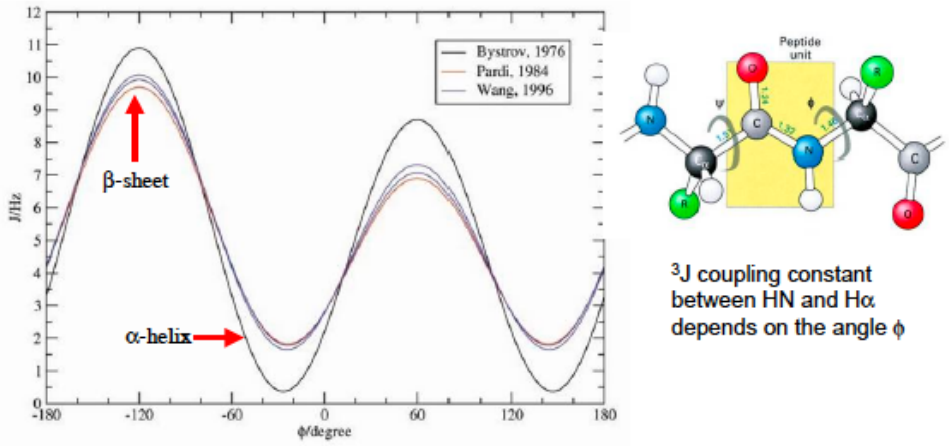
Ensembles and sampling, leading to molecular dynamics simulations

In this lecture, we will discuss the basics of molecular dynamics simulations. Starting from the link between a molecular structure and the (potential) energy of a system, we will discuss various algorithm to modify the structure in physically meaningful ways. This will lead to the definition of ensembles and the computational tools to generate ensembles of complex biomolecular systems. We can subsequently use the statistical mechanical ensembles to calculate averages of molecular properties which may be compared to experimental data directly. Finally, we will hint at the methods to define ensembles at different thermodynamic state points.



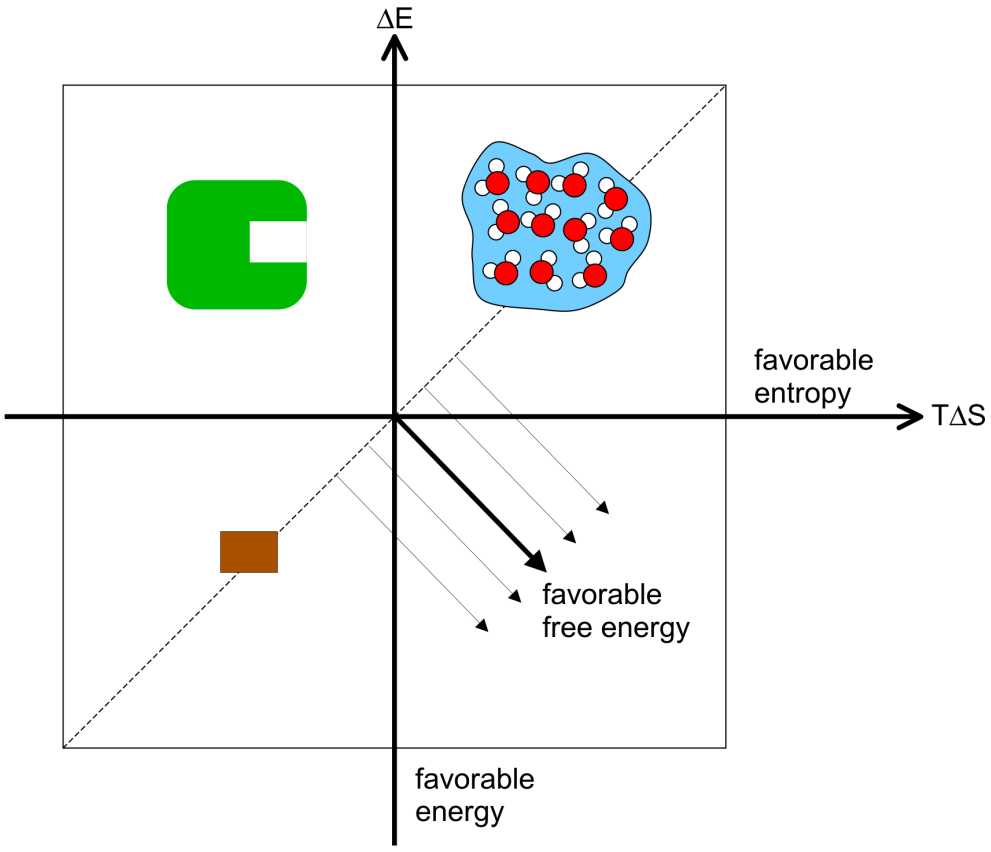
Structure refinement using molecular dynamics simulations (NMR observables)

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 3J-coupling constants as examples, the various ways of restraining the molecular structure will be discussed.



Calculation of free energies from molecular simulation

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



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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 physicochemical rationales, which led to the development of code's structure, may still be relevant in present-day cells.

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? Here, I will discuss these questions with a particular focus on nuclear magnetic resonance, X-ray scattering methods and different types of spectroscopy and address their individual susceptibility to conformational (motional) averaging.

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Structure-based ligand discovery & chemoinformatics

In these lectures, I will introduce the basic concepts and possibilities of protein structure-based drug design (SBDD). A prototypical approach in this area is docking. Docking and related methods are based on force fields, describing molecular interactions with biophysical or empirical terms. In the absence of an experimentally determined protein structure, such force fields can also be used to calculate 3D structures through homology modeling. The combination of all techniques constitutes a powerful tool set that can be employed to search for ligands with tailored properties.

I will also highlight key lessons learned from docking multi-million compound libraries to different G protein-coupled receptors (GPCRs), the protein family most frequently targeted by present-day drugs. The most prominent example is the first unbiased screen we did with the β_2-adrenergic receptor, which produced six novel binders – some of them with chemotypes previously undescribed for this target – and a most potent compound with an affinity of 9 nM [1]. Further examples include the chemokine receptors CXCR3 and CXCR4, where we identified potent ligands with tailored selectivity profiles with high hit rates [2]. The malleability of GPCRs seems to make multi-conformation screenings a good strategy, as we have shown for the A_1 subtype of the adenosine receptors [3]. At the low-throughput end, I will talk about the docking-based in-depth analysis of four ligands of the orexin receptor subtype 2 [4]. The challenge in this system were the unusual binding mode of the crystallographic ligand as well as the comparative scarcity of binding site features. These learnings have now been translated into additional ligands of this highly investigated system.

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Principles of biological solid state NMR

Lecture Synopsis:

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

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

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[2] Grage SL and Watts A (2007) Applications of REDOR for distance measurements in biological solids. Annual Reports in NMR *60:192-228.*

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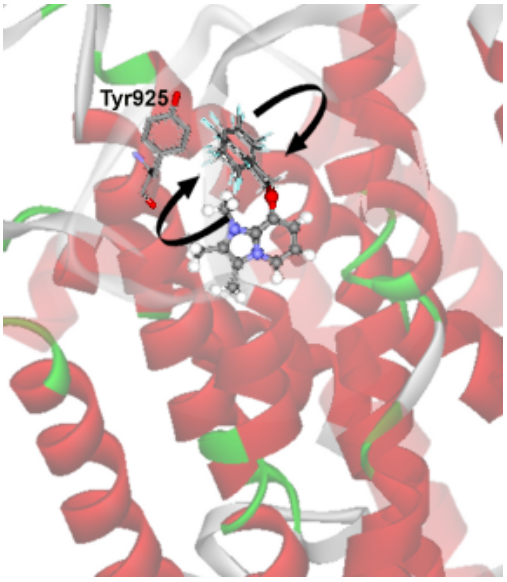
NMR of membrane proteins - Drug targeting



Systems studied

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

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

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

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Molecular Structure and Interactions by Nuclear Magnetic Resonance

The scientific advances of the last decades have allowed to reach a molecular level understanding of an increasing number of (bio)chemical processes. This is possible due to the development of numerous methodologies that allow to rationalize, at the atomic level, the relationship between structure and function, and the role of intermolecular interactions in mediating all (bio)chemical processes. My research explores the application and development of solution state NMR techniques for the study of intermolecular interactions in biological and chemical systems, and it has been conducted in the context of the functional analysis of catalytic systems and the understanding of molecular recognition processes. We have been applying NMR to study intermolecular interactions in three main areas: (a) in chemical systems relevant to the rationalization of asymmetric transformations or catalysis[1]; (b) in non-conventional solvents (ionic liquids and CO2) to rationalize solvation phenomena[2]; (c) in biological systems for the rationalization of biocatalysis or in the context of drug design[3]. To explore these subjects using NMR it is necessary to develop methods that allow to obtain information about molecular structure, dynamics, and interaction in different experimental conditions, in particular high pressure NMR[4], diffusion NMR[5] and techniques for the study of protein-ligand interactions[6].

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Nuclear Magnetic Resonance and the study of Protein Ligand Interactions

NMR is an essential analytical method in chemistry and one of the most prominent applications of solution NMR is probably the characterization and structure determination of biological macromolecules, such as proteins and nucleic acids. However, the specific interactions between molecules in solution through non-covalent forces represent the fundamental basis of molecular recognition processes. On the other hand, the detection and investigation of the interaction between proteins and small ligands becomes more and more important as a screening method in drug discovery and drug design. In all cases, the specificity of the molecular recognition relies on both structural and functional complementarities between the chemical groups of the interacting molecules. NMR spectroscopy has demonstrated its suitability to provide such structural information at an atomic level, and many experiments have been developed and applied, in particular, to study ligand–receptor interactions of biological relevance. In principle, any NMR parameter that changes when a molecule interacts with the target can be used to describe an intermolecular interaction. In general, for the study of ligand–receptor interactions by NMR, two approaches can be distinguished: (i) observation of the receptor signals (for strong binding) or (ii) monitoring the signals from the small ligand (primarily in the intermediate to fast binding regime, corresponding to dissociation constant, KD, in the millimolar to micromolar range). This lecture introduces the general NMR concepts necessary to understand both approaches and presents several NMR methods and examples: from the mapping of the interaction site in the surface of the protein[1] to particular type of NOE (nuclear Overhauser effect) experiments such as TR-NOE (transferred-nuclear Overhauser effect) and STD (saturation transfer difference spectroscopy)[2] or the measurement of diffusion coefficients with NMR.

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Biological mass spectrometry

1. **Ions and ion sources**
   (a) The nature of the ions
   (b) Sources of ions, the historical electron impact
   (c) ESI, MALSI and related sources
   (d) Ion formation mechanism and the so called soft ionization
   (e) Native or not?
2. **The mass spectrometers**
   (a) Scanning and non-scanning devices
   (b) The different time scales of the experiment
   (c) High resolution, high accuracy
   (d) Ion mobility separation
3. **Going up in mass**
   (a) The isotopes
   (b) The isotopic pattern
   (c) The non-covalent interactions
4. **Activation methods and MS/MS**
   (a) Statistical model of energy distribution
   (b) Rules of fragmentation
   (c) The special case of peptides
5. **Separation methods: LC, CE, Size exclusion**
6. **Quantitative aspects of mass spectrometry**
   (a) Proteomics
   (b) Metabolomics
   (c) The use of databases

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A Novel Positive and Negative Mass Spectrometry Chemically Activated Fragmentation for Genome Fingerprinting Scanning

A novel method for peptide amino acid sequence determination upgraded with genome fingerprint scanning (GFS) was designed to investigate gene expression of the organisms which proteome is still not well known. Unknown proteomes can be used as models in these experiments (e.g. congenital diseases, cancer cells). The method links proteomic data, consisting of determined tryptic peptides amino acid sequences obtained by negative and positive ion mode MALDI tandem mass spectrometry (MS/MS) matched against sequenced genome of the observed organism and conformed by mass spectrometry (MS) data of elucidated peptide mass fingerprints sequences derived from the annotated genome (Figure 1)[1]. The idea that lies behind this new technology is enhanced de novo sequencing of unknown peptide amino acid sequences in negative (enabled by mild overnight derivatization by carrier that contains two negatively charged groups without observed side reactions or peptide degradation) and positive ion mode of the same peptide used as quality control (MS/MS of derivatized or underivatized precursor ions). Furthermore, only 20% of tryptic peptides are derivatized by this method and are detectable only in negative ion mode. Therefore, classical MS and MS/MS proteomics analysis by mass spectrometry can be performed in positive ion mode without any significant differences in overall database score results. The method can be compared to DIGE (Differential Gel Electrophoresis)[2] when fluorescence tag would be compared to negatively charged N-terminal tag in negative ion mode mass spectrometry. The enhancement of negatively charged ions' ionizability (similar to fluorescence effect in spectroscopy) and dissociation in negative ion mode ensures data confidence without ion adduction or in-source decay interferences characteristic for positive ion mode peptide mixture analysis. This technology enables unambiguous determination of at least five to six amino acids and often more in a row from a MS/MS experiment only in one ion mode, but it can be combined as b-ion series from N-terminus (negative ion mode) and vice versa y-ion series from C-terminus (positive ion mode), even though derivatized ions are "invisible" as precursor ions in positive ion mode. Short peptide of five to six amino acids in a series (it is important to create a continual series without gaps or statistical uncertainties which could influence the scoring data) can be used to detect gene that codes for the analyzed protein. Protein BLAST search, against non-redundant data bases (nr), can point directly to the protein observed, to protein homology; it can indicate wrong genome annotation or even find mutation (Figure 2). On the other hand, the new software is developed to translate peptides into nucleotide sequence and coding regions of the organism which genome is not annotated. The software creates all the possible combinations of nucleotide bases that could code for given peptide and then it searches sequenced genome for the unique match (e.g. frameshifting mutations). The result discovers peptide coded genome locus. The sequence

that is located on the detected locus can be further investigated using nucleotide BLAST against assessable data bases to define the gene which codes for the expressed protein.

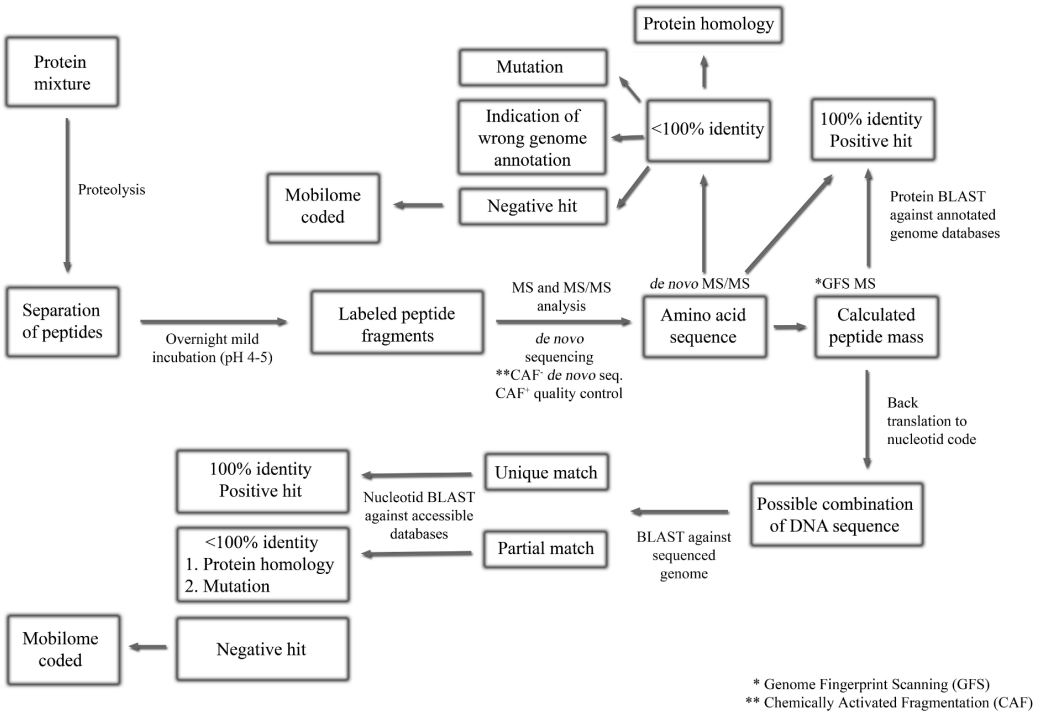

Figure 1: **Illustration of the matching process.** Masses derived after CAF-de novo sequencing and CAF+ quality control of the same peptide are matched within a tolerance % against the any annotated genome (BLASTp or BLASTn). The matching sequence (at least 6 amino acids) from the digested protein are then mapped back onto the chromosome whence they were derived.

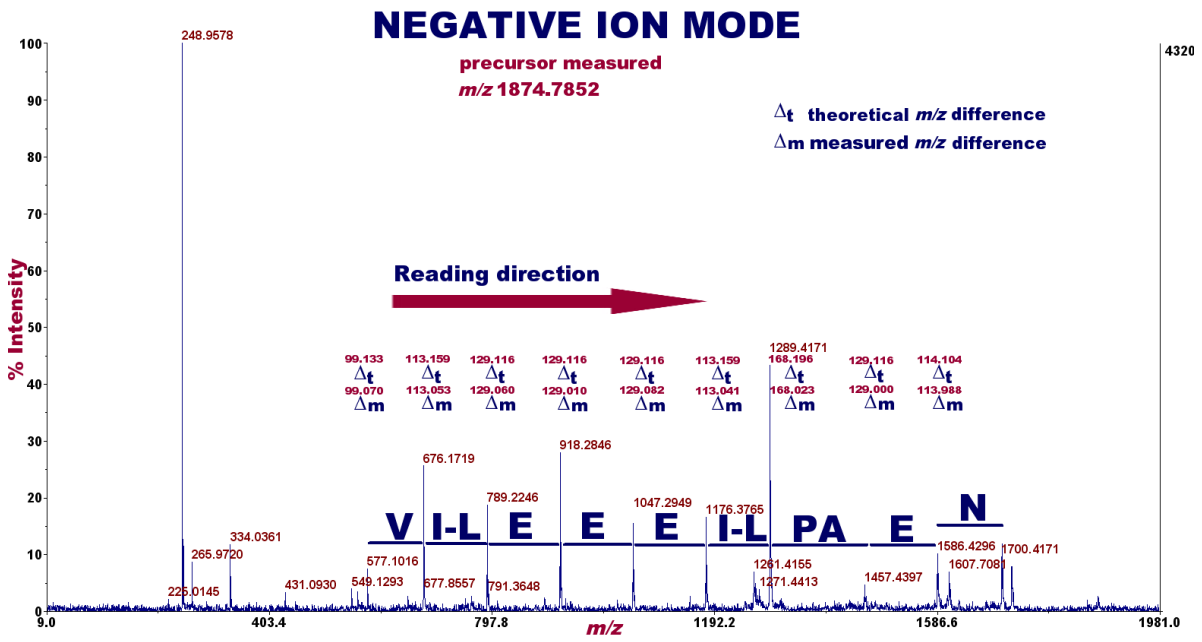

Figure 2: Illustration of the protein annotation after MALDI-MS/MS in negative ion mode of precursor ion m/z 1884. The protein is not annotated in protein sequence databases (searched by Mascot), but it is easily annotated by reading the peptide sequence and by matching the precursor mass against Lacctobacillus brevis annotated genome (BLASTp, NCBI). Matched protein is Glycerol-3-phosphate dehydrogenase (gi|116098639).

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Protein Mass Spectrometry: Defining proteoforms and Going native

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

Dynamic protein structure: From protein disorder to membrane pores

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

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Review of solid-state nanopores and their applications

General: I will give a general introduction to biomolecule analysis with nanopores, explaining the general concept and the variations of this techniques used in biophysics experiments. Nanopore experiments comprise the ultrafast, all-electronic detection and analysis of biomolecules by driving them through tiny holes – or nanopores – in thin membranes, including the efforts towards mapping a human genome under 10 min. As molecules are driven through nanopores in solution, they block the ion current flow resulting in current reductions from which molecule's physical and chemical properties are inferred. DNA, proteins, microRNA and other biomolecules can be analyzed. This lecture will focus on solid-state nanopores, that are fabricated on silicon or other solid-state chips, using a variety of methods. I will overview the solid-state nanopores explored so far, review the materials aspects as well as discuss what nanopores are able to reveal for biophysics. I will attempt to give an objective, comparative overview of the various nanopore results as well. The discussion will include nanopores in two-dimensional membranes including graphene and other more recent 2D materials. The temporal, spatial resolution and sensitivity in these experiments have been improved over the last few years thanks to advanced materials, device designs and new electronics.

Techniques: I will give a lecture on nanofabrication and measurement tools used in solid-state nanopore experiments. This will include a tutorial on solid-state nanopore fabrication starting with wafer growth to chip and membrane preparation. I will discuss the influence of chip parameters (wafer composition, membrane and nanopore dimensions, chip capacitance, nanopore conditions, etc.) on the characteristics and quality of the measured signals. Additionally, I will explain and discuss the electronics requirements for the experiments. These are not frequently highlighted in detail in nanopore papers, but are essential for the optimal signal-to-noise ratios required to achieve the best spatial and temporal resolution in detecting and analyzing biomolecules. I will show photos of the measurement setups and discuss the relevant details, to make it useful to students who may be setting up these experiments. This discussion will complement the hands-on demonstrations and discussions that we will be carried out at the school.

