Workshop on the
Biomolecules and Nanostructures – Będlewo 3

Będlewo (Poland), September 4-8, 2011

Organized by
Institute of Physics, Polish Academy of Sciences, Poland,
University of Warsaw, Poland,
and École Polytechnique Fédrale de Lausanne, Switzerland

Under the auspices of the
National Multidisciplinary Laboratory of Functional Nanomaterials – NanoFun,
financed by Innovative Economy Operational Programme 2.2.
and the Division of Physics in Life Science of the European Physical Society

Editors:
Marek Cieplak, Anna Niedźwiecka, Andrzej Sienkiewicz

ABSTRACTS

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Preface

The 2011 Workshop on Biomolecules and Nanostructures continues the tradition of the two previous Workshops on the Structure and Function of Biomolecules that were held at the conference center in the village of Bedlewo near Poznan in 2004 and 2006. The current title of the Workshop reflects a markedly broader range of the covered topics and also the fact that the primary funding consortium is now the National Multidisciplinary Laboratory of Functional Nanomaterials - NanoFun.

The overall goals, however, remain similar. One of them is to provide an active forum for cross-disciplinary interactions between specialists who are active in different fields related to the science of biomolecules and bio-related aspects of nanotechnology. Another is to generate an overview of the current subjects of this very active and rapidly evolving branch of research.

Various experimental and theoretical methods used in the bio- and nano-area will be described. Still another goal is to develop inspiring scientific and personal contacts in the quiet setting of the village of Bedlewo.

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Conference program

Sunday

chairperson: **Marek Cieplak**			
15:00 - 22:00	*Registration*		
19:30 - 20:30	I-1	**David B. Searls**, University of Pennsylvania, USA	*Macromolecules, Languages, and Automata*
20:30 - 20:40		**Anna Niedźwiecka**, Institute of Physics, PAS; University of Warsaw, Poland	*The NanoFun Project*
20:40 - 22:00	*Dinner at bonfire*		

Monday

chairperson: **Marek Cieplak**			
9:00 - 9:40	I-2	**Bertrand Garcia-Moreno**, Department of Biophysics, Johns Hopkins University, Baltimore, USA	*Charges in the Hydrophobic Interior of Proteins*
9:50 - 10:25	I-3	**Mariusz Jaskólski**, Faculty of Chemistry, Adam Mickiewicz University, Poznan, Poland	*Structure and Function of Chitooligosaccharide* N*-Methylase: a Small Step Towards Understanding Plant-Bacterium Symbiosis*
10:25 - 10:50	*Coffee break*		
chairperson: **Mariusz Jaskólski**			
10:50 - 11:25	I-4	**Ard A. Louis**, Institute of Theoretical Physics, Oxford University, UK	*Coarse-grained Model for Self-assembling DNA*
11:30 - 12:05	I-5	**Mariano Carrión-Vázquez**, Cajal Institute, Madrid, Spain	*The Nanomechanics of Neurotoxic Proteins*
12:10 - 12:45	I-6	**Michał Dadlez**, Institute of Biochemistry and Biophysics, Warsaw, Poland	*Ion Mobility Separation Coupled with MS Detects Two Structural States of Alzheimer's Disease Aβ*1-40 *Peptide Oligomers*
12:45 - 15:00	*Lunch*		

chairperson: **Cornelia G. Palivan**			
15:00 - 15:35	I-7	**Dror Noy**, Weizmann Institute of Science, Israel	*Light Energy Harvesting and Dissipation in* de novo *Designed Bacteriochlorophyll Proteins*
15:40 - 16:15	I-8	**Wiesław I. Gruszecki**, Institute of Physics, Maria Curie-Sklodowska University, Lublin	*Regulation of Excitation Density at the Level of Single Photosynthetic Antenna Complex LHCII*
16:15 - 16:45	*Coffee break*		
chairperson: **Bertrand Garcia-Moreno**			
16:45 - 17:20	I-9	**Daniel H. Reich**, Department of Physics, Johns Hopkins University, Baltimore, USA	*Probing Sub-Cellular Force Dynamics with Arrays of Magnetic Microposts*
17:25 - 18:00	I-10	**Harald Janovjak**, Institute of Science and Technology Austria, Klosterneuburg, Austria	*Optical Control of Cellular Signaling*
18:05 - 18:40	I-11	**Sophie Jackson**, Department of Chemistry, Cambridge University, UK	*A Tangled Problem: the Structure, Function and Folding of Knotted Proteins*
18:40 - 19:40	*Dinner*		
20:00 - 22:00	*Poster Session I* *P1 - P20*		

