11th Greta Pifat-Mrzljak International School of Biophysics

BIOMOLECULAR COMPLEXES AND ASSEMBLIES

BOOK OF ABSTRACTS

Primošten, Croatia, 30.9.-9.10.2012.

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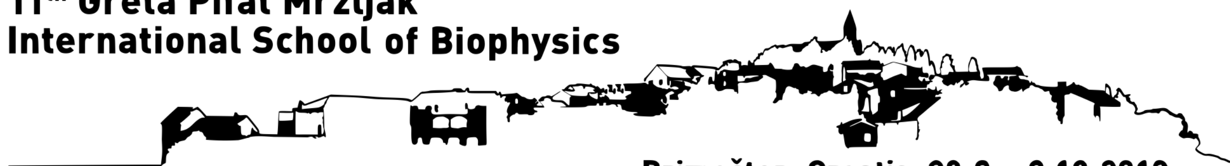

The 11th Greta Pifat Mrzljak International School of Biophysics
Biomolecular complexes and assemblies / Book of Abstracts
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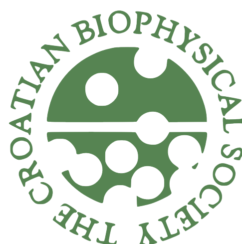

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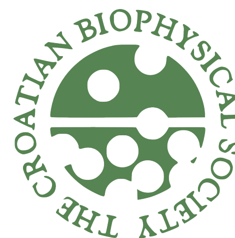

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FOREWORD

The 11th Greta Pifat-Mrzljak International School of Biophysics school is intended for young scientists 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.

For more than 30 years, the school was chaired by the late Prof. Greta Pifat-Mrzljak, an eminent Croatian biophysicist and recipient of the American Biophysical Society's Emily M. Gray Award, and is generally regarded as one of the best of its kind in Europe. Through the years, more than one thousand Ph.D. students and postdocs interested in becoming acquainted with the state-of-the-art in biophysics have attended it. The lecturers have always been of the highest quality, including top scientists in their respective disciplines and several Nobel laureates.

Therefore, in addition to the school's inherent role in the transfer of knowledge and ideas, it has always had a catalytic role in arranging future research collaborations, joint projects, visits and postdoc positions.

With the legacy of Prof. Pifat-Mrzljak in mind, it is the intention of the organizers to position this school as a biennial event, complementary to, rather than competing with, relevant international activities, e.g., the Regional Biophysics Conference or the EBSA Biophysics Course.

Tomislav Vuletić

Chair

Sanja Tomić

President of the

Croatian Biophysical Society

SCHOOL 1981 - TODAY

This session of the Greta Pifat-Mrzljak International School of Biophysics is the eleventh in a series held for 30 years in Dubrovnik and Rovinj, Croatia. The previous sessions were held in 1981, 1984, 1987, 1990, 1994, 1997, 2000, 2003, 2006 and 2009.

Throughout this period, the school was chaired by the late Prof. Greta Pifat-Mrzljak, an eminent Croatian biophysicist, recipient of the American Biophysical Society's Emily M. Gray Award, and member of the International Union for Pure and Applied Biophysics (IUPAB) Council.

It is widely agreed that a regular school of biophysics for young scientists within Europe is still needed. Continuing this series formerly organized by Prof. Pifat-Mrzljak is a perfect solution.

Therefore, the Croatian Biophysical Society and Ruđer Bošković Institute have committed their resources in order to assure the future of the school. 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. Indeed, previous sessions of the school have 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.

The lecturers at the International School of Biophysics have always included highly prominent figures in this area of research, which spans the borders dividing biology, chemistry and physics. The prospect of top senior scientists interacting with enthusiastic young scientists, some of whom will ultimately go on to work in these quickly changing and challenging areas of biophysics, is nearly unique in the world. The interfaces of the traditional disciplines are being broken down within biophysics. Teaching dozens of young and inquisitive minds is a thrilling experience, enhanced by the environment of the past sessions.

School Methodology

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 of the Croatian Biophysical Society (CBS) and the Ruđer Bošković Institute.

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 (Table 1). From then on, a two-week course was regularly held every third year as a permanent activity of the CBS and RBI.

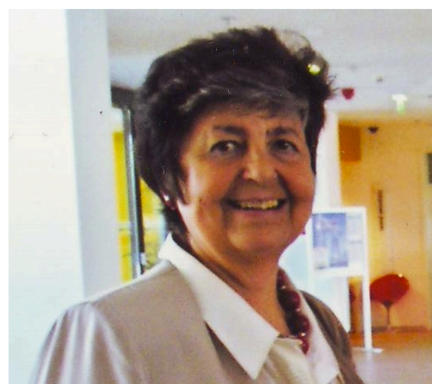
One week of the core curriculum, with basic biophysical knowledge presented by prominent lecturers, is followed by the second week, devoted to topics specific to each session. The particular scope of the school has enabled the participants to become acquainted with state-of-art problem-oriented and/or methodology-oriented approaches to biological systems. Major topics are presented in a series of lectures, which include inter- and intra- molecular interactions in biological systems; the structure, organization and function of biological macromolecules (proteins, nucleic acids, lipids, sugars) and supramolecular assemblies (membranes, ribosomes, etc.); major techniques in biophysics (X-ray crystallography, NMR, EPR, FTIR, fluorescence, mass spectrometry, microscopic techniques, etc.) and the evolutionary implications of molecular interactions, as well as molecular biology and biotechnology.

There are a few facts paramount to the scientific success and continuity of the International Summer School of Biophysics. 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.

To maintain the peripatetic character of the Greta Pifat-Mrzljak International Summer School of Biophysics, the number of students has been limited to 100. They come from all over the world, from Nigeria to Estonia, Cuba to Taiwan, participating very actively in discussions during or after the lectures, at poster sessions, in school discussion clubs or even on the beaches. About 30 prominent lecturers give two to three lectures each per session (Table 2).

Most of the sessions have resulted in proceedings published under the title *Supramolecular Structure and Function* by Plenum Press, London 1983 (eds. G. Pifat and J. N. Herak); Springer Verlag, Heidelberg 1986 (ed. G. Pifat); World Publishing Co., Singapore 1988 (ed. G. Pifat); Balaban Publishers, Rehovot 1992 (ed. G. Pifat) and Balaban Publishers, Rehovot 1995 (ed. G. Pifat-Mrzljak); Periodicum Biologorum, Zagreb (ed. G. Pifat-Mrzljak); Kluwer Academic/Plenum Publishers 2001 (ed. G. Pifat-Mrzljak); Kluwer Academic Publishers 2004 (ed. G. Pifat-Mrzljak); and Springer 2007 (ed. G. Pifat-Mrzljak).

PROF. GRETA PIFAT-MRZLJAK (1939 - 2009)



Personal views

I first met Greta in the last School held in Kupari-Dubrovnik in 1990. A group of people, including lecturers of previous schools, suggested her to include infrared spectroscopy in the techniques to be shown at the School and I was invited to give those lectures. In arriving I was impressed by Greta the level of the School and by Croatia. And after 22 years 6 Schools and many activities with Greta I still am. She became interested in the application of infrared to LDL. It was a good model to study lipid-protein interactions and a good technique for dilucidating structural changes produced in the particle e.g. after oxidation, that could lead to pathological situations. Later Greta came to our laboratory as guest investigator, together with her daughter, to work with infrared and lipoproteins. We obtain nice results that were published in Journal of Biological Chemistry and were seeing nice ways of combining infrared and lipoproteins but she was appointed Deputy Minister of Science and Technology of the Republic of Croatia.

José Luis R. Arrondo
Departamento de Bioquímica y Unidad de Biofísica, Bilbao, Spain

I do not remember when I first met Greta, but it was surely at the first Biophysics summer school I attended. It is hard to remember life before Greta and even harder to imagine future summer schools without her forceful presence. She hovered over all the speakers and students like a mother hen with an exceptionally unruly brood, but it was always their science and education that was foremost in her mind. By amazing persistence, foresight, and with a talented group of other Croatian scientists, she kept this summer school moving for decades in the face of continual financial hardship that always threatened to scuttle it. It is right to question how I was able, like so many other lecturers in spite of a constantly overcommitted schedule to participate in so many of these meetings. First and foremost, once I met Greta I could never let her down. It was not that I feared disappointing her, since nothing could disappoint her, but it was the anticipation of the broad and sincere smile and exuberant hello that crossed her face every time, in spite of all the odds, that I reappeared at one of the summer schools. There were two other reasons. These schools were always infused with first rate lectures about first rate science. Even more important they were populated with an amazingly enthusiastic and dedicated group of students from countries throughout eastern Europe including countries like Albania, Bulgaria, Romania where I had never

managed to travel myself. Greta always rewarded us with her enthusiasm, lots of great food and good wine. Occasionally we got other rewards –one of my proudest possessions is a sample of her husband's art that one year she presented as a gift to the speakers. It still hangs in my office, as do photographs from previous summer schools.

Charles R. Cantor
Chief Scientific Officer, Sequenom, Inc., San Diego, USA

Greta Pifat-Mrzljak set the standard for schools teaching biophysics. Indeed, she started these in 1981 before biophysics became as popular as it is today, and as a result must be credited for enlarging the biophysics community, certainly within Europe. As well as being ahead of the times in her choice of school and the subject, she realized that high quality science and tutoring needed to be coupled with a conducive environment in which to present it, and where better than on the beautiful Dalmatian Coast. Students have been enthused about the subject as a result of these schools for many years now, and the networking that inevitably followed, has perpetuated.

At a personal level, it was impossible to refuse any invitation from Greta to teach at these schools. She had a charm and gift of persuasion that extended not only to lecturers, but also to committees of international organizations to whom she looked for, and succeed in gaining, support. For senior scientists looking to recruit the best graduates or post-docs, as well as younger participants looking for research opportunities, these schools offered a perfect, relaxed and perfect environment to foster personal interactions and realize future ambitions. Greta, and her dedicated team, gave their time in an unrestricted and unselfish way, ensuring the schools ran without stress or tension to the participants. All of this resulted from Greta's own temperament and efficiency, and it is a fitting tribute to her that the schools are continuing, as I am sure the community would want for some time to come.

I wish the venture every success, and am delighted to have been asked back to lecture, once again.

Anthony Watts
Biochemistry Department, University of Oxford, UK

(Managing Editor, European Biophysics Journal, 2000 – on;
Chair, British Biophysical Society, 1993 – 1997; 2007-on;
European Biophysical Societies Association, Executive Committee, 2000 - on)

Obituaries

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Obituary

Prof. Greta Pifat-Mrzljak (1939–2009)*

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To the memory of Greta Pifat-Mrzljak
She was a citizen of the world
and in her heart a true citizen of Croatia.
Greta personified Croatian culture,
humanities, arts and sciences.

The words of the Nobel Laureate Manfred Eigen and Prof. Ruthild Winkler-Oswatitsch, engraved on the memorial alongside the Canadian maple-tree which they planted at the Ruđer Bošković Institute in memory of Prof. Greta Pifat-Mrzljak, who died on the 11th December 2009. Their words fully summarise the personality and professional work of Prof. Pifat-Mrzljak. As a person of exceptional energy and talent, in segments of her work she bridged the gap between visionariness and reality and in many ways went beyond the border of the Republic of Croatia and indebted world science. Although she collaborated and was on friendly terms with Nobel Laureates and many other world renowned individuals in the field of science and politics, she greeted everyone with equal pleasure, always ready to help privately or professionally. Consequently many turned to her for help. She always placed the common good before her own. She overcame all obstacles with pedagogic uncompromising sincerity and thus showed how an honourable human being can overcome the boundaries of his restricted physical existence.

The scientific opus of Prof. Greta Pifat-Mrzljak is extremely abundant (http://www.irb.hr/hr/events/confpages/biophysics). It consists of over 60 source publications, printed in journals cited by *Current Contents*, 16 professional publications, 64 invited lectures, 68 presentations at congresses and symposia. She was editor of 27 books, organised 31 symposia, principal investigator of 12 scientific projects, she was also a guest scientist in many world centres. For her work she received 6 awards.

In this short presentation we will select just a few interesting details from her tireless work, which demonstrate that Prof. Pifat-Mrzljak went beyond the scope of her parent institution and homeland.

Prof. Greta Pifat-Mrzljak's interest in biophysics, incited by the influence of Dr Siniša Maričić through research of haemoglobin, was demonstrated in her first scientific study, which was published in 1964 in the distinguished biophysics journal Biochimica et Biophysica Acta. Biophysics is an interdisciplinary science in which the collaboration of scientists of different backgrounds is essential, and therefore the organisation of a monthly meeting of the Croatian Biophysics Society was the logical task of the President, Prof. Pifat-Mrzljak. It was a novelty at that time in the Institute, and lectures, at which foreign scientists frequently

Figure 2. *Group photograph of participants in the International Summer School on Biophysics, Red Island, 2006.*

took part, were very well attended. Very soon members of other institutions began to attend. At that time biophysics in Croatia was in its infancy, and it was necessary to educate young investigators. As the President of the Association of Biophysics Societies in former Yugoslavia, Prof. Pifat-Mrzljak became involved in a new adventure, which in time will become her greatest achievement and her favourite activity. In 1981 with great resoluteness she succeeded in arranging for the Ruđer Bošković Institute and Croatian Biophysics Society to organise together the international summer school in Biophysics, which in time became a Croatian scientific *brand*. With great persistence and negotiation she was given the opportunity of holding the School in the hotel complex of the Yugoslav National Army (JNA) in Kupari. With her personal acquaintances she succeeded in bringing together currently the most well known names in the field, and ensured sponsorship of the world biophysics organisations IUPAB and UNESCO for the programme. During the period from 1981 to 2009 ten schools were held with intervals of three years. Because of the Homeland War the School which was envisaged for 1993 was held in 1994 on the Red Island at Rovinj. As it was impossible to return to the devastated Kupar the School remained on the new loca-

Figure 1. *Prof. Greta Pifat-Mrzljak in 2004 during the Day of Open Doors of the Ruđer Bošković Institute with Prof. Stjepan Marčelja, Director of the Institute, and in 2008 at the entrance of the Ruđer Bošković Institute waiting for visitors.*

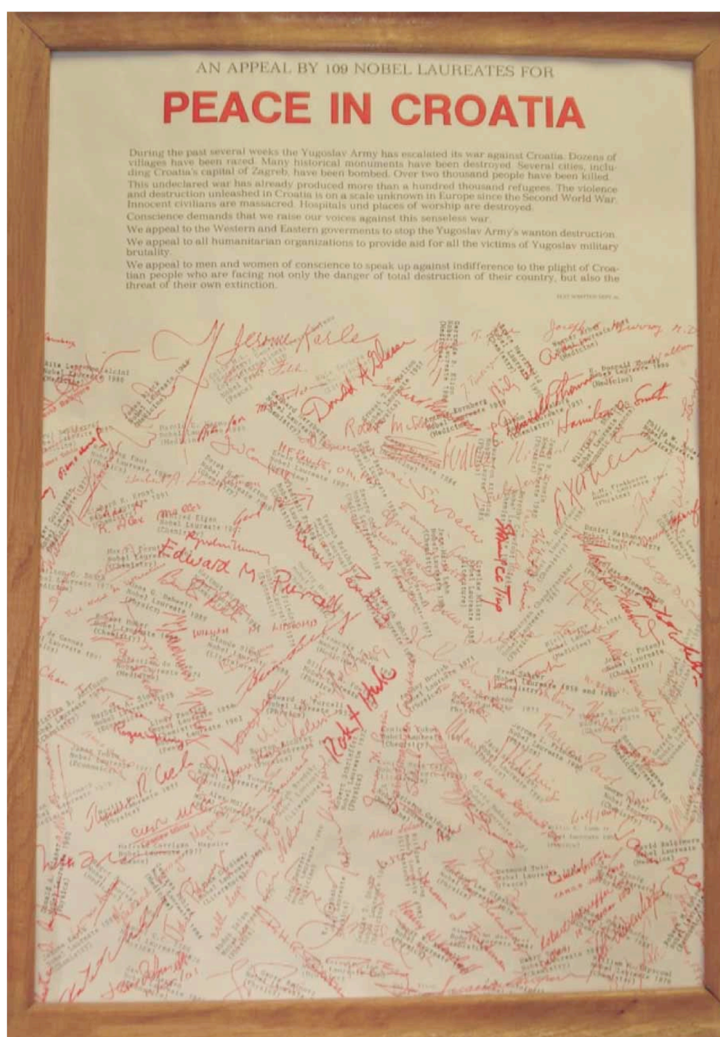

Figure 3. *Appeal for Peace in Croatia which was signed by more than one hundred Nobel Laureates. The Action organised by the Nobel Laureates M. Eigen and R. Huber was coordinated by Prof. Greta Pifat-Mrzljak.*

tion. During the 30-year period more than 300 lecturers participated gratis in the work of the School, all eminent world scientists or Nobel Laureates. The School always included »sensitive« themes. The basic idea of Prof. Pifat-Mrzljak was to enable young scientists from Croatia to meet and actively associate with eminent scientists and young biophysicists from the whole world during lectures, seminars and discussion clubs, which her concept of the School achieved. Each School was attended by at least 25 young Croatian scientists. It was for all an unforgettable scientific experience. They transferred the atmosphere of this association to their own institutions. Thus it is of no surprise that the School quickly became well known and acknowledged in the international scientific world. It was awarded the UNESCO School Certificate, and many universities in Europe included the School in post-graduate *curriculum*. Prof. Greta Pifat-Mrzljak, organiser, director and good spirit of all the Schools, was presented with the award »Emily M. Gray« for 2010, by the American Biophysical Society, for her contribution to the promotion of scientific research and education in the field of biophysics.

Prof. Greta Pifat-Mrzljak spread the truth about occurrences during the 1990's and the threat of war in Croatia throughout the world by writing letters to well known scientists, encouraging our scientists abroad to take some action. Consequently, at the beginning of the

Homeland War, with the help of two Nobel Laureates, Professores Manfred Eigen and Robert Huber, a unique appeal was organised under the name *Appeal for Peace in Croatia*, which was coordinated by Prof. Pifat-Mrzljak. More than a hundred Nobel Laureates signed the appeal for peace, and Prof. Pifat-Mrzljak collected and documented all activity of co-workers in the Book *Nobel Laureates for Peace in Croatia*, University edition, Zagreb, 1992. In fact the appeal was unique in the history of Nobel Laureates, who gathered together with the aim of preventing military aggression in a country.