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Squeezing New Information out of DNA Using Adjustable Nanoconfinement

Our goal is to understand the physical principles that determine the structure and dynamics of DNA; principles that are central to researching essential information storage and replication functions which underlie life. This goal has motivated the development of single-molecule methods that allow us to visualize spatiotemporal dynamics of biopolymers. Existing methods often face challenges in gently formatting, chemically modifying, and visualizing delicate DNA polymers and protein-DNA complexes in nanofluidic environments. Here, we present a new high-throughput platform for gently loading and reacting delicate biopolymers and complexes in sub-50 nm nanostructures, which we use for long-DNA mapping as well as for direct visualization of DNA interactions and dynamics. Our single-molecule manipulation and visualization platform uses the principle of "Convex Lens-induced Confinement" (CLiC). In CLiC, we can continuously adjust the height of a nanofluidic imaging chamber to gently and dynamically unravel long DNA polymers into embedded open-face nanostructures, from above (Berard et al, PNAS 2014). Recently, we have integrated controlled, in-situ chemistry procedures within the CLiC nanofluidic device, allowing us to first format DNA polymers in embedded nanostructures, and subsequently introduce reagent molecules into the nanofluidic chamber with exquisite temporal control, such as proteins. We use this platform to study DNA condensation along the nanogrooves in response to inserting reagent molecules including crowding agents, proteins, enzymes and surfactants.

How DNA do the twist? Visualizing supercoil-induced site-unwinding and site-invasion in DNA loops

In living prokaryotic cells, DNA experiences constant torsional strain due to its supercoiled state. On the one hand, this topological property of DNA leads to dense storage of genetic code. On the other hand, DNA supercoiling is conjectured to play a key role in mechanically regulating the local unwinding of sites of DNA transcription, replication, and repair. While our understanding of the effects of supercoiling on these essential cellular processes has been developing for decades, the kinetics of local DNA unwinding and DNA strand-invasion have remained largely unaddressable with existing microscopic techniques. Advances in our methodologies have been required to visualize weak and slow interactions between unwinding sites on untethered supercoiled DNA and DNA oligonucleotide probes that can invade unwinding sites. In this work, we demonstrate a new method and assay for visualizing supercoiling-induced site-unwinding, and consequent site-invasion by small molecules, which overcomes these challenges. Our approach uses Convex Lens-induced Confinement (CLiC) microscopy to trap and visualize Cy3B-conjugated probe DNA molecules and plasmid DNA in micron-sized pits etched into a glass coverslip. We demonstrate trapping of DNA molecules ranging from several-kB plasmids to few-bp oligonucleotides in pits and we watch their dynamics and interactions over several minutes. The pits are much larger than the trapped molecules, allowing them to explore accessible conformations. As a model for supercoiled DNA, we use a DNA plasmid with a known unwinding site, prepared in different topoisomer states. We use a suite of short DNA oligonucleotides, which are complementary to different target sequences within the unwinding site, as a model for small molecules invading unwinding sites. In this work, we study how supercoiling, applied temperature and solution conditions, as well as oligonucleotide sequence and length influence site-unwinding and site-invasion. Beyond this study, the flexibility of our microscopy assay opens the door to performing new measurements of weak and slow molecular interactions in a wide range of biophysical, biochemical, and biotechnological contexts.

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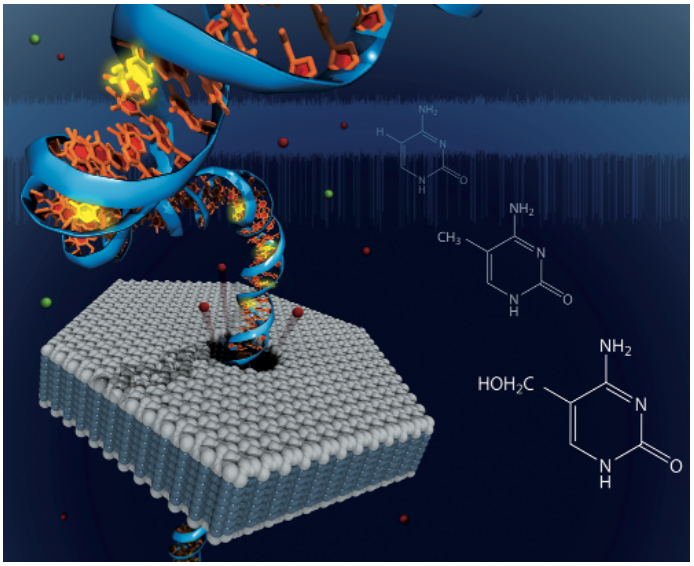
Biomolecular Capture and Transport Through Synthetic Nanopores

Nanopores are miniaturized electrical sensors with arguably the smallest detection volumes (sub-yoctometers, or below 10^{-24} m^3). Detection of molecules using nanopores involves electrical monitoring of ion current flow through a pore using a pair of electrodes placed across the nanopore-containing membrane. Our group focuses on the use of nanopores that range from 1 to 10 nm in all dimensions (diameter and thickness). We fabricate such nanopores using a combination of state-of-the-art ultrathin membrane fabrication and focused electron beam irradiation using a transmission electron microscope. Recently, we have found that nanopore dimensions critically determine the quality of detection and discrimination of biomolecules. I will talk about our efforts to distinguish different types of tRNA molecules, RNA-drug complexes, and proteins. In addition, I will mention our efforts to control DNA transport through nanopores, useful for genomic mapping. Finally, I will mention our studies that probe nucleosomal interactions and influence by epigenetic factors, as well as our latest efforts in combining nanopores and optical waveguides for direct DNA sequencing from picogram-level genetic material.



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Biophysics at the interface between academia and pharmaceutical industry

The development of new technologies can be driven either by a technology driven process where new methods are developed and later enable innovative research, or by research questions that require the development of new methods. Pharmaceutical industry has been influential in the development and application of biophysical tools, and now has a broad repertoire of technologies and methods for the discovery of new drugs. The first lecture will focus on the development of surface plasmon resonance biosensor technology has developed and become an essential technology for state-of-the-art drug discovery and how it has not only transformed drug discovery by introducing kinetics as an essential parameter for optimization of drugs, but how it has also increased the basic science of molecular recognition. My experience of interacting with biotech industry in the development of novel instruments and methods, and with pharmaceutical industry in applying the methods, while remaining at the university where I carry out basic science will be described. The second lecture will focus on how the discovery of drugs has increased our understanding of the fundamentals of molecular interactions. Case studies will be used to illustrate the research.

PARTICIPANT CONTRIBUTIONS

Identification of small molecular NPR-A agonists for the treatment of resistant hypertension

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Natriuretic peptides (NPs) play an important role in the regulation of blood pressure. Atrial (ANP) and brain NP (BNP) activate NP receptor-A (NPR-A), causing production of cyclic 3',5' guanosine monophosphate (cGMP) resulting in the reduction of blood pressure. Hypertension (HT) is associated with an impaired NP system and a reduced NPR-A activation. Resistant hypertension (RHT) is associated with increased risk of cardiovascular and renal events, and there are currently no good treatment options available. Replacement therapy with recombinant BNP has been shown to significantly reduce blood pressure in uncontrolled HT, suggesting BNP as potential treatment of uncontrolled HT and RHT[1]. However, peptides as BNP are challenging and expensive to administer to patients, thus we aim to find small molecular NPR-A agonists with better drugability.

We performed high throughput screening (HTS) of about 30,000 low molecular compounds by using the AlphaScreen assay for cGMP (Perkin Elmer). We identified one compound that enhanced the cGMP production by ANP and BNP in NPR-A expressing HEK293 cells, however our compound did not cause cGMP increase alone. This suggests positive allosteric modulation of the receptor by our lead compound. We are now testing new ligands based on our lead compound to improve its pharmacological properties. Our goal is to combine both ligand based and structure based methods to strengthen our knowledge about the structure-and activity relationship of small molecular compounds that activate the NPR-A receptor.

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Anisotropic SAXS in flow as a probe of protein structure and function

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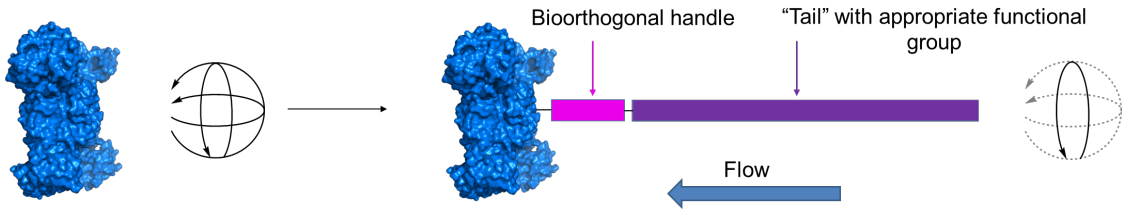
Small-angle X-ray scattering is a solution based technique often used complementarily to crystallography to study the structure and function of biomolecules in physiological buffers. This allows them to be studied in "near-native" conditions, however the freedom of motion the particles have in solution leads to it being a low resolution technique as the data must be averaged over all possible orientations for the protein. The aim of my project is to try and improve the quality of the data obtained from SAXS experiments by reducing the number of degrees of freedom the protein has in solution. We aim to do this by site-specifically incorporating a tail group on to the protein to try and achieve anisotropy in flow. In order to achieve this, a biorthogonal probe, bicyclononyne, will be site-specifically incorporated into the protein using Sortase A[1]. This will then be reacted by "click" chemistry with an azide-functionalised piece of DNA which will act as the tail.



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Characterization CueR metalloregulatory protein and its interaction with „soft" metal ions

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Selective recognition of toxic metal ions may attract wide interest due to its potential medical and environmental applications. Understanding the details of bacterial metal ion regulatory mechanisms may forward the design of molecules for selective metal ion binding/accumulation or the development of sensitive metal ion-detection techniques. Our study was inspired by the bacterial metal ion selective transcriptional metalloregulators. CueR is a fascinating example of such proteins that regulates the intracellular level of Cu^I-ion in several strains of bacteria. The regulation by CueR is based on the conformational change of the protein upon metal ion coordination, influencing the structure of the protein-bound DNA. The effector metal ion binds to the protein in its metal binding loop, close to the C-terminus, where the sidechains of two cysteine residues restrict linear coordination geometry around the metal ion. According to in vivo experiments CueR provides a transcriptional response to single-charged, but not to double-charged metal ions.[1] In order to better understand the mechanism of the selective metal ion recognition and regulation of CueR we expressed and purified the wild type CueR from E. coli for subsequent structural and activity investigations.[2] The initial characterization of the purified protein was performed by tandem mass spectrometry, circular dichroism spectroscopy and electrophoretic mobility shift assay. Next we are going to study the metal binding properties of CueR by means of different methods. Beside the crystallographic studies we aim at understanding the solution structure and dynamics of the protein in the presence/absence of its interacting partners.

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The role of microtubule pivoting in formation of antiparallel microtubule bundles

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During cell division, the genetic material is divided into two equal parts by the mitotic spindle. This complex dynamic micro-machine is made of chromosomes, microtubules extending from two poles and a variety of accessory proteins. Microtubules extending from one spindle pole bind to those from the other spindle pole by motors and other cross-linking proteins, forming antiparallel bundles that comprise the spindle. Formation and stability of these structures is mediated by motor proteins. However, the physical principles behind the formation of antiparallel microtubule bundles are still not known. In this work we develop a theoretical description of the formation of antiparallel microtubule bundles motivated by the newest experimental work done by our collaborators. Our model includes the angular movement of microtubules around spindle poles[1] and attractive forces exerted by motor proteins[2] walking along the microtubules and cross-linking them. When two microtubules are found in close proximity to each other, motor proteins cross-link them and move towards the poles creating forces, which move the microtubules towards an antiparallel configuration. This model shows that thermal fluctuations and forces exerted by motor proteins facilitate formation of antiparallel microtubule bundles.

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Hypermanganesemia-Related Mutation in ZnT-10 Shows a Domain Structural Loss

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SHORT TALK

The cation diffusion facilitator (CDF) protein family is a highly conserved, metal ion efflux transporter family that maintains divalent transition metal cation homeostasis. Human CDF proteins are named Zinc Transporters (ZnT) 1-10. Different mutations within ZnT proteins were shown to enhance or cause a variety of diseases, such as zinc deficiency and parkinsonism. The missense mutation L349P in the manganese transporter ZnT-10 was shown to be related to high levels of whole-blood manganese (hypermanganesemia), hepatomegaly and dystonia. In this study, we characterized ZnT-10 L349P via a CDF protein from magnetotactic bacteria, MamM, an iron-transporter that can serve as a model system to study human CDF-related disease mechanisms. We used a variety of computational, biophysical and molecular techniques to study the synonymous mutation in MamM, M250P. Our results show that the M250P mutation causes severe structural changes to the MamM cytoplasmic domain and reduces MamM function in the MTB strain Magnetospirillum gryphiswaldense MSR-1. Based on this work, we suggest a mechanism for the effect of the ZnT-10 L349P mutation and explain how it leads to the related symptoms in humans.

Contribution of cross-linking and ion-mobility for the study of protein and complex structure

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Tridimensional structure of proteins and map of protein-protein interactions is a precious source of information for the understanding of their function. Different techniques exist nowadays such as X-ray crystallography or nuclear magnetic resonance to achieve this goal. In the field of mass spectrometry, several tools were developed. Those used in this research are on one hand cross-linking and on the other hand ion-mobility.

The first one allows to obtain local information thanks to a cross-linker, a molecule that binds two parts of one or two proteins. Using cross-linking coupled with mass spectrometry makes it possible to probe interaction zones between two proteins. In a first attempt, the cross-linking of the cAb-HuL6 – lysozyme complex was realised to determine the proximity between two residues. To find out if the procedure used is efficient, we compared our results with the already known 3D structure. The results demonstrate that the majority of the identified cross-links are in disagreement with the 3D structure of this complex (PDB:1OP9 [1]). Several hypotheses have been put forward to explain this disagreement. Firstly, there may exist non-specific interactions between V_HH (the binding site of cAb-HuL6) and lysozyme. Secondly, the addition of DMSO to solubilise the cross-linker may have altered the structure of the complex. Finally, most of the residues involved in a cross-link are found on loops, supple and flexible elements that could allow cross-links that are theoretically forbidden by distance restraints [2].

The second tool provides global structural information thanks to collisional cross sections. With cross-linking and ion-mobility coupled to mass spectrometry, we can study the folding of proteins in the gas phase. We perform our analyses on cytochrome c with two different cross-linkers. We highlight that adding cross-linkers to cytochrome c holds the protein in a more compact form than without cross-linker. We also demonstrate that cross-linking allows to fix the shape of the protein in solution and left it intact when analysed in the gas phase.

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The dynamics of kinesin-8 motor proteins is correlated with the kinetochore oscillatory movements

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Mitosis is a part of the cell cycle, in which DNA material is passed on to two daughter cells. During mitosis, mitotic spindle forms a complex protein structure which provides a structural basis for chromosome segregation. Sister chromatides bind to kinetochore proteins in the central part of the spindle (equatorial plate). Each sister kinetochore is bound to the k-fiber, microtubule bundle that connects it to the nearer centrosome, and such configuration is named bi-orientation. When sister kinetochores reach bi-orientation, they oscillate around equatorial plate of the mitotic spindle. Kinetochores bind to microtubule plus ends and their movements depend on the microtubule dynamic, which includes microtubule growth and shrinkage. These microtubule traits are regulated by proteins like motor protein kinesin-8 which is known to stimulate microtubule shrinkage. In addition, kinesin-8 increases the distance between sister kinetochores regulating the tension forces among them. The dynamics of kinesin-8 and its relation with the kinetochore oscillations has not been investigated. We used live imaging confocal microscopy to research the dynamics of the kinesin-8 and kinetochore oscillations in metaphase of human U2OS cell line. Our preliminary data show that GFP-tagged signal of kinesin-8 appears as comet-like shape with it's peak at the very end of k-fiber. This signal changes with time, dependently of the length of the k-fiber and whether the k-fiber grows or shrinks. Interestingly, the signal of kinesin-8 rises as the polymerization of k-fiber progresses and it drastically lowers when k-fiber starts to depolymerize. In addition, this signal dynamics occur concurrently with kinetochore oscillations, as kinesin-8 accumulates at the trailing sister kinetochore and it decreases at the leading sister kinetochore. To further investigate influence of kinesin-8 to the kinetochore oscillations, we plan to combine the laser ablation of k-fibers (changing the force balance), with the silencing and overexpression of the kinesin-8 in cells.

Using EPR spectroscopy and MST to analyse protein-protein interaction at high concentration

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Therapeutic monoclonal antibodies (mAbs) are often administered at high protein concentrations (> 100 mg/mL) which can lead to unwanted solution behaviour, such as liquid-liquid phase separation, aggregation and viscosity [1]. Protein-protein interactions (PPI) are expected to play a major role in the origins of these solution properties. In order to formulate mAbs at the most stable buffer conditions it is therefore important to determine the effect of PPI on concentration-dependent solution behaviour. With most common methods, PPI are analysed at dilute conditions and used to predict solution behaviour at high protein concentrations. In this work we want to evaluate and adapt physicochemical methods to measure PPI directly at high protein concentration and at different buffer conditions to gain a better understanding of the origins of macroscopic solution behaviour. Electron Paramagnetic Resonance (EPR) spectroscopy and Microscale Thermophoresis (MST) are powerful tools, that have the potential to measure PPI at high concentrations. Both have a high sensitivity and information content combined with a low sample consumption. Also they are not limited to specific protein concentrations, buffers or solution viscosities. Our preliminary results show that both EPR spectroscopy and MST are able to analyse the behaviour of mAbs in a large concentration range, at various pH-values and at different temperatures. These results indicate that EPR and MST are feasible techniques to analyse PPI directly at highly concentrated protein solutions.

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Development of Sub-Cellular Mass-Spectrometry Imaging by Impulsive IR Laser Ablation

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Mass spectrometry imaging (MSI) is a label-free method for obtaining protein concentrations along with their spatial distributions in biological samples. The quality of the proteomic data is determined by the instrument's mass resolution, spatial resolving power and, most importantly, its sensitivity. Two techniques serve as the basis for most MSI studies: Secondary ion mass spectrometry (SIMS) offers high spatial resolution (<100nm), but leads to intense fragmentation when applied to molecules larger than a few hundred Dalton due to the high energy associated with the ion beam. Matrix assisted laser desorption ionization (MALDI) using ultraviolet (UV) light on the other hand is more suitable for large biomolecules, although it is less soft than electrospray ionization (ESI). It additionally suffers from sample heterogeneities introduced by the crystalline matrix. Improving upon this concept, infrared laser desorption ionization (IR-LDI) reduces fragmentation and is capable of analyzing water rich samples without the requirement for an often detrimental matrix by targeting the O-H stretching vibration of water for efficient energy deposition and ablation. Employment of an IR laser source in the picosecond regime, recently demonstrated by our group [1, 2] as desorption by impulsive vibrational excitation (DIVE), allows for the ablation event to occur under thermal and stress confinement conditions. This minimizes damage to surrounding sample areas, reduces the laser fluence necessary for ablation, and decreases the time molecules spend in the initial, dense ablation plume where most fragmentation takes place.

We have developed a reflectron time-of-flight (TOF) mass spectrometer with a cryogenic in-vacuum DIVE ion source to characterize the new ultra-soft imaging technique. In order to extend the spatial resolution to the cellular level, we implement an ion-microscope-coupled DIVE-TOF system. Placement of the ion source in vacuum enables efficient extraction of charged molecules while the absence of back pressure minimizes collisional fragmentation.

At the same time we are developing methods for the characterization of laser-desorbed material (the ablation plume) via time resolved interferometric microscopy, a method sensitive to minute changes in the index of refraction and therefore capable of determining the particle velocity and directionality even for small amounts of desorbed gas phase water. While knowledge of the ejecta's axial velocity distribution and the timeline of the ablation event – i.e. the onset of desorption, recoil ejection, etc. – aids in optimizing the mass resolving power of the TOF mass spectrometer, the lateral velocity distribution is of specific significance for mass spectrometry imaging. Neither has been characterized for DIVE ablation events in vacuum before.

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It's not easy being flexible - the curious tale of NEMO and the importance of the intracellular milieu for protein flexibility

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Protein flexibility is key to the function of many regulatory proteins and enzymes. In particular, 'hub' proteins, that mediate a large number of molecular interactions, are thought to require a natively flexible structure to achieve both specificity and affinity for multiple targets. However, protein flexibility is extremely sensitive to the molecular environment and so studies of protein flexibility with recombinant protein may not accurately capture the true in cellulo situation. We have previously found that the master regulator of much of the human immune system, NF-κB essential modulator (NEMO), is a highly flexible protein. We have developed the use of red edge excitation shift (REES) spectroscopy to capture information on protein flexibility1. We have found that this probe can accurately capture subtle changes to protein flexibility and dynamics, and discern subtle difference between different ligand bound states. Further we have used high-pressure fluorescence measurements to capture thermodynamic information on NEMO intrinsic flexibility and dynamics. Our findings suggest that the intrinsic flexibility and dynamics of NEMO are key to mediating its functional interactions. We now use these experimental probes to explore the effect of the intracellular environment on 'native' flexibility of NEMO by mimicking the intracellular environment including high viscosity, macromolecular crowding, high ionic strength and the presence of osmolytes. We find that in the intracellular environment NEMO is significantly stabilised but counterintuitively also becomes more flexible. Our data suggest that the intracellular environment does not simply cause proteins to be 'more folded' but instead promotes the functionally important flexibility whilst simultaneously improving stability. These findings offer a significant advance in the understanding of functional protein flexibility provide a new paradigm for understanding complex molecular interactions.