Tuesday

chairperson: **Harald Janovjak**			
9:00 - 9:35	I-12	**Joanna Sułkowska**, University of California, San Diego, USA	*Folding and Unfolding of Proteins with Knots*
9:40 - 10:15	I-13	**Emanuele Paci**, Institute of Molecular and Cell Biology, School of Physics and Astronomy, Univeristy of Leeds, Leeds, UK	*Thermodynamics, Kinetics and Mechanics of Structured and Unstructured Polypeptides*
10:20 - 10:55	I-14	**Pilar Cossio**, SISSA, Trieste, Italy	*Enhanced Sampling of Proteins' Conformational Space beyond the PDB*
10:55 - 11:15	*Coffee break*		
chairperson: **Andrzej Sienkiewicz**			
11:15 - 11:50	I-15	**Cornelia G. Palivan**, Department of Chemistry, University of Basel, Switzerland	*Enzyme - Containing Nanoreactors*
11:55 - 12:30	I-16	**Marcin Nowotny**, International Institute of Molecular and cell Biology in Warsaw	*Structural Studies of DNA Repair*
12:30 - 13:30	*Lunch*		
13:30 - 17:15	*Excursion*		
17:15 - 17:30	*Coffee break*		

chairperson: **Anna Niedźwiecka**			
17:30 - 18:05	I-17	**Andrzej Dziembowski**, Institute of Biochemistry and Biophysics, Warsaw, Poland	*Biochemical and Structural Analysis of Protein Complexes Involved in RNA Metabolism*
18:10 - 18:45	I-18	**Ryszard W. Adamiak**, Institue of Bioorganic Chemistry, Poznań, Poland	*HIV-2 Leader RNA Structure: the Interplay Between Experiment and Modeling*
18:50 - 19:25	I-19	**Ryszard Stolarski**, Univeristy of Warsaw, Poland	*eIF4E Recognition Specificity for Mono- and Trimethylated Structures of mRNA 5'cap - an Open Question*
19:30 - 20:15	*Dinner*		
20:15 - 22:00	*Poster Session II* *P21 - P37*		

Wednesday

chairperson: **Daniel H. Reich**			
9:00 - 9:40	I-20	**José N. Onuchic**, University of California, San Diego, USA	*Exploring the Landscape for Protein Folding: from Function to Molecular Machines*
9:45 - 10:20	I-21	**Christopher M. Johnson**, MRC Laboratory of Molecular Biology, Cambridge, UK	*Fluctuation Correlation Spectroscopy: from Peptides to Molecular Machines*
10:25 - 11:00	I-22	**José Maria Valpuesta**, Centro Nacional de Biotechnologia, CSIC, Madrid, Spain	*Molecular Chaperones: Nanomachines in the Protein Folding Assembly Line*
11:00 - 11:30	*Coffee break*		
chairperson: **Emanuele Paci**			
11:30 - 12:05	I-23	**Thomas Weikl**, Max Planck Institute of Colloids and Interfaces, Potsdam	*Discrete Energy Landscapes in Protein Folding and Function*
12:10 - 12:45	I-24	**Marek Langner**, Institute of Physics, Wroclaw Technical University, Poland	*Lipid Bilayer - the Simplest Biological System*
12:50 - 13:25	I-25	**Paolo De los Rios**, Ecole Polytechnique Lausanne, Switzerland	*Protein Rehabilitation by the Hsp70 Chaperone*
13:25 - 15:00	*Lunch*		
chairperson: **Sophie Jackson**			
15:00 - 15:35	I-26	**Dimitrios Fotiadis**, Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland	*Membrane Protein Structure Determination by High-resolution Microscopy Techniques*
15:40 - 16:15	I-27	**Ramunas Valiokas**, Center for Physical Sciences and Technology, Vilnius, Lithuania	*Nanopatterning of Soft Surfaces for Organizing Proteins and Cells*
16:20 - 16:55	I-28	**Piotr Szymczak**, Faculty of Physics, University of Warsaw, Poland	*Protein Translocation Through a Pore*