As the Deputy Minister of Science and Technology of the Republic of Croatia, Prof. Pifat-Mrzljak worked intensively on two important projects: a catalogue of Croatian scientists outside the homeland and an exhibition *Science in the Croat, Natural Sciences and its Application.* She was Editor-in-Chief of the edition *Scientific Research in Croatia* of the Ministry of Science and Technology, Zagreb, 1995, and two catalogues which accompanied the exhibition in 1996. A great wish of hers, sadly never realised, was for parts of the exhibition to remain as permanent parts of a future museum of natural sciences.

The leading idea of Prof. Greta Pifat-Mrzljak, even in the early days, when as a young doctor of chemistry she returned from a study visit to Prof. Manfred Eigen, her role model, tutor and co-worker in the Max Planck Institut fur Biophysikalische Chemie in Cottingen, was for science to become familiar and accessible to everyone, because understanding leads eventually to individual opinion. She was surprised at the level of disinterest and neglect of the »public« for science and the latest achievements. Scientists were considered odd characters who »amuse themselves« behind the closed walls of institutes and who spend money for no useful purpose. Their success in science, about which it was occasionally possible to read in the newspapers, was presented in an unintelligible and boring language. At that time she decided to permanently and conscientiously with great determination and enthusiasm to create communication between scientists and the public, which she continued to do throughout her whole working life. With this intention, by a revival of events at the Ruđer Bošković Institute under the name *Open Door Days of the Ruđer Bošković Institute* a new concept was given. With special care and inexhaustible ideas she encouraged young scientists to arrange presentation of their work to pupils and interested public, prompted by her wish to create communication and understanding at all levels and in different circumstances. In this she succeeded as scientists are no longer considered odd characters, and science is written and talked about, people listen, some even hear, science is necessary, and the fact that financing is still insufficient, is not the failure of Prof. Pifat-Mrzljak, but rather that some have still not heard.

From 1996 Prof. Greta Pifat-Mrzljak was President of the Croatian-Austrian Society (HAD) for the advancement of cultural, scientific and economic relations of the two countries. In this context she arranged, as she was fond of saying, capillary collaboration of the two countries. She organised a meeting in Graz under the title *Croatia is Europe*, in which a large number of Croatian and Austrian scientists, economists and politicians took part. She was also the main organiser of *Scientific Days of RH and RA (Republic of Croatia and the Republic of Austria),* which, under the organisation of HAD, IRB and the Joanneum Institute in Graz was held on two occasions in Zagreb and once in Graz, during in the interval of two years. The meeting of scientists from Croatia and Austria was intended to facilitate inclusion of our scientists in projects in the European Programme. For this tireless work in 2002 she was awarded the high recognition of the Government of Austria, *Osterreichische Ehrenkreuz fur Wissenschaft und Kunst I Klasse*.

Prof. Greta Pifat-Mrzljak was a person of firm beliefs and moral values, a gentle friend and demanding co-worker, and at the same time a person endowed with the particular attitude and marked quality of a leader. She invested great effort in everything she did and was deeply involved in her work. She did not spare herself and expected the same of her co-workers. The contribution of Prof. Pifat-Mrzljak is much more than just the numbers of her comprehensive scientific biography. She directed all her activity to science and youth. She spent the whole of her working life at the Ruđer Bošković Institute and she regarded all her achievements as those of her parent institution. Her desire was for knowledge, ability, diligence and honesty to be the only criteria in Croatia. The many activities of Prof. Pifat-Mrzljak were based on these values. This is the heritage she left us.

IUPAB
CONNECTING THE WORLD OF BIOPHYSICS

INTERNATIONAL UNION for PURE and APPLIED BIOPHYSICS

IUPAB NEWS

No. 55, December 2009

Editor: Louise Matheson
Email: mail@iupab.org

Greta Pifat-Mrzljak 1939-2009

Greta Pifat-Mrzljak was born in Zagreb, Croatia and died after an illness on December 11, 2009. She was educated from primary school through post-graduate studies in Zagreb, receiving her Ph.D. in 1971 in Chemistry. At the time of her death, Greta was director of the Ruder Boskovic Institute, and the current recipient of the Emily M. Gray Award for 2010, which is made annually for exceptional contribution to education in biophysics. Greta was the first non-U.S. recipient in the thirteen year history of the award, and she was in distinguished company. It is most fitting that her achievements were recognized in this forum. The 10th International Summer School in Biophysics held in Croatia last year was another fine example of Greta's effo

PROGRAM

Sunday September 30 / SCHOOL DAY 1

15:00 REGISTRATION

OPENING OF THE SCHOOL

17:00 INTRODUCTION

CHARLES R. CANTOR, Sequenom, Inc. USA

17:15 Noninvasive Personalized Genomics

18:15 Overcoming fundamental obstacles to produce useful tools and reagents

19:00 WELCOME DRINK

19:30 DINNER

Monday October 1 / SCHOOL DAY 2

NENAD BAN, ETH Zürich, Switzerland

9:00 Springs, tethers and switchblades: mechanistic insights into the functioning of large macromolecular assemblies involved in fatty acid synthesis

10:00 Crystal structures of the eukaryotic ribosome and insights into the regulation and initiation of protein synthesis

10:45 COFFEE BREAK

C. OOSTENBRINK, University of Natural Resources and Life Sciences, Vienna, Austria

11:15 Ensembles and sampling, leading to molecular dynamics simulations

12:15 Structure refinement using molecular dynamics simulations (NMR observables)

13:00 LUNCH

J. L. R. ARRONDO, Universidad del País Vasco/Euskal Herriko Unibertsitatea, Bilbao, Spain

15 :00 Infrared spectroscopy of lipoproteins

NENAD BAN, ETH Zürich, Switzerland

16 :00 Discussion

C. OOSTENBRINK, University of Natural Resources and Life Sciences, Vienna, Austria

17:00 Calculation of free energies from molecular simulation

18:00 DINNER

20-22 POSTERS TALKS & POSTER SESSION I

20:00 **M. Montes-Saralegui:** Cluster formation of amphiphilic polymer chains

20:10 **J. A. Martinez:** The reaction of the hepatitis C virus NS3/NS4A protease with its main substrate. A QM/MM investigation.

20:20 **M. Žganec:** DMD simumulations of stefin folding and early stages of oligomerization

Tuesday October 2 / SCHOOL DAY 3

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

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

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

10:45 COFFEE BREAK

A. WATTS, University of Oxford, UK

11:15 Principles of biomolecular solid state NMR

12:15 Solid state NMR of biomembranes

13:00 LUNCH

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

16:00 Code within code: sequence signatures of direct complementarity between mRNAs and cognate proteins on multiple levels

A. WATTS, University of Oxford, UK

17:00 Drug targeting to membrane receptors

18:00 DINNER

20-22 SHORT POSTERS TALKS & POSTER SESSION II

20:00 **K. Jodko-Piórecka:** The thermodynamics of dopamine / membrane interactions

20:10 **C. Marasini:** Thermodynamical and structural changes in two functional states of regulatory domain of CFTR

20:20 **K. Renngli:** Controlled radical polymerization in protein nanoreactors and with native enzymes

Wednesday October 3 / SCHOOL DAY 4

P. ZIHERL, University of Ljubljana and Jozef Stefan Institute, Ljubljana, Slovenia

9:00 Mechanical models of structure of animal tissues I

10:00 Mechanical models of structure of animal tissues I

10:45 COFFEE BREAK

E. SACKMANN, Technical University Munich, Germany

11:15 Thermoelasticity of the self organisation and biological function of composite cell membranes

12:15 Microviscoelasticity and viscoplasticity of semiflexible biomacromolecular networks and cells

13:00 LUNCH

P. ZIHERL, University of Ljubljana and Jozef Stefan Institute, Ljubljana, Slovenia
16:00 Mechanical models of structure of animal tissues III

E. SACKMANN, Technical University Munich, Germany
17:00 The infinite manifold of life - Evolution as interplay of physics and genetics

18:00 DINNER

20-22 SHORT POSTERS TALKS & POSTER SESSION III

20:00 **A. Muratov**: Self-organization of microtubules in plant cell cortex under the influence of pressure

20:10 **N. A. Ghazaryan**: Molecular mechanism of GUVs from high density lipoproteins under the influence of *MLO* venom

20:20 **H. Salehi:** Detection of anticancer drug paclitaxel in MCF-7 cells by confocal Raman microscopy

20:30 **A. L. Torosyan:** Estimation of the bilayer lipid membrane permeability for TButPyP4 porphyrins

Thursday October 4 / SCHOOL DAY 5

R. PODGORNIK, University of Ljubljana, Slovenia
9:00 DNA-DNA electrostatic interactions

10:00 Long range order in DNA mesophases

10:45 COFFEE BREAK

G. A. VOTH, University of Chicago, USA
11:15 Theory and simulation of biomolecular systems: surmounting the challenge of bridging the scales

12:15 Proton transport in proteins: insights and surprises from multiscale computer simulation

13:00 LUNCH

14:00 EXCURSION to Krka National Park

20:00 DINNER

Friday October 5 / SCHOOL DAY 6

R. C. WADE, Heidelberg University, Germany
9:00 Brownian dynamics simulation of biomacromolecules (Part I)

10:00 Brownian dynamics simulation of biomacromolecules (Part II)

10:45 COFFEE BREAK

R. PODGORNIK, University of Ljubljana, Slovenia
11:15 Ordering and condensation of DNA in bacteriophages

H. STARK, MPI for Biophysical Chemistry, Goettingen, Germany
12:15 Introduction to single particle electron cryomicroscopy

13:00 LUNCH

H. STARK, MPI for Biophysical Chemistry, Goettingen, Germany
16:00 3D structure determination of dynamic macromolecules by cryo-EM

R. C. WADE, Heidelberg University, Germany
17:00 Discussion

18:00 DINNER

20-22 SHORT POSTERS TALKS & POSTER SESSION IV

20:00 **M. Berynskyy:** Protein-protein docking: a combined all-atom and coarse grained approach

20:10 **S. Sukenik:** Diversity in the mechanisms of cosolute action on peptide folding

20:20 **A. Roychoudhury:** Stabilization of membrane proteins by compatible solutes: single molecule force spectroscopic study

20:30 **I. Mihaljevic:** Functional characterization of novel uptake transporters in zebrafish (*Danio rerio*): Oat2a and Oat2d

Saturday October 6 / SCHOOL DAY 7

H. GRUBMÜLLER, Max Planck Inst. for Biophysical Chemistry, Goettingen, Germany
9:00 Forces and conformational dynamics in biomolecular nanomachines (Part I)

10:00 Forces and conformational dynamics in biomolecular nanomachines (Part II)

10:45 COFFEE BREAK

H. GRUBMÜLLER, Max Planck Inst. for Biophysical Chemistry, Goettingen, Germany
11:15 Discussion

A. PERCZEL, Eötvös Loránd University, Budapest, Hungary
12:15 Peptide and protein folding as seen by NMR (and ECD)

13:00 LUNCH

A. PERCZEL, Eötvös Loránd University, Budapest, Hungary
16:00 In cell NMR of intrinsically dynamic proteins

17:00 Quantitative ECD analysis of peptides and proteins: the CCA+ method

18:00 DINNER

20-22 SHORT POSTERS TALKS & POSTER SESSION V

20:00 **B. L. Aekbote:** Two-photon polymerized and functionalized 3D microstructures for biological applications

20:10 **I. Vlahović**: Finding tandem repeats in Tribolium Castaneum using computational method Global Repeat Map

20:20 **B. Glisic:** Phylogenetic and tissue distribution analysis of GST superfamily in zebrafish (*Danio rerio*)

Sunday October 7 / SCHOOL DAY 8

H.- J. STEINHOFF, University of Osnabrück, Germany

9:00 Site-directed spin labeling and electron paramagnetic resonance spectroscopy: an introduction

10:00 Inter- and intramolecular distance measurements using cw and pulse EPR spectroscopy

10:45 COFFEE BREAK

A. - S. SMITH, Universität Erlangen-Nürnberg, Germany

11:15 Relating the conformational space of a peptide with its circular dichroism spectra by computational methods

12:15 The binding affinity ‡ effects of reactant confinement

13:00 LUNCH

H.- J. STEINHOFF, University of Osnabrück, Germany

16:00 Multi-frequency EPR spectroscopy of membrane proteins

A. VAZIRI, University of Vienna, Austria

17:00 Recent frontiers of molecular biophysics (Part I)

18:00 DINNER

20-22 SHORT POSTERS TALKS & POSTER SESSION VI

20:00 **D. Klose**: Simulation vs. reality: a comparison of in silico distance predictions with DEER and FRET measurements

20:10 **L. V. Kulik:** Sodium-dependent movement of covalently bound FMN residue(s) in Na^{+}-translocating NADH:quinone oxidoreductase

20:20 **S. Marion:** Low-frequency impedance spectroscopy: role of microscopic phase separation in gelation of aqueous gelatin

Monday October 8 / SCHOOL DAY 9

A. VAZIRI, University of Vienna, Austria

9:00 Recent frontiers of molecular biophysics (Part II)

10:00 Recent frontiers of molecular biophysics (Part III)

10:45 COFFEE BREAK

I. M. TOLIĆ NØRRELYKE, MPI of Molecular Cell Biology & Genetics, Dresden, Germany

11:15 Microtubules and motor proteins

12:15 Microtubules and the mitotic spindle

13:00 LUNCH

A. TOSSI, University of Trieste, Italy

16:00 Membrane-active helical antimicrobial peptides

17:00 Methods of studying AMPs (antimicrobial peptides)

19:00 GALA DINNER

Tuesday October 9 / SCHOOL DAY 10

A. ŠIBER, Institute of physics, Zagreb, Croatia

9:00 Mean-field electrostatics explained through application to viruses (Part I)

10:00 Mean-field electrostatics explained through application to viruses (Part II)

10:45 COFFEE BREAK

A. ŠIBER, Institute of physics, Zagreb, Croatia

11:15 Mean-field electrostatics explained through application to viruses (Part III)

12:15 CLOSING REMARKS

13:00 LUNCH

DEPARTURE

LECTURES

Noninvasive Personalized Genomics

Charles R. Cantor

Chief Scientific Officer, SEQUENOM, Inc., San Diego, CA USA

We now have the power to determine virtually any aspect of an individual genome sequence but still have much less ability to interpret this data in a medically useful way. Measurements of germ line DNA from blood or sputum inform about disease risk but for most complex disease these estimates are not terribly informative. The exceptions are one complex disease, age related macular degeneration, and many aspects of individual responses to particular drugs, so called pharmacogenetics. Somatic mutations and epigenetic modifications must usually be measured in a specific tissue such as a tumor, but these frequently do have high predictive value. Such measurements are becoming a common and an effective step in selecting effective anti-cancer medications. However they require a tumor biopsy and thus cannot easily be used to monitor the course of therapy. In the future it should be possible to detect these mutations noninvasively at low concentrations in plasma. The precedent for this comes from the development of non-invasive prenatal diagnostics which uses sequence measurements of DNA fragments from the fetus that circulate in the mother's blood. Nearly perfect detection of the common aneuploidies can now be achieved routinely with absolutely no risk to mother and fetus. It is even possible to determine the full genome sequence of an unborn fetus from these fragments, but the current cost of doing this currently prohibits wide spread use.

Overcoming fundamental obstacles to produce useful tools and reagents

Charles R. Cantor

Chief Scientific Officer, SEQUENOM, Inc., San Diego, CA USA

Successful implementation of a new method frequently requires grappling with basic issues in chemistry and physics. Here three examples of such obstacles will be described. (a) Plasma DNA fragments are present at extremely low concentrations. This compromises the accuracy of DNA sequencing and obliterates the ability to quantify single locus copy numbers. However these issues can be solved by exploiting large numbers of equivalent near-by loci through haplotype analysis. (b) Detection at high contrast of the abundance and spatial localization of specific molecules in living cells is an essential tool in cell biology. The availability of naturally fluorescent proteins like the green fluorescent proteins greatly facilitates such measurements at the protein level but it is more difficult to execute such strategies with nucleic acids. However, by using nucleic acids to direct the assembly of halves of fluorescent proteins high sensitivity and high contrast in vivo detection of specific RNA species has become possible. (c) Oxidative damage is implicated in many disease processes, especially those involved in the central nervous system. Antioxidants are not efficient in combating the damage because it is not possible to deliver them at high enough concentrations to tissues of interest to scavenge short lived reactive oxygen species before they can do their damage. However it is possible to reinforce the targets of these oxygen species with specific deuterium substitutions so that the rates of oxidative damage are slowed considerably by the kinetic isotope effect without otherwise perturbing normal cell biochemistry. By using deuterated essential nutrients the problem of delivery to specific tissues is obviated since the body apparently cannot discriminate between these compounds and ordinary hydrogen containing nutrients.

Springs, tethers and switchblades: mechanistic insights into the functioning of large macromolecular assemblies involved in fatty acid synthesis

Nenad Ban

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Many important cellular processes are catalyzed by multifunctional proteins where many individual enzymes are brought together to form a "molecular assembly line". As a paradigm for such multienzymes we have investigated the structures of mammalian and fungal fatty acid synthases, giant complexes that contain multiple catalytic domains and catalyze all steps of fatty acid synthesis. The fungal and mammalian systems reveal two different architectural solutions that allow these multienzymes to hand over the products of one enzymatic active site to the next. This work offers mechanistic insights into substrate shuttling and delivery in such megasynthases, with direct implications on our understanding of polyketide synthases and non-ribosomal peptide synthases[1-3].

[1] Jenni S, Leibundgut M, Boehringer D, Frick C, Mikolásek B, Ban N (2007) Structure of Fungal Fatty Acid Synthase and Implications for Iterative Substrate Shuttling. *Science* 316: 254-261.

[2] Leibundgut M, Jenni S, Frick C, Ban N (2007) Structural Basis for Substrate Delivery by Acyl Carrier Protein in the Yeast Fatty Acid Synthase. *Science* 316: 288-290.

[3] Maier T, Leibundgut M, and Ban N (2008) The Crystal Structure of a Mammalian Fatty Acid Synthase. *Science* 321(5894):1315-22.