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Using the maximum entropy principle to enforce NMR data on RNA simulations

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SHORT TALK – EBSA

Molecular dynamics (MD) is a powerful tool that can be used as a virtual microscope to investigate the dynamics of proteins and nucleic acids at atomistic resolution. However, recent extensive simulations of small RNA systems have shown that current force field parameterizations are not accurate enough to reproduce solution experiments[1]. These results suggest that MD should be complemented with available experimental data, when available. The maximum entropy (MaxEnt) procedure, where ensemble averages are constrained, is a natural framework to enforce ensemble-averaged data[2]. We propose a modfied maximum entropy method which tolerates noisy data as well outliers in dataset and wrong estimation of forward model parameters. We tested this method enforcing ^{3}J scalar couplings on all RNA nucleosides obtaining a significant improvement in the overall agreement with experimental data. We also propose a procedure that can be used to self consistent fit different systems. We show an application of this by simultaneously fitting several nucleosides and dinucleoside monophosphates. The procedure allow us to construct a single force field that is by construction in agreement with a large number of experimental data.

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Light induced conformational changes in transmembrane protein complex NpSRII/NpHtrII

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The transmembrane signaling complex NpSRII/NpHtrII plays a key role in negative and positive phototaxis of Natronomonas pharaonis archaea. Structural information in terms of X-ray crystallographic data exists for the transmembrane part of this protein complex as well as for the rod shaped cytoplasmic part of the transducer due to its high homologies with chemoreceptors. Photon absorption induces structural changes in NpSRII, which are conducted to the transducer NpHtrII. The transducer in turn regulates the phosphorylation level of a bound histidine kinase in the cytoplasm which modulates the rotation of the flagellum. The aim of this study is to understand the signaling mechanism within the sensory rhodopsin – transducer complex. To trace the kinase-activating signal along the extended transducer, we use site directed spin labeling in conjunction with EPR spectroscopy and photoactivation to probe the complex for conformational and dynamical changes starting from the transmembrane region. In order to establish a correlation to the initial light signal, we monitor the photoinduced all-trans to 13-cis isomerization of NpSRII's retinal chromophore and its subsequent relaxation back to the ground state by transient optical absorption spectroscopy, which allows following the transitions between the so called photocycle intermediates[1]. Conformational changes of NpSRII and NpHtrII reconstituted in purple membrane lipids were observed during the photocycle[1]. Here, the influence of the lipid environment on the propagation of the signal and the relation between light intensity and signal strength is studied with NpSRII-NpHrII complexes reconstituted in nanodiscs and styrene maleic acid lipid particles (SMALPs) which are disc-shaped lipid assemblies on the nano-scale.

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Dynamic small heat-shock protein interactions with non-aggregating clients

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SHORT TALK

The small heat-shock proteins (sHSPs; HSPBs) are a family of molecular chaperones that are involved in stress response. Their canonical function is prevention of irreversible aggregation of denaturing proteins in the cell [1]. Most sHSPs form large oligomeric ensembles, adopting multiple interconverting stoichiometries and architectures. The dynamic heterogeneity of sHSPs and the propensity of their clients to aggregate present challenges for many biophysical techniques. Binding partners have been identified that interact with sHSPs without prior denaturation, suggesting a function separate from that of holdase chaperone. Several of these partners are involved in mechanical translation of chemical signals. They are key components in systems that determine cell or tissue viscoelastic properties, such as the muscle sarcomere and the actin cytoskeleton. Though sHSP association with the cellular support network is long known to be widespread [2], we understand little about the purpose or mechanism of interactions with these targets. Here we discuss the biophysical methods we employ to study the interactions between sHSPs and mechanosensitive non-aggregated clients, with a focus on native mass spectrometry of large noncovalent complexes, X-ray crystallography of targeted sub-domains, and microrheology via optical trapping. We highlight select results that demonstrate a propensity for stabilisation at the atomic level which leads to network stiffening at the macro scale, with implications for cardiovascular health. Through characterisation of these novel sHSP-client interactions, we aim to contribute to an emerging more complete understanding of chaperone function.

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Single Molecule localisation and Discrimination of DNA-Protein Complexes with a Nanocapillary

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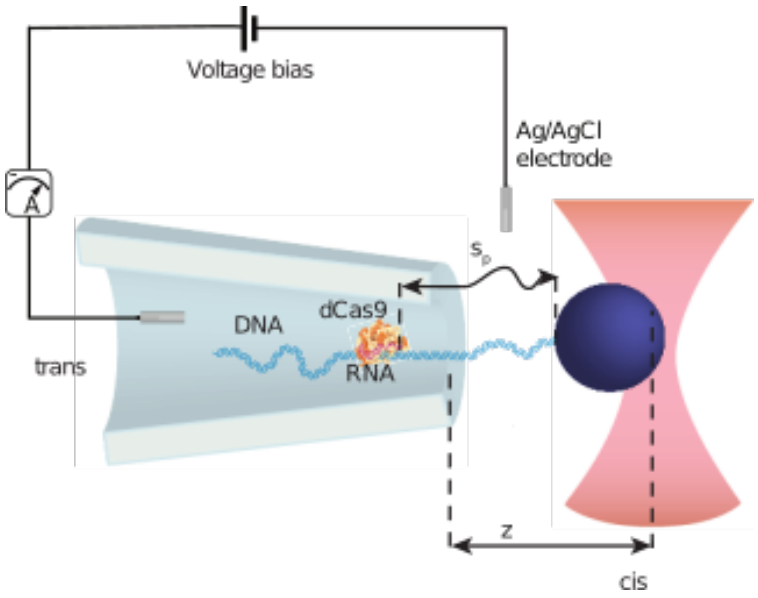
DNA-protein interactions ubiquitously regulate almost all aspects of cellular function, such as DNA replication, chromosome maintenance, DNA repair, and transcriptional regulation. Most of these interactions occur after complex protein search and binding to a sequence specific DNA target. Because of this wide spectrum of interactions it is crucial to gain a better understanding of DNA-protein interactions, specifically the intricacies of binding.

Over the years, numerous experimental and computational methods have been developed to elucidate the role of DNA-protein interactions in cellular processes. Experimental methods can broadly be separated into bulk or single molecule measurements. Single-molecule techniques are suitable to characterize rare DNA-protein interactions with high sensitivity and reveal interesting phenomena due to the complex mechanisms and inhomogeneous dynamics of DNA-protein interactions.

In this single molecule world nanopres are of high interest due to the possibility of translocating molecules through the pore one at a time. Recently we developed a method to combine nanocapillaries and optical tweezers in order to controllably translocate a single DNA molecule or DNA-protein complex.

In this study we extend this method by focusing on more precise localisation of DNA bound proteins as well discriminating them. We use two proteins of interest, RNAP which is critical to gene transcription and dCas9 which can easily form complexes on DNA thanks to the single RNA guide technique. Localisation is based on a correction for an observed shift in position measurements thanks to robust analytical and numerical modelling, while discrimination is obtained through two separate methods. The first of these uses the characteristic of nanocapillaries of obtaining current traces simultaneously to force traces to compare protein size, while a non-equilibrium work analysis using the Jarzynski equality allows discrimination based on effective charge.



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2D materials based nanotemplates for biomolecular arrays

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Hybrid systems consisting of a solid-state substrate functionalized or decorated with biomacromolecules bridge the gap between living matter and technology [1]. The aim of present work is to develop a method for production of an array of gold nanoclusters on a single-atomic layer, two-dimensional (2D) materials. Of interest here are 2D materials grown epitaxially on mono-crystalline substrates that often exhibit moiré effect. This is an additional periodic corrugation of the 2D material with a period which is an order of magnitude larger than its the lattice constant. E.g. graphene grown on the (111) face of iridium monocrystal has a hexagonal moiré lattice with a lattice constant of 2.5 nm, while graphene lattice constant is 0.24 nm [2]. Moiré pattern is a very suitable template for deposition of metallic clusters. That is, metals deposited on corrugated 2D material will often form lattice of clusters which follows the moiré - thus forming a nanopattern [2]. Here, we recognize a similarity of the moiré length scales and DNA diameter (or protein sizes) and also the possibility to use cluster lattice as a nanotemplate for ordered attachment of thiolated DNA chains to 2D material. We also discuss the possibility to attach a DNA origami structure, DNA tetrahedra, whose symmetry and size (5 nm) fits the nanotemplate. For the practical use of the nanopattern, stability of the produced structures under ambient conditions and in solutions, and additionally after the graphene lift-off is tested in this work. The novel structures could have applications e.g. in (bio)chemical sensing where different biomacromolecular pixels could be addressed or read directly by the underlying 2D electronic material instead by the biochemical or optical methods used for standard DNA chips [3, 4, 5]. The overall goal is to complement the classical approach of DNA constructs/origami as templates for metamaterials with an approach where a metamaterial is a template for DNA or other macromolecules.

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EPR characterisation of key outer membrane proteins required for metal respiration

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EPR spectroscopy, especially in combination with site-directed spin labelling (SDSL), is a versatile tool to investigate proteins in native-like or even native environment. In the Henry Wellcome Unit for Biological EPR, a research focus is set on investigation of membrane transport systems. Membrane proteins are often challenging to study and with SDSL-EPR it is possible to gain structural data as well as invaluable information on interaction and mechanisms of the complex molecular machinery that membrane proteins represent. The outer membrane complex MtrCAB from Shewanella oneidensis is one such efficient nanomachine. It is part of the major electron transport chain that is responsible for the (bidirectional) electron transfer across the outer membrane to the final electron acceptor in metal respiration. The extracellular domain, a decahaem cytochrome (MtrC), has already been structurally characterised but little is known about the interaction between MtrC and the porin-cytochrome complex MtrAB. Continuous wave (CW-) EPR combined with SDSL at intrinsic cysteine residues in MtrC and MtrAB was used to examine reconstituted spin labelled MtrCAB and allowed for structural insight into the domain interaction.

Functional characterization of organic anion transporters in zebrafish (Danio rerio): Oat2a-d

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Polyspecific SLC22 (Solute carriers) membrane transporters are responsible for the uptake of various endogenous and exogenous compounds into the cell. Organic anion transporter (OAT) subfamily constitute roughly half of the SLC22 transporter family. Organic anion transporter 2 (OAT2) plays an important role in uptake and distribution of physiological compounds, as well as anionic toxins and drugs. Mammalian OAT2 was characterized in human, rat and mouse with highest tissue expression in liver and kidney. Typical substrates of OAT2 are salicylate, acetylsalicylate, prostaglandin E2 (PGE2), dicarboxylates, and PAH. Five members of Oat2 (a-e) subfamily were identified in zebrafish. OAT2 is well characterized in mammals, however OAT2 co-orthologs have been poorly studied in nonmammalian species. Therefore, the goal of our study was to determine phylogenetic relationships, tissue distribution and substrate specifities of five zebrafish co-othologs: Oat2a, Oat2b, Oat2c, Oat2d and Oat2e. Phylogenetic analysis of OAT/Oat genes points to certain similarities among mammalian and zebrafish Oat transporters. Zebrafish Oat2 members (Oat2a-e) are highly expressed in kidney, intestine and testes and moderately expressed in brain. Functional characterization of zebrafish Oat2 members is still in progress and is being performed using the transiently transfected HEK293T cells as heterologous expression system and fluorescent substrates lucifer yellow and 6- carboxyfluorescein. We plan to test a number of compounds previously known to interact with human OAT2 and determine their IC50 values.

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Parallel subfunctionalisation of PsbO protein isoforms and their GTPase activity

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PsbO, the manganese-stabilising protein, is an indispensable extrinsic subunit of photosystem II. It plays a crucial role in the stabilisation of the water-splitting Mn4CaO5 cluster. PsbO proteins from spinach and Arabidopsis were reported to have weak GTPase activity, which might be involved in a regulation of the D1 protein turnover[1]. Our analysis of psbO sequences from a wide range of plant species showed that many angiosperm species express two psbO isoforms. Phylogenetic analysis of these pairs of paralogs revealed that psbO duplication occurred many times independently, frequently in ancestors of modern angiosperm families. In spite of this, the level of isoform divergence is similar in different species. Moreover, mapping of the differences on the protein tertiary structure showed that the isoforms in individual species differ from each other on similar positions, suggesting that similar subfunctionalisation of PsbO isoforms occurred parallelly in various lineages. Interestingly, the differences between PsbO isoforms are located mostly on the luminally exposed end of the β-barrel structure, where the GTP-binding site was predicted[2]. It was reported that PsbO isoforms from Arabidopsis differ in GTPase activity[1]. However, the observation that PsbO is a GTPase have been still under dispute. Recently, we have measured GTPase activity of PsbO isoforms from potato. We have shown that PsbO is able to hydrolyse GTP. The potato PsbO isoforms differ in GTPase activity similarly as Arabidopsis isoforms, despite independent duplication of psbO gene in these two species. We suggest that the presence of two PsbO isoforms with different GTPase activity helps the plants to finely adjust the photosynthetic apparatus in response to variable conditions.

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Dendrimer-based Cancer Gene Therapy

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During transformation, normal cells start to propagate uncontrollably, loosing the ability to undergo apoptosis, thus resulting in malignancies formation. Expression of apoptosis inhibitors can be selectively down regulated by means of RNA interference (RNAi). RNAi effectors - siRNA (small interfering RNA) or microRNA undergo degradation by endogenous enzymes and are unable to penetrate cellular membranes. Thus, the major limit of such gene therapy application is absence of effective delivery of nucleic acids siRNA into the target cells. Dendrimers are synthetic tree-like polymers 3–10 nm in diameter and highlighted for gene delivery. Cationic PAMAM, carbosilane (CBD) and phosphorous-containing (CPD) dendrimers were tested as carriers for 3 anticancer siRNA: siBcl-xL, siBcl-2, siMcl-1 and in cocktail
citeCitationLabel98, CitationLabel98. It was shown complexes are upto 1μm in size and are insensitive for nucleases action, protecting siRNA structure[1]. All complexes are able to penetrate into cytosol after 3h of incubation with Hela and HL-60 (human leukemia) cells. Viability test of HeLa and HL-60 cells reveled synergetic action of siRNA cocktail. The difference between efficiency of dendrimers depended on their (1) generation, (2) nature, and (3) stability. CPD of 3rd and 4th generations were the most effective [2]. Cationic dendrimers (PAMAM, CPD, CBD) are useful as vectors for anticancer siRNAs. Phosphorous and PAMAM dendrimers were shown to facilitate siRNA intracellular penetration at a high rate, but reveled also high toxic effect, while carbosilane dendrimers were moderate in internalization efficacy with low toxic side effect. The cytotoxicity of siRNA/dendrimer complexes has a dual nature based on three mechanisms: apoptosis, autophagy and necrosis, siRNA induced apoptosis and dendrimers themselves may induce all above cell deaths depending on dendrimer's nature.

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Joint analysis of SAXS and SANS data of asymmetric lipid vesicles

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SHORT TALK

Mammalian plasma membranes consist of an asymmetric lipid distribution along the two leaflets, in other word the inner leaflet is compositional different from the outer. The asymmetry of the bilayer is expected to affect various membrane properties, such as membrane potential, permeability, surface charge, and stability. Bilayer asymmetry is also hypothesized to affect structural properties of the membrane, like bilayer thickness and thickness of the single leaflets for example. However, due to the difficulty of preparing asymmetric vesicles the majority of model membrane studies have been performed on symmetric bilayers, where inner and outer membrane leaflets are identical in composition. Of recent, we developed new protocols for the construction and characterization of asymmetric vesicles amiable for scattering and NMR experiments with a well-defined inner and outer leaflet composition. Quantification of bilayer composition and degree of asymmetry enables the determination of transverse structural parameters, such as, area per lipid and the bilayer thicknesses of the various phases in each leaflet. We are able to determine these structural parameters through a joint analysis of small angle neutron scattering (SANS) data exploiting D/H contrast variation and small angle X-ray scattering (SAXS). Here we report on the first probe-free analysis yielding insights into a transbilayer coupling mechanisms. First results have shown a decrease in lipid packing density at room temperature of the DPPC-rich phase (outer leaflet) compared to typical gel phase packing, indicating a disordering effect from coupling to the fluid inner leaflet.

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Optical Manipulation of Liquid Crystals in Microfluidic Environment

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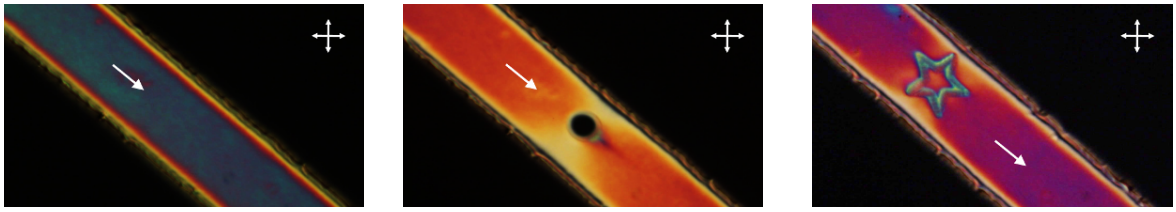
Liquid crystals are well-studied anisotropic materials that combine properties of liquids and solids. The nematic phase that is most widely used in display applications has recently attracted considerable interest in the field of microfluidics [3, 4]. It has been demonstrated that nematic microflows undergo various dynamic regimes due to a complex coupling between hydrodynamics, viscosity and orientational order that is absent in conventional flows of isotropic liquids [3, 4, 5, 6]. We explore the dynamics of nematic mesophases by using a combination of polarising optical microscopy, external pressure control and optical tweezers. The conventional microfluidic setups are prepared by standard soft lithography methods and various light-induced distortions in the nematic field are controlled by arrays of optical traps. We apply time series sequences to control laser light position and intensity to generate and manipulate complex textures in the flowing nematic [7]. We believe that these new concepts of optically-addressed topological microfluidics can be applied to different classes of soft materials.



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Raman microspectroscopy of stem cells: approach to monitor the CBCT radiation effect

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Stem cell research has gained much appreciation in recent years due to the beneficial potential of stem cells to transform the modern clinical medicine when dealing with difficult diseases. The current approaches to characterise stem cells are invasive, time-consuming, and are estimations of morphological features, which are insufficient to give a proper insight into the intracellular biomolecular processes. Raman microspectroscopy has been successfully applied for the investigation of live and fixed cells[1, 2], due to the advantages that it offers such as rapid measurement (a Raman image of an entire cell of 20 by 20 μm takes about 30 min), no damage to the cells when a suitable laser wavelength is used[3], and non-invasive, as no labels or markers are required[4]. Cell studies applications of Raman spectroscopy include the investigation of biochemical changes related to the exposure of cells to different toxic chemicals and various conditions[5], the identification and characterisation of the cell and nucleus cycle, and cell death related molecular specific modifications[6]. With the development of modern technologies there has been an increased interest in identifying the biological markers specific to irradiation. The scientific literature shows that ionizing radiations induce modifications at the cellular and molecular level, by modifying the biological functions[7, 8]. We present here label-free Raman microspectroscopy investigations of human stem cells and we intend to identify biomarkers characteristic to the biochemical modifications induced in stem cells by the CBCT (cone beam computed tomography) irradiation used in orthodontics treatments. The results can be important for our general understanding of the variations of the molecular properties of stem cells and the influence of various environmental conditions on the biomolecular dynamics.

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Coulomb blockade in ion transport

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SHORT TALK

Emergent behaviour from electron-transport properties is routinely observed in systems with dimensions approaching the nanoscale[1]. However, analogous mesoscopic behaviour resulting from ionic transport has so far not been observed, most probably because of bottlenecks in the controlled fabrication of sub-nanometre nanopores for use in nanofluidics [2, 3] . Here, we report measurements of ionic transport through a single sub-nanometre pore junction, and the observation of ionic Coulomb blockade: the ionic counterpart of the electronic Coulomb blockade observed for quantum dots4. Our findings demonstrate that nanoscopic, atomically thin pores allow for the exploration of phenomena in ionic transport, and suggest that nanopores may also further our understanding of transport through biological ion channels.

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Pulse EPR with Gd(III)-spin labels to determine distance distributions in a large protein/RNA complex

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SHORT TALK

Electron Paramagnetic Resonance (EPR) pulse dipolar spectroscopy represents a popular method to obtain structural information of large biological macromolecules and complexes by measuring long range distance constrains by double electron-electron resonance (DEER) [1]. Nitroxide radical pairs of S=1/2 spins are well studied and broadly used for this purpose.

Spin labels based on chelate complexes of Gadolinium ions (Gd^{3+}, S=7/2) provide good sensitivity at high magnetic fields and reveal no orientation selection [2]. Importantly, spectroscopic properties of Gd^{3+} ions are very different from those of nitroxide radicals, thus allowing spectroscopic selection experiments. Gd^{3+} chelate complexes can be used as tags for distance distribution measurements in protein samples, protein/RNA complexes and even for In-Cell EPR, where nitroxide labels are inapplicable due to the reducing milieu inside a cell [2, 3]. The combination of Gd^{3+} chelate complexes and nitroxide radicals provides the possibility to characterize the environment of each paramagnetic spin label separately. This orthogonal spin labeling approach shows a comparable sensitivity to nitroxide-nitroxide DEER and is attractive for biological complexes [4].