Crystal structures of the eukaryotic ribosome and insights into the regulation and initiation of protein synthesis

Nenad Ban

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ban@mol.biol.ethz.ch

A long term interest in my laboratory is to study structures of prokaryotic and eukaryotic ribosomes, ribosomal subunits and their complexes with various factors involved in protein synthesis, with the aim to better understand this process. Although basic aspects of protein synthesis are preserved in all kingdoms of life, 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 recently determined crystal structures of the 40S eukaryotic ribosomal subunit in complex with initiation factor 1 and of the 60S eukaryotic ribosomal subunit in complex with initiation factor 6[1,2]. These results provide detailed structural information on the entire eukaryotic ribosome, reveal novel architectural features of this ribonucleoprotein complex and offer insights into the various eukaryotic-specific aspects of protein synthesis and ribosome evolution.

[1] Rabl J, Leibundgut M, Ataide SF, Haag A, Ban N (2011) Crystal structure of the eukaryotic 40S ribosomal subunit in complex with initiation factor 1. *Science* 331(6018):730-736.

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

Ensembles and sampling, leading to molecular dynamics simulations

Chris Oostenbrink

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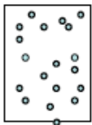
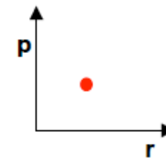
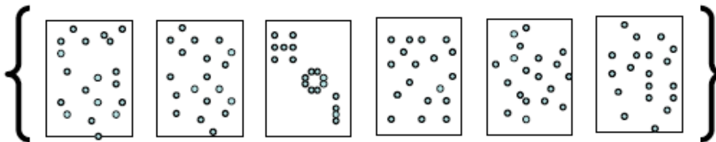
chris.oostenbrink@boku.ac.at

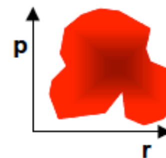
In this lecture, we will discuss the basics of molecular dynamics simulations. Starting from the link between a molecular structure and the (potential) energy of a system, we will discuss various algorithms to modify the structure in physically meaningful ways. This will lead to the definition of ensembles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



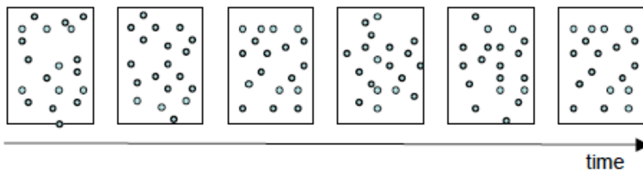


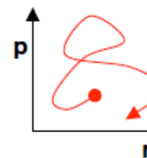
A point in phase space





A probability distribution in phase space





A continuous trajectory in phase space

Structure refinement using molecular dynamics simulations (NMR observables)

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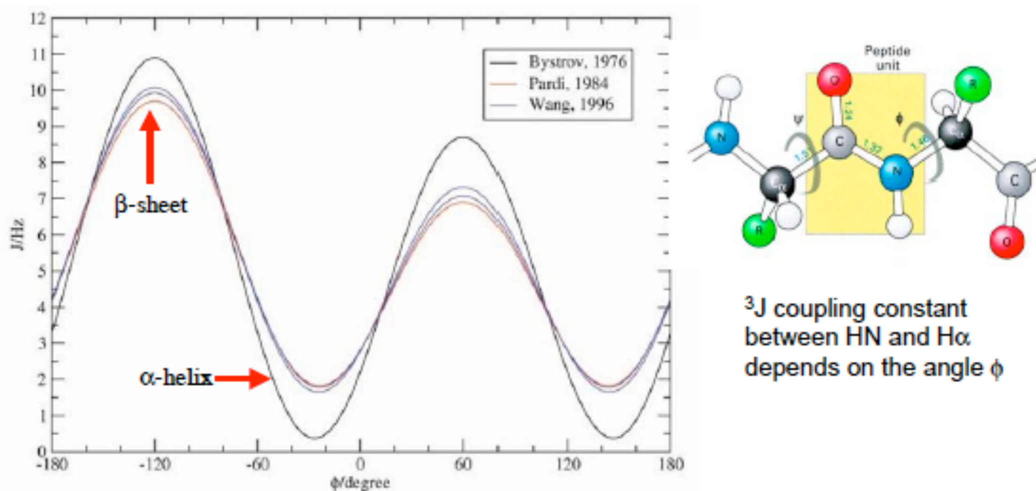
In the previous lecture, we have seen how molecular simulations can be used to generate conformational ensembles and how averages over such ensembles may be correlated to experimental properties. In the current lecture, we will discuss possibilities to do the inverse: use experimental observations as boundary conditions for molecular simulations, in order to e.g. refine molecular structures. Using NMR parameters like NOE distance restraints or ^{3}J-coupling constants as examples, the various ways of restraining the molecular structure will be discussed.



Infrared spectroscopy of lipoproteins

José Luis R. Arrondo

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Lipoproteins, the major carriers of lipids in human plasma, are a good topic to study by infrared spectroscopy. The components of the particles, lipids and proteins, give rise to independent bands in the infrared spectrum that allow the study of both moieties in the same sample. Three major lipoprotein classes are found in fasting human blood plasma, VLDL (very low density lipoprotein), LDL (low density lipoprotein) and HDL (high density lipoprotein).

LDL is the principal carrier of cholesterol and play an important role in the development of atherosclerotic lesions. It is described as a spherical particle containing a hydrophobic core of cholesteryl esters and triglycerides surrounded by an amphipatic monolayer of phospholipid and cholesterol in which a single molecule of protein (apoB) is located. Infrared spectroscopy has been applied to characterize the secondary structure of apoB and its topology in the LDL particle. A special feature of the apoB spectrum is a band at 1618 cm^{-1} with a high wavenumber component at 1693 cm^{-1} that have been attributed to β-strands embedded in the phospholipid monolayer. Thermal profiles of the different bands allow the study of the surface-core relationships by looking at the variations in the thermal profiles produced by alterations in the ionic strength of the medium[1].

The hypothesis that oxidative modification of both the lipid and protein components of LDL plays a substantial role in the initiation and progression of atherosclerosis is now well substantiated. Studies effectuated at the early stages of oxidation does not show changes in secondary structure in the lag phase of oxidation, where antioxidants are still present, or in the propagation phase where dienes are formed in the lipid, but the particle is not altered. However, looking at the thermal profiles, it is shown that in the propagation phase the profile corresponding to the band attributed to α-helix is shifted with respect to the control showing that even if secondary structure is not affected, the tertiary structure has been modified[2]. The studies can be made by conventional IR and 2DCOS, an analytical tool that we are developing and gives a better insight in the small differences that sometimes are observed.

[1] Bañuelos S., Arrondo JLR, Goñi FM, Pifat G (1995) Surface-Core Relationships in Human Low Density Lipoprotein as Studied by Infrared Spectroscopy. *J Biol Chem* 270:9192-9196.

[2] Chehin R, Rengel D, Milicua JCG, Goñi FM, Arrondo JLR, Pifat G (2001) Early stages of LDL oxidation: apolipoprotein B structural changes monitored by infrared spectroscopy. *J Lipid Res* 42:778-782.

Calculation of free energies from molecular simulation

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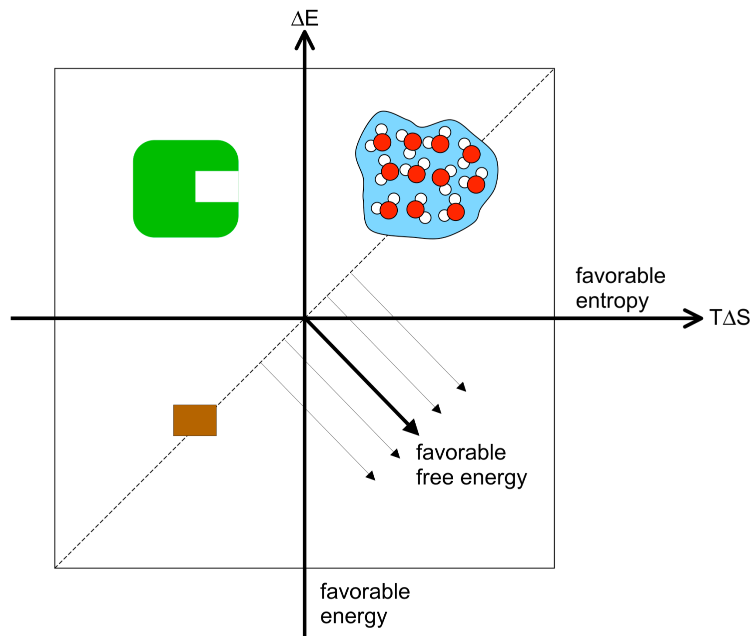
The free energy forms the driving force of any molecular process. Intrinsically containing both enthalpic and entropic contributions, the accurate estimation of free energies is possible from statistical mechanical principles. This lecture will use the free energy of ligand binding as a representative 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.



More dynamic than we think?
On conformational averaging in structural biology
Part I

Bojan Zagrovic

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

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

Part II

Bojan Zagrovic

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

Principles of biomolecular solid state NMR

Anthony Watts

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Solid state NMR is a versatile methodology for examining molecular structure in a non-perturbing way, and with a widely versatile form of sample. There are no molecular weight limits, and the wealth of information complicates the data by often being too prolific and approaches are needed to simplify the data obtained to reveal the required information. By judicious approaches, specific sites of action and interest can be studied, to give biologically relevant detail that can complement other structural information.

Solid state NMR of biomembranes

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One of the successes of solid state NMR is in its application to the challenging area of biomembranes, both of the lipid and the protein components.

Because of the extensive molecular weight range of the method, and versatility of sample form, solid state NMR has found widespread application to many systems, including small trans-membrane peptides as ion channels, large polytopic proteins and receptors, as well as drug targets.

Here, examples of a range of systems will be presented, and the methodology used to resolve the details explained.

Code within code: sequence signatures of direct complementarity between mRNAs and cognate proteins on multiple levels

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

Drug targeting to membrane receptors

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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. This information is obtained by isotopically (^{2}H, ^{13}C, ^{19}F, ^{15}N, ^{17}O) labeling selective parts of either a ligand, or the protein understudy, and observing the nucleus in non-crystalline, macromolecular complexes.

References to selected reviews (see also: http://www.bioch.ox.ac.uk/~awatts/):

- Watts A (2005) Solid state NMR in drug design and discovery. *Nature Drug Discovery* 4:555-568.
- Higman V & Watts A (2012) "CHAPTER 13 Recent Developments in Biomolecular Solid-State NMR", in *Recent Developments in Biomolecular NMR*, (eds M. Clore and J. Potts) (in press)
- Judge PJ & Watts A. (2011) Recent contributions from solid-state NMR to the understanding of membrane-protein interactions. *Current Opinions in Chemical Biology*, 15:690-695.
- Watts A, Straus SK, Grage S, Kamihira M, Lam Y-H, Xhao Z (2003) Membrane protein structure determination using solid state NMR. In: *Methods in Molecular Biology – Techniques in Protein NMR Vol. 278* (ed. K. Downing)
- Grage SL & Watts A (2007) Applications of REDOR for distance measurements in biological solids. *Annual Reports in NMR* 60:192-228.

Mechanical models of structure of animal tissues I

Primoz Ziherl

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Many biological tissues are marked by some kind of spatial regularity or order. We review the various mechanical models of structure of simple animal tissues, especially epithelia. We restrict the discussion mainly to conservative, energy-minimizing theories of tissue structure. We begin with models based on the differential adhesion hypothesis and on the surface tension of cells, and we discuss their predictions, scope, and shortcomings.

Mechanical models of structure of animal tissues II

Primoz Ziherl

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After introducing cell cortex tension as an mechanism of fairly clear microscopic origin, we present a few variants of cortex-based models. Such models are particularly useful in reproducing the various features of the in-plane structure of single-cell epithelia. Also described are some related empirical findings which may point to the universality observed in many epithelial tissues and other 2D tilings, and the application of cortex-elasticity-based models to the surface tension of tissues.

Thermoelasticity of the self organisation and biological function of composite cell membranes

Erich Sackmann

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Elastic forces and structural phase transitions control the architecture and function of bio-membranes from the molecular to the microscopic scale of organization. The multi-component lipid bilayer matrix behaves as a pseudo-ternary system. Together with elastically and electrostatically mediated specific lipid–protein interaction mechanisms, fluid–fluid phase separation can occur at physiological temperatures. This can drive the transient generation of microdomains of distinct composition within multi-component lipid–protein alloys, enabling cells to optimize the efficiency of biochemical reactions by facilitating or inhibiting the access of enzymes by distinct substrates or regulatory proteins.

Together with global shape changes governed by the principle of minimum bending energy and induced curvature by macromolecular adsorption, phase separation processes can also play a key role for the sorting of lipids and proteins between intracellular compartments during the vesicle mediated intracellular material transport.

Cell adhesion is another example of mechanical force controlled membrane processes. By interplay of attractive lock and key forces, long range disjoining pressures mediated by spacer molecules (repellers) or membrane undulations and elastic interfacial forces, adhesion induced domain formation can play a dual role for the immunological stimulation of lymphocytes and for the rapid control of the adhesion strength.

The present picture of the thermo-elastic control of membrane processes based on concepts of local thermal equilibrium is still rudimentary and has to be extended in the future to account for the intrinsic non-equilibrium situation associated with the constant restructuring of the cellular compartments on a time scale of minutes.

References:

Sackmann E (2006) Thermo-elasticity and adhesion as regulators of cell membrane architecture and function. *J Phys: Condens Matter* 18:R785–R825.

Bruinsma R, Behrisch A, Sackmann E (2000) Adhesive switching of membranes: experiment and theory. *Phys Rev E* 61:4253-4267.

Smith AS and Sackmann E (2009) Progress in mimetic studies of cell adhesion and the mechanosensing. *Chem Phys Chem* 10:66 –78.

Microviscoelasticity and viscoplasticity of semiflexible biomacromolecular networks and cells

Erich Sackmann

Physics Department, Technical University Munich, Garching, Germany
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The mechanical behaviour of cells is determined by the viscoelastic and viscoplastic properties of the actin-based cytoskeleton which is coupled in an interactive way to the composite cell envelope and the microtubule (MT) network. Many cellular processes (ranging from adhesion to cell locomotion) and mitosis are controlled by the interactive cross-talk between the action cortex and the aster-shaped MT-network. The lecture consists of four parts.

The first part deals with the self organisation of the actin network by an army of actin-binding proteins (ABP); with special emphasize of the regulation of the ABP activity by cell signalling.

Then the viscoelastic properties of the actin network as studied by magnetic tweezer microrheometry are related to the single filament dynamics. In this context it is shown that shear field mapping allows local measurements of the mechanical properties of cell envelopes and cytoplasms. The viscoelastic impedance of cell envelopes reflects the rheological signature of the actin networks enabling real time studies of structural reorganization of cell cytoskeleton by cell stimulating agents. In the fourth part evidence is provided that the cytoplasmic space of cells behaves as an active viscoplastic body.

In the third part I introduce the concept of tensional homeostasy and the role of mechanical impedance matching of cells and tissue for the mechanical stabilisation and differentiation of cells.

References:

Ditchtl M and Sackmann E (2002) Microrheometry of semiflexible actin networks through enforced single filament reptation: Frictional coupling and heterogeneities in entangled networks. *Proceed Nat Acad Sci* 99:6533-6538.

Bausch AR, Möller W, Sackmann E (1999) Measurement of local viscoelasticity and forces in living cells by magnetic tweezers. *Biophys. J* 76:573-579

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Sackmann E (2010) How cells feel the force. *(Biophysics) Nature Physics* 6: 407-408.

Mechanical models of structure of animal tissues III

Primoz Ziherl

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The final aspect discussed is the 3D shape of epithelia such as their shape and their bending elasticity. We describe physical models of crypts and villi in intestine epithelia as well as theories of morphogenetic processes such as the formation of ventral furrow. Also introduced are selected models of bulk tissues.

The infinite manifold of life - Evolution as interplay of physics and genetics

Erich Sackmann

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We are fascinated and overwhelmed by the sheer infinite manifold of life forms which stretch over 10 orders of magnitude in size. This enormous diversity is in particular stunning since we know that the genetic blueprint of life is determined by the DNA double helix. Since the groundbreaking book of Schrödinger "What is life", an ongoing discussion is whether the information on the DNA is sufficient to control the development of animals from the fertilized egg to the fully developed animals. In this general lecture I intend to show that the diversity of life can be explained as interplay of genetic information physics and chemistry.

In fact, the situation may be simpler than we think, since Mother Nature managed to generate the manifold of life forms by an astonishing small number of molecular building blocks. We discuss a number of examples providing some evidence that three major principles guided the biological evolution:

- The self-organisation of supra-molecular machines by shape–dependent intermolecular forces.
- The hierarchical design of living matter by very similar, specific functions fulfilling modules
- The scaling laws of physics

DNA-DNA electrostatic interactions

Rudi Podgornik

Dept. of physics, Faculty of Mathematics & Physics, University of Ljubljana, Slovenia
rudolf.podgnornik@fmf.uni-lj.si

I will present the basic theory of electrostatic interactions between DNA molecules in arrays. I will describe the effect of conformational fluctuations on these interactions and show how they lead to enhanced electrostatic interactions. I will describe the effect of polyvalent counterions on the nature of interactions and show that they can lead to a reversal of sign of the interaction force, giving rise to attractive interactions between helices. I will examine different descriptions of these attractive interactions.

Long range order in DNA mesophases

Rudi Podgornik

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rudolf.podgnornik@fmf.uni-lj.si

I will describe the nature of known mesophases of DNA at high densities (cholesteric, line hexatic, hexagonal colunar, hexagonal and orthorhombic) and invoke some recent advances in their understanding. Concerted rotations and translations of long helical molecules around their long axes in bulk samples lead to a new mesophase with a screw-like order. This screw-like order actually expels the cholesteric twist from a line hexatic phase and allows it to show a typical sixfold lhexatic scattering intensity. I will also show how the azimutal interactions lead to crystallization of the fiber with an orthorhombic unit cell which can be observed in DNA at high density.

Theory and simulation of biomolecular systems: surmounting the challenge of bridging the scales

Gregory A. Voth

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A multiscale theoretical and computational methodology will be discussed for studying biomolecular systems across multiple length and time scales. The approach provides a systematic connection between all-atom molecular dynamics, coarse-grained modeling, and mesoscopic phenomena. At the heart of the approach is a method for deriving coarse-grained models from protein structures and their underlying molecular-scale interactions. This particular aspect of the work has strong connections to the theory of renormalization, but it is more broadly developed and implemented for heterogeneous biomolecular systems. A critical component of the methodology is also its connection to experimental structural data such as cryo-EM or x-ray, thus making it “hybrid” in its character. Important applications of the multiscale approach to study key features of large multi-protein complexes such the HIV-1 virus capsid, actin filaments, and protein-mediated membrane remodeling will be presented.

Proton transport in proteins: insights and surprises from multiscale computer simulation

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The behavior of hydrated excess protons passing into and through transmembrane proton channels will be described based on the results of a novel multiscale computer simulation methodology. The unique electrostatics related to the dynamic delocalization of the excess proton charge defect in water chains and amino acid residues will be elaborated, as well as the effects of these complex electrostatics on the channel proton transport and selectivity properties. The often opposing and asymptotic viewpoints related to electrostatics on one hand and Grotthuss proton shuttling on the other will be reconciled and unified into a single conceptual framework. Specific simulation results will be given for several proton channel systems, including the M2 channel of influenza A, proton selective mutant aquaporin-1 channels, and the ClC Cl^-/H^+ antiporter. Comparison to experimental results will be discussed where possible.

Brownian dynamics simulation of biomacromolecules

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Brownian dynamics (BD) is a computational technique that allows the diffusive motion of molecules to be simulated. The solute molecules may be modelled in atomic detail, with a coarse-grain model, or simply as spheres; a continuum model is used to represent the solvent. A classical application of BD is to compute bimolecular diffusional association rate constants by simulating the relative diffusional motion of two solute molecules[1]. BD is also a useful tool for macromolecular docking and for simulating protein and nucleic acid conformational dynamics. It can be applied to simulate macromolecular solutions consisting of hundreds to thousands of macromolecules. Such simulations can, for example, help to investigate how macromolecular crowding in cellular environments affects the properties of individual molecules.

In the first lecture, I will introduce the BD technique and key aspects of the methodology for simulating protein diffusion and protein interactions. In the second lecture, I will discuss recent applications of the BD technique to study protein binding properties[2-4], to model large macromolecular complexes[5,6], and to simulate crowded environments with many diffusing proteins[7-9]. The third session will be devoted to informal discussion.

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Ordering and condensation of DNA in bacteriophages

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I describe the ordering of DNA inside a bacteriophage capsid based on a theory of liquid crystalline ordering of polymer nematics, such as DNA. I will show how tight DNA packing leads to a large osmotic pressure that is released when the virus infects the cell. Later I will show that DNA can also condense inside a viral capsid which leads to a deformed toroidal aggregate. The nature of interactions between this deformed aggregate and the protein wall of the capsid will be addressed and shown to have a pronounced effect on DNA packing within the viruses.

Introduction to single particle electron cryomicroscopy

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Single particle electro cryomicroscopy (cryo-EM) is the method of choice for 3D structure determination of large macromolecular complexes that are difficult to purify in the amounts and quality needed for X-ray crystallography. At least two to three orders of magnitude less material is required for single particle cryo-EM than for any other structure determination method (X-ray crystallography or NMR). This makes even low abundant macromolecular complexes accessible to structural research by cryo-EM. There is also no need for crystallization in single particle cryo-EM and in addition to low overall amounts, the concentrations of purified material can also be very low.

In single particle electron microscopy a macromolecular complex (typically in the range from ~200 kDa to xx MDa) is being prepared for electron microscopic imaging by flash freezing a suspension into liquid ethane on an electron microscopic grid. The rapid freezing process prevents water from crystallization and leads to an embedding of molecules in a thin layer of vitrified water (an amorphous phase of water). Molecules can thus nicely be prepared in their close-to-native buffer conditions. Images are then taken in a cryo electron microscope at low temperature. Several ten thousand of molecular images are usually collected making use of automated high-end transmission electron microscopes.

The images we obtain in a transmission electron microscope are two-dimensional (2D) projection images of a three-dimensional (3D) object. Similar to x-ray tomography in medical applications such 2D images obtained from different viewing angles can be combined computationally to reconstruct the 3D structure of the imaged object by computational image processing analysis. In cryo-EM the different orientation are usually obtained by the possibility of a macromolecule to orient itself almost randomly in the ice layer. The orientation of each molecule in the ice is, however unknown and needs to be determined a posteriori by computational means.

We employ advanced computational image processing tools to calculate the translational and rotational parameter of each imaged molecule. Since the signal-to-noise ratio in cryo-EM images is extremely low, we use pattern recognition procedures and average similar image to improve the signal-to-noise-ratio in the images significantly. Once we know all orientation parameters we can use 3D reconstruction algorithms to calculate the structure of the macromolecule in 3D which then needs to iteratively refined.

The lecture will describe all the individual steps from sample preparation, electron microscopical imaging and computational image analysis. it will also cover the possibilities, strength and weaknesses as well as possible pitfalls of the technique and some outlook into future perspectives.

3D structure determination of dynamic macromolecules by cryo-EM

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Originally, single particle cryo-EM was developed as a method to determine the 3D structure from a large set of 2D projection images of a macromolecular complex. This makes the important assumption that all imaged molecules are derived from the same 3D object. However, in the last couple of years we had to learn that this is a very simplified view and for many macromolecular complexes this is indeed not the case. We rather have to think about macromolecular machines that are embedded in solution and are thus free in adopting numerous conformations.

The presence of multiple conformational states of a macromolecule in one sample requires an additional computational image processing step, namely the sorting of molecules into populations with similar conformation (conformational sorting). We have developed various computational tools to perform these computational sorting steps at different levels of conformational heterogeneity. This possibility allows us to compute several structures from the same sample and thus provides insight into the structural flexibility of a macromolecular complex. Such measurements can even be carried out in a time and temperature dependent manner which provides access to kinetic data and the determination of conformational landscapes of macromolecular complexes and how they can be modified by temperature.

Structural studies of the dynamics of the 70S ribosome during tRNA translocation will be presented that illustrate the possibilities in obtaining structural insights even for dynamic macromolecules. We have recorded more than 2 million images of retro-translocating 70S E. coli ribosomes that provide a molecular movie of how tRNAs translocate through the ribosome. Additionally, we were able to determine the rate limiting step of the retro-translocation reaction and the entire conformational landscape of the ribosome by calculating 50 different structures that were all present in the same sample. Since we can monitor how the population of certain states changes along the reaction pathway, we can also determine the energy landscape of the translocating ribosome which provides us important insights into ribosome function.

Forces and conformational dynamics in biomolecular nanomachines

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

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Peptide and protein folding as seen by NMR (and ECD)

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The 20-residue long Trp-cage peptide is the smallest folded protein yet known and thus, has been the subject of several *in vitro* and *in silico* studies of folding and unfolding. The atomic details of a multi-state thermal unfolding will be presented. Different intermediates (I, U' and U") obtained by temperature dependent NMR measurements of ^{15}N and $^{13}C/^{15}N$ labeled molecules will be presented, both at neutral and acidic pH conditions. Even sophisticated experimental conditions are unsuitable to reveal fully fold (100%) and fully unfold (100%) states, thus we have developed a deconvolution technique to characterize these "invisible" states. Using non-linear mathematical fitting methods we have determine both the thermodynamic parameters (DH^{F-I}, T_m^{F-I}, DC_p^{F-I} and DH^{I-U}, T_m^{I-U}, DC_p^{I-U}) and the NMR chemical shifts of the multi-state unfolding process, a prototype of more complex protein foldings. *In silico* MD studies in conjunction with heteronuclear relaxation analysis show the molecular nature of such transition. The ability to detect structural information about folding intermediates *in vitro* provides an excellent opportunity to gain new insights into the structural and energetic aspects of the energy landscape of protein folding.

In cell NMR of intrinsically dynamic proteins

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Structurally disordered proteins (IDPs) can be classified into several functional categories. Based mainly on bioinformatic analysis, we have suggested a couple of years ago that disordered regions of traditional chaperones or even fully disordered proteins can have potent chaperone activity, probably by an "entropy transfer" mechanism. To carry out detailed structure-function analysis of this phenomenon, we studied two dehydrins of *A. thaliana*. Dehydrins are fully disordered stress proteins of plants, the expression of which increases critically upon dehydration elicited by water stress, high salinity or cold. In a range of chaperone assays with distinct substrates (e.g. alcohol dehydrogenase, firefly luciferase, human citrate synthase), we have shown that this IDPs are potent chaperones *in vitro*, with activities commensurable with that of Hsp90. In-cell NMR of overexpressed proteins in *E. coli* show that three of these regions (conserved K-segments) undergo further ordering, probably due to binding partner molecules in the cell. Overexpressed ERD14 provides significant protection to cells against stress conditions elicited by freezing, high salt (2M NaCl) or severe dehydration (6M gylcerol). These studies show that ERD14 is an IDP largely disordered *in vivo*, with local elements undergoing function-related local folding; the implications of these findings are discussed in detail.

Quantitative ECD analysis of peptides and proteins: the CCA+ method

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Electronic Circular Dichroism (ECD) spectroscopy is traditionally used for rapid, non-atomic level structure analysis of natural products such as peptides and proteins. Unlike globular proteins, peptides less frequently adopt a single 3D-fold in a time average manner. Moreover, they exhibit an ensemble of conformers composed of a multitude of substantially different structures. In principle, both ECD- and VCD-spectroscopy are sensitive enough to pick up structural information on these dynamic ensembles. However, the interpretation of the raw spectral data of these highly dynamic molecular systems can be cumbersome. The herein presented Convex Constraint Analysis Plus method, or CCA+ for short (http://www.chem.elte.hu/departments/protnmr/cca/), provides a unique opportunity for spectral ensemble analysis of peptides, glycopeptides, peptidomimetics and other foldamers. The precision and accuracy of the approach is presented here through different peptide model systems. An interesting temperature and pH dependent folding and unfolding of a miniprotein (*e.g.* Tc5b variant) will also be described. Analysis of CD-spectra sets strongly affected by solvent and ion type is also introduced to account for severe environmental induced structure influencing effect(s). The deconvolution makes always possible the quantitative data analysis even when the interpretation of the deconvolution resulted in pure CD-curves is complex.

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

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Electron paramagnetic resonance (EPR) spectroscopy in combination with site-directed spin labeling (SDSL) has emerged as a powerful method to study the structure and conformational dynamics of membrane proteins. The lecture presents the basics of this method and covers the following paragraphs:

1. Principles of EPR spectroscopy and site directed spin labeling
   1.1. Site directed spin labeling
   1.2. The Zeeman effect
   1.3. Hyperfine interaction
   1.4. The EPR powder spectrum of a nitroxide spin label
   1.5. Motional narrowing: isotropic and anisotropic reorientational motion of a nitroxide spin label

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

Suggested reading:

[1] Steinhoff H-J (2002) Methods for study of protein dynamics and protein-protein interaction in protein-ubiquitination by el1ectron paramagnetic resonance spectroscopy. *Frontiers in Bioscience* 7:c97-110.

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

Inter- and intramolecular distance measurements using cw and pulse EPR spectroscopy

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The second lecture of the series is dedicated to the application of site directed spin labeling to determine inter- and intramolecular distances[1,2]. The basics of cw EPR and pulse electron-electron double resonance (DEER) methods to determine interspin distances are presented and illustrated with studies of the structure and dynamics of soluble and membrane bound proteins: the Rpo4/7 stalk module of RNA polymerase[3], vinculin and the (ABC) maltose importer $MalFGK_2$. Molecular modeling approaches will be introduced which relate inter-spin distances measured between NO groups of nitroxide spin labels to the protein structure (MMM 2011package[4]). We will then focus on DEER studies on the spin labeled halobacterial phototaxis receptor sensory rhodopsin (pSRII) in complex with the receptor specific transducer (pHtrII). This complex is considered as a general model system for transmembrane signal transduction. Inter-spin distances determined from pairs of interacting nitroxide spin labels lead to a unique structural model of the dimeric complex[5]. Time resolved detection of inter-spin distance changes after light activation reveals conformational changes of pSRII and uncovers the mechanism of the signal transfer from pSRII to the associated transducer pHtrII. Conformational changes in the first HAMP domain of pHtrII are shown to play an essential role in the signal transfer[6].

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Relating the conformational space of a peptide with its circular dichroism spectra by computational methods

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Circular dichroism (CD) spectroscopy is an experimental method employed to characterize the structure of optically active molecules such as peptides and proteins. However, as is the case with all spectroscopic techniques, the CD spectrum reflects an average of the entire molecular population. In case of flexible molecules, the interpretation of these spectra might be challenging. In this lecture, I will describe a method[1] to calculate a CD spectrum of a flexible peptide, which allows to associate parts of a conformational space with a particular CD signal.

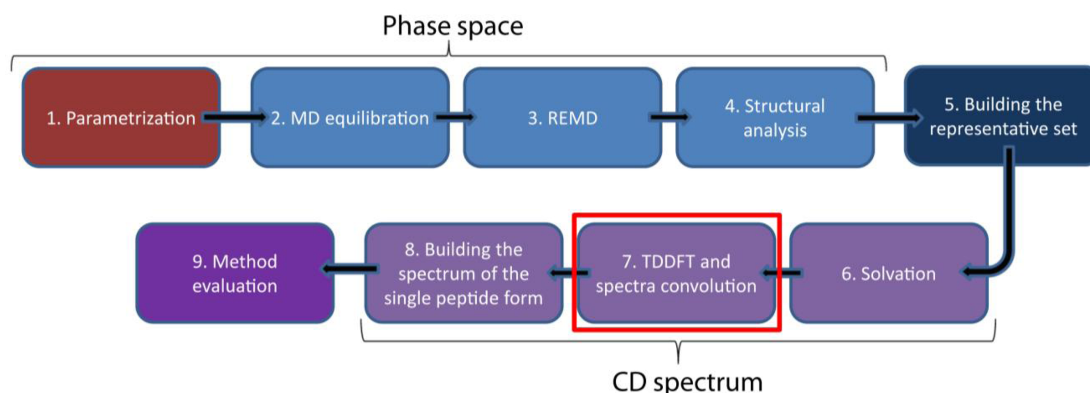

Figure 1. Key steps in building the CD spectra of a flexible solvated molecule

[1] Brkljača Z, Čondić-Jurkić K, Smith A-S, Smith DM (2012) Relating the conformational space of a peptide with its circular dichroism spectra by computational methods: The case of Metenkephalin and its unnatural analog. *J Chem Theory Comput* **8**, 1694–1705.

The binding affinity – effects of reactant confinement

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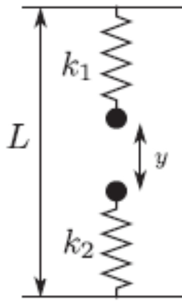

In this lecture, I will introduce the general concepts driving the linetics of chemical reactions between two species of molecules. I will consider the standard case of molecules in solution and develop the mass-action law of chemical kinetics. In the following, I will explain the idea of association and dissociation rates, and their relations to the detailed balance. In the last part of the lecture, I will introduce the effective binding affinity[1] for reactants confined to surfaces and study the effects of thermal fluctuations by introducing a variety of toy models for chemical reactions.

[1] Schmidt D, Bihr T, Seifert U, Smith A-S (2012) Coexistence of dilute and densely packed domains of ligand-receptor bonds in membrane adhesion. *EPL* 99:38003.

Multi-frequency EPR spectroscopy of membrane proteins

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The enhanced resolution of high-field (95 GHz, 3.4 T) EPR spectroscopy provides new insight into the structure and dynamics of spin labeled membrane proteins. At low temperature (T < 200K) the components of the hyperfine- and of the g-tensor can be resolved and provide a measure of the polarity and proticity of the spin-label environment. This has been used, e.g., to characterize hydrophobic barriers across the membrane[1,2], to study conformational changes[3,4] and to determine the polarity profile within the interface between two proteins in a membrane protein complex (pSRII-HtrII)[5].

Polarity profiles measured along transmembrane helices can be used as a ruler for the location of the helix with respect to the lipid - water interface. This is exemplified with site-directed spin labeled colicin A, a bacterial toxin. The water soluble conformation of colicin A is known. In the presence of lipids colicin A forms a voltage gated ion channel. The conformations of the closed and open channels are not known, however, it is generally agreed that membrane binding and channel formation are accompanied by large conformational changes. Accessibility measurements for paramagnetic quenchers and the behavior of the g-and A-tensor components as determined by high-field EPR reveal two different conformations of the membrane bound colicin A. The application of EPR spectroscopy on spin labeled proteins in living cells will finally be demonstrated.

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Recent Frontiers of Molecular Biophysics

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In this lecture series I will present and discuss a few recent biophysics methodologies that have opened up the way to study a series of new biological questions ranging to single molecule studies to control and read out of neuronal network activity. The topics will include such-super resolution microscopy, single molecule techniques and optogenetics. Each lecture will be structured to introduce students into the basics of the methods followed by case examples of their application in specific biological or biophysical questions. In addition I will point out to a few recent developments a physics based approach to biological questions has led to the discovery of new principles that are now leading to the new field of quantum biology, where non-trivial quantum ef-fects such as quantum coherence are thought to be generated through dynamic interactions with relevance for biological function.

Microtubules and motor proteins

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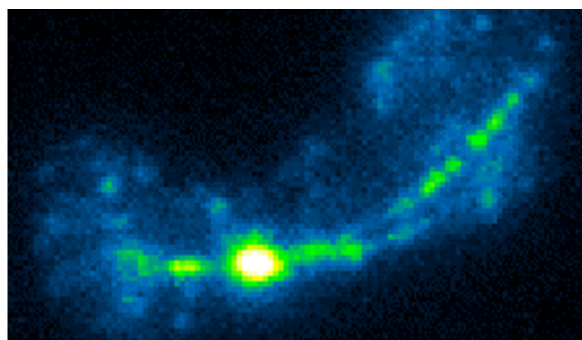
A living cell is not a bag full of randomly distributed bits and pieces – molecules, molecular assemblies, and organelles. The cell interior is instead neatly organized in a dynamic yet controlled manner. Dynamic organization of the cell interior requires constant exploration of the intracellular space to adjust the position of cell components in a response to changes such as cell growth, progression through the cell cycle, and signals from the environment. To this aim the cell uses microtubules and actin filaments, motor proteins, and other cytoskeleton-associated proteins.