In this project, we aim to obtain inter- and intra-domain distance distributions between Gd^{3+}-Gd^{3+} labeled Polypyrimidine Tract Binding Protein 1 (PTBP1) in its free state. Furthermore, we use an orthogonal labeling approach to measure distance distributions in a complex between PTBP1 and Encephalo-MyoCarditis Virus RNA. Here PTBP1 is singly or doubly labeled with Gd^{3+} chelate complexes whereas the RNA sequence is labeled with one nitroxide radical at a certain nucleotide. The obtained long range distance constrains should give structural information on the arrangement of this nearly 100 kDa protein/RNA complex, which will be combined in future with short-range NMR constraints for structure calculations.

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Single-layer MoS_2 nanopores as nanopower generators

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Making use of the osmotic pressure difference between fresh water and seawater is an attractive, renewable and clean way to generate power and is known as 'blue energy'. Another electrokinetic phenomenon, called the streaming potential, occurs when an electrolyte is driven through narrow pores either by a pressure gradient or by an osmotic potential resulting from a salt concentration gradient. For this task, membranes made of two-dimensional materials are expected to be the most efficient, because water transport through a membrane scales inversely with membrane thickness. Here we demonstrate the use of single-layer molybdenum disulfide (MoS_2) nanopores as osmotic nanopower generators. We observe a large, osmotically induced current produced from a salt gradient with an estimated power density of up to 106 watts per square metre - a current that can be attributed mainly to the atomically thin membrane of MoS_2. Low power requirements for nanoelectronic and optoelectric devices can be provided by a neighbouring nanogenerator that harvests energy from the local environment - for example, a piezoelectric zinc oxide nanowire array or single-layer MoS_2. We use our MoS2 nanopore generator to power a MoS2 transistor, thus demonstrating a self-powered nanosystem.

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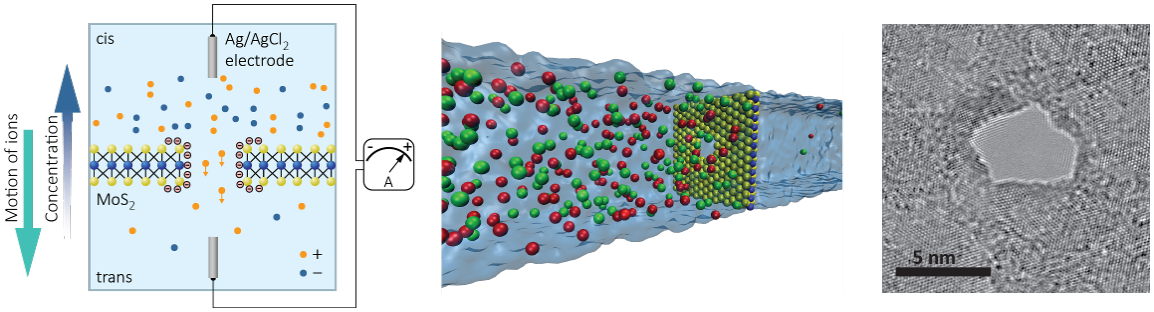

Self-assembled biomimetic nano-structures based on stimuli-responsive block-copolymers

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Microscopy and malaria rapid diagnostic tests (MRDTs) are the most common methods for malaria detection

1. However, they do not have the sensitivity which is needed for the mass detection of asymptomatic reservoirs of the infection in Malaria eradicating countries

2. More sophisticated and sensitive methods include, e.g., polymerase chain reaction (PCR). However, PCR tests involve complex instrumentation and expensive chemicals

3. We have developed a new assay for the detection of malaria based on the amplification of the metabolite Hemozoin (HZ). This reaction is the combination of a biocatalytic atom transfer radical polymerisation $(ATRP)_4$ and a precipitation polymerisation, HZ being the catalyst for such reaction. A correlation function between the quantity of HZ and the maximum rate of turbidity formation throughout the polymerisation was obtained, making this test a quantitative method for the detection of malaria. Here we present a highly sensitive malaria diagnosis method involving stable and cheap chemicals.

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Dynamics and structure of DNA: influence of counterion valency

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DNA solution structure is studied with dielectric spectroscopy (DS) at least since 1976[1]. Despite such a long run the researchers still do not agree about the nature of counterion relaxation in MHz range and the origin of the observed length scale L - whether the relaxation is perpendicular or parallel to the polyion and whther the free or Manning condensed counterions are the relaxing entities [2, 3, 4, 5, 6, 7, 8, 9]. Basically what was know was only that for semidilute solution $L \propto c^{-0.5}$ and for dilute $L \propto c^{-0.33}$.

As a control, for semidilute solutions of biopolelectrolytes we measured de Gennes correlation length (polymer mesh size) ξ by small angle X-ray scattering (SAXS) and found that it expectedly scales as $L \propto c^{-0.5}$ but is 5-6 times larger [10]. We tested also the scenario of denaturation of DNA. Here we observe a decrease in characteristic length scale L obtained with DS - this goes well with an expected decrease in ξ due to the simple fact that the number of chains in solution doubles. Consistent picture to be built from these findings is that the free counterions relax perpendicular to the polyion and the characteristic diameter of the relaxation volume L is the distance from the polyion at which the potential felt by the counterions falls to kT, as shown in the figure. In parallel, in order to be able to deal with the free counterion concentrations and estimate the counterion extent around the polyion we measured the Manning condensation coefficient via conductometry and also the osmotic coefficient related to the Manning coefficient we obtained by SAXS [11, 12].

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Characterization of the globin coupled sensor from Geobacter sulfurreducens using native mass spectrometry

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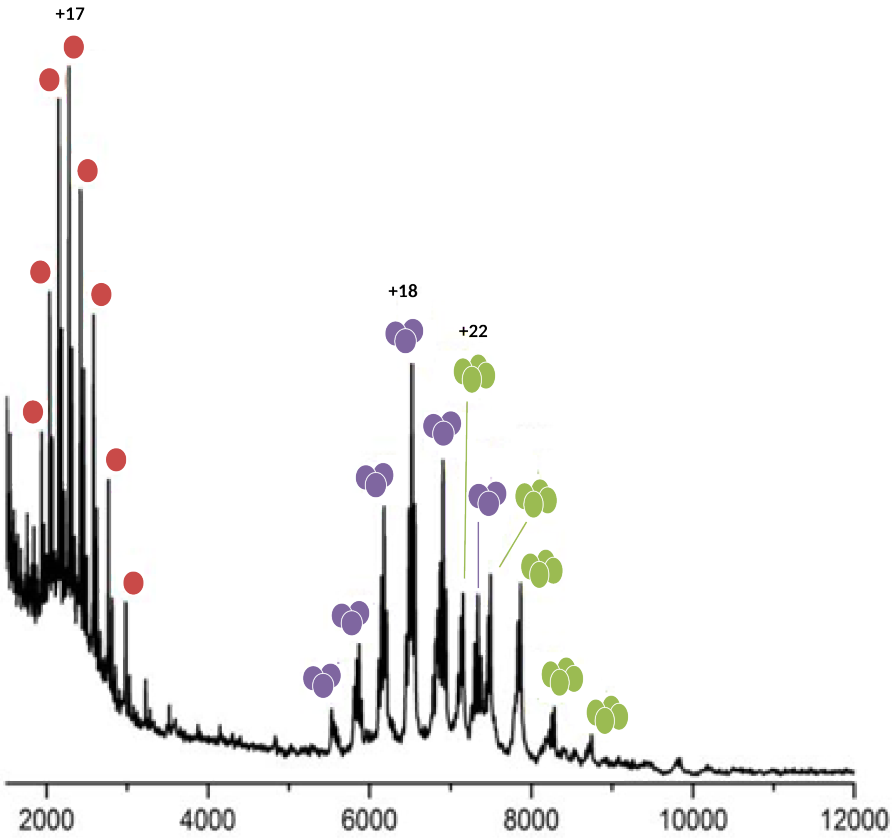
Globins are a family of globular proteins, present in all kingdoms of life, that share a distinctive structure, the globin fold. This globin fold incorporates a prosthetic heme group with a central iron atom responsible for the functionality. Bacteria and Archaea express globin coupled sensors (GCS), where a globin fold is attached to an additional transmitter region which can be (in)activated depending on certain changes in the environment of the organism. In this study we focus on characterizing GCS from Geobacter sulfurreducens (GsGCS), with a globin domain linked to a putative four transmembrane helical region. Common biophysical and structural techniques, such as crystallography, are still a bottleneck for analyzing integral membrane proteins (IMP). On the one hand, we suffer from poor expression levels compared to soluble proteins. On the other hand, IMPs require a hydrophobic environment, either in form of detergent micelles or nanodiscs, to solubilize the protein.

Recent efforts in native mass spectrometry, however, have demonstrated major progress for IMP characterization. The most critical step here is probably the electrospray ionization process, where the detergent- or lipid-embedded complexes are transferred from solution to the gas phase, where the IMPs can be released from the native-like environment by collisional activation. For this purpose, the ions are accelerated in a collision cell filled with inert gas such as argon. When critical parameters – the acceleration voltage together with the gas pressure in the cell – are tuned properly, the membrane proteins can be detected. The spectrum shown in figure 1 was acquired by using micelles of the detergent Triton X-100. In this spectrum we can clearly see monomers, trimers, and even tetramers. However, another spectrum acquired by applying micelles of the sugar-based detergent n-Dodecyl β-D-maltoside (DDM) highlights a charge state distribution only for monomers. Additional to that, a broad peak appearing in the m/z region between 5,000 and 6,000 indicates that there might be some oligomeric forms as well. This comparison highlights the importance of using the right detergent/lipid system for the embedded protein, as we were mostly only able to release monomers. Applying the highest collision energies (400 V) possible in the instrument probably leads to unfolding, indicated by higher charged monomers compared to the oligomeric forms. In future experiments, we will screen different detergents with GsGCS and study the behavior in the gas phase and the ability to release GsGCS under more gentle conditions, with the aim to obtain a better idea of the protein higher order structure.



Spectrum of GsGCS in Triton X-100 micelles, acquired on a Synapt G2 HDMS instrument.

Using MD simulations to improve the description of FRET labels on biomolecules

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Molecular dynamics (MD) simulations can provide detailed information about biomacromolecular dynamics on the atomic level.

Using MD simulations, we investigated a complex network of tertiary interactions in a guanine-sensing riboswitch aptamer domain (Gsw). Riboswitches are short genetic regulatory elements, which are commonly found in the 5' untranslated region of bacterial mRNA. Their aptamer domain can bind small ligand molecules with high specificity, thereby inducing a conformational change in the downstream expression platform. This conformational change results in alternative expression of the genes under the riboswitch's control. Even though the unbound state plays an important role in the gene regulation decision, detailed structural knowledge of this state and its dynamics is still scarce. We employed MD simulations with an aggregate simulation time of more than 10 μs in order to investigate the complex network of long-range interactions found in the Gsw. For this, we investigated the wildtype Gsw and a mutant with destabilized tertiary interactions in the loop region. Our results suggest a dynamic coupling between these tertiary interactions and the ligand binding region which is located $\sim$ 25 Åaway. Furthermore, we found this coupling to be dependent on the presence of Mg^{2+} ions.

As a next step, we will employ all-atom MD and coarse-grained simulations of biomolecules together with dye labels used in Förster Resonance Energy Transfer (FRET) experiments. Even though, parameters for such FRET dyes exist, modelling of their behavior on complex protein surfaces is still challenging. This is due to the different environments a label can encounter around a biomolecule. In order to help improving this description, we will also perform MD simulations of RNA, proteins in solution and membranes together with FRET labels. Comparing the results of the different simulation techniques with FRET experiments will help to optimize and speed up the label simulations.

Investigation of tautomerisation and protonation equilibria of nucleic acids with lambda dynamics

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SHORT TALK

Several nucleic acid bases exist in various tautomerisation and/or protonation states in physiological conditions, which can affect structure through the loss or gain of hydrogen bond, which in turn may affect functions. Conventional molecular dynamics simulation cannot take into account these equilibria since the protonation/tautomerisation states are fixed, but a special type of simulation called lambda dynamics can[1]. In this study, we focus on the protonation equilibrium of cytidine and tautomerisation equilibrium of pseudoisocytidine, in the formation of triplex helical structure. The work is still in preliminary stage as calibrations are needed to properly include the equilibria in the dynamics. The investigation will then be extended to tautomerisation equilibrium of a modified uridine in the ribosome decoding site[2].

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Sub-nanometer Carbon Nanotube Porins as Biomimetic Nanopore Sensors

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Here, we show that ultra-short 0.8-nm-diameter carbon nanotube porins, can spontaneously insert into lipid bilayers to form cation selective channels that exhibit unitary conductance of 7-10 picosiemens in the presence of 1M NaCl. These channels, which promote the formation of one-dimensional water wires, can support proton transport rates exceeding those of bulk water by an order of magnitude, while displaying small ionic current values, and high levels of permselectivity. We also show that the ionic conductance of these channels can be modulated by the presence of Ca2+ ions, in similar fashion to the protein channel GramicidinA. Our results illustrate the potential of small-diameter carbon nanotube porins as a proton conductor material and suggest that strong spatial confinement is a key factor in enabling efficient proton transport. We also show that by using a solid-state nanopore to act as a support and form a completely solvent-free bilayer system, we can observe extremely stable ionic currents through these nanotubes, in contrast to previous reports with solvent containing bilayers. These results establish these carbon nanotube porins as a promising biomimetic platform for developing cell interfaces, creating stochastic sensors, and water desalination.

A GPCR as a Michalis-Menten enzyme: Understanding biased signalling of vasopressin V2 receptor

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SHORT TALK

Many GPCRs signal through several different G protein subfamilies as well as β-arrestins. Agonist-induced preference for one pathway over another depends on the cellular context of the receptor and the properties of the agonist-bound GPCR. Here we present a simple approach to model G protein activation based on the Michaelis-Menten formalism. It shows the influence of different system properties such as receptor amount and activity (k_{cat}), the Michaelis constant K_m of the receptor for a specific G protein and the conversion of active Gα back to inactive heterotrimeric G protein on the signalling output. Our model improves the understanding of signalling data by assigning molecular explanations to the observed signalling behaviour. We chose Vasopressin V2 receptor (V2R) as a model and used BRET biosensors to measure ligand-induced activation of the different G protein pathways (Gαs, Gαi, Gαq, Gαz, Gα13...). We found that V2R activates all G protein subfamilies but not all proteins within these families with its native ligand vasopressin. However, substitution of single amino acids in the peptide ligands gave rise to decreased ligand affinities, efficacies and functional selectivity at different G proteins.

Free energy calculation on stability of the 14-3-3 protein

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Proteins of the 14-3-3 family are important modulators of signalling proteins, regulating critical biological activities such as cell cycle control, cell growth, proliferation, and apoptosis [1, 2, 3]. 14-3-3s are associated with oncogenic proteins, such as Raf-1, Bcr-Abl, Bcr, and polyoma middle T-antigen, and are particularly abundant in the mammalian brain where they have been associated with the pathophysiology of various neurological and neurodegenerative disorders, including Creutzfeldt-Jakob, Alzheimer's, Parkinson's and polyglutamine repeat diseases [4, 5, 6].

Free-energy calculations in the framework of classical molecular dynamics simulations are nowadays used in a wide range of research areas including thermodynamics of solvation, molecular recognition, and protein stability and folding [7].

In this work, we were able to observe the stability of the 14-3-3ζ protein in its natural homodimeric and monomeric form using plain molecular dynamics simulation. Alchemical free energy calculations were used to predict the stability of the protein upon a mutation in one of the central helices of the protein. Our predictions largely agree with experimental melting temperatures. Hydrophobic residues at the dimerization interface play a crucial role in dimer formation. Experiments have suggested that a decreased hydrophobicity at the interface has a significant impact on sustaining the dimeric form. The changes in dimerization free energy were calculated after introducing the double mutations L12E and M18K at the interface.

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Binding mode prediction using free energy perturbations

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SHORT TALK

Our group has recently published a protocol for the computation of the effect of site directed mutagenesis (SDM) on ligand-binding affinities, based on the free energy perturbation (FEP) methodology [1, 2, 3]. The protocol was thoroughly applied to characterize both agonist (NECA) and antagonist (ZM241385) binding to the adenosine A_{2A} receptor, with excellent results. We used this protocol to characterize the binding mode and SAR of novel A_{2A} antagonist scaffolds recently published[4, 6]. The in silico exploration is integrated with all available experimental data publicly available for the compound series reported by Heptares. This includes crystal structures, pharmacological data/SAR and biophysical mapping (BPM) data on three ligand series for 8 alanine mutations. We considered different binding modes obtained with GLIDE-SP[6] , as illustrated for the triazine derivative in the figure (green and grey for both pose and bars). The binding modes proposed in the original publication[5] (in grey) was revealed as the most promising based on correlation with the mutagenesis data. Finally we used our protocol to rationalize the binding mode and affinities of a series of chromones, which core scaffold has low similarity to traditional xanthine and triazine based A_{2A} antagonists.

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CEED: A Novel Biophysical Approach to Altering Protein Stability and Enzyme Activity

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Enzyme thermal stability can potentially be engineered through site-directed mutagenesis. This is desirable not only for the industrial use of enzymes, but also to study fundamental concepts in the biophysics of protein folding and enzymology. Increased stability is achieved through semi-rational approaches, such as large scale robotics screens. Results from these approaches are typically poor, and the most common outcome is protein with only a small increase in thermal stability and a significant loss of activity. We are developing a novel protein engineering approach based on understanding intramolecular protein dynamics. The CEED (Computational Enzyme Engineering with Dynamics approach combines in-silico structure calculation and simulation with recent advances in experimentally detecting the contribution of protein dynamics to enzyme catalysis. The approach predicts and detects intramolecular protein dynamics and seeks to optimise the rigidity of the protein whilst retaining the native flexibility of the active site. The process involves an initial screen using all atom flexible motion calculations from a known starting structure using FIRST (Floppy Inclusions and Rigid Substructure Topography) which will be validated with molecular dynamics simulations. This identifies site-specific variants that alter thermal stability but do not impact on enzyme activity or substrate binding. We then experimentally verify the dynamic alterations by monitoring the difference in heat capacity (ΔCp) between the ground and transition state. We have validated our approach with two different experimental systems including a terpene synthase (tau-muurolol synthase, SSCG_03688) and a monoamine oxidase (MAO-B). We find that by monitoring and manipulating protein dynamics not only are we able to optimise the thermal stability of the protein, but also the optimum temperature of the enzyme catalysed reaction. These findings suggest CEED will be a powerful biophysical tool for protein and enzyme engineering, and will contribute to the ongoing debate regarding the role of protein dynamics in enzyme catalysed reactions.

Preparation and Structural Characterization of Human NK Cell Activating Complex NKp80:ACIL

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Natural killer cells (NK cells) play a key role in recognition and elimination of infected, stressed or malignantly transformed cells. Recognition is promoted by surface NK cell receptors. NKp80 and its myeloid-specific activating ligand AICL are both C type lectin-like receptors with C-type lectin-like domain (CTLD)[1]. Activation of NK cells through AICL:HER2-scFv, while HER2 receptor is hidden and AICL, as NK cell activating ligand, is shown on tumor surface, was described recently[2]. Structure solution of this receptor-ligand complex and structural description of its interaction interface might be helpful for design of novel anticancer immunotherapeutics based on NK cell cytotoxicity.

We used mammalian expression system of modified human embryonic kidney cell line 293 (HEK293) to produce glycosylated NKp80 ectodomain. With PiggyBac system we were able to create stable cell lines expressing soluble extracellular parts of NKp80 in inducible way. AICL ectodomain contains odd cysteine which is not conserved, compared to other CTLD receptors. After mutation of this cysteine to serine (C87S mutation) the yield as well as stability of prepared protein are greatly enhanced compared to wild-type construct.

Thanks to these approaches, we are able to produce both proteins in sufficient amount to initiate structural studies using analytical ultracentrifuge, dynamic light scattering and finally crystallization of this immunocomplex.

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Resolving the Architecture of the Integrin Proximal Adhesome Complex

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Integrins are α/β heterodimers that mediate cell adhesion to the extracellular matrix (ECM) and thereby control cell polarity, motility, proliferation, differentiation and survival. Each integrin subunit has a large ectodomain, a single transmembrane (TM) domain and a short cytoplasmic tail (13-70 amino acids), which lack enzymatic and actin-binding activities and hence rely on the recruitment of adaptor and signaling molecules for signal propagation[1]. The entirety of these proteins is termed the integrin adhesome[2]. It is generally believed that the assembly of the adhesome is triggered upon binding of certain core proteins such as talin, kindlin, integrin-linked kinase (ILK), PINCH and α-parvin to the integrin tails, forming an initial integrin proximal complex. It is less clear, however, how these proteins interact with the different integrin tails and with each other to induce integrin type-specific functions. To address this issue, we produce a selected number of adhesome proteins in E. coli, High Five insect cells or HEK293 cells and purify and then characterize them biochemically and biophysically. We determine binding affinities of the individual components towards each other by microscale thermophoresis (MST) and analytical ultracentrifugation (AUC) and assemble them into large multi-protein complexes to gain information about structure and interacting interfaces. To this end, these complexes are chemically crosslinked, proteolytically cleaved and analyzed by LC-MS/MS. Our long-term objective is the reconstitution and structural analysis of complexes consisting of the innermost adhesome components, centered around β1 or β3 integrin tails and to determine potential differences in structure and/or composition.