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



Microtubules and the mitotic spindle

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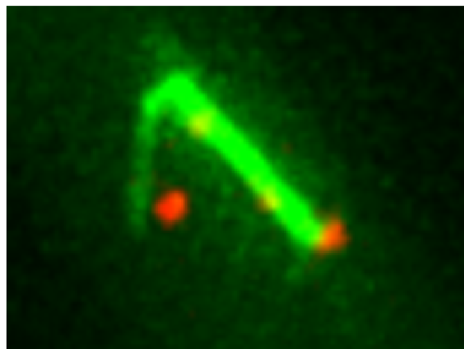
For a mother cell to divide its genetic material equally between the two daughter cells, the chromosomes have to attach to microtubules, which will pull them apart. The linkers between chromosomes and microtubules are kinetochores, protein complexes on the chromosome. The central question, how microtubules find kinetochores, is still under debate. According to the pioneering idea termed "search-and-capture," numerous microtubules grow from a centrosome in all directions and by chance capture kinetochores. The efficiency of search-and-capture can be improved by a bias in microtubule growth towards the kinetochores, by nucleation of microtubules at the kinetochores, by nucleation at preexisting spindle microtubules, by kinetochore movement, or by a combination of these processes. Recent experiments in fission yeast show that kinetochores are captured by microtubules pivoting around the spindle pole, instead of growing directly towards the kinetochores. This pivoting motion of microtubules does not depend on ATP-driven motor activity. In addition to the microtubule movement, movement of the kinetochores can be observed. By introducing a theoretical model, it is possible to show that the measured random angular movement of microtubules and random movement of kinetochores are sufficient to explain the process of kinetochore capture. This model predicts that the speed of the capture process depends mainly on how fast microtubules pivot. This prediction was confirmed experimentally by speeding up and slowing down microtubule pivoting. Thus, pivoting motion allows microtubules to explore space laterally, which accelerates their search for intracellular targets such as kinetochores.



Membrane-active helical antimicrobial peptides

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Antimicrobial peptides are ancient and ubiquitous components of innate host defence, found in all organisms. Amongst the many different types of AMPs, those with an amphipathic helical structure are amongst the most numerous, and are present in both vertebrate and invertebrate animals, probably representing cases of convergent evolution to a successful and robust molecular scaffold. Their structure-activity relationships are likely the most studied amongst AMPs. Much useful information can be gathered on their mode-of-action using both rational design methods to selectively and independently control the peptides' different physico-chemical attributes, and to determine how these affect antimicrobial potency, as well as the selectivity for bacterial versus host cells. Alternatively, one can follow the evolution of natural helical peptides such as the cathelicidins in closely related species, to reveal how evolution has modulated these characteristics for its own purposes, in some cases to favour direct inactivation of microbial pathogens, in others apparently to favour a stimulatory interaction with host cells. The combination of these methods has increased our understanding of how aspects such as the overall charge, hydrophobicity and amphipathic nature of these peptides, as well as their capacity to aggregate, affects membrane interactions and ultimately the cytotoxic or stimulatory activities.

Methods of studying AMPs (antimicrobial peptides)

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Antimicrobial peptides are ancient and ubiquitous components of innate host defence, found in all organisms. They have a direct capacity to inactivate microbes, including bacteria and fungi, and often also indirectly stimulate cellular components of immunity. Their interaction with biological membranes seem to be central to both these types of activities, but in some cases this then leads to membrane breaching and damage, underlying a direct cytotoxic activity. In other cases it leads to receptor interaction, or assisted transport into the cellular cytoplasm, where they interact with other molecular targets. The study of AMP thus requires the combined and integrated application of many techniques, ranging from biophysical methods for studying membrane interactions and their role in the mode-of-action as well as microbiological, biochemical, microscopic and genetic methods for determining functional effects and the consequences of membrane interactions or penetration. Some of these methods will be described and discussed.

Mean-field electrostatics explained through application to viruses

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I will expose the standard Poisson-Boltzmann (PB) and Debye-Huckel (DH) approaches to electrostatic interactions in salty solutions. These will be illustrated through elaborated examples. In particular, I will show elementary application of PB and DH approaches to viruses. The electrostatic interactions are known to be important for the assembly[4] and structure[1,5,6] of viruses which come in two essential "flavors" – ones containing the, usually, single-stranded RNA molecule, and the others containing the, usually, double-stranded DNA molecule. Both type of genomes are strongly negatively charged in the solution, and their encapsulation in the viral protein coating (capsid) may sometimes require specific form of electrostatic "screening", such as positive protein tails on the capsid interior, or the encapsulation of the histone-like, positively charged proteins, together with the genome.

The worked-through exercises will show the strength and deficiencies of mean-field approaches and indicate the situations where more advanced approaches to electrostatics are needed.

The lecture will be based on the following papers authored by the lecturer:

[1] Šiber A, Lošdorfer Božič A, Podgornik R (2012) Energies and pressures in viruses: contribution of nonspecific electrostatic interactions. *Phys Chem Chem Phys* 14:3746.

[2] Lošdorfer Božič A, Šiber A, Podgornik R (2011) Electrostatic self-energy of a partially formed spherical shell in salt solution: Application to stability of tethered and fluid shells as models for viruses and vesicles. *Phys Rev E* 83:041916.

[3] Šiber A, Zandi R, Podgornik R (2010) Thermodynamics of nanospheres encapsulated in virus capsids. *Phys Rev E* 81:051919.

[4] Šiber A, Majdandžić A (2009) Spontaneous curvature as a regulator of the size of virus capsids. *Phys Rev E* 80:021910.

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POSTER TALKS AND POSTERS

P1: Cluster formation of amphiphilic polymer chains

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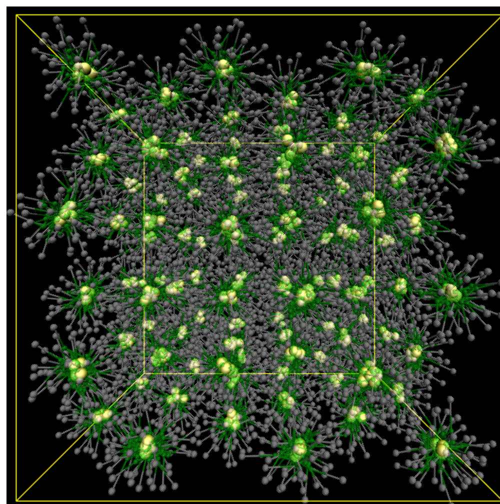
Motivated by a recently synthesized single-chain polymer that is able to fold into a supramolecular nanoparticle[1] we have designed an in silico model for these particles, built up by solvophilic and solvphobic monomers. Using standard interactions proposed in literature for these units[2], we could extract effective interactions between the nanoparticles via a method proposed by Voth et al.[3]. Based on a criterion put forward some time ago in literature[4], we can conclude from the shape of this effective potential that the polymer chains are able to form clusters of overlapping interpenetrating macromolecules. Using monomer-resolved Molecular Dynamics simulations we could demonstrate that these clusters are able to form – under suitable external conditions (i.e., temperature and density) – stable fcc cluster crystals (see figure), as predicted on the level of effective particles[5]. We explore the range of stability of these clusters and provide on the monomeric level a detailed analysis of the their internal structure.



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P2: The reaction of the hepatitis C virus NS3/NS4A protease with its main substrate. A QM/MM investigation.

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3% of the world population is infected by the hepatitis C virus (HCV), that infects 3-4 million of people every year, and long-term carriers of this virus are at risk of developing a life-threatening liver disease. The development of small-molecule inhibitors for the main therapeutic target (HCV NS3/NS4A protease) was very hard to achieve. Fortunately, several inhibitors were developed recently and some of them have or are being progressed into clinical trials in humans.[1,2]

Computational Chemistry is an useful tool in the fight against this disease[2] to look for new inhibitors of the HCV replication. Here, we have performed combined quantum mechanics and molecular mechanics (QM/MM) calculations to characterize the reaction mechanism of NS3/NS4A with its main substrate (NS5A/5B), extending previous efforts of our group on this problem[2].

We used the SCC-DFTB[3] method and the CHARMM22 force field to locate the stationary points that are present along the distinguished coordinate path involved in the title process. Temperature effects were taken into account by means of umbrella sampling molecular dynamics calculations, which lead to free energy profiles. These free energy paths were corrected, within the VTST framework, to include quantum effects.

This work was supported by the Spanish Ministry of Science and Innovation (projects CTQ2008-06805-C02-01 and CTQ2011-27857-C02-01). Thanks are also given to the "Generalitat de Catalunya" (Autonomous Government of Catalonia, ref. 2009SGR 17) and Autonomous Government of La Rioja (Martinez's fellowship) for some support.

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P3: DMD simulations of stefin folding and early stages of oligomerization

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We study folding and early stages of oligomerization of stefin A, B [C3S] and B[C3S, E31Y] by discrete molecular dynamics simulations. For each protein 8 trajectories each comprising of 16 protein chains in implicit solvent were simulated, neglecting effective electrostatic interaction between the side-chains. After doing the first 20 M time steps the results have not converged as the total potential energy was still decreasing with the simulation time.

Preliminary secondary structure analysis of the first 40 M time steps shows elevated coil propensity between amino acid residues 24 and 37. Intra-residue monomer contact map of stefin B [C3S, E31Y] indicates a hairpin structure around residue 80. Inter-residue contact map of stefin A suggests that the chains in contact may contain anti-parallel strands. It has been shown[1] experimentally that a solution of stefin B [C3S, E31Y] contains more dimers than monomers. We are currently in the process of calibrating implicit solvent parameters of the discrete molecular dynamics approach to stefins by varying the strength of effective electrostatic interactions. However, we did observe an increased number of stefin B [C3S] tetramers which seems to be consistent with the experimental data.

[1] Jenko Kokalj S, Gunčar G, Štern I, Morgan G, Rabzelj S, Kenig M, Staniforth RA, Waltho JP, Žerovnik E, Turk D (2007) Essential role of proline isomerization in stefin B tetramer formation. *J Mol Biol* 366:1569–1579.

P4: The thermodynamics of dopamine / membrane interactions

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Beside specific binding to receptors, dopamine can modulate synaptic transmission through weak interactions with neuronal membranes[1]. There are some evidences that this neurotransmitter also acts as an endogenous antioxidant - the pathological changes in dopamine homeostasis (detected in Parkinson disease and schizophrenia) may be associated with its high reactivity towards Reactive Oxygen Species (ROS).[2] Since lipids of cellular membranes are primary targets for ROS, the antioxidant activity of dopamine should be correlated with its ability to penetrate lipid membranes and to interact with phospholipid functional groups being hydrogen bond acceptors.

We employed Isothermal Titration Calorimetry (ITC) and Differential Scanning Calorimetry (DSC) to obtain detailed thermodynamic description of dopamine binding to lipid membranes and its impact on the lipid phase behavior. The calorimetric studies were performed on unilamellar phospatidylcholine vesicles assembled from 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylglycerol (DMPG) in order to obtain series of vesicles with varied surface charge. Experiments proved a clear relationship between thermodynamic parameters and charge of the membrane what suggests a dominant electrostatic component of dopamine-membrane interactions. The intrinsic binding constant, considered as a thermodynamic quantitation of hydrophobic effects, was also determined. On the basis of experimental results, a simple model for the binding of dopamine to lipid membranes is proposed, indicating that dopamine largely partitions into lipid headgroup region and is probably able to anchor through the electrostatic interaction to the membrane surface.

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[2] Yen GC, Hsieh CL (1997) Antioxidant Effects of Dopamine and Related Compounds. *Biosci, Biotechnol, Biochem* 61(10):1646-1649.

P5: Thermodynamical and structural changes in two functional states of regulatory domain of CFTR

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The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated anion channel involved in cystic fibrosis. CFTR consists of two membrane-spanning domains, each followed by a nucleotide-binding domain; these two pairs are linked by a unique regulatory domain (RD), that contain a multiple phosphorylation sites that determine the activation of the channel. The RD is an intrinsically disordered protein, characterized by lack of stable or unique tertiary structure. Bioinformatics verification of the disordered nature of the RD was done with a set of analysis programs. The lack of a defined and stable structure results in a considerable limitation when trying to in build a unique molecular model for the RD. The aim of our work is to provide data to describe the structural and thermodynamical properties of the RD in i two functional states, native and phosphorylated. Circular dichroism (CD) spectra from samples with a different degree of phosphorylation, from the no-phosphorylated (native) state to completely phosphorylated state showed an modification of the secondary structure composition with the polypeptide phosphorylation: random coil structure decrease at expenses of an increase of alpha-helix and beta-sheets. We this observation as an favoring of a more structured state by phosphorylation. We studied the thermal denaturation curves of RD monitoring the ellipticity changes at 222 nm between 20 °C from 90 °C. The thermodynamical analysis of the denaturation curves showed that the maximum phosphorylation of the protein induces a low stability of the RD, characterised by a lower transition temperature and a smaller Gibbs free energy. The structural differences in the two states are confirmed by small-angle X-ray scattering (SAXS) data that show two different gyration radius: 3.4 nm for the native RD and 2.92 nm for the phosphorylated peptide. This data will be useful to understand the molecular mechanisms of normal and pathological CFTR functioning, and the action of potential CFTR drugs.

This work was supported by the Italian Cystic Fibrosis Research Foundation grant #7/2010, with the collaboration of Delegazione FFC di Cosenza 2, Work in Progress Communication "Sapore di Sale 2010", Gruppo di Sostegno di Monterotondo (RM), Delegazione FFC di Genova, Delegazione FFC "Il Sorriso di Jenny", LIFC Comitato provinciale di Livorno.

P6: Controlled radical polymerization in protein nanoreactors and with native enzymes

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Atom transfer radical polymerization (ATRP) has emerged as one of the most powerful synthetic techniques in polymer science. Similarly to other controlled radical polymerization (CPR) methods, it allows the synthesis of polymers with predetermined molecular weight, narrow molecular weight distribution, as well as desired composition and molecular architecture[1]. ATRP is a metal complex-mediated reaction with copper-based catalysts being most commonly used. However, the catalysts are considered mildly toxic, environmentally problematic and difficult to remove from the polymer product. An environmentally friendly alternative to conventional catalysts in many areas of synthetic chemistry are enzymes. However, no natural protein or enzyme was known to be able to catalyse ATRP, despite the fact that Nature offers a wide variety of metal-containing enzymes and proteins.

We are studying conjugates of ATRP catalysts and proteins, e.g. in order to confine ATRP to the cavity of a protein cage. We successfully grafted ATRP catalysts into the thermosome (THS), an archaeal chaperonin, and used the resulting conjugates as nanoreactors for ATRP polymerization. The resulting nanoscale catalyst enables ATRP in pure water and greatly enhances control over the polymerization. In the course of this project, we discovered that the heme proteins hemoglobin (Hb) and horseradish peroxidase (HRP) catalyse the polymerization in aqueous solution in the presence of an ATRP-initiator and the reducing agent ascorbic acid.[2-3] This novel biocatalytic activity of hemo-proteins has been termed ATRPase activity. ATRPases could become green alternatives to the transition metal complexes that are currently used as catalysts for ATRP, because proteins are non-toxic, derived from renewable resources, and cheap and abundantly available.

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[2] Sigg S, Seidi F, Renggli K, Silva T, Kali G, Bruns N (2011) Horseradish peroxidase as catalyst for atom transfer radical polymerization. *Macro Rapid Commun* 32:1710–1715.

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P7: Self-organization of microtubules in plant cell cortex under the influence of pressure

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In plant cells microtubules are organized in parallel arrays which are adjacent to the cell's membrane. These arrays are called cell cortex. They reorganize in response to the changes in the environment.

Microtubules of the cell cortex play significant role in the production of the cellulose fibrils in the cell wall. These fibrils are rigid enough to convert turgid pressure into directed growth. Thus, the orientation of microtubules define the distribution of the intracellular pressure and consequently the axis of the growth of the plant cell. Meanwhile, this process is more complex; cell wall and turgid pressure were also reported to have influence on the orientation of the microtubules' array[1].

We investigate the connection between growth and microtubule orientation. Using our mathematical model[2] we show that the distribution of intracellular pressure may lead to the reorientation of cortex microtubules.

[1] Hamant O, Traas J (2010) The mechanics behind plant development. *New phytologist* 185:369–385.

[2] Baulin VA, Marques CM, Thalmann F (2007) Collision induced spatial organization of microtubules *Biophys Chem* 128: 231-244.

P8: Molecular mechanism of GUVs from high density lipoproteins under the influence of *MLO* venom

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The venom as medicine has interested the scientists since ancient times, but it is only in the recent years that important achievements have been described. Snakes have toxins in their venom, which have unique features due to which they are widely used for scientific and medical purposes (lebetoxs which can stop bleeding). The aim of our study is to find out how the lipid membrane condition changes under the influence of zootoxin like *Macrovipera lebetina obtuse (MLO)*. The information about membrane-venom interaction provides an ideal platform for the design of potent inhibitors which are useful in the development of prototypes and lead compounds with potential therapeutic applications.

Materials and Methods: Giant unilamellar vesicles (GUVs) were formed from the high density lipoproteins (HDL) fraction from bovine brain by the electroformation method (Angelova and Dimitrov, 1987). We used ANS and pyrene as fluorescence probes, which allow us to quantify the fluidity changes in the membrane by measuring of the fluorescence intensity. GUVs we modified with *MLO* venom added to the sample chamber before the liposomes were formed. Venom is made in the same buffer as liposomes. Fluorescent spectra were measured on a *Varian* fluoremeter instrument.

Results: Unlike ANS, pyrene interacts with lipoprotein liposomes and this hydrophobic interaction is an interesting information source for deciphering of mechanisms of lipid/protein interaction. As a result, we get two peaks with high intensity which are monomer and dimmer fluorescence peaks in the lipid/lipid and lipid/protein contact points. Pyrene monomer fluorescence is more intense, which is due to the great number of lipid/protein contacts. It is natural, as according to the chemical structure of the lipoproteins, there are only few free lipid bilayer areas in these vesicles, as these phospholipids are hydrophobic. If we compare pyrene interaction in the liposomes modified by venom (as we shown earlier) and in lipoprotein GUVs, we can see that having protein properties the components of the venom go into the membrane themselves and play the role of membrane protein. But in case of negatively charged lipoproteins the venom binding mechanisms are superficial.

P9: Detection of anticancer drug paclitaxel in MCF-7 cells by confocal Raman microscopy

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Confocal Raman microscopy, a non-invasive, labels free and high spatial resolution imaging technique is used to study apoptosis in living MCF-7 cells (Fig.1A). The images are obtained based on the Raman spectra of cells components and drug and are treated by K-mean cluster analysis method (Fig.1C) to localize drug in cell. Distribution of paclitaxel in cells is verified by calculating the correlation coefficient between the reference spectrum of the paclitaxel (Fig.1B) and the Raman spectra of the whole image. Our results show that the drug is distributed all over in the cytoplasm (Fig.1D), that is in good agreement with other recent researches suggesting a new picture of the pharmaceutical action of this drug based on rapid binding of crystallized paclitaxel to free tubulin.[1,2]

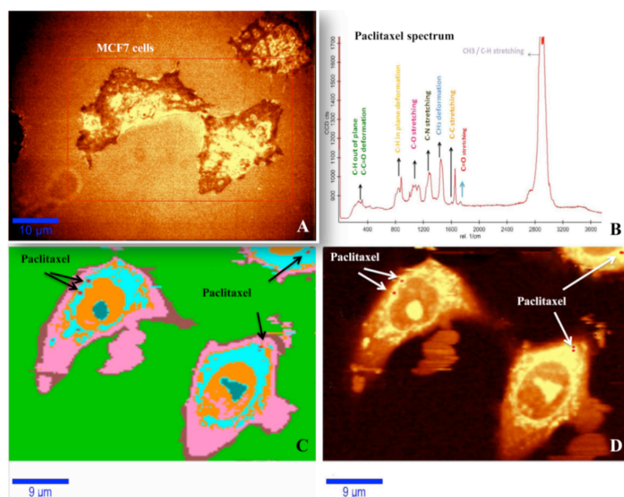

FIG. 1
(A) Bright-field microscopic image of MCF-7 cell in buffer solution, 60× objective.
(B) Predominant bands in the Raman spectra of clinical paclitaxel solution from Teva Ind.
(C) Raman image of various cell components and paclitaxel and drug obtained via KMCA.
(D) Integrated Raman intensities in the 2800-3000 cm -1 region (C-H stretching) of the cells marked with paclitaxel.

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P10: Estimation of the bilayer lipid membrane permeability for TButPyP4 porphyrins

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In the modern literature there is information about the permeability of membranes for ions, and no information on the application of the method for measuring permeability of bilayer lipid membrane for porphyrins. Porphyrins are a group of organic compounds widely spread in nature. They have antiviral, antibacterial and antitumoral properties. They also are successfully used in photodynamic therapy of tumors. It is well known that the biological effect of porphyrins depends on their interaction with DNA and other cell organelles. Due to this it is very important to study the interaction of porphyrins with membranes, as well as to examine membrane permeability in the presence of porphyrins.

In this work penetration of meso-tetra-4N-butylpyridyl [TButPyP4] porphyrins is studied through the bilayer lipid membrane (BLM). The special cell adapted to spectrophotometer is made for the investigation of porphyrin penetration through BLM. Experiments are conducted on BLM derived from phosphatydilserine, which is suspended in nonane. BLM is forming by the method of Muller. The interaction of porphyrins with membrane has characterized by considering kinetic aspects. The passage of porphyrin through the membrane is investigated by absorption in the Soret band as a function of time. As a result the time dependence of the porphyrin's concentration is obtained. From this dependance the permeability coefficient of bilayer lipid membrane is determined for TButPyP4 porphyrins.

Obtained results allow to estimate and understand the mechanisms of porphyrins passage through the cell membranes, which is important in estimation of efficiency of the substance in the field of medicine.

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[2] Avdeef A (2003) Absorption and Drug Development: Solubility, Permeability, and Charge State, eds. (J. Wiley&Sons Inc., Hoboken, New Jersey) pp 116-235.

P11: Protein-protein docking: a combined all-atom and coarse grained approach

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Despite the computer power available today, simulations to predict protein-protein interactions still provide a challenge. Whenever a large number of atoms or conformational changes are involved, long simulation and sampling times are required. Such conformational changes can include loop flexibility, domain motion or side chain rearrangement. As an example, the inhibitor fasciculin undergoes a local loop motion of residues 5-15 of up to ~6.5Å upon binding to its receptor acetylcholinesterase. Atomic detail simulation of this loop flexibility during protein-protein docking would be very computationally demanding due to the long sampling time. In such cases, the transformation of the full atomic structure to a coarse grained representation can be done, to reduce the number of degrees of freedom. Here, we present a coarse grained approach which is part of a multi-scale work-flow consisting of 5 steps: all atom rigid body Brownian dynamics simulation of protein-protein diffusional association, conversion of protein structures to a coarse grained representation, coarse grained simulation to bring the proteins to bound conformations, back transformation to all atomistic representation and short refinement by atomic detail molecular dynamics simulation. The focus of this work is on transformation and simulation of the coarse grained structures. Each amino acid residue is represented by one bead in the coarse grained representation. Based on elastic network analysis, an elastic network is used to connect the beads within a protein structure. Protein-protein interactions are described by a Lennard-Jones term and Coulombic charge interactions. We show that the docking of fasciculin to its receptor, using their unbound structures can be efficiently modeled using this coarse grained approach. To finally obtain all-atom structures, the coarse grained prediction is used in a targeted molecular dynamics simulation of the unbound structures.

P12: Diversity in the mechanisms of cosolute action on peptide folding

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On the molecular level, life is established through the specific interaction between and within macromolecules in an aqueous environment. It is increasingly realized, however, that even modest changes in solution conditions, brought on by the presence of many cellular cosolutes, can profoundly affect the balance of forces underlying the specificity of interaction, with consequences that can be severe if not fatal. We have followed the thermodynamic effect of several cosolute classes, including polymers, cellular osmolytes, and inorganic salts, on the stability of biomolecular folding. By comparing changes in free energy, enthalpy, and entropy upon cosolute addition for this process, we identify several thermodynamically distinct mechanisms. Surprisingly, even while many cosolutes display similar scaling of the change in free energy with cosolute concentration, a breakdown of this free energy into enthalpic and entropic contributions distinguishes between different families of cosolutes. We show how these "thermodynamic fingerprints" differentiate between different cosolute families, and can give conclusive evidence for the existence of different mechanisms by which they act.

[1] Sukenik S, Sapir L, Gilman-Politi R, Harries D (2012) Diversity in the mechanisms of cosolute action on biomolecular processes. *Faraday Disc* 106 doi: 10.1039/C2FD20101A.

P13: Stabilization of membrane proteins by compatible solutes: single molecule force spectroscopic study

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The single molecules without inherent symmetry can directly be monitored in their physiological conditions using atomic force microscopy (AFM). Recent developments in AFM enable us to go beyond the ensemble average and measure the behavior of single molecules. Compatible solutes (organic osmolytes) are used for protecting cells against high osmotic stress. They are compatible with cell metabolism even at molar concentrations. The influence of Ectoine, Betaine and Taurine on the mechanical properties of Bacteriorhodopsin has been studied by single molecule force spectroscopy. Different concentrations of these three compatible solutes were used (1mM, 10mM, 100mM, 1M) to get an in depth insight into the concentration dependency of protein unfolding by using external forces. Unfolding experiments taking BR as a model system revealed that these compatible solutes increase the tendency of the polypeptide to coil, thus decreasing its persistence length significantly. This allows us to conclude the mechanism of interaction between the unfolded polypeptide chain and the osmolyte. The osmolytes are expelled from protein surface due to the increase in chemical potential of the stretched state forcing the protein into a more compact structure. This information provide basis for our further studies regarding the effects of compatible solutes on other membrane proteins. In most cases however, the protein needs to be studied in its natural environment, a bilayer lipid membrane. This can be achieved by incorporating the protein into an artificial tethered bilayer lipid membrane (tBLM). The formation of a tBLM on a quartz-glass surface and the incorporation of a Rhodopsin with defined orientation by forming a His-tag and Tris-NTA interaction were studied by atomic force microscopy. Using a Rhodopsin as a model protein this approach now can easily be modified to study other membrane proteins. The goal of this work is to establish a final protocol combining the advantage of concentration-controlled protein density and the knowledge about protein orientation due to specific coupling and is set to provide exciting possibilities in the field of drug development including in vitro rescue of the misfolded proteins and to directly analyze and correlate their structural and functional properties at the sub-molecular level.

P14: Functional characterization of novel uptake transporters in zebrafish (*Danio rerio*): Oat2a and Oat2d

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Multi-specific transport proteins of SLC22 family (solute carriers) are responsible for uptake of various endogenous and exogenous compounds across the plasma membrane. Organic anion transporter 2 (OAT2) is member of SLC22 family and plays important role in uptake and distribution of physiological compounds, as well as anionic toxins and drugs. Mammalian OAT2 was characterized in human (OAT2), rat and mouse with highest tissue expression in liver and kidney. Physiological role of human OAT2 is based on sodium-independent uptake of hormones: estrone-3-sulfate (E3S) and dehydroepiandrosterone sulfate (DHEAS); eicosanoids: prostaglandin E2 (PGE2) and F2α (PGF2α); bile salts: taurochenodeoxycholate (TCDC) and cholate; citric acid cycle intermediate α-ketoglutarate; fatty acid propionate and signal molecules cAMP and cGMP. OAT2 is well characterized in mammals, however OAT2 co-orthologs have not been studied in non-mammalian species. Therefore, the goal of our study was to determine phylogenetic relationships, tissue distribution and substrate specificities of two zebrafish co-orthologs Oat2a and Oat2d. qPCR tissue expression revealed similarities among Oat2a and Oat2d, but differences in comparison with OAT2. We have found highest expression of Oat2a and Oat2d in testes, followed by brain and gills. Functional characterization of zebrafish Oat2 members was performed using the transiently transfected HEK293 cells and fluorescent substrates lucifer yellow (Km=5.7 μM) and 6-carboxyfluorescein (Km=0.3 μM) for Oat2a and Oat2d, respectively. We tested 47 compounds previously known to interact with OAT2. Oat2a showed highest affinity for physiological substrates 19-norethindrone (Ki=13.6 μM) and bilirubin (46.3 μM), followed by DHEAS (Ki=84 μM), cGMP (134 μM), triiodthyronine (210 μM) and E3S (216 μM). Oat2d showed different affinities then Oat2a: highest interaction was found with DHEAS (Ki= 11.7 μM) , E3S (Ki= 19.5 μM) and PGE2 (Ki= 28.3 μM) followed by TCDC (Ki= 50 μM), folic acid (45.3 μM) and bilirubin (58.4 μM), while no interaction was found with 19-norethindrone, cGMP and T3. In conclusion, our results indicate that Oat2a and Oat2d play crucial role in transport of physiological and non-physiological compounds in zebrafish testes and brain, and significantly differ from their human co-ortholog OAT2.

P15: Two-photon polymerized and functionalized 3D microstructures for biological applications

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There is an increasing interest in functionalized complex 3D microstructures for micro-and nanotechnology applications in biology. Our aim is to make such microtools that can be used in optical tweezers to manipulate biological objects. The tools are made of SU-8 photoresist by two photon polymerization which is capable of making practically any arbitrarily-shaped 3D structures with sub-micrometer features. In order to make the tools biocompatible their surface needs to be functionalized. In order to demonstrate the feasibility of this goal, the polymerized tools were coated with streptavidin and gold nanoparticles via an aminosilane–based methodology. The surface functionalization chemistry, the effect of the chemical modification on the surface morphology and the surface protein density determined via single molecule fluorescence microscopy will be presented[1].

[1] Aekbote BL, Jacak J, Schütz GJ, Csányi E, Szegletes Z, Ormos P, Kelemen L (2012) Aminosilane-based functionalization of two-photon polymerized 3D SU-8 microstructures. *Eur Polym J* (in press).

P16: Finding tandem repeats in Tribolium Castaneum using computational method Global Repeat Map

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We construct complete GRM [1-2] diagram for Tribolium Castaneum (identification of repeat units and distances between dispersed repeat copies) from 1 bp to 100 kb. In previous reports of Tribolium Castaneum genome it was shown that much of DNA is composed of tandem repeats, of which 17% are satellite monomer 360 bp long [3]. Using our novel computational algorithm Global Repeat Map (GRM), we find in Tribolium Castaneum genome tandem repeats based on 102 bp, 123 bp, 127 bp, 144 bp, 258 bp, 310 bp, 319 bp, 322 bp, 142 bp, 143 bp, 166 bp, 171 bp, 180 bp, 360 bp, 558 bp, 563 bp, 688 bp, 1118 bp, 1351 bp, ~1446 bp, 1859 bp and many others. In the next step of analyzing tandem repeats we indentified possible higher order repeats (HORs) in status of formation, as a new phenomenon. We think that those sequences are important in understanding of selective constraints on satellites that are probably related to interaction with specific proteins and role in control and regulation of gene expression [2].

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[2] Paar V, Glunčić M, Rosandić M, Basar I, Vlahović I (2011) Intra – gene higher order repeats in neuroblastoma breakpoint family (NBPF) Genes distinguish humans from chimpanzees. *Mol Biol Evol* 28(6):1877.

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P17: Phylogenetic and tissue distribution analysis of GST superfamily in zebrafish (*Danio rerio*)

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Glutathione-*S*-transferases (GSTs) are crucial part of the cell detoxification system. They catalyze the nucleophilic attack of glutathione (GSH) on electrophilic substrates to produce less toxic and more water soluble compounds. Since GSTs are involved in detoxification of environmental pollutants, such as heavy metals, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), the measurement of GST activity in aquatic organisms has been frequently used as an ecotoxicological biomarker. The expression pattern of GSTs represents an important factor in determining the sensitivity of tissues/organs in response to environmental toxicants. The aim of our study was initial characterization of GST superfamily in zebrafish (*Danio rerio*) as an important model species in biomedical and ecotoxicological research, using phylogenetic analysis and tissue mRNA expression profiling. Genome databases were searched for GST members in mammalian, bird, reptilian, amphibian and several teleost genomes including zebrafish in order to determine orthology relationships and assign provisional annotations to zebrafish GST genes. Phylogenetic tree of GST superfamily was constructed using Maximum Likelihood method, while qRT-PCR was employed to investigate the expression of GST genes in six tissues of adult female and male zebrafish (liver, kidney, gills, intestine, brain and gonads). Phylogenetic analysis revealed that GST superfamily in zebrafish consists of three families: cytosolic (Gst alpha, mu, pi, omega, theta, zeta and rho); mitochondrial (Gst kappa) and microsomal (Mgst) family. Altogether, we have identified 23 zebrafish GSTs on the genome level, and quantified transcript levels of 20 chosen genes. Phylogenetic analysis revealed the presence of three new genes, members of different Gst classes. Tissue distribution showed very different expression profiling. In summary, the described comprehensive analysis of GST superfamily in zebrafish represents the first and necessary step towards characterization of toxicologically most relevant GST members in teleosts.

P18: Simulation vs. reality: a comparison of in silico distance predictions with DEER and FRET measurements

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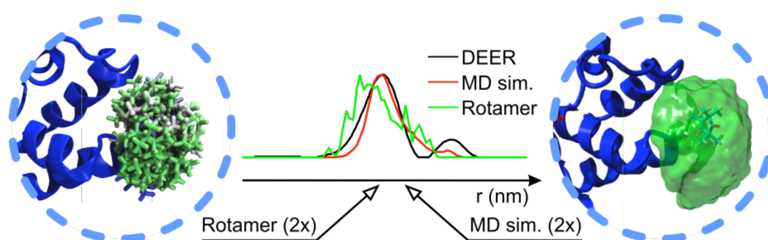
Structural elucidation based on inter label distance determination by DEER or FRET relies on a translation of inter label distances (or distributions) to constraints in a structural model. Here we present results on this problem using a model system, the two subunits Rpo4/7 from the archaeal RNA polymerase[1]. We focus on a comparative evaluation of experimentally determined and simulated distances for DEER and FRET. We determined experimental distances for spin- and fluorescent labeled Rpo4/7[2] and simulated orientation distributions of the spin- or fluorescent labeled side chains using conformational sampling by molecular dynamics- (MD) as well as by Monte Carlo (MC) simulations and, for the spin labels, by a rotamer library analysis[3]. This versatile approach not only provides a comparison of experimental inter-label distances (or distributions) to the simulated orientation distributions for both EPR- & FRET labels, but additionally, it allows for cross validation of the different simulation techniques and protocols.

As exemplified in the figure, for spin labels the comparison shows reasonable agreement, especially in terms of the mean distances.



This comparative study is aimed at developing the simulation methodology in order to increase the efficacy of structural modeling based on experimental constraints.

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P19: Sodium-dependent movement of covalently bound FMN residue(s) in Na^+-translocating NADH:quinone oxidoreductase

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Na^+-translocating NADH:quinone oxidoreductase (Na^+-NQR) is a component of respiratory electron-transport chain of various bacteria, generating transmembrane electrochemical Na^+ potential. The species producing the EPR signal in Na^+-NQR are flavin mononucleotide residues (FMN) bound to the NqrB and NqrC subunits (FMN_{NqrB} and FMN_{NqrC}, respectively) and [2Fe-2S] cluster. These species are the main candidates for the role of the intermediates of transmembrane electron transport. We found that the change in Na^+ concentration in the reaction medium has no effect on the thermodynamic properties of prosthetic groups of Na^+-NQR from *Vibrio harveyi*, as was revealed by the anaerobic equilibrium redox titration of the enzyme's EPR spectra. On the other hand, the change in Na^+ concentration strongly alters the EPR spectral properties of the radical pair formed by the two FMN residues bound to the NqrB and NqrC subunits. At the same time, no Na^+-dependent change in pulse ENDOR spectra of FMN radicals of Na^+-NQR is detected. Therefore, no substantial spin density redistribution within FMN radical occurs. In the presence of Na^+, the interspin distance between FMN_{NqrB} and FMN_{NqrC} of about 21 Å was determined from pulse X-band ELDOR [2]. The ELDOR trace change dramatically upon Na^+ removal, and dipolar modulation disappears. From the simulation of X- and Q-band EPR spectra the distance between FMN_{NqrB} and FMN_{NqrC} 15.5 Å in the absence of Na^+ was estimated. Thus the distance between the covalently bound FMN residues can vary on more than 5 Å upon changes in Na^+ concentration. Using these results, we proposed a scheme of the sodium potential generation by Na^+-NQR based on the redox- and sodium-dependent conformational changes in the enzyme[1].