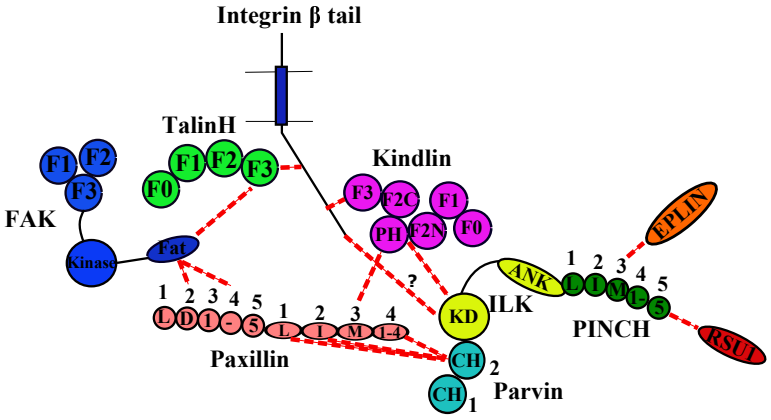

Fig. 1: Components of the integrin proximal complex and their reported interaction sites.
Talin and kindlin interact with the cytoplasmic tail of the intergin β subunit. ILK, PINCH and Parvin (the IPP complex), focal adhesion kinase (FAK) and paxillin are also recruited to the early adhesome complex. These proteins, together with numerous others, form an intricate network that is strongly influenced by the integrin β tails.

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Computational design of histone deacetylase inhibitors as epigenetic agents targeting cancer

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Cancer belongs between the most prevalent causes of death in developed countries and its therapy is not sufficient. Thus, development of new cancer therapeutics is of utmost importance. Histone deacetylase (HDAC) inhibitors represent new class of anticancer therapy[1]. 11 HDAC isoforms take part in epigenetic regulation, influencing transcription of genes, often oncogens. Intriguingly, tumor cells are more responsive on HDAC inhibitor treatment than normal cells, enabling selective tumor cells targeting. In my dissertation, we have focused on HDAC4 isoform which plays roles in renal carcinoma, several breast cancers and acute promyelocytic leukemia and its inhibition slows down angiogenesis[2]. In the pursuit of designing new HDAC4 inhibitors, we have computationally explored chemical space around trisubstituted diarylcyclopropane-hydroxamic acids, molecules similar to those synthesized by Bürli et al.[3].

We have created three different quantitative structure-activity relationship (QSAR) models that correlated computationally calculated interaction energy with experimental inhibitory activities of the molecules synthesized by[3]. First QSAR model was based on molecular docking. The second on calculation of interaction energy between ligand and receptor by means of molecular mechanics (MM) and the third was based on combination of MM with quantum mechanics (QM/MM). Afterwards, combinational library of $\sim$ 20 thousand molecules similar to [3] was docked into the HDAC4 structure. The best scoring analogues were further processed to predict their binding affinities using MM and QM/MM methods. Five compounds with low nanomolar inhibitory activities were finally recommended for synthesis as potent HDAC4 inhibitors.

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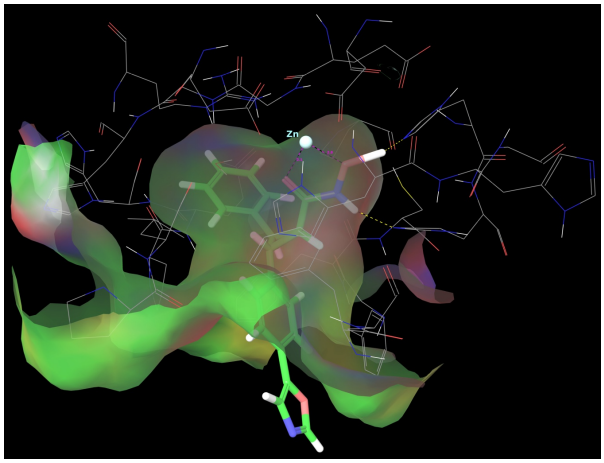

Active site (with its surface) (represented as lines) of histone deacetylase 4 co-crystallized with reference synthesized inhibitor (represented as tubes)

Exploring NTS1 helix 8 conformation and dynamics in lipid environments

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SHORT TALK

G protein-coupled receptors (GPCRs) are the largest class of cell surface receptors encoded in the human genome, and are of considerable biological and pharmacological significance. They exhibit high levels of structural plasticity, which gives rise to poor thermostability outside of a membrane environment and hampers crystallisation efforts, necessitating the use of thermostabilising modifications. Crystal structures of thermostabilised neurotensin receptor 1 (NTS1) constructs are inconsistent on the presence and extent of the secondary structure element helix 8 [1]–[3], an element that has been implicated in G protein & β-arrestin activation for other GPCRs. Here, the dynamics and secondary structure of the relevant sequence is analysed using a non-thermostabilised receptor in a functionally supporting membrane environment of native-like lipid composition, by employing site-directed spin labelling and continuous-wave electron paramagnetic resonance (CW-EPR). The data reveal the ability of the lipid environment to promote helicity in regions of the sequence, and how the dynamics of this sequence are modulated by the activation state of the receptor. These data are supported by circular dichroism and molecular dynamics simulations in different lipid environments, showing that a native-like mixture of lipids gives rise to the greatest helical content for the helix 8 sequence, and revealing specific protein-lipid contacts that account for this lipid dependence.

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Nanopores in MoS2 opened atom by atom

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Ultrathin nanopore (NP) membranes based on 2D materials have demonstrated ultimate resolution toward DNA sequencing. Among them, molybdenum disulfide (MoS2) shows long-term stability as well as superior sensitivity enabling high throughput performance. The traditional method of fabricating NP with nanometer precision is based on the use of focused electron beam in transmission electron microscope (TEM) which is a time-consuming, expensive and not scalable process. Recent improvement was NP formation by controlled dielectric breakdown of a 20nm thick Si3N4 membrane immersed in an electrolyte solution[1], the method which may be executed by the NP measurement setup itself. We developed an analogous method for controllable NP creation in single-layer MoS2 with subnanometer precision where an electrochemical reaction (ECR) opens the pore in an atom-by-atom manner [2]. ECR as a scalable, low-cost and accessible technique represent the enabling concepts for the design of a functional DNA sequencing device which will require parallel integration of NPs into arrays.

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Nucleic acids and condensing proteins in viral confinement

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SHORT TALK

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]. Similar packing mechanisms have been found in adenoviruses where DNA-condensing proteins encoded in the viral genome condense the DNA in the capsid[2]. The packing of the DNA and protein mixture inside adenoviruses lacks any icosahedral order characteristic of the virus protein coating, and indicates that the DNA and proteins form a strongly confined and dense phase of soft particles (adenosomes) without a strictly defined DNA backbone[3]. We use molecular dynamics simulations to probe packing of DNA with condensing proteins in confinement. We study the effects of genome length, protein content and DNA-protein attraction on packing. Results indicate different regimes of DNA and protein mixing with implications for achieving optimal packing of DNA.

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Design of artificial (β/α)8-barrel proteins

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The de novo protein design is an emerging field in biochemistry in which artificial proteins are first designed in silico and then validated experimentally. This allows to expand the general protein knowledge, (mainly based on natural proteins), taking in consideration aspects as folding, stability and solubility.

My group is a pioneer in the design of artificial $(\beta/\alpha)_8$-barrel proteins (called Octarellins[1]). This fold is extremly interesting because it is widespred in nature (10

First, 4000 artificial backbone structures were created with the use of modeling packages Rosetta and Modeller. 54 out of them were selected as targets for the following steps of sequence design and energy minimization (10 cycles), in order to find the best sequence to fit each target. More than 10000 different artificial sequences were created and different selection steps were performed, taking in account parameters as amino acid composition, secondary structure prediction, molecular dynamic simulation and ab initio folding.

Five models were finally chosen for gene synthesis and experimental validation, and are been tested for expression in E. coli and preliminary purification.

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The structural basis for the recognition of the „Phosphorylation Barcode“ of the G protein-coupled receptors by arrestin

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SHORT TALK

G-protein coupled receptors (GPCRs) play a crucial role in transmembrane cell communication. In addition to activating G proteins, receptors are regulated and also signal via arrestins. These proteins recognise phosphorylated active states of GPCRs. There are four arrestins, of which two are specific for the visual system and two are “universal” for all other receptors. This leads to the questions: How do these proteins couple to over 800 different GPCRs and activate various independent signaling pathways? Are there multiple specific conformations which arrestins may adopt by recognising a specific phosphorylation pattern, the so called “phosphorylation barcode”? We are addressing these questions by a combination of biophysical and structural methods. We identified important phosphorylation positions on the C-terminal end of rhodopsin and vasopressin V2 receptor using a peptide array containing every possible permutation for up to three phosphorylations. Subsequently, we are investigating by nuclear magnetic resonance if different phosphorylation patterns induce conformational changes and distinct conformations in the arrestin.

Sliding of microtubules in the bridging fiber segregates chromosomes by creating poleward flux of the attached k-fibers

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At the onset of mitosis microtubules form a spindle, which is responsible for proper segregation of chromosomes between two daughter cells. During anaphase, transport of chromosomes towards opposite spindle poles is facilitated by depolymerization of k-fibers, either on their plus ends or on their minus ends (poleward flux). We have recently shown that microtubule bundles termed bridging fibers link sister k-fibers and balance the forces between them in metaphase [1, 2]. However, the role of bridging fibers in chromosome segregation has not been investigated. Here we show, by using photoactivation of tubulin tagged with photoactivatable GFP in human U2OS cells, that antiparallel microtubules in the bridging fiber slide apart, resulting in poleward flux of the bridging fiber. The associated k-fiber undergoes poleward flux at the same velocity as the bridging fiber. Spindle poles separated at a velocity that was slower than sliding of bridging microtubules, as expected in the presence of poleward flux. These data together suggest that the sliding of microtubules in the bridging fiber generates poleward flux of the k-fibers and separation of the spindle poles. In addition, we found that in early anaphase the spindle length increased while its width decreased, which was associated with a slower extension and faster poleward flux of the outer bundles consisting of sister k-fibers and the bridging fiber, compared to the inner bundles. In late anaphase, poleward flux velocities were similar in all bundles. We conclude that sliding of microtubules in the bridging fiber is responsible for chromosome segregation by causing poleward flux of the attached k-fibers and separation of the spindle poles.

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Functional and structural characterization of human Cannabinoid CB2 receptor

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G protein-coupled receptors (GPCRs) are membrane proteins that transmit extracellular chemical and visual signals across the plasma membrane. When activated by a ligand GPCRs undergo a conformational change leading to activation of G protein, arrestin and other signaling pathways. GPCR malfunction is often associated with disease, which makes them a prime target in drug discovery. [1] Human cannabinoid CB2 receptor is a GPCR involved in regulatory processes in our body and therefore holds the potential for treating various inflammatory conditions, such as pain, osteoporosis and Alzheimer's disease.[2] My research is focused on determination of CB2 structure and understanding its signaling pathways. The receptor is expressed in mammalian expression system and purified on small scale using affinity chromatography. Conditions for CB2 isolation from the membranes were optimised by main protein peak quality evaluation by fluorescence assisted size-exclusion chromatography (FSEC). In order to make it more susceptible for structural and biophysical studies, CB2 will be stabilized using both rational and systematic site-directed mutagenesis. Additionally, signaling events upon CB2 activation will be studied by bioluminescence-based biosensors and a selection of CB2 ligands will be screened for biased signaling.

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Phycocyanobilin, a bioactive tetrapyrrolic compound, binds to human serum albumin and enhances its stability

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Human serum albumin (HSA) is an important regulator of the pharmacokinetic properties of bioactive compounds. Phycocyanobilin (PCB) is a blue tetrapyrrole chromophore of C-phycocyanin with proven health-promoting activities. The aim of our study was to examine binding of PCB for HSA and to investigate its effects on protein stability. Based on a computational approach, we demonstrated two putative high-affinity binding pockets on HSA of virtually identical energies (subdomains IB and IIA). Results obtained by protein and pigment fluorescence measurements, circular dichroism (CD), and bilirubin (structural analog of PCB)-displacement experiments confirmed high affinity (binding constant of 2.2×10^6 M^{-1}), stereoselective binding of PCB M-conformer to HSA and its competition with warfarin (subdomain II A marker) and hemin (subdomain IB marker). Fluorescence and UV/VIS absorption spectra indicated that PCB underwent conformational change from cyclic to more stretched conformation, upon binding to HSA. CD and fluorescence melting curves of HSA in the presence of PCB showed increased thermal stability of HSA upon chromophore binding. Trypsin digestion study showed that HSA-PCB adduct was more resistant to proteolysis than free HSA. Fourier transform infrared spectroscopy and CD spectra have revealed slightly higher alpha-helical content in HSA-PCB adduct than in free protein. The present results provide valuable information for the transportation and distribution of PCB in vivo, which may be of importance for the understanding of its numerous beneficial effects, including partial stabilization of HSA as a consequence of PCB binding.

Mechanistic study on catalase activation by curcumin

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SHORT TALK

Being tailor of biological molecule, curcumin (diferuloylmethane) is the important antioxidant flavor which has been widely reported as an effective component in treating and reducing complications of many diseases. Studying the effect of curcumin as an effective antioxidant on proteins and enzymes which are involved in diseases has not only theoretical significance but also clinical applications. In our study, while curcumin has been shown to significantly increase catalase activity, its mechanism of action was examined. Here, using a series of biochemical, biophysical techniques and computational methods, we demonstrated that curcumin activates bovine liver catalase (BLC) by modifying hydrophobic pocket of enzyme. Docking studies indicated best position of ligand binding based on binding energy then molecular dynamic simulation data showed that curcumin increases the size of the bottleneck in the narrow channel of BLC and induces conveniently access of substrate to the active site. On the other hand, the increase in emission of intrinsic fluorescence in modified BLC ruled out possibility of less quenching in the presence of curcumin which may be due to increase distance between tryptophan and quenching groups. Also we used Circular dichroism (CD) spectroscopy to determine how curcumine may alter secondary structure of enzyme. Catalase spectra in the presence of various concentrations of curcumin showed an increase in amount of α-helix content.

Coupling cellular membrane shapes with localization of viral proteins

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The effect of shape on the distribution and function of proteins in living cells has so far received little attention from physicist and could benefit from a thorough investigation. Cellular morphology has the potential to affect cellular functions at different scales. Positive and negative curvatures are found in structures like filapodia and invapodia or during the release of progeny viruses from infected host cells[1]. Membrane proteins play a key role in the proliferation of disease and the affinity of these proteins for lipid ordered phases and for high curvature regions can play an important part in the recruitment of proteins and the formation of the viral bud. One such protein is the neuraminidase (NA), which is responsible for enzymatically hydrolyzing sialic acid residues to facilitate release of the progeny virus from the infected cell after completion of the viral assembly. Using a quantitative assay developed in the research group[2] we can asses the concentration of NA on membrane tubes pulled from HEK293T cells overexpressing GFP-NA. By including a curvature insensitive membrane dye we can quantify the curvature sensing of GFP-NA by comparing the ration of NA-GFP to dye intensity on the tube as well as on the flat part of the cell membrane.

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Understanding structures & functional dynamics of membrane proteins using EPR spectroscopy

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SHORT TALK

Work in the Henry Wellcome Unit for Biological EPR is focused on elucidating structural features and functional dynamics of membrane proteins, particularly membrane transport systems. Membrane proteins assemble into nano-machineries which function through a concerted action with high specificity observed in both time and space. Our aim is to unravel the principles underlying the architecture and mechanisms of these macromolecular systems. Our approach focuses on the use of Electron Paramagnetic Resonance (EPR) spectroscopy techniques in combination with complementary molecular biological, biochemical and biophysical methods including theoretical MD approaches.

Here, the recent results from a range of collaborative projects involving medically-relevant membrane proteins and membrane transporters are presented. These projects draw on the use of a variety of EPR methodologies, from room and low temperature continuous wave (cw-)EPR at various microwave frequencies to advanced pulsed EPR techniques. These studies deliver detailed structural and mechanistic insight into the systems, including observation of conformational changes within membrane proteins and identification of the molecular determinants of substrate binding.

The collaborative efforts include potential antibiotic target outer membrane proteins LptDE and BamABCDE (Prof Dong, University of East Anglia), the substrate binding protein of the manganese-specific ABC transporter in S. pneumoniae PsaA (Dr McDevitt, University of Adelaide) and the substrate binding domain SBD2 of the essential bacterial amino acid importer GlnPQ (Dr Cordes, University of Groningen).

Fluorescence kinetics of flavin adenine dinucleotide in different microenvironments

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Introduction Flavin adenine dinucleotide (FAD) is a fluorescent coenzyme with substantial functions in catalyzing redox reactions. The fluorescence originates from the isoallaxazine group of the molecule and its decaying kinetics depends highly on the interaction with the neighbouring adenine group as well as other factors in its microenvironment[1, **?**]. The aim of this work was studying extensively the fluorescence kinetics of FAD in different enviroments measured on wide time and spectral range. **Method** Fluorescence kinetics measurements were carried out on $1.5\text{x}10^{-3}$ M FAD dissolved in water and its 1:1 mixture with different organic solvents and on flavocytochrome C sulfide dehydrogenase enzyme (FCC). The samples were excited at 400 nm by the second harmonic of a Ti:sapphire oscillator, the fluorescence was detected by a hybrid setup, combining the technique of fluorescence up-conversion and time-correlated single photon counting. **Results and Discussion** In water solution the preferred conformational state of the molecule is a stacked one, the fluorescence is highly quenched by the adenine group, interacting via an electron transfer process, leading to a fast decay[1, 3, 4]. In the mixture of water and ethanol, dioxane or DMSO the overall decay time of the FAD fluorescence markedly increased. In these solvents the weight of the planar conformation of the FAD molecule is higher than in pure water, resulting in less interaction between the two groups and hence in long-lived excited state. In contrast to this, when the FAD molecule is bound to FCC the slowest component attributed to the open conformation indicating further interactions with the amino acid residues.

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Density effects in entangled solutions of ring polymers

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Here, we employ extensive Molecular Dynamics computer simulations in order to provide a detailed description concerning the equilibrium and dynamical properties of ring polymers in solutions of different densities[1]. For comparison, we also discuss the same properties for their linear counterparts. It is recently argued[2] that the physical behavior of ring polymers in solution could reveal connections to the experimentally observed territorial segregation behavior of interphase chromosomes in eukaryotic nuclei. The fact that segregation is nonetheless observed points to the existence of mechanism beyond polymer chain entropy and confinement that affect the spatial distribution of chromatin. By construction, it is topologically impossible for unlinked ring polymers to interpenetrate each other, thus for long linear chromosomes, topological constraints play the same role as for ring polymers. Since, there is rapidly growing evidence that folding of the chromatin fiber inside the interphase nucleus has an important role in the regulation of gene expression, it was important to outline and understand elaborately the properties of rings solutions which have been implicated as a model for the generic behavior of interphase chromosomes.

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Positive/negative ion mode nano-electrospray ionization mass spectrometry of metallated peptides

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Mass spectrometry (MS) techniques have emerged as a powerful tool for the investigation of metallodrug-protein interactions. The aim of this study was to investigate the full potential of nano-electrospray ionization (ESI) MS for characterization of the binding of three Ru(II) complexes, synthetized as potential anticancer agents, to human serum albumin (HSA). The protein was incubated with each complex in 1:5 molar ratio for 24 h. After the incubation, the unbound complexes were removed by ultrafiltration and the ruthenated protein was subjected to tryptic digestion. The obtained peptides were separated on nanoAcquity Ultra Performance Liquid Chromatography and analyzed using Synapt G2-Si mass spectrometer. MSE as well as MS/MS of individual metallated peptides were acquired in positive and negative ion modes and analyzed manually or by ProteinLynx Global Server (PLGS). Several metallated peptides were detected in both, positive and negative ion modes for each HSA-complex adduct. In the positive ion mode, the peptides were detected primarily as multiply charged species while the negative ion mode almost exclusively favored single charged ions. Since ruthenium has a characteristic isotopic profile similar to multiply charged species, the differentiation between ruthenated and non-ruthenated peptides was significantly simplified in the negative mode. The target sequences were confirmed by elucidation of the MS/MS spectra. MSE data analyzed by PLGS revealed some of the sequences after each complex mass was marked as a variable side chain modification. To the best of our knowledge, this is the first successful study that includes investigation of metallated peptides in the negative ion mode and the first attempt towards automated single-run metallodrug target sequence identification. This work provides important structural informations and highlights the novel approach to using ESI MS for investigation of metallodrug-protein interactions. We believe the described MS E method and data processing can be further utilized for identification and discovery of potential metallodrug cellular targets that would help in elucidating mechanism of action of various metallodrugs.