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P20: Low-frequency impedance spectroscopy: role of microscopic phase separation in gelation of aqueous gelatin

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We present results of impedance spectroscopy on gelatin solutions using a specially constructed four-electrode impedance spectroscopy cell[1]. Our method enables impedance measurements of conductive liquid samples down to the sub-Hertz frequencies, avoiding electrode polarization effects that usually cripple standard impedance analyzers. This enables conductivity studies of self-diffusion and collective phenomena in biological samples (macromolecules, cells, ...).

Our results in aqueous solutions of gelatin reveal two relaxation processes in the dilute and semi-dilute solutions. Using PFG NMR diffusiometry we identify the faster process as a self-diffusion of gelatin molecules. Scaling relations for the self-diffusion indicate that we have a microscopic phase separation distinct from spinodal decomposition. After the formation of triple-helix junction sites and the onset of gelation, these hydrophobic triple-helices aggregate due to the attractive helix-helix interactions. This leads to the formation of microphase separation between gelatin molecules which are part of the gel macrostructure (gel phase) and those which are free (sol phase). The slower process indicates a collective phenomena with anomalous diffusion. This suggests that between the single helical segments joining three chains and the macroscopic gel there exists additional levels of organization. The scales inferred from these experiments lie in the micron range and on the same scale that TEM micrographs reveal polypeptide bundles forming a network.

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POSTERS

P21: An infrared spectroscopy study of fibril formation

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Neurodegenerative diseases are an increasing problem in our society. A huge array of these diseases seems to be caused by abnormal clusters of protein known as amyloids. These amyloidosis are characterized by the abnormal self-assembly and deposition of proteinaceous material into insoluble ordered aggregates. In many proteins, when heated at high temperature and low pH, a series of structural changes resulting in the formation of fibrillar structures are produced. Insulin is a model of fibril formation that has produced a wealth of biochemical and structural data. The time-course of fibril formation can be followed by infrared spectroscopy looking at the appearance of a characteristic band in the amide I region. The kinetics is triggered by temperature at 65 ºC and pH at 2.3. The infrared spectrum shows, that after a lag time (concentration-dependent), the α-helical band decreases and the random coil component increases subsequently. Random coil increases up to a percentage and later a band in 1626 cm^{-1}, associated with extended chains, replaces the random coil component. Infrared 2D-COS has been applied to different stages of the process. We have developed an approach that we call “moving lapse window” and allow us to follow the process without being obscured by the aggregation band. Maps are presented at different incubation times: before random coil formation and at different stages in the random coil-fibril exchange. Also a movie is shown. Lipids have been proposed as a factor influencing fibril formation. The effect of different lipid composition, including anionic lipids, sphingomyelin and cholesterol has been studied to see changes in kinetics according to the lipid charge.

P22: Characterization of different aggregate species of the Alzheimer's disease associated amyloid beta peptide

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A key feature of Alzheimer's disease is the pathogenic self-association of the amyloid beta (Aβ) peptide, leading to the formation of diffusible Aβ oligomers and extracellular amyloid plaques. The characterization of different assemblies of the Aβ peptide is essential for the understanding of its structures, mechanism of aggregation and for target selection in drug development.

The aim of our study was to establish an advanced matrix free assay for the fractionation of different aggregate species of the Aβ peptide making them accessible for further characterization by atomic force microscopy (AFM). The AFM imaging conditions for each of the separated Aβ assemblies should be optimized in order to preserve the solution based shape of the Aβ assemblies.

Pre-incubated Aβ solutions were fractionated by density gradient centrifugation and single fractions were visualized by AFM carried out in buffer, avoiding drying artifacts. We could demonstrate that different types of Aβ assemblies like oligomers, fibrils and protofibrils were enriched in different fractions of the density gradient. With this combination of methods a specific oligomeric Aβ assembly exhibiting a high degree of homogeneity could be detected and characterized. Furthermore the method turned out to be suitable for determining the influence of aggregation modulators on the Aβ aggregation.

Our conclusion is that the combination of AFM and density gradient centrifugation is a very useful method to separate and characterize different assemblies of the Aβ peptide. It can be applied to analyze the influence of ligands, solution conditions or Aβ concentrations on the aggregation behavior of the peptide.

P23: Integrating complementary high resolution microscopy techniques for visualizing casein micelles and aggregates

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Fundamental understanding of the behavior of proteins during the processing of food is of critical importance to the food industry. In the dairy industry knowledge of casein micelle aggregation behavior, in products such as cheese, yoghurt and ice cream, is significant in product development and for improvement of existing processes. For investigating this, different high resolution microscopy techniques are able to provide a direct visualization of casein micelles as well as more complex dairy products.

During the project we utilize state-of-the-art microscopy techniques for characterizing soft nanostructures: Atomic Force Microscopy (AFM), Field Emission Scanning Electron Microscopy (FESEM) and Brewster Angle Microscopy (BAM). Using complementary techniques which produce high resolution microscopic data will verify previous studies and provide new structural evidence.

AFM is able to provide detailed topographical information directly in aqueous media without using fixation and dehydration in sample the preparation. This enables the visualization of individual casein micelles. FESEM is suitable for imaging aggregated casein micelles in already processed milk. The interfacial properties of casein micelles can be studied in Langmuir monolayers by BAM. Implementation of more than one technique helps alleviate discrepancies in relying on only one. Here we present examples of imaging individual and aggregated casein micelles as well as pure caseins.

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[2] Dalgleish D G (2011) On the structural models of bovine casein micelles – review and possible improvements. *Soft Matter* 7:2265-2272.

P24: Actin self-assembly in micro-confinements

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Actin is a versatile cytoskeletal protein playing a crucial role in cell mechanics and motility by the virtue of self-assembly (monomers – filaments – bundles - networks). Actin bundling and bundling disassembly ('de-bundling') are important biological processes that occur in various plant and animal cells or in specialized regions of the cell, such as filopodia, microvilli and growth cones of axons. Using microfluidics, we create quasi two-dimensional micro-confinements (h = 0.5 - 2 μm) of different geometries (squares and circles, 10 - 100 μm) termed 'microchambers'. The microchambers act as flow-free diffusion dominated regions and are connected to an advection dominated controlling channel. With such a set-up, modifying the fluid composition in the controlling channel is used to change the chemical environment of microchambers, without inducing any flow fields. We show the functioning of the device by carrying out repeated cycles of bundling and consequent de-bundling of actin filaments by respectively adding and depleting Mg^{2+} ions from the system.

Using PDMS' permeability to water, we steadily increase the concentration of actin and Mg^{2+} ions inside the microchambers, which result in complex networks of actin bundles without any cross-linking proteins. We find that the properties of these networks depend on the geometry they are confined within; rectangular meshes are predominantly found in squares while less elongated, triangular meshes are formed in circles. The average link length stays constant regardless the geometry. Interestingly, link orientations show a 4-fold symmetry in square microchambers and a possible 6-fold or multiple of 6-fold symmetry in circular microchambers. We provide a plausible explanation for these properties on the basis of a bundling process, which is predominantly initiated at the microchamber walls.

P25: Protein-based ultrafast all-optical switching

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All-optical data processing is considered to be the most promising method to fulfil the high demands of the continuously growing internet traffic. The concept of Tbit/s telecommunication represents the expectations both in capacity and speed of data processing.

The state-of-the-art photonic integration technology is ready to provide the passive elements of integrated optical circuits based either on silicon, glass or plastic materials. The bottle-neck is to find a proper nonlinear optical material that is supposed to be the cladding medium in waveguide-based photonic applications, performing light-controlled active functions. Several organic and inorganic materials have been proposed for this special application but none of them is considered to be the optimal solution[1-2].

Based on our earlier results[3-4], we present the first demonstration of a subpicosecond photonic switch with an alternative approach, where the active role is performed by a biological material, the chromoprotein bacteriorhodopsin. The results may serve as a basis for the future realization of protein-based integrated optical devices that can eventually lead to a conceptual revolution in the development of telecommunication technologies.

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P26: Comparative study of native and denatured MgDNA pure water solutions

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After exploring Na-DNA[1], we studied Mg-DNA pure water solutions. For divalent Mg counterions it is conceivable that DNA may show different properties. We have prepared Mg-DNA samples by dialyzing Na-DNA (Sigma D1626) against $MgCl_2$, and against water as a final step. The degree of polymerization of DNA samples is 2k-20k base pairs which corresponds to the contour length of L_c = 0.7 – 7 µm. In order to determine the conformation of the Mg-DNA in pure water conditions (concentration range c = 0.01-5 mg/mL; semidilute regime) we have also prepared and tested the denatured MgDNA. The samples were heated for 20 minutes at 97°C and then quenched to 4°C. Subsequently, UV spectrophotometry (at 260 nm) and dielectric spectroscopy (DS) at 25°C were done and these results were compared with the results measured on the native samples. A quantitatively different behaviour between native and denatured Mg-DNA shows that Mg-DNA in pure water solutions is in the double-stranded conformation in the whole measured concentration range. DS results revealed two relaxation modes only for native Mg-DNA samples. The characteristic length scale of HF mode ($f>10^5$ Hz), scales with DNA concentration as $c^{-0.5}$ in the whole measured range of concentrations. Thus, we associate it with de Gennes correlation length ξ[2]. This is partly in contrast with behaviour previously found on Na-DNA[1] where that scaling changes to $c^{-0.33}$ for samples with concentrations smaller than 0.6 g/L. The latter we ascribed to locally exposed hydrophobic cores due to conformational fluctuations. The next important result was that this characteristic length is 1.6 times larger than for denatured Mg-DNA. This is in accordance with the expression predicted by de Gennes theory $\xi = (bc)^{-1/2}$, where b is monomer size. Characteristic length scale of LF mode ($f<10^5$ Hz) scales with DNA concentration as $c^{-0.25}$. This behaviour was also found previously for Na-DNA and it was associated with the single chain property, i.e. with the average end to end distance of a single chain modelled as a random walk of correlation blobs. Another important result was that for denatured Mg-DNA we did not detect LF mode probably since it shifted to higher frequencies and merged with the much larger HF mode.

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P27: An integrated approach to the study of complexation of alkali-metal cations by calix[4]arene amide derivative

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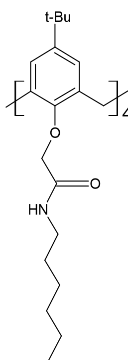
Complexation of alkali-metal cations by calix[4]arene derivative (**L**) in benzonitrile was studied by means of microcalorimetric titrations, molecular dynamics simulations and single-crystal X-ray diffraction. The inclusion of acetonitrile molecule in the calixarene hydrophobic *cone* was also studied using the same methods.



Structure of **L**.

The stability constants of the Li**L**$^+$, Na**L**$^+$, Li**L**MeCN$^+$ and Na**L**MeCN$^+$ complexes in benzonitrile were determined along with the enthalpies and entropies of complexation reactions. All investigated reactions were found to be enthalpy driven. In the case of Li**L**$^+$ complex the inclusion of benzonitrile molecule in the calixarene *cone* was observed, and the corresponding molecular and crystal structures were determined. This finding was in accordance with the results of molecular dynamics simulation of Li**L**$^+$ complex in benzonitrile.

P28: Using 180GHz spectrometer for molecular motions study by stimulated electron spin echo

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A few years ago a new method of molecular motions investigation by stimulated spin echo (π/2-τ-π/2-T-π/2-τ-echo) technique was proposed[1].

So far, it was successfully used to study nanosecond time scale librations and microsecond reorientations of nitroxide probes in organic glasses[2], spin-labeled lipids[1,3] and membrane antimicrobial peptides[4] in lipid bilayers using X-band machine.

Nitroxide G-band (180 GHz) spectrum has much higher anisotropy what allows one to study the symmetry of molecular motions in contrast to X-band. Also high-field EPR machines have much lower "dead time", what is extremely required for stimulated echo relaxation investigation.

Stimulated spin echo experiment was performed on a G-band spectrometer to study nanosecond time scale librations and microsecond reorientations and its symmetry in lipid bilayers containing spin-labeled lipids.

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P29: Melanin nanoparticles - biomacromolecular antioxidant

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Melanin, a biopolymer very common among the living organisms, expresses a variety of important biological functions, including photoprotection, chelation of metal ions, antibiotic and potential antioxidant activity. [1-2] Despite many studies on its physicochemical properties, the exact structure of melanin is yet to be determined. Presumably, studies on model systems composed from synthetic melanin nanoparticles will expand our knowledge about the properties of this biopolymer, including its antioxidant activity and will result in a structure-activity relationship (SAR).

In this research, the size-controllable nanoparticles were synthesized with a recently reported method: through neutralization of dopamine hydrochloride with sodium hydroxide, followed by spontaneous dopamine oxidation. [3] The scanning electron microscope (SEM) was used to determine the size and shape of obtained melanin nanoparticles.

Numerous analytical studies were conducted, including infrared spectroscopy (IR) and nuclear magnetic resonance (NMR), to define the structure of synthesized melanin. Moreover, the real time measurements of NMR spectra were performed to follow a dopamine oxidation to melanin.

The ability of melanin nanoparticles to inhibit peroxidation of model zwitterionic and anionic lipid bilayers was evaluated. Melanin antiradical activity was related to membrane charge. Revealing the structure of melanin nanoparticles will allow to fully understand melanin-induced inhibition of lipid peroxidation.

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P30: Interactions of hydrophobically modified polycations with lipid membranes studied by molecular dynamics

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The interactions between synthetic polycations and lipid or cell membranes play an import ant role in many biophysical applications, such as gene delivery[1], antibacterial usage[2] or obtaining stabilized vesicles by covering liposomes surface with multilayer films[3]. The molecular mechanisms of these processes are yet unknown, however much empirical evidence suggests that positively charged polymers can disrupt the cellular and/or nuclear membrane and increase membrane permeability. Recently, polycations containing quaternary ammonium or alkyl pyridinium moieties have been extensively studied for these purposes, and quaternary amino groups are believed to be responsible for membrane disruption[2].

Herein, we present the results of Molecular Dynamics (MD) simulations that were performed to investigate interactions between two poly(allylamine) hydrochloride (PAH) derivatives and phospholipid bilayer, which is an appropriate model of a biological membrane and/or a liposome. Simulations allowed to get insight into the systems at the molecular level, typically not accessible to the experimental observation. They reveal that polymer side chain substitution drastically changes the polimer's behavior, ranging from almost no effect on the bilayer structure to its substantial modification, such as formation of hydrophilic pores in the membrane.

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P31: Surfactant-like designer-peptides as solubilizing agents for Apolipoprotein B-100

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Surfactant-like designer-peptides constitute a new class of detergents. With their amphipathic character they structurally mimic natural phospholipids. Recently these peptides were successfully used to solubilize and stabilize membrane proteins[1]. Our project aims to test different surfactant-like designer-peptides regarding their delipidation-efficiency and to further use them instead of classical detergents in order to solubilize, stabilize and crystallize Apolipoprotein B-100 (Apo B-100). Apo B-100 is one of the largest monomeric proteins know. It is the sole protein component of low density lipoprotein (LDL), the main cholesterol-transporter in human circulation[2].

The delipidation-efficiency of different surfactant-like designer-peptides has been tested with a variety of biophysical techniques. Out of the different candidates the peptide ac-V_4WD_2 showed the highest propensity to act on membranes. Small angle x-ray scattering studies showed that ac-V_4WD_2 was able to remodel dipalmitoylphosphatidylcholine (DPPC) multilamellar vesicles. Electron spin resonance spectroscopy measurements gave information on the mobility of an incorporated spin-label and on the penetration depth of the peptide. With differential scanning calorimetry we were able to see a dramatic change in the phase transition behavior of DPPC when incubated with ac-V_4WD_2. When the same peptide was applied to LDL the characteristic core-melting transition temperature seemed unchanged whereas the protein denaturation temperature was highly affected. Interestingly, we could observe a stabilizing effect on the LDL protein moiety.

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P32: Tissue distribution and gene expression regulation of MRP9 (abcc12) in rainbow trout and zebrafish

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MRP9 is the last discovered member of the MRP subfamily. It has predicted topology of 12 transmembrane helices arranged in two membrane spanning domains and is similar in structure to short MRP's sharing the highest degree of amino acid sequences identity (42%) with MRP5 (ABCC5). In humans, MRP9 is expressed in various tumor tissues and cell lines, with negligible expression in normal tissues while in mice dominant expression is in testis with modest expression in other tissues. No physiological substrates of MRP9 have been identified so far. MRP9 has not been studied in fish and its possible role in aquatic species is not known. Main goals of our work were to determine the gene expression levels in different tissues of two fish species and to obtain first insights into regulation of Mrp9 gene expression.

Using the qPCR we found ubiquitous expression of abcc12 with the same tissue distribution pattern in both fish species. High expression was found in intestine while the expression in other tissues was moderate to low (intestine >> testis/ovaries > kidney > liver >> brain > gills > muscle). We found no sex related differences in abcc12 mRNA levels. In addition, to study regulation of abcc12 in fish we used rainbow trout liver cell line R1, which showed high expression of various nuclear receptors. R1 cells were exposed for 24 h to 30 different model ligands of nuclear receptors. We found that fibrates, dexamethasone and fluoxetine induce abcc12, suggesting the involvement of peroxisome proliferator-activated receptors (PPARs) and glucocorticoid receptor (GR) in regulation of abcc12. Furthermore, we found that aryl hydrocarbon receptor (AhR) ligands like TCDD and benzo[*a*]pyren, significantly elicit expression of abcc12.

In conclusion, our results showed that abcc12 is under complex regulation of both, physiological as well as receptors related to xenobiotic metabolism. Determination of substrate specificity of Mrp9 will be focus of our further research.

P33: Centrally acting oximes in reactivation of tabun-phosphoramidated AChE

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Organophosphates (OP) inhibit acetylcholinesterase (AChE, E.C.3.1.1.7), both in peripheral tissues and central nervous system (CNS), causing adverse and sometimes fatal effects due to the accumulation of neurotransmitter acetylcholine (ACh). The currently used therapy, focusing on the reactivation of inhibited AChE, is limited to peripheral tissues because commonly used quaternary pyridinium oxime reactivators do not cross the blood brain barrier (BBB) at therapeutically relevant levels. A directed library of thirty uncharged oximes that contain tertiary amine or imidazole protonable functional groups that should cross the BBB as unionized species was tested as tabun-hAChE conjugate reactivators along with three reference oximes: DAM (diacetylmonoxime), MINA (monoisonitrosoacetone), and 2-PAM. The oxime RS150D [*N*-((1-(3-(2-((hydroxyimino)methyl)-1*H*-imidazol-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)methyl)benzamide] was highlighted as the most promising reactivator of the tabun-hAChE conjugate. We also observed that oximes RS194B [*N*-(2-(azepan-1-yl)ethyl)-2-(hydroxyimino)acetamide] and RS41A [2-(hydroxyimino)-*N*-(2-(pyrrolidin-1-yl)ethyl)acetamide], which emerged as lead uncharged reactivators of phosphylated hAChE with other OPs (sarin, cyclosarin and VX), exhibited only moderate reactivation potency for tabun inhibited hAChE. This implies that geometry of oxime access to the phosphorus atom conjugated to the active serine is an important criterion for efficient reactivation, along with the chemical nature of the conjugated moiety: phosphorate, phosphonate, or phosphoramidate. Moreover, modification of the active center through mutagenesis enhances the rates of reactivation. The phosphoramidated-hAChE choline-binding site mutant Y337A showed three-times enhanced reactivation capacity with non-triazole imidazole containing aldoximes (RS113B, RS113A and RS115A) and acetamide derivative (RS194B) than with 2PAM.

P34: Evaluation of scoring functions use in case of acetylcholinesterase inhibitors

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Molecular modelling tools are recognised as helpful in drug design and medicinal chemistry. Proper evaluation of receptor-ligand interaction is one of the main goals in drug design and development. Predicting receptor-ligand interaction with respect to binding affinity represents the next step, as this is what is required to optimise the ligand structure. Recognition of key interactions is essential for developing selective and high affinity ligands and scoring functions have been introduced to this end. They are developed by correlating interactions from numerous crystal structures of various different receptor-ligand complexes with the matching inhibition constant. In this study, we evaluated several high affinity acetylcholinesterase inhibitors using scoring functions to determine the correlation between the reported inhibition constants for the following inhibitor complexes PDB ID: 1E66, 1EVE, 1H22, 1H23, 1U65, 1ZGB, and 1ZGC, and score values derived from scoring functions. We used the following scoring functions available in Accelrys Discovery Studio software: PLP1, PLP2[1], PMF, PMF04[2], Jain[3], LigScore1, and LigScore2[4]. Deviation between inhibition constants was 10 % while deviation between matching scores ranged between 12 and 40 %. Scoring functions LigScore2 and PLP2 provided a sound evaluation of acetylcholinesterase-inhibitor complexes with the lowest relative score deviation. Although LigScore2 predicted a logarithmic value of affinity in two cases (1H23 and 1ZGC), some disagreement was noticed due to 20 % lower value of predicted affinities in average.

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P35: Substrate selectivity of atypical methanogenic-type Seryl-tRNA synthetases – computational approach

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Atypical, methanogenic seryl-tRNA synthetases are homologues to catalytic domain of seryl-tRNA synthetases. Our study focuses on two Ser-tRNA synthetases homologues, from *B. japonicum* and from *A. tumefaciens*. These two homologs, with 63% similar sequences, display different amino acids specificity. Only *A. tumefaciens* homolog is able to activate serine, while homologue from *B. japonicum* shows only glycine activation[1]. Amino acid recognition other than serine is puzzling since the amino acid specificity of aminoacyl-tRNA synthetases is evolutionary strictly conserved.

In order to understand the altered homolog specificity we have performed molecular dynamics simulations of the two homologues. Since the crystal structure of *A. tumefaciens* homolog has not been available, we applied the comparative modeling (program Modeller[2]) using the crystal structure of *B. japonicum* homologue Bll0957[1] as a template. Molecular dynamics (MD) simulations were performed using the ff99SB force field[3]. Electrostatic potential (ESP) derived atomic charges using the HF (6-311G(p,d)) have been calculated for the Zn ion, amino acids from its first coordination sphere and the substrate. The MD simulations and the quantum mechanical calculations were performed by the Amber10[4] program package and by the Gaussian09 program[5], respectively. The MD simulations revealed different subunits behavior of these homodimeric proteins. In order to better understand difference in the substrate selectivity we shall perform QM/MM calculations based on molecular modeling studies presented here.

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P36: Thermodynamics of interactions driving functioning of bacterial toxin-antitoxin modules

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Toxin-antitoxin (TA) modules are bacterial genetic systems encoding two proteins: stable well structured toxin and unstable partially unstructured antitoxin. The toxin attacks its cellular target (e.g. DNA gyrase) which may cause cell death or growth arrest. The antitoxin inhibits the action of the toxin by forming the toxin-antitoxin complex.[1-2]

In this work we studied molecular mechanism of functioning of *ccdAB* TA module from bacteria *Vibrio fischeri*. We investigated binding of the unstructured fragment of the antitoxin, $CcdA_C$, to the toxin, CcdB, and binding of CcdB to the fragment of its target gyrase, GyrA. Thermodynamic information on molecular recognition of the proteins was obtained by the global model analysis of calorimetric titration curves. Fluorescence emission and CD spectroscopy were employed to obtain additional information on the nature of the observed binding processes. In our presentation thermodynamic driving forces of the monitored protein-protein interactions will be discussed in terms of available structural information and mechanism of functioning of the chromosomal TA module.

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P37: Investigation of antioxidative mechanisms of poly-hydroxy-phenols

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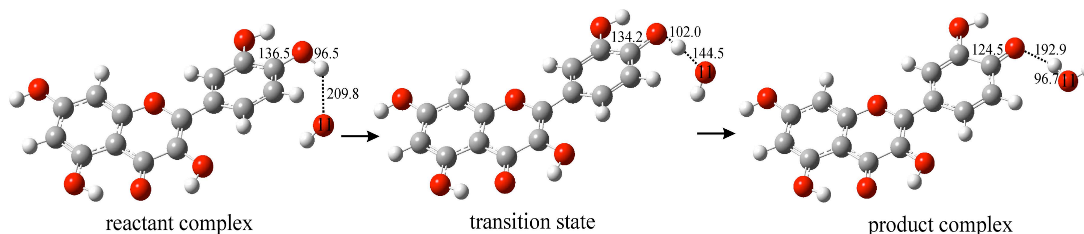
Flavonoids are natural phenolic compounds recognized as potent external defense factors against oxidative damage. In the radical scavenging mechanisms reactive radical species are inactivated by accepting a hydrogen atom from a hydroxyl group of the flavonoid. This transfer can be visualized through at least three mechanisms: Hydrogen Atom Transfer (HAT), Single Electron Transfer followed by Proton Transfer (SET-PT) and Sequential Proton Loss Electron Transfer (SPLET). Quercetin is consistently ranked as one of the most powerful antioxidants in the flavonoid class of compounds. Investigation of the reaction of quercetin with the hydroperoxy radical showed that this reaction is governed by the HAT mechanism. According to the activation energies, the reaction in the 3'OH position is fastest, followed by the reaction in the 4'OH position[1,2]. This outcome slightly disagrees from the BDE results, since the 4'OH radical form has a lower BDE value.

To explain the SET-PT mechanism, the reactions of the quercetin radical cation with base OH^- were investigated in gas and water. It was found that in all cases the most reactive site of quercetin is 4'OH. The reaction with OH^- in the gas phase takes place via the HAT mechanism, while the SET-PT mechanism is dominant in the aqueous phase.[3]



Reaction path for the H atom transfer from OH at C-4' of quercetin to OH^- radical

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P38: Activation of a CNBD upon cAMP-binding: concerted / sequential event?

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Cyclic nucleotide-gated ion channels (CNG) are tetrameric proteins; each subunit comprises six transmembrane segments and a cyclic nucleotide-binding domain (CNBD) at the C-terminus. Unlike its mammalian counterparts, the bacterial CNG channel MloK1 is activated by the binding of 3'-5'-cyclic adenosine mono-phosphate (cAMP) to the CNBD in a non-cooperative fashion.

Structural studies on the *apo*- and the *holo*-CNBD of MloK1 revealed major conformational changes from ligand-free to ligand-bound state. Recent kinetic and NMR studies indicate that the structural transitions follow the "induced-fit" mechanism, i.e. these are a direct consequence of ligand binding. However, the detailed mechanism of these structural rearrangements leading to receptor activation remains elusive. We use time-resolved electron paramagnetic resonance spectroscopy (EPR) combined with site-directed spin labeling (SDSL) to resolve the dynamics of the conformational changes in CNBD of the MloK1 channel. We intend to create a temporal map of the structural transitions during receptor-ligand interaction which will provide us a better understanding of the dynamics of the process.

P39: Characterizing the structure and dynamics of α-synuclein oligomers using H/D exchange monitored by MS

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by neuronal loss and the occurrence of intraneuronal fibrillar inclusions in the substantia nigra pars compacta of the brain.[1] Soluble prefibrillar and oligomeric aggregates of α-synuclein (α-syn) have been proposed to be promoters of neuronal cell death in PD[2].

While α-syn incorporated in fibrils has been characterized using various methods [3-4] little is known about the structure of α-syn found in the putatively cytotoxic oligomeric aggregates. The heterogenic nature of the oligomers represents a great analytical challenge as most traditional spectroscopic techniques cannot resolve such populations. These techniques yield population average data, making them incapable of resolving individual conformers. We attempt to resolve and characterize these aggregates using hydrogen/deuterium exchange (HDX) monitored by MS. The oligomer protection patterns deviated from previous characterizations of α-syn fibrils using HDX-NMR, and interestingly revealed protected regions unique to the oligomeric aggregates. These regions offer clues about the formation and further aggregation states of α-syn oligomers.

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P40: Rationalization of stereospecific binding of propanolol to cytochrome P450 2D6 by free energy calculations

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Cytochrome P450 2D6 is a major drug metabolising enzyme with a wide substrate range. A single point mutation introduced in this enzyme induces stereo-selective binding of R- and S-propranolol, while the wild type shows no such preference. The system was studied previously both experimentally and computationally [1], but the *in silico* study reported hysteresis and significant deviations from the closure of thermodynamic cycles, probably due to a lack of sampling. With prolonged simulation time and enhanced sampling methods such as Hamiltonian replica exchange, we reduced these problems, and improved the precision of free energy calculations. Here we rationalize the results at a molecular level and compare data to experimental finds as well as the previously estimated free energies.

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P41: Flow free microfluidic device for studying bacterial chemotaxis

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Motility helps bacteria explore spatially heterogeneous environments. By a mechanism called chemotaxis bacteria are able to accurately detect the concentration gradient of chemoeffectors and migrate towards high attractant concentrations (and/or away from repellents). In order to study chemotaxis one has to precisely generate chemical gradients and follow the migration of cells in it. Here we present two microfluidic devices that may be used for this purpose. A device typically used in the literature is based on the diffusional mixing of constantly flowing culture media: the chemical gradient is formed in the first part of the device, and bacteria are added in the mixing channel. While the bacteria are flowing along this channel they also swim around and as a response to the chemical gradient they may accumulate on one side of the channel. Although very sharp and smooth gradients are generated in the device, bacteria are only exposed to it for a short time (~20 s): the time the cells spend flowing along the mixing channel. To overcome this limitation we designed a new device in which there is no flow: bacteria swim in a completely static but chemically heterogeneous environment. In this device we are able to observe the behavior of the culture for an extended period of time (~12 h) therefore we can study small variations in chemotaxis in various chemical environments. We are also able to detect how the cells change the chemical composition of their environment and how they react to these changes.

P42: Finding conformational transition pathways from discrete molecular dynamics simulations

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We present a new method for estimating pathways for conformational transitions in macromolecules from the use of discrete molecular dynamics and biasing techniques based on a combination of essential dynamics and Maxwell–Demon sampling techniques. The method can work with high efficiency at different levels of resolution, including the atomistic one, and can help to define initial pathways for further exploration by means of more accurate atomistic molecular dynamics simulations. The method is implemented in a freely available Web-based application accessible at http://mmb.irbbarcelona.org/MDdMD.

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P43: Low temperature electron-spin relaxation in molecular solid exhibiting frozen-in disorder

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In the multidisciplinary quest for understanding of glasses, the study of molecular glasses provides an adequate experimental framework to investigate dynamics in states characterized by frozen-in disorder. The advantage of applying electron paramagnetic resonance (EPR) spectroscopy is in the sensitivity of the electron-spin coupling with the disorder modes in the observed system. Since radiation chemistry of saccharides has been developed for numerous reasons, radiation-induced defects in sugar compounds are well described for number of sugar species. Therefore, we made use of such paramagnetic defects to study the coupling of the electron spin with the excitations of the lattice exhibiting glassy state. The results of continuous-wave and pulsed X-band EPR measurements (electron spin-lattice relaxation time) are to be presented along with the theoretical analysis in the context of extracting the contribution of disorder modes from the experimental data.

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P44: Membrane skeleton deformation of the aspirated red blood cell adhered to a flat surface

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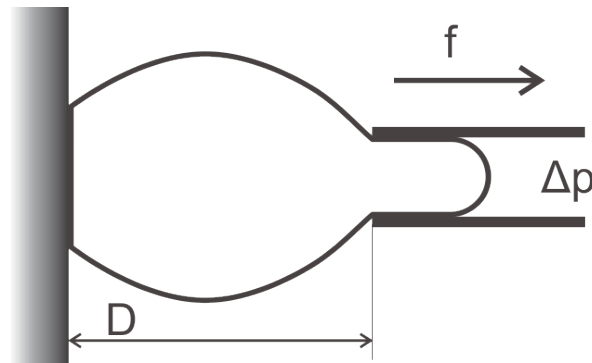

Deformation of the red blood cell (RBC) depends on its membrane which is composed of a lipid bilayer, an almost incompressible two-dimensional liquid, and an underlying skeleton, a two-dimensional network of elastic spectrin tetramers. At the mechanical stress of the RBC the bilayer defines its deformed shape and the skeleton redistributes in the plane of the membrane. At some deformations, like micropipette aspiration of the cell, the deformed shape of the cell is geometrically known because it is defined by solid walls of the pipette and increased membrane tension. For such deformations of the cell the corresponding response of the elastic skeleton can be determined. With the approximation that spectrin tetramer bonds behave in a harmonic (linear) manner, we determined for a given deformation the elastic energy of individual bonds. Lateral distribution of bonds was obtained by the minimization of this energy. The skeleton elastic energy and its lateral distribution was employed in the simulation of the experiment considering the enforced detachment of RBCs adhering to surface.[1] We studied at a given suction pressure Δp the effect of the skeleton on the dependence of the force f on the cell extension D.

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P45: Further study of the zinc coordination in the active site of the human DPP III - ONIOM calculations

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The first step in understanding the chemical reactions in the DPP III active site is to determine the coordination of the central zinc ion. Discrepancy between the experimental data (PDB ID: 3FVY) and the results of MD simulations[1,2] indicate that this question is still to be answered. The main issues are: a) does the Glu451 participates in Zn2+ coordination (MD simulations indicate that it does), and b) how many water molecules coordinate the metal ion (one or two, according to the crystal structure or the structures obtained from MD simulations, respectively).

To address this problem we performed series of ONIOM (Our own N-layered Integrated molecular Orbital and molecular Mechanics) QM/MM calculations. The initial structures were either taken from the MD simulations or from the experimental data. For this purpose the protein was divided into two layers which were handled at different levels of theory. The quantum mechanical (QM) layer (consisting of His450, Glu451, His455 and Glu508 side chains, the zinc ion and two water molecules) was treated by the DFT method using B3LYP functional and different basis sets (from 3-21g to 6-311++g(d,p)). The molecular mechanic (MM) part (the rest of the protein) was treated by the AMBER force field (param96). To take into account solvation, additional 50 water molecules, close to the central zinc ion, were considered as a part of the MM layer as well. Some of these water molecules were fixed during the minimization.

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P46: DNA knotting and shell ordering inside viral capsids, a computational study

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The packing of DNA inside bacteriophages arguably yields the simplest example of genome organisation in living organisms[1-3]. Cryo-em studies on bacteriophages epsilon-15[4] and phi-29[5] showed that DNA is neatly ordered in concentric shells close to the capsid wall, while an increasing level of disorder was measured when moving away from the capsid internal surface. Another, indirect, indication of how DNA is packaged is provided by the detected spectrum of knots formed by DNA that is circularised inside the P4 viral capsid[6-7]. The experimental knot spectrum indicates that packaged DNA tends to be knotted with high probability, forming complex knots characterized by a bias toward torus knots and against achiral ones.

Here we show, using stochastic simulation techniques, that both the shell ordering and the knot spectrum can be reproduced quantitatively if one accounts for the preference of contacting DNA strands to juxtapose at a small twist angle, as in cholesteric liquid crystals. The DNA knots we observe are strongly delocalized and, intriguingly, this is shown not to interfere with genome ejection out of the phage[8].

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P47: The human cathelicidin LL-37: effect of self-aggregation on the mechanism of action

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LL-37 is the only member of the cathelicidin family of the antimicrobial peptides found in humans, and is an important multifunctional component of the innate immune system. Its direct antimicrobial activity is reportedly due to its ability to interact with and to permeabilize bacterial membranes by a detergent-like "carpet" mechanism or to the formation of discreet channels. Studies on the evolution of LL-37 orthologues in primates[1] indicate an important role for oligomerization and aggregation that need to be further investigated to elucidate its mode of action. Therefore, we have synthesized a photoreactive analog by replacing the Phe in the 5th position with the unnatural amino acid, p-Benzoyl-L-Phenylalanine (Bpa). Irradiation with UV light enables covalent cross-linking, so that self-assembly of the LL-37 monomer in PIL buffer could be somehow quantified.

Another strategy was to synthesize three different disulfide-linked LL-37 dimers by adding a Cys residue to either the C- or N-termini, (C- or N-terminal-linked parallel, and antiparallel dimers) as obligatorily aggregated forms. SAR studies revealed that the dimeric forms have an increased propensity to form stacked helices in bulk-solution and when in contact with model membranes, and act differently on both bacterial and host cells. We found a lower antimicrobial activity for dimers against both Gram-positive and Gram-negative bacteria, so that the generally lower antimicrobial activity of LL-37 with respect to *mmu*RL-37 may derive from its greater tendency to aggregate. The haemolytic effect was different amongst the diverse dimeric forms and particularly high for C-terminal dimer.

Surface Plasmon Resonance was used to study both its self-aggregation and interaction with zwitterionic or anionic LUVs. Membrane interaction was found to be quite different from that of the *mmu*RL-37, which tends not to aggregate under the conditions used. This confirmed the importance of conformation and self-aggregation on the mode of action and biological activity of LL-37.

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