Self-assembly of DNA-based anisotropic soft-patchy particles

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Patchy colloids constitute a relatively new class of materials in the area of soft matter which exhibits a wide variety of anisotropy in chemical functionality, size and shape. These new soft matter materials have attracted intensive attention mostly due to their potential to open novel programmed self-assembly pathways that are unknown for usual colloidal particles. Despite the substantial progress on the modelling and experimental aspect of self-assembly behaviour of spherical patchy colloids, the knowledge about their anisotropic counterparts is considerably less comprehensive, mostly due to significant synthetic challenges. Inspired by the hierarchical self-assembly concept with block copolymers and exploiting the unique DNA's physico-chemical properties, a novel family of DNA-based soft-patchy rods is constructed in this work, consisting of a rigid charged DNA and a neutral, thermo-sensitive flexible polymeric segment that are covalently connected. Small angle X-ray scattering (SAXS) measurements have been carried out in aqueous solutions of the DNA-based soft-patchy particles and revealed a formation of a rich variety of self-assembled structures with increasing complexity.

Calculation of Protein-Protein Binding Free Energies using Molecular Dynamics Simulations.

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Virtually all cellular processes involve interactions between proteins at some point. Furthermore, development of modern therapeutics focuses more and more on biopharmaceuticals, like antibodies. Therefore, it is highly advantageous to quantify the strength of protein-protein interactions *in silico*.

Despite recent methodological advances in the field of molecular dynamics (MD) simulations [1, 2], calculations of standard binding free energies of protein-protein interactions that are comparable to experimental results remain both complex and computationally demanding. This can partly be attributed to the apparent dilemma of introducing restraints to limit sampling to phase-space regions of interest, which in turn raises the need for numerical corrections of the result based on further simulations and theoretical considerations.

We present a novel approach to this problem using perturbed hidden restraints [3], which aims to simplify calculations by eliminating the need for complex corrections of the resulting free energy values. Given the fundamental path-independence of the free energy state function, desirable reaction pathways can easily be explored using this method. Moreover, the use of a collective coupling parameter λ for all restraints allows for the application of Hamiltonian replica exchange molecular dynamics (HREMD), a method commonly employed to enhance sampling in free energy calculations.

To demonstrate the presented method, we calculate standard binding free energies of model systems with experimentally determined complex structures, including the interaction of ubiquitin with a ubiquitin-binding motif of the human DNA polymerase ι [4]. We compare our results to experimental findings and discuss future challenges.

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The microcsopic structure of hot and cold alcohol mixtures

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SHORT TALK

Water and associating liquids constituate the environment where the vast majority of biological processes take place. Also, the physical principles that govern large scale associations and assembly of biological complexes are also responsible for the local structuring in liquid mixtures. In this work, we're looking into the ordering in aqueous alcohol mixtures. It is well known that, under ambient conditions, those mixtures have a pronounced micro-heterogeneous structure where water and alcohol form locally segregated domains [1]. What happens in colder conditions? How does this micro-heterogeneous structure evolve? By using computer simulations, we investigate the changes in microstructure with temperature and concentration and show that it depends both on the topology of the methyl groups and the evolution with temperature of the charge ordering of the charged atomic sites. This evolution with temperature is followed through the static atom-atom distribution functions as well as Kirkwood-Buff integrals and atom-atom structure factors.

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Position dependent fluorescent properties of coupled fluorescent dyes in RNAs and proteins

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High-precision Förster-Resonance-Energy-Transfer (FRET), used in combination with FRET-restrained structural modeling, electron paramagnetic resonance (EPR) and molecular dynamics (MD) simulations, is an effective multidimensional approach to determine the structure of nucleic acids and proteins. It has been shown that the characteristics of the dye linkers[1] and the dye environment[2] influence the fluorescent properties of the dyes in FRET. Here, the study is carried out on the helical junctions in RNAs and the model protein, Lysozyme of the Bacteriophage T4 (T4L) to assess the effect of the local environment on the fluorescence properties of the dyes; which in turn affect the accuracy of distance determination. The labeling sites in RNA were evaluated by studying 73 single labeled molecules and quantitative FRET studies were conducted on 273 FRET pairs with Alexa488 as donor and Cy5 as acceptor to characterize the position dependent fluorescent properties of coupled fluorescent dyes. The results of our study show that labeling at the ends of the helices and at the junction region lead to unexpected behavior of donor and acceptor dyes. In the next part of our study, the T4L protein variants, with the acceptor Alexa647 and one of the donors, Alexa488, Atto488 or BODIPY dyes with different linker lengths, are investigated. It can be seen that properties like dye linker length, dye distribution and mobility affect the determination of distances. Thus a hybrid approach involving FRET, EPR and MD techniques is expected to increase the resolution and accuracy of label-based techniques.

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Simple and complex disorder in binary mixtures

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We aim to classify binary mixtures according to whether they contain hydrogen bonds forming liquids (water, alcohols) or not (benzene, cyclohexane, toluene, carbon tetrachloride), into complex and simple disorder. We define complex disorder as the presence of microheterogeneous spatial associations of molecules with similar interactions, like hydrogen bonding ones, where simple disorder represents the totally homogeneous mixture. For that purpose we compare experimental and simulation results for thermodynamic, structural (radial distribution factor, structural factor and Kirkwood-Buff integral) and dynamical (diffusion coefficient) data. This analysis clarifies the role of the concentration fluctuations versus that of micro-heterogeneous structures. This is done mainly analyzing the k=0 values of the structure factors S(k) and those corresponding to the pre-peak. The existance of structure factor prepeaks at small k, arising from periodicity in radial distribution functions should imply the microheterogeneous structural ordering. The structure factor value at k=0 tells us about the concentration fluctuations which are, with the help from statistical physics, related to the experimentally measureable quantities such as isothermal compresibility. Understanding the microheterogeneity and structural behavior on molecular level can be useful in understanding more complex biological structures and processes.

Revisiting organometallic chemistry by ion mobility

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The classes of shape of molecules or ions containing a central metal atom have been historically organized using qualitative methods such as Valence Shell Electron Pair Repulsion (VSEPR) or "Gillespie" rules. In a more theoretical manner, the Crystal Field Model describes the electronic structure and the geometry of such complexes. Even if the geometry is the basis for classification, very few direct measurements are available. The geometry of the complexes is deduced from indirect spectroscopic measurements, or, is obtained from crystallographic data.

The aim of this project is to revisit the geometric classification of central metal complexes in the absence of solvent, when only intrinsic properties of the partners play a role, among which the formal oxidation state of the cation, the steric hindrance of the ligands, their binding energies, are key factors governing the reactivity of systems essential for catalysis. All these properties can be accessed using 'Ion Mobility' coupled with mass spectrometry (MS) supported by action spectroscopy of trapped ion and theoretical calculations.

In the case of carboxylate ligands, the preliminary results show a linear relation between the CCS of the complex and the mass of the ligand. This smooth increase indicates the absence of steric hindrance. From energy resolved MS/MS, the V50 values are similar, as expected for ligands with similar electronic properties. Comparing the evolution of the CCS of the complexes with the CCS of the ligands alone, a predictive incremental value can be determined. In the case of inorganic ligands (chlorine, bromine, iodine) different trends in the CCS in terms of m / z, associated with the number of ligands of a complex, suggests a distinction between the tetrahedral, trigonal planar, linear and bent geometries. In the case of complexes based on organic monodentate (carboxylates) and polydentate (oxalate and citrate) ligands, different trends were observed as compared to the inorganic complexes. This should result from the density effect compared to their halogenated counterparts and from the number coordination bonds. This work has also revealed changes in the oxidation state of the metal center of the complex either in solution or in the gas phase after activation.

Structural characterization of the transiently populated, redox-sensitive HSP27 monomer

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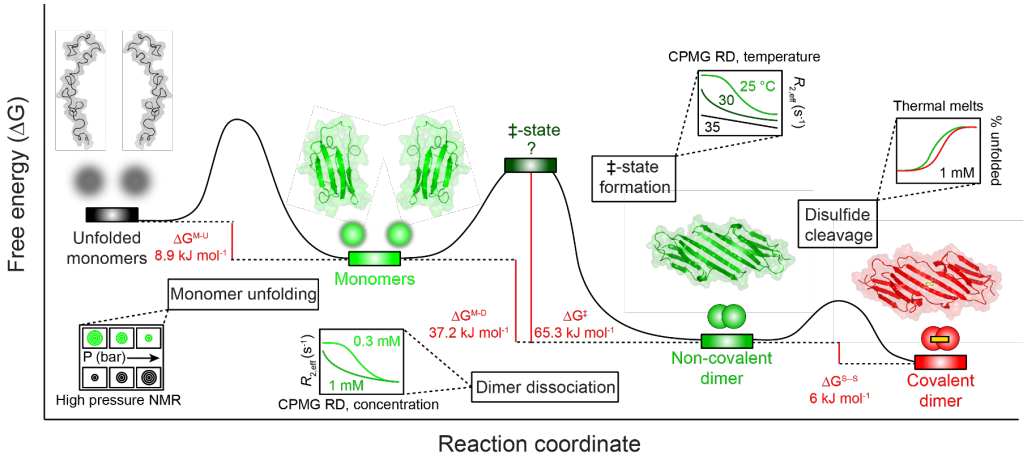
Ubiquitously expressed throughout the human body, the small heat shock protein (sHSP) HSP27 is an essential molecular chaperone that forms an ensemble of dynamic, polydisperse oligomers ranging from 45 to 1000 kDa in mass. HSP27 is a known regulator of intracellular redox state, and an intermolecular disulfide bond within the protein enables accession of reduced (non-covalent) and oxidized (covalent) dimers. However, detailed investigations of the structure and dynamics of HSP27 have been limited by the heterogeneity of this system. Here, we analyzed redox regulation of the structure, conformational dynamics, and molecular function of both full-length HSP27 and its excised α-crystallin domain (ACD). We found that the chaperone activity of full-length HSP27 is modulated by both the redox state and total chaperone concentration. With high-pressure NMR and ^{1}5N Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion (RD) measurements, we provide the first structural and kinetic characterization of the transiently populated HSP27 ACD monomer and a complete thermodynamic analysis of the ACD dimer-to-monomer energy landscape. Based on experimental NMR data, our structural model of the HSP27 ACD monomer reveals the formation of partially disordered conformations near the broken dimer interface. Collectively, our results demonstrate that the disulfide bond in HSP27 regulates molecular function through control of conformational dynamics that restrict the amount of monomer.



The new vitamin B12 degradation product transporter BtuM

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SHORT TALK

Cobalamin (vitamin B12) is an essential co-factor in all kingdoms of life (with the exception of plants), but can only be synthesized by a handful of bacteria. Therefore, the ability to take up cobalamin is indispensable for most organisms, including the majority of prokaryotes. The only characterized prokaryotic uptake system is the Escherichia coli ABC-transporter BtuCD-F. However, many prokaryotes that require cobalamin for their metabolism and are devoid of cobalamin de novo synthesis-capacities lack a BtuCD-F homologue and therefore must take up the vitamin by means of yet unknown uptake systems. Genome mining has identified one potentially new prokaryotic uptake system exclusive to Gram-negative bacteria, BtuM, which does not share sequence homology to any known transporter and has five predicted TMs. On the chromosome the gene encoding BtuM is constitutively associated with the cobinamide adenosyltransferase BtuR and the outer membrane active transporter BtuB. Additionally, translation is controlled by a B12-regulatory element suggesting a role in vitamin transport. We tested if BtuM from Thiobacillus denitrificans, hence called BtuMTd, is involved in transporting vitamin B12. We show that an E. coli knock-out strain that needs a functional vitamin B12 transporter for survival, can grow when expressing BtuMTd. This means that BtuMTd is a new cobalamin uptake system. Purification of BtuMTd in the presence of cobalamin shows that the substrate co-elutes with the protein, however the affinity is too low to be measured by isomeric titration calometry (ITC). Because BtuMTd exhibits a KD of 700 nM to cobinamide, a vitamin B12 derivative, we propose that this is the real substrate of BtuMTd. We further demonstrate that BtuMTd, is a monomer in vivo and used static light scattering analysis to confirm the monomeric state and molecular weight of 22 kDa in detergent solution. BtuMTd is the first prokaryotic cobinamide transporter that does not belong to the ABC transporter superfamily, which makes it an intriguing new uptake system from a phylogenetic, structural and mechanistic point of view. The genetic organization with BtuR suggests that the mean of transport is facilitated diffusion with subsequent trapping. Cobalamin and cobinamide transporters might also be targets for new antimicrobial drugs because scavenging the co-factor is essential for many pathogens and humans take up the vitamin by means of endocytosis.

Site-Directed Spin Labelling of the Splicing Regulator hnRNP A1 for the Study of its Intrinsically Disordered C-terminal Domain

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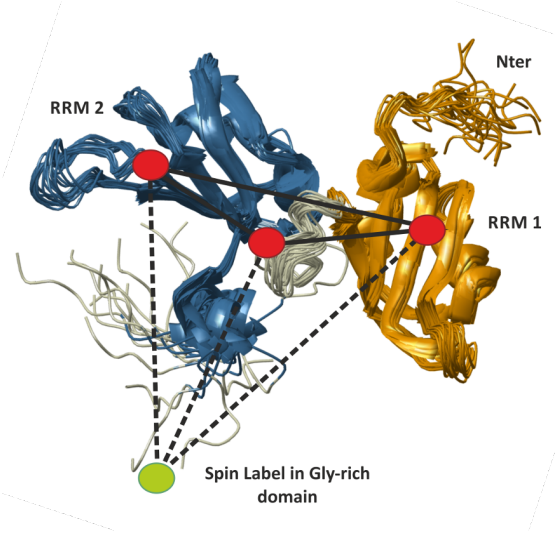
The human protein hnRNP A1 is known for its regulatory function in splicing [1]. Cooperative binding of individual hnRNP A1 units upon target RNA recognition has been proposed as a regulatory mechanism and the intrinsically disordered C-terminus (residues 197-320) is believed to be the propagator of this process [4, 5]. Due to its flexibility, structural information on this domain remains difficult to obtain with commonly applied structure determination methods, such as NMR or X-ray crystallography. The structure of a proteolytic fragment (residues 1-196), which by itself is known as UP1, has been solved [6, 7]. Based on this core structure we develop a trilateration approach for understanding the function of the disordered C-terminal domain in multimerisation. Using site-directed spin labelling and DEER-based distance measurements [8] we aim to construct a coarse grained model of this domain. Sites in the structured domain suitable for SDSL have been selected based on multi-scale modelling [9] and single cysteine mutants were expressed and labelled with a nitroxide spin label with high labelling efficiencies. The suitability of these positions for DEER distance measurements towards the intended trilateration procedure will be discussed.



Site-Directed Spin Labelling strategy for the trilateration of the unstructured domain of hnRNP A1. Three labelling positions are chosen in the structured domain for DEER based distance measurements (schematically displayed in red). The dashed lines indicate that these positions are then intended as a basis for trilateration to a spin label in the disordered domain (green dot) (*pdb*: 2LYV).

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Allelic Modulation of Global Dynamics of HLA-B*27:05 and HLA-B*27:09 Molecules

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Highly polymorphic Major Histocompatibility Complex (MHC, also termed Human Leukocyte Antigens, or HLA in humans) proteins bind peptide fragments generated as a result of the action of the proteasome machinery in Antigen Presenting Cells and present them on the cell surface to T-cell Receptor molecules. The flexibility profile of the molecular surface presented to the TCR may influence the response of T-cells. However, peptide-binding and polymorphisms in HLA may also influence more general properties of the peptide-loaded MHC (pMHC) complex, such as global dynamics or stability, as well. In this work, we performed classical Molecular Dynamics simulations and compared global dynamics profiles of eight different pMHC systems: four nonamer peptides bound to HLA-B*27:05 and HLA-B*27:09 alleles, which differ by a single amino acid exchange (D116H) and are differentially associated with the Ankylosing Spondylitis disease. Our results point to an allele-dependent effect on the global dynamics of pMHC. In addition, we also characterized the influence of D116H on the whole complex via residue-residue interaction energy calculations and found that the effect of the polymorphism may be relayed to the interface with the beta-2-microglobulin, a component of pMHC which is particularly important for the stability of the whole complex.

Study of the biological effects of oral diagnostic irradiation with Raman spectroscopy

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Accurate diagnostic imaging is an essential tool in orthodontics and dentofacial orthopedics. Cone beam computed tomography (CBCT) is a modern radiological technique used in clinical orthodontics for the tridimensional analysis of maxillofacial structures[1]. While the benefits of CBCT examination have been reported widely[2], the radiation dose to the patient is also becoming a major concern. The absorbed radiation dose arising from CBCT examination is relative low, but the accumulated effect on young orthodontic patients must be taken into consideration. Several literature reports have shown that distinct biological functions and damage patterns are triggered in cells in response to different doses of ionizing radiation [3, 4, 5] . Children are more sensitive to radiation than adults because the number of dividing cells promoting DNA mutagenesis is higher and they have more time to express any radiation induced effects, such as cancer[6]. Raman microspectroscopy can be successfully used for the investigation of live and fixed cells, offering a rapid measurement, it is non-invasive for the cells and no labels or markers are required[7]. The salivary glands are among the organs at risk during oral and maxillofacial radiology because they are often within the primary beam and because they are one of the most radiosensitive organs in children. Label-free surface-enhanced Raman spectroscopy SERS of saliva, a vibrational spectroscopic technique that has been shown to be useful in biomedical applications, can be used for diagnostic purposes because of its high sensitivity, specificity and accuracy[8] . We study label-free Raman microspectroscopy of human dental follicle stem cells (DFSC) and SERS of saliva. The biochemical changes induced by the CBCT irradiation in DFSC and in saliva can be important in understanding the effects of ionizing radiation used in the orthodontic diagnosis of pediatric patients.

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Nanoparticle wrapping via receptor-mediated adhesion

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Biological cells internalize cargo via different pathways. In all cases, the cargo is encapsulated within a carrier that interacts with the plasma membrane of a cell via wrapping. Understanding the mechanisms involved in this internalization process is important both from a fundamental science and from an application point of view. For example, in drug delivery and nanomedicine, nanoparticles can be used as carriers. Furthermore, nanoparticles are used for food processing and in technological applications, therefore a better understanding of their toxicity is required. Towards understanding the complex wrapping process, we study wrapping of a spherical nanoparticle decorated with ligands that interacts with a lipid-bilayer membrane via receptor-ligand bonds.

Theoretical calculations have shown that the wrapping state of nanoparticles depends both on particle properties, such as size and shape, the membrane properties, such as bending rigidity and tension, and the strength of the adhesive interaction [1, 2, 3]. We calculate the deformation energy of the fluid membrane using the Helfrich curvature Hamiltonian. We take into account receptor-ligand binding energy and receptor entropy [4]. For a given fraction of a particle being wrapped by the membrane, we obtain an optimum number of bound receptors at equilibrium. For low-receptor densities and high receptor-ligand bond energies, we find stable partial-wrapped states. We show that in this regime the kinetics of receptor-mediated wrapping can determine the number of nanoparticles that attach to a cell, which is important to understand dosage effects for nanoparticle-cell interaction.

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In vitro and in vivo changes in lipids erythrocyte membrane affected aluminum ions

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The aim of this study was evaluation of structural and functional state erythrocytes membrane of subjects who do not occupied in industrial production of aluminum and its salts under different level of aluminum load which was formed latently in natural environment of living.

Group of observations consists of 27 almost healthy adults 27-54 ages, comparison group composed of healthy donors 18-45 ages. Level of depositing chemical elements in hair was established using atomic emission spectroscopy; aluminum accumulation in human erythrocytes after incubation with AlCl3 was established using ICPE Fluorescence measurements on haemoglobin-free erythrocyte membranes (ghosts) were performed using a luminescent spectrophotometer. Three different lipophilic fluorescent probes (TMA-DPH, laurdan and pyrene) were used for investigation the physico-chemical state of membranes. Evaluation results of group of observation were based on comparative assessment of investigate fluorescent parameters of probes incorporated in erythrocyte membranes under different aluminum level deposition in hair of subjects.

Firstly, *in vitro* we studied aluminum influence on structural state of lipids in erythrocytes membrane. Collectively, the results obtained using lipophilic fluorescent probes indicate that Al accumulation in human after incubation with sublytic concentrations of $AlCl_3$ (2.7 – 27 mg/l) leads to dose-depended significant ($p<0.05$) decreasing of steady-state anisotropy of TMA-DPH and significant ($p<0.05$) increasing of generalized polarization (GP) of laurdan and excimerization coefficient of pyrene, which reflects changes in the bilayer microviscosity of red blood cells. Besides, using atomic force microscopy, we showed that 3h of incubation with 27 mg/l $AlCl_3$ leads to smoothing surface irregularities typical to normal erythrocytes.

In *in vivo* experiments at the first stage level of aluminum ions in subject's hair of group of observation was established. We took 1-10 μg/g as a mean biologically acceptable level aluminum content in hair. While analysing the results we divided group of observation into two parts: statistically normal aluminum deposition and relative high concentration. Comparison of two groups have shown that in erythrocytes membrane of subjects with high aluminum concentration GP of laurdan was statistically significant ($p<0.05$) increased.

Thus, comparison of studied biophysical parameters which characterized erythrocytes membrane showed monodirectional way of in vitro and in vivo alteration under chronicle low-dose accumulation aluminum in human organism.

Pantothenate Transport via Energy Coupling Factor Transporter ECF-PanT

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Energy Coupling Factor (ECF) transporters are a subgroup of ATP-Binding Cassette (ABC) transporters involved in the uptake of vitamins and micronutrients in prokaryotes[1]. ECF transporters employ integral membrane proteins for substrate binding (named S-components). S-components form active translocation complexes with the ECF module, an assembly of two nucleotide-binding domains (NBDs, or EcfA/EcfA') and a second transmembrane protein (EcfT)[2]. In many cases the ECF module can interact with several different S-components which are unrelated in sequence and bind diverse substrates. The modular organization with exchangeable S-components on a single ECF module enables the transport of chemically different substrates via a common route. The recent crystal structures of complete ECF complexes revealed that the orientation of the S-components in the membrane is highly unusual[3, 4, 5]. Whereas in the absence of the ECF module S-component substrate-binding site is located close to the outer surface, in the presence of ECF module the S-components topple over, with the α-helices lying almost parallel to the membrane plane, exposing the binding site to the cytoplasm. This unexpected toppling of the S-components suggests a unique mechanism that allows ECF transporters to translocate solutes across the membrane. Here, we study the mechanism of the pantothenate transport by ECF transporter ECF-PanT in vitro. The whole transporter was expressed in E.coli whereas solitary S-components were expressed in L.lactis, purified and reconstituted in liposomes subsequently the uptake of the radiolabeled substrate was assayed. We characterized the transporter and demonstrate that the two different S-components competed for the common ECF module.

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Study of viscoelastic properties of different biological systems with optical tweezers

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Knowing the mechanical properties of different biological systems have shown to be very important for better understanding of system structure, functioning and/or its formation. First, we used optical tweezers technique to obtain visco-elastic properties of homogeneous and inhomogeneous leva-DNA mixtures. DNA and levan are two important components of the biofilm. At critical DNA concentration levan clusters of a few microns in size are appiered. We performed micro- and macro-rheological measurements, both inside and outside the clusters [1]. We have also observed development of wild type and double mutant type Bacillus subtilis biofilms in the early stage of its formation under different conditions by observing the correlated motion of two trapped bacteria. Furthermore, we apply optical tweezers technique to perform micro-rheological measurements of human whole saliva and compared them with macro-rheological measurements obtained with rheometer. Structural and rheological characterisation of human whole saliva can lead to better understanding the biological role of this fluid in oral cavity and could be used in diagnostics or targeting the site for drug delivery [2]. The last but not the least experiment, is experiment connected with determining Young's modulus of the cells (HUVEC) in order to test surface effects on the cell elasticity. Applying the optical tweezers on all of those systems we have shown that optical tweezers are powerful tool to study viscoelastic properties of different biological systems.

[1] Stojković B., Sretenovic S., Dogsa I., Poberaj, I., Stopar, D. (2015). Viscoelastic Properties of Levan-DNA Mixtures Important in Microbial Biofilm Formation as Determined by Micro-and Macrorheology. Biophys J, 108(3), 758-765.

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Micro and macro-rheology study of DNA-levan mixtures

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SHORT TALK

Levan and DNA are important components of the extracellular matrix of bacterial biofilms. Levan is a non-ionic fructan polymer. In sucrose rich growth media Bacillus subtilis produces big amounts of this very soluble, polydisperse and low viscosity polymer. We performed rheological measurements of homogeneous and inhomogeneous levan-DNA mixtures using optical tweezers and a rotational rheometer. Due to the large difference in viscosities between levan and DNA fluids, we addressed phase separation at a critical DNA concentration. Due to the capability of optical tweezers to locate micron sized particles on a desired place in the sample, we performed microrheology measurements both within and outside the levan aggregates. This phase behaviour additionally was studied with fluorescence and DIC microscopy. Microrheology measurements give comparable results to the bulk rheology measurements in pure polymer solutions or homogenous mixtures. The results of active microrheology, however, are invaluable when microheterogeneous material is studied. In such cases the biophysical properties and, therefore its biological function maybe completely missed by averaging material viscoelastic properties. The results obtained highlight the importance of microrheology in the study of formation of microheterogeneous matrices such as those found in microbial biofilms. Although microbial biofilms are much more complex structures than the simple binary mixtures studied in this work, the results emphasize the importance of DNA in the formation of biofilms [1].

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Stability of proteins and viruses determined by differential scanning fluorimetry

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Differential scanning fluorimetry (DSF) is readily employed high-throughput method for probing protein stability. Typically, DSF monitors thermal unfolding of proteins using a fluorescent reporter dye (e.g. Sypro Orange) and a real-time PCR instrument[1]. Recently, a new method, nanoDSF, was developed which enables measuring of intrinsic tryptophan and tyrosine fluorescence[2, 3], its main advantage being that no fluorescent dye is needed, thus avoiding any detrimental dye-protein interactions. Both in DSF and nanoDSF, the fluorescence intensity increases with the exposure of hydrophobic protein parts. When plotted as a function of temperature, it gives a value of Tm at which the concentrations of folded and unfolded proteins are equal. Effect of different compounds on protein stability is seen as a change in Tm. Stability of human immunoglobulin G (hIgG) and human serum albumin (HSA) is very interesting because of their therapeutical use. DSF and nanoDSF were used to examine the stability of hIgG, HSA and ovalbumin (OVA) in different formulations (e.g. pH, salts, amino acids, sugars) and the methods showed excellent correlation. Formulations containing ammonium sulphate and sucrose proved as best stabilizers, while pronounced destabilizing effects were observed with imidazole, arginine and at low pH (pH 4). Thermal stability of measles and mumps virus was also probed, but several problems occurred – acquiring freshly concentrated viruses, damage to the viruses during concentration and lack of clear interpretation of the obtained Tm. Protein stability was also monitored by measuring protein aggregation by SEC-HPLC and virus stability by CCID50. Obtained results correlate well with DSF results.

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Analysis of intrinsically disordered region of human topoisomerase I using hydrogen/deuterium exchange mass spectrometry

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SHORT TALK

Intrinsically disordered proteins (IDPs) and proteins with intrinsically disordered protein regions (IDPRs) are a large and still not fully understood group of biological macromolecules. Their most characteristic features are lack of stable tertiary structure under conditions considered native and remarkable conformational dynamics. IDPs play crucial roles in many regulatory pathways and because of their unfolded regions they are known to have various interaction partners. One of such proteins is human topoisomerase I (topo I), enzyme that has primarily been known for its DNA relaxation activity. Topo I is also known to interact with a number of proteins such as p53[3], nucleolin[2] and splicing factors which belong to SR proteins.[5] It has also been shown that in adult human dermal fibroblasts, topoisomerase I stimulates cell migration via G-protein-coupled receptor (CCR7).[1] Apart from DNA relaxation topo I plays a significant role in phosphorylation of serine/arginine-rich splicing factor 1 (SRSF1).[5] Whereas the structure of topo I complexed with DNA has been known for almost twenty years, the structure of topo I in the complex with SRSF1 remains unknown. The main obstacle in resolving the structure is a contribution of unfolded domains of topo I and SRSF1 in formation of the complex. Studies have shown that N-terminal domain and cap subdomain are mainly involved in this interaction.[4] For further studies of the complex the mentioned domains were expressed in Escherichia coli and purified. Since previous attempts of topo I-SRSF1 crystallization were not successful we employed an alternative strategy. Complex formation dynamics were studied using hydrogen deuterium exchange (HDX) mass spectroscopy which revealed regions involved in this interaction. Because variety of topo I functions are linked with presence of unfolded domain, HDX experiments in combination with further NMR analysis of the topo $I_{(IDPR)}$-SRSF1 complex will give insight into mechanisms responsible for interaction of IDPs with their molecular partners and dynamics of these processes.

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Biophysical characterization of recombinant pernisine from Streptomyces sp.

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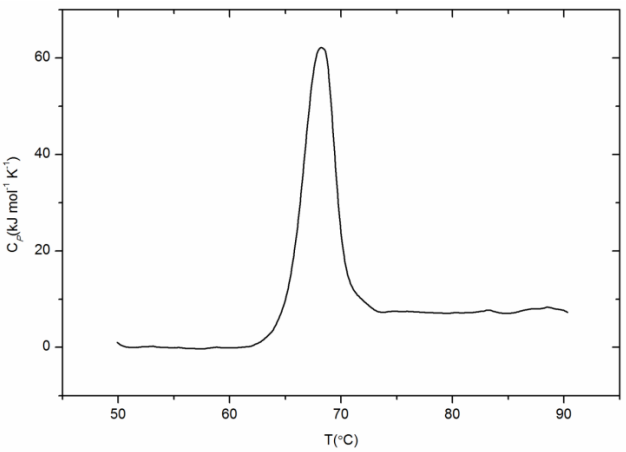
Recombinant protease designated pernisine was purified from extracellular growth medium of Streptomyces sp. and further biophysically characterized. Pernisine originates from hyperthermophilic archaea Aeropyrum pernix K1. There is an important industrial applicability of thermostable proteases[2].

Codon optimized pernisine was cloned in bacterial expression vector under constitutive promoter. Purification was done by using affinity chromatography and lyophilisation. Spectroscopic methods help us to determine conformational stability and analysing conformational changes of proteins as a function of temperature. The temperature of denaturation (T_d) of pernisine at pH 8.0 was determined at 68°C by differential scanning calorimetry (DSC). Intrinsic tryptophan fluorescence emission spectroscopy was used for analysing pernisine at different pH (2.1, 8.0, 12.6) and temperature range from 20 to 95°C. At pH 2.1 protein was completely denaturated, at pH 12.6 denaturation process starts at (48.0 ± 0.5)°C and at pH 8.0 protein starts to change its conformation at (66 ± 0.5)°C. Further biochemical characterization of recombinant pernisine showed that pernisine retained similar characteristics as native one [2]. The temperature and the pH optima of the enzymatic activity of the recombinant pernisine, evaluated by azocasein assay, were at (98 ± 1)°C and pH 7.0, respectively.



DSC thermogram of recombinant pernisine using Nano DSC (TA Instruments)

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SHORT TALK – EBSA

Looking for a generic inhibitor of amyloid-like fibril formation among flavone derivatives

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A range of diseases is associated with amyloid fibril formation. Despite different proteins being responsible for each disease, all of them share similar features including beta-sheet-rich secondary structure and fibril-like protein aggregates. A number of proteins can form amyloid-like fibrils in vitro, resembling structural features of disease-related amyloids. Given these generic structural properties of amyloid and amyloid-like fibrils, generic inhibitors of fibril formation would be of interest for treatment of amyloid diseases. Recently, we identified five outstanding inhibitors of insulin amyloid-like fibril formation among the pool of 265 commercially available flavone derivatives. Here we report testing of these five compounds and of epi-gallocatechine-3-gallate (EGCG) on aggregation of alpha-synuclein and beta-amyloid. We used a Thioflavin T (ThT) fluorescence assay, relying on halftimes of aggregation as the measure of inhibition. This method avoids large numbers of false positive results. Our data indicate that four of the five flavones and EGCG inhibit alpha-synuclein aggregation in a concentration-dependent manner. However none of these derivatives were able to increase halftimes of aggregation of beta-amyloid.

Investigation of selected amino acids influence on calcium carbonate precipitation - simple model of biomineralization

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SHORT TALK

Calcium carbonate is the main inorganic component of biominerals found in many invertebrate organisms and is present either as a specific polymorph (calcite or aragonite), hydrated or in amorphous form. The calcite skeletal elements regularly contain small amounts of proteins which are either incorporated or adsorbed on the single crystals of calcite. Previously it has been shown that isolated fragments of proteins extracted from mineralised tissue, or their synthetic macromolecular analogues, exert a significant influence on the morphology and crystal structure of calcium carbonate when precipitated in the appropriate model systems[1, 2, 3].

The aim of this research is to investigate a role of the selected amino acids as a simple models of biomacromolecules supposed to be responsible for specific precipitation of calcium carbonates in biomineralising systems. In addition, possible crystallographic distortions of the calcite lattice will be investigated. For that purpose amino acids having distinct chemical and physical properties were selected: asparagine, aspartic acid and lysine were chosen because of differently charged side chains, while tyrosine and phenylalanine, as well as serine and alanine have different polarity. The results of structural (EPR)[4] and kinetic analyses indicated an overall inhibition of calcite growth in the presence of all amino acids. The inhibition is probably caused by a slower transformation of initially formed vaterite into the calcite[5]. Since the selected amino acids are charged under the applied experimental conditions, some surface interactions are assumed to be responsible for the observed effect.

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Interactive modelling of missing segments in proteins

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The RCSB Protein Data Bank (PDB) is an established source for atomistic tertiary structure models. However, in more than one half of all entries segments are missing. These segments are often located in flexible and functionally important regions of proteins such as loops, turns or binding pockets, not resolved by X-ray crystallography or single particle cryo-electron microscopy (cryo-EM). These missing segments have to be modelled to obtain complete structures for further analysis, e.g. for molecular dynamics simulations. Here, we provide an interactive tool for modelling of missing segments in proteins.

We implemented a fragment-based approach for our web server SuperLooper2 (SL2 [1]), which allows fast prediction of missing segments from a fragment database and instant visualization. Visualization is performed with the NGL viewer [2], which adopts the capabilities of modern web browsers without the need of third-party plug-ins or additional installations to visualize even large molecular complexes. The user selects a suitable conformation from a list of pre-calculated fragments after visual inspection, which improves prediction quality compared to purely automated approaches. The fragments are derived from a database containing over 9 hundred million fractions extracted from the PDB and sorted by a fast search algorithm based on sequence similarity and geometrical fingerprints. Finally, we show that using additional conformational constraints derived from cryo-EM density maps, 'FragFit' [3] further optimizes prediction of missing segments in proteins.

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Chaperone Mechanism of αB-Crystallin

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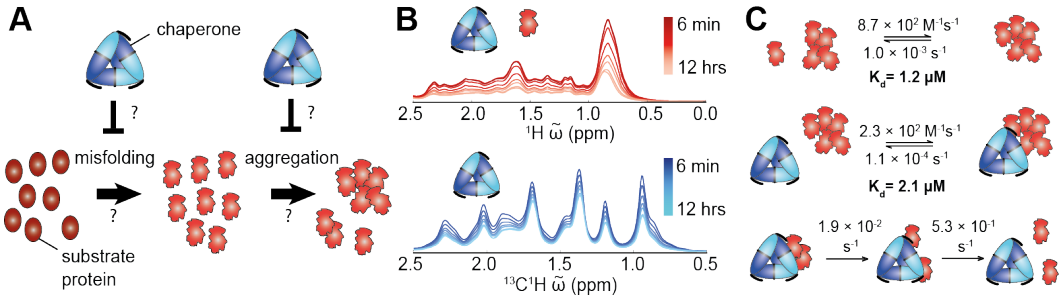
Protein aggregation is associated with many debilitating diseases such as Alzheimer's and Parkinson's. The human body is not without defences, and small heat-shock protein αB-crystallin, closely associated with both diseases in vivo, directly inhibits aggregation in vitro. Despite their biological and medical importance, the heterogeneity and large size of aggregate-chaperone complexes and the dynamic nature of these interactions have posed severe challenges to investigating chaperone function using traditional biophysical techniques in vitro. There is therefore no consensus on how the sHSP chaperones, in particular αB-crystallin perform, their protective role (Fig. 1A).

We address this deficiency with a new, quantitative methodology based on combining nuclear magnetic resonance spectroscopy (NMR) and chemical kinetics to monitor interactions between individual species in real time (Fig. 1B). We apply the method to both full-length ^{13}C-labelled αB-crystallin and its isolated ^{15}N-labelled core α-crystallin domain, studying their interactions with an unlabelled model substrate α-lactalbumin. The NMR data afford a great deal of power to distinguish between many possible mechanisms of aggregation and chaperone action. We test a total of 51 models to find ones that adequately describe the entire dataset and derive kinetic and thermodynamic parameters for the underlying network of interactions (e.g. Fig. 1C). We show that the data justify a chaperone mechanism based on competitive association with substrate, with full-length αB-crystallin being more selective as to which species it binds than the core domain. Remarkably, we determine that the chaperone enhances the dissociation of small substrate oligomers (Fig. 1C, bottom). This explains how αB-crystallin might reduce the reported toxicity of such oligomers in neurodegenerative disease.



In order to determine the mechanism of interaction (A) we perform real-time differentially labelled NMR experiments (B). Quantitative modelling analysis allows us to extract thermodynamic and kinetic parameters (C).

The Influence of Sodium Dodecyl Sulfate on Stability of Bilayer Lipid Membranes

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Sodium dodecyl sulfate (SDS) is a commonly used detergent to solubilize membranes and to isolate and purify membrane proteins and membrane lipids[1]. Because of its intensive use and release into the environment the volume of water pollution by SDS comes with great speed. SDS has the ability to absorb and accumulate in the body, causing disorders. It has allergenic properties, even when a small amount of it gets into the body[2]. Let us note that there are some molecules (such as fatty acids, lysophospholipids) in the cell, which have similar structures like SDS molecule, and it's necessary to know their influence on the membrane stability.

Therefore it is important to investigate the influence of SDS on the cell membrane. But as the study of stability on the living cell is too hard, it seems more expedient to do a detailed study on the widely used experimental system for modeling of cell membranes, which are bilayer lipid membranes (BLM).

The influence of SDS on planar bilayer lipid membranes was investigated. The membranes were formed according to the Muller–Rudin method[3] in the solution of electrolyte in a special teflon cell. For measurements of parameters of the BLM, two silver-chloride electrodes were located in two compartments of the experimental cell, which were separated by a membrane. The electrodes were connected to the ADC (E14–140M) and controlled by a computer, using the computer program LabVIEW.

It is shown that the presence of SDS and it's concentration increasing leads to the loss of stability of the BLM, which is associated with the decrease in the value of the linear tension of pore edge in BLM, because of positive spontaneous curvature of the SDS molecule. It is also shown that the number of lipid pores on the BLM increases as a result of increase in probability of the pore formation, with reduction of the value of linear tension.

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Modelling and simulations of glycans and glycosylated proteins

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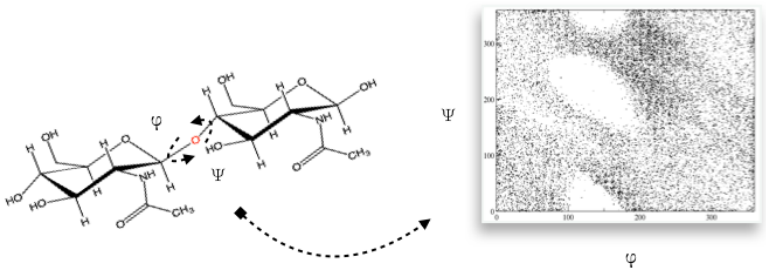
Glycosylation is a critical part of postgenomic studies due to their significant role in numerous biological processes such as immune defence, cellular growth and infection through influencing the properties of biomolecules. The number of attached glycans, their size, sequence, charge and the position to which they are attached are some of the parameters that are involved in modulating protein behaviour [1]. However, there is a lack of reliable structural information by means of experimental techniques because of their highly flexible nature and high degree of coordination with water. In these situations, computer simulation of biomolecular complexes emerge as a powerful tool, complementary to experimental exploration. The aim of our study is to model relevant glycan structures in the absence of experimental structural data and study their effects in the glycoprotein environment on protein dynamics.

In this context, the solution conformations of all disaccharides constituting glycan units are studied using the local elevation umbrella sampling method (LEUS). The first phase of this method relies on building up a memory-based biasing potential energy term which penalizes the revisiting of the space [2]. A subsequent umbrella sampling phase uses this biasing potential for production simulation. The adaptable nature of the LEUS method allows modelling of the carbohydrate moiety of the glycoproteins by using biasing potentials for glycosidic linkages of all dissacharide fragments in the glycan unit and enhancing the sampling in the production phase. Here we show preliminary results of our approach.



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SHORT TALK

Optical and Electrical Characterization of Voltage Sensitive Dyes in Lipid Bilayers

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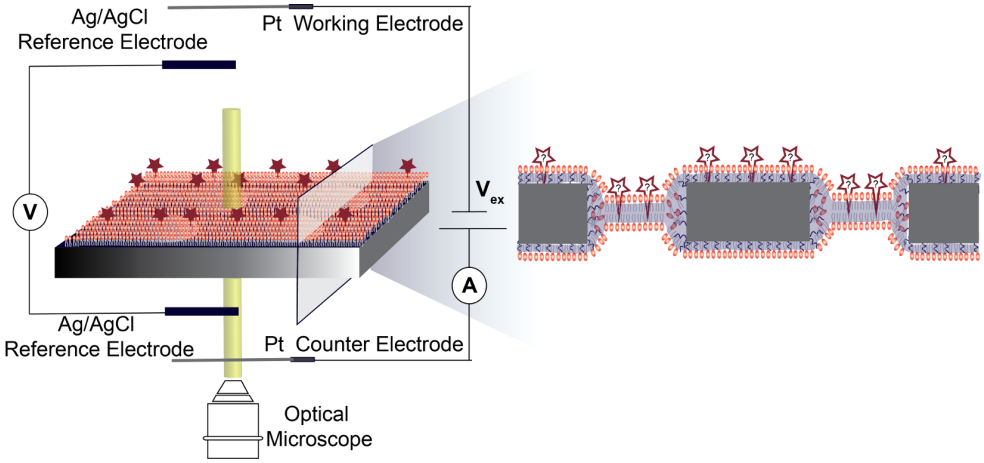
Voltage sensitive dyes (VSDs) are powerful optical tools for membrane potential monitoring. Their main characteristic is the change in their inherent fluorescence upon membrane potential changes. In this work, we demonstrate a new approach for the characterization of VSDs based on VSDs incorporated free-standing lipid bilayers spanning on a micro-porous Si3N4 membrane. The developed platform, after being mounted in a home-made optical compatible electrochemical cell, enables the possibility 1) to apply any voltage sequences, 2) to modify bilayer composition freely, and 3) to acquire two-dimensional mapping of the VSD activities, allowing more detailed studies of the most famous VSDs (e.g. di-4-ANEPPS).



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Immobilization of glyphosate antibodies onto quartz surfaces

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Glyphosate (N-(phosphonomethyl)glycine; trade name RoundupTM) is a non-selective postemergence herbicide for weed and vegetation control in farms and gardens, currently with the highest production amount of all herbicides. Although it is officially concluded that "glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet", glyphosate has been since 2015 classified as probably carcinogenic based on the evidences of carcinogenicity in humans and experimental animals[1]. At present glyphosate is determined by HPLC methods. This method is sensitive, but time-consuming and expensive. For the conduction of real time analyses we propose a fiber-optical biosensing system with glyphosate antibody for selective biorecognition of glyphosate. The aim of the present study is to immobilize glyphosate antibodies onto quartz, find optimal conditions for the immobilization and to develop a bioselective system for the detection of glyphosate. In order to avoid nonspecific binding on the surface, we used pegylation. The commercial glass slides (Ted Pella, Inc., USA) were pre-cleaned and activated in plasma chamber before silanization. Silanized slides were functionalized with the mixture of biotin-PEG and methoxy-PEG (mass ratio 1:50), providing a certain number of specific binding sites for streptavidin molecules. The binding was studied with total internal reflection fluorescence microscope (TIRFM), using phycoerythrin conjugated streptavidin. The slides showed less than 1 Biotin-streptavidin interaction was also used for the attachment of glyphosate antibodies. As the sensitivity and stability of biosensors is determined by the immobilized antibody, we used site-directed immobilization via oxidized oligo-saccharide moieties of the antibodies. IgY-type glyphosate antibody was oxidized with periodate solution and biotinylated with biotin-PEG-hydrazide. Anti IgY marked with FITC was used to determine the number of bound glyphosate antibodies. We found that over 250 000 IgY per 1 mm2 was available for glyphosate detection.

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In silico investigation of *Helicobacter pylori* purine nucleotide phosphorylase inhibitors

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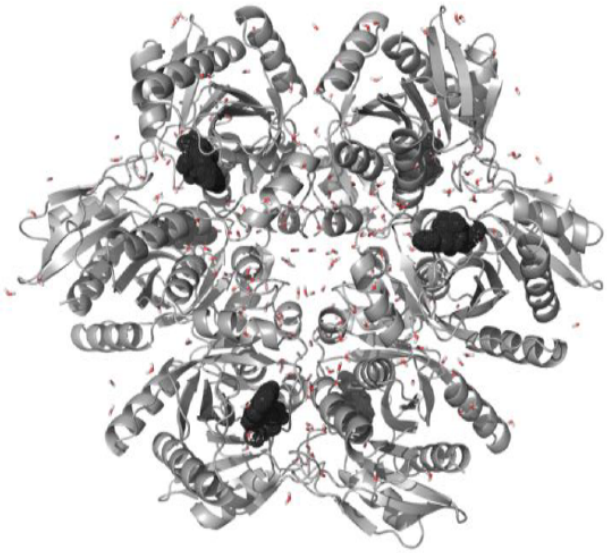
Purine nucleotide phosphorylase (PNP) is a key enzyme in the purine salvage pathway. Bacterial and eucaryotic PNP are significantly different, making the bacterial enzyme an excellent potential target for rational antibacterial drug design. The recently solved *Helicobacter pylori* PNP crystal structure allows the application of *in silico* methods for gaining an insight into the molecular mechanisms of enzyme activity, which can be used to design efficient and selective inhibitors. The most common interactions of both the natural substrate and experimentally tested inhibitors in the PNP active site were determined by molecular docking. These interactions were correlated with experimentally obtained biological activity. Molecular dynamics simulations were used to study ligand placement in PNP active site with the goal of establishing the most important ligand binding interactions. The results suggest that π–π interactions of ligands with phenylalanine 159 and hydrogen bonds with threonine 90 and glutamate 181 are the key interactions for the design of selective and efficient inhibitors. New inhibitors will be designed and experimentally tested based on these results.



Coupling fluctuations with multicellular motion

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Morphogenesis and wound healing both require migration of a large number of constituent cells. This still unresolved problem of collective cell migration is addressed by using MDCK II model epithelium grown on collagen I coated glass substrates. We look at the global development of an initially droplet seeded system of cells which is allowed to expand freely over time. Large scale experiments spanning days and multiple connected fields of view are analyzed with particle image velocimetry of live fluorescent samples. This approach allows for both microscopic and macroscopic (millimeter) scales as cell clusters are investigated all the way from the border to the contact inhibited centre. We analyze the correlations between these scales and the perpetually increasing velocity of the colony border. Our recent findings push the limit of cooperative cell motion numbers further than expected to the regime where thousands of cells act simultaneously in a coordinated fashion.

Angstrom-Sensitivity Protein Sizing and Conformation Detection using Solid-State Nanopores

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SHORT TALK

We present here nanopore-based measurements of the size, charge, conformational changes, and rigidity of individual proteins in their native state. The size of various proteins in solution is estimated within angstrom sensitivity using mean fractional current blockade values in high-bandwidth ion current measurement through crystallized hafnium oxide and silicon nitride nanopores. The relative charge of the proteins is derived from the direction of electrophoretic and electroosmosis forces under applied bias to trans side, taking isoelectric points of proteins/pores and working pH values into account. We further present here a new model to measure rigidity of native proteins in solution, which is also correlated to the secondary structure of proteins, based on width of fractional current blockade distributions. Finally, we detect conformational changes in Calmodulin, a protein that changes its conformation upon calcium-ion binding, at the single-molecule level.

Characterization of physicochemical properties of model lipid membranes modified by 5-n-alkylresorcinols

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5-n-alk(en)ylresorcinols (ARs) are naturally occurring compounds and can be found in cereal bran. They are absorbed from digestive tract, transported with proteins in blood as well as they can be incorporated into erythrocyte membrane and stored in the adipocytes. ARs are amphiphilic compounds of very low critical micelle concentration and are thus practically insoluble in water. The preference of ARs for a hydrophobic environment is also reflected in the high octanol/water partition coefficient. Therefore, they have the ability to incorporate into natural and model lipid bilayers and alter their properties and functions. ARs showed a stabilizing effect on phospholipid membranes by making the bilayers less permeable to small solutes, and liposomes are more resistant to osmotic stress[1]. The aim of this studies was to understand how cereal ARs affect biophysical properties of phospholipid bilayers? Is it dependent on the length of alkyl tail? How do ARs distribute within a bilayer? For characterization of the alterations caused by ARs on lipid bilayer Laurdan and Prodan fluorescence, Attenuated total reflectance Fourier transfer infrared spectroscopy (FTIR-ATR), Differential Scanning Calorimetry (DSC), $^{3}1$P nuclear magnetic resonance spectroscopy (NMR) and dynamic light scattering (DLS) were employed. As model membranes we used dipalmitoylphosphatidylcholine (DPPC) - pure or enriched with cholesterol (Chol) - modified with long chain homologs of ARs (from C15 to C25). Chain length-dependent changes in DPPC:AR and DPPC:Chol:AR membranes were investigated as a function of temperature. Our data suggest strong interaction between ARs homologs with DPPC and DPPC:Chol membranes. We have shown that incorporation of ARs lead to a shift in the gel-liquid crystalline phase transition of ARs-mixed bilayer towards higher temperatures. Additionally, with a further increase in the alkyl-chain length and membrane concentration of doped compound, we observed lower cooperativity of the chain-melting phase transition which is sign of increase stiffness within the lipid acyl chains. Furthermore, ARs decrease the hydration of the lipid headgroups. We also shown that -OH groups of ARs molecules form hydrogen bonds with the phosphate groups of DPPC lipids, which may explain the stabilizing effect of ARs on phospholipid membranes.

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The mechanism of DNA deactivation by hydrogen peroxide action

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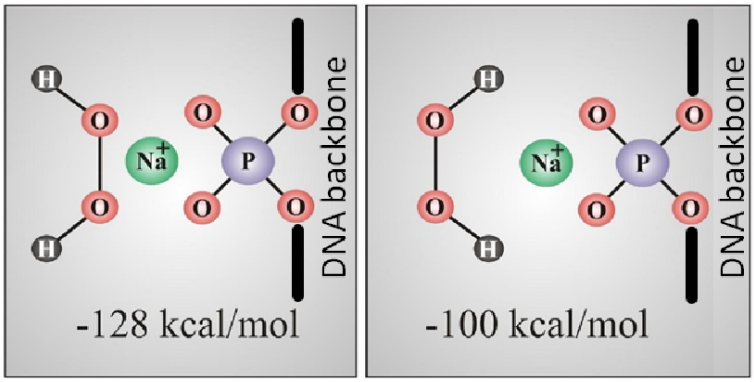
Among the most known mechanisms of action of heavy ion radiation on cell DNA are influence of secondary electrons and free radicals which are produced in the medium [1]. But up today it is not paid essential attention to the structural changes of biological medium and processes of molecular fragmentation in cell environment under heavy ion radiation. Particularly in the process of water radiolysis the essential concentration of hydrogen peroxide molecules (H_2O_2) is accumulated in the medium [2]. In the present work interactions between peroxide molecules and cites of non-specific recognition of DNA macromolecules (backbone phosphate groups) in the presence of water and counterions are studied. Complexes of DNA phosphate group (PO_4) with H_2O_2 molecule, water molecule and Na^+ counterion are investigated. Binding energies of these complexes are estimated. Calculations are made using atom-atomic potential functions method taking into consideration electrostatic, van der Waals and H-bond interactions [3]. Dual (hydrogen peroxide molecule or water molecule with phosphate group) as well as trial complexes (dual complexes in the presence of sodium counterion) are studied. It is shown that in the presence of Na^+ ion hydrogen peroxide may create stable complexes with DNA (see Figure). The lifetime of this complex is more than of the complex of DNA phosphate group with water and counterion Na^+. The formation of such complex on DNA sugar phosphate chain should block the processes of DNA transcription and genetic information transition.



Two stable configurations of peroxide-DNA complex and their binding energies.

[1] Obolensky O, Surdutovich E, Pshenichnov I, Mishustin I, Solov'yov A, Greiner W (2008) Ion beam cancer therapy: Fundamental aspects of the problem. Nucl. Instr. Meth. Phys. Res. B *266:1623–1628.*

[2] Kreipl M S, Friedland W, Herwig G P (2009) Time- and space-resolved Monte Carlo study of water radiolysis for photon, electron and ion irradiation. Radiat Environ Biophys *48:11–20.*

[3] Pyatnitskyi D, Zdorevskyi O, Perepelytsya S, Volkov S N (2015) Understanding the mechanism of DNA deactivation in ion therapy of cancer cells: hydrogen peroxide action. Eur Phys J D *69:255.*

Silicon nanopore array: fabrication and DNA length measurement

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Nanopore sensing is an emerging field where nano-fabrication technology meets bio-molecular applications. Here, a nanometer-sized pore in a free-standing membrane that separates a buffer and an analyte solution is used. Bio-molecules, such as DNA, RNA or proteins, with sizes in the same range as the nanopores are dragged through the pores by an electrical field which is applied across the membrane. This temporary isolation of the single molecules in the nanopore gives us a platform to perform single molecule studies without immobilization of molecules. In order to achieve a high throughput, parallel sensing, fluorescence detection on a large array of nanopores seems a preferable way over the electrical detection on a single pore.

In our group, we have developed an electrochemical etching method to obtain nanopores in a large array in a silicon membrane with controlled pitch distance and relatively small pore size distribution. Pores with an average diameter down to 18 nm are etched through 2.5 μm thick membranes with inverted-pyramidal shaped etching pits. 20000 pores are made at the same time in a batch of 10 minutes etching. As a proof of concept, these nanopores are subject to DNA translocation experiments. Wide-field optical microscopy and a CMOS camera are employed to detect fluorophore-labeled DNA passing through the array of pores simultaneously. The translocations of dsDNA are observed on every pore position when adequate bias is applied. A high threshold bias (1V for 450 bp dsDNA, 3V for 3360 bp dsDNA) indicates a large energy barrier probably associated with the high aspect ratio of the pores. Interestingly, we have observed that the translocation decay time decreases substantially with increasing excitation laser power, probably due to the photoinduced effect on surface charge of the pore wall. Further, dsDNA with length of 450 bp and 3360 bp are distinguishable by statistical analysis of the decay constant of the dwell time.

Structural basis of non-muscle α-actinin regulation by calcium

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Actinin functions as scaffolding protein crosslinking actin filaments into actin bundles. This is achieved via a particular antiparallel dimeric topology in which each subunit comprises an N-terminal actin binding domain (ABD), a connecting segment (NECK), a central rod domain built by spectrin-like repeats, and a C-terminal calmodulin-like domain with four EF hand motifs (EF12-EF34). The non-muscle isoforms (1 and 4) are regulated by calcium binding to EF hands [1]. Accordingly, calcium concentration >10–7 M reduces or even abolishes their actin bundling capacity. However, the molecular mechanism of this regulation remains unknown. To address this question we focused on α-actinin isoform 2 from parasite Entamoeba histolytica (ehACTN2), which shows high sequence identity with human non-muscle homologs [2]. It also contains a shorter rod domain (two spectrin-like repeats vs. four in human proteins), thus representing an ancestral form of α-actinin. Low-speed actin co-sedimentation assays using ehACTN2 revealed a significant decrease of actin bundling activity when the experiment was performed in the presence of calcium. We further used the recently published crystal structure of hACTN2 to design mutations on ehACTN2 disrupting contacts between NECK and EF34, which abolished completely its bundling capacity. The same result was obtained for a protein deletion variant lacking EF34. Circular dichroism showed identical secondary structure conformation for both "NECK" mutant and EF34 deletion variant when compared to that of wild type protein. In addition, these experiments evinced a substantial increase in stability of the wild type ehACTN2 upon calcium binding. The crystal structures of calcium-bound wild type ehACTN2 and of calcium-insensitive mutant were determined, showing an overall architecture similar to that of hACTN2. Most interestingly, unlike in hACTN2, EF12 (which coordinates calcium) is sandwiched between the two spectrin-like repeats, while the EF34 wraps around NECK. In addition, the calcium-insensitive mutant evinces flexibility for ABD and certain regions of EF12-EF34. To sum up, we hypothesize that calcium binding introduces a conformational change that is transmitted from EF12 to EF34, which subsequently affects the flexibility of NECK and reduces orientation sampling for ABD. These results highlight the importance of both the proper positioning and flexibility of ABD for actin bundling.

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[2] Virel A, Addario B, and Backman L (2007), Characterization of Entamoeba histolytica alpha-actinin2, Mol. Biochem. Parasitol. 154, 82-89.

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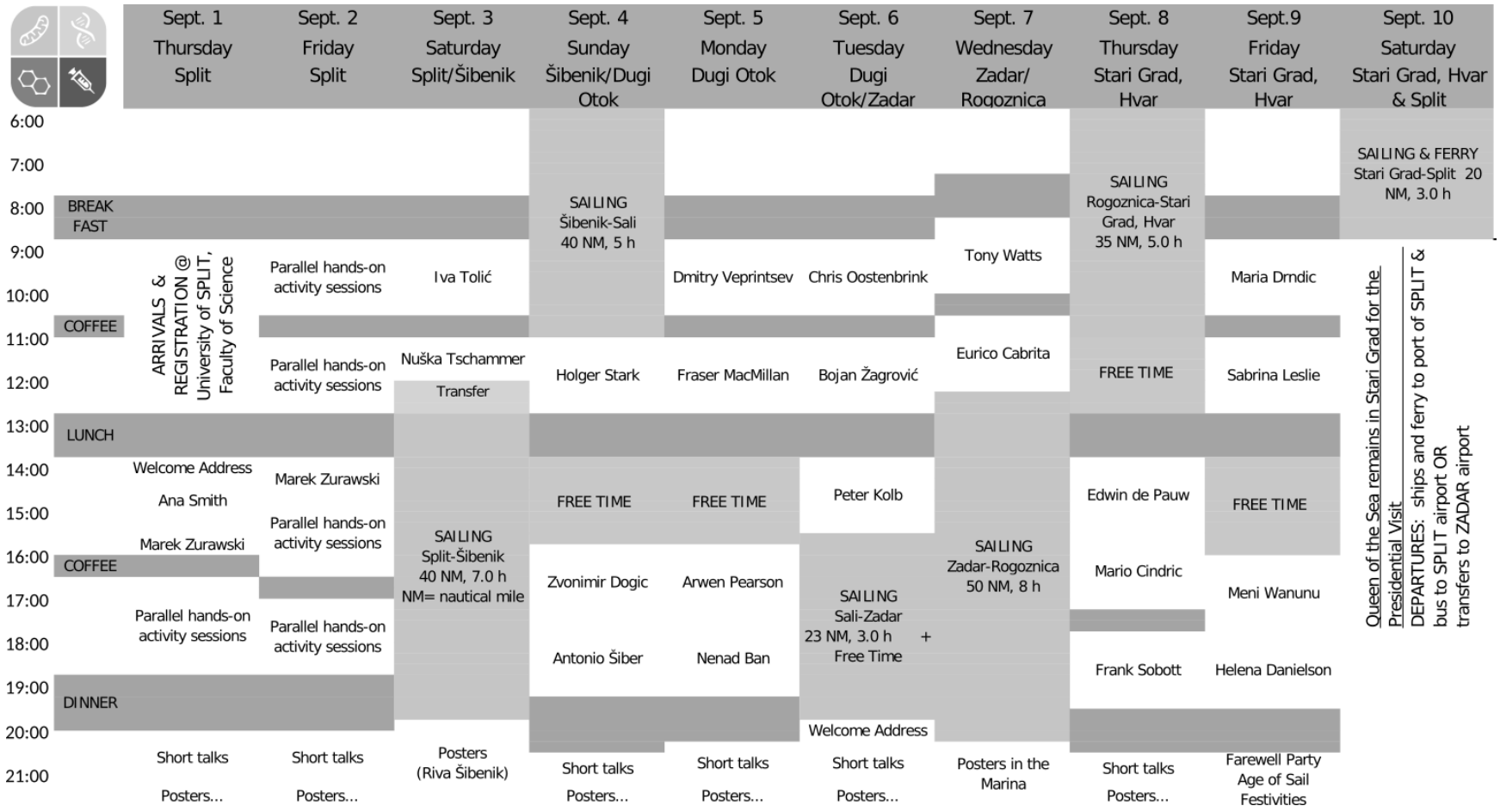
Sept. 1 Thursday Split
Sept. 2 Friday Split
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Sept. 4 Sunday Šibenik/Dugi Otok
Sept. 5 Monday Dugi Otok
Sept. 6 Tuesday Dugi Otok/Zadar
Sept. 7 Wednesday Zadar/ Rogoznica
Sept. 8 Thursday Stari Grad, Hvar
Sept.9 Friday Stari Grad, Hvar
Sept. 10 Saturday Stari Grad, Hvar & Split
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BREAK FAST
COFFEE
LUNCH
COFFEE
DINNER
ARRIVALS & REGISTRATION @ University of SPLIT, Faculty of Science
Welcome Address
Ana Smith
Marek Zurawski
Parallel hands-on activity sessions
Short talks
Posters...
Parallel hands-on activity sessions
Marek Zurawski
Parallel hands-on activity sessions
Parallel hands-on activity sessions
Short talks
Posters...
Iva Tolić
Nuška Tschammer
Transfer
SAILING Split-Šibenik 40 NM, 7.0 h NM= nautical mile
Posters (Riva Šibenik)
SAILING Šibenik-Sali 40 NM, 5 h
Holger Stark
FREE TIME
Zvonimir Dogic
Antonio Šiber
Short talks
Posters...
Dmitry Veprintsev
Fraser MacMillan
FREE TIME
Arwen Pearson
Nenad Ban
Short talks
Posters...
Chris Oostenbrink
Bojan Žagrović
Peter Kolb
SAILING Sali-Zadar 23 NM, 3.0 h + Free Time
Welcome Address
Short talks
Posters...
Tony Watts
Eurico Cabrita
SAILING Zadar-Rogoznica 50 NM, 8 h
Posters in the Marina
SAILING Rogoznica-Stari Grad, Hvar 35 NM, 5.0 h
FREE TIME
Edwin de Pauw
Mario Cindric
Frank Sobott
Short talks
Posters...
Maria Drndic
Sabrina Leslie
FREE TIME
Meni Wanunu
Helena Danielson
Farewell Party
Age of Sail
Festivities
SAILING & FERRY Stari Grad-Split 20 NM, 3.0 h
Queen of the Sea remains in Stari Grad for the Presidential Visit
DEPARTURES: ships and ferry to port of SPLIT & bus to SPLIT airport OR transfers to ZADAR airport

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