16:55 - 17:30	*Coffee break*		
Main lecture place		chairperson: **Ryszard Stolarski**	
17:30 - 18:05	I-29	**Dorota Wolicka**, University of Warsaw, Faculty of Geology, Warsaw, Poland	*Mineralogical Processes under Sulphate Reducing Condition in Soils Contaminated by Crude Oil*
18:10 - 18:45	I-30	**Joanna Wesoly**, Biology Department, Adam Mickiewicz University in Poznań, Poland	*Signal Transducers and Activators of Transcription in Renal Transplantation and Renal Cell Carcinoma*
18:50 - 19:30	I-31	**Mikołaj Olejniczak**, Institute of Bioorganic Chemistry PAS, Institute of Biochemistry and Molecular Biology, Adam Mickiewicz University, Poznań, Poland	*The Kinetic and Thermodynamic Framework of the Chaperone Protein Hfq Interactions with Bacterial RNAs*
Poster room place		chairperson: **Ard A. Louis**	
17:30 - 17:55	I-34	**Ulrich H.E. Hansmann**, Department of Physics, Michigan Technological University, USA	*Enhanced Sampling in Protein Simulations*
18:00 - 18:25	I-32	**Andrzej Koliński**, Chemistry Department, Warsaw University, Poland	*Modeling of Protein Folding and Docking Pathways - a Multiscale Approach*
18:30 - 18:55	I-33	**Kay-Eberhard Gottschalk**, Department of Experimental Physics, University of Ulm, Germany	*Molecular Dynamics Simulations of Interactions of Amino Acids and Proteins with Gold Surfaces*
19:00 - 19:25	I-35	**Paul Barker**, University of Cambridge, Chemistry Department, UK	*Control of Protein Fibril Superstructure by the Conformation of Pendant Domains*
19:45 -	*Conference banquet*		

General Information

Lectures
Speakers of the morning sessions are asked to provide their presentations to Dr. Michał Wojciechowski in the Lecture Room immediately after the last session of the day before, and at the beginning of the lunch time in the case of the afternoon sessions.

Posters
Presenting authors are requested to place their posters before 9:00 a.m. on the indicated day and dismount the posters not later than at 12 p.m. on the same day.

WiFi
Network access will be available in the conference venue. Information will be provided by the hotel reception desk.

Workshop Office

- Registration desk opening hours

September 4^{th}, 2011 (Sunday)	15:00 – 22:00
September 5^{th}, 2011 (Monday)	8:30 – 19:30
September 6^{th}, 2011 (Tuesday)	8:30 – 19:30
September 7^{th}, 2011 (Wednesday)	8:30 – 19:30

- The registration fee includes admission to the conference, conference materials, coffee breaks, accommodation, meals, banquet and an excursion.
- There is also a fee for accompanying persons 380 PLN for Polish citizens and 220 EUR for foreigners to be paid on arrival by cash or credit card at the hotel reception desk. The fee covers the expenses except the conference materials.

Useful Phone Numbers

Hotel reception desk of the Bdlewo Conference Center		+48 61 813 51 87
Registration desk of the Workshop		+48 532 831 818
	or	+48 532 831 819

Emergency Calls
From mobile phone 112 for all emergencies.
From stationary phone:

Ambulance	999
Police	997
Fire department	998

Phone Calls From Poland
From a stationary phone: 00 Your Country Code The Number Abroad
From a mobile phone: + Your Country Code The Number Abroad

Insurance
The organizers do not take responsibility for individual, travel or personal insurance. Participants are advised to have their own insurance policies. Please, let us know about any emergencies.

History of Będlewo

The village of Będlewo became the property of the Polish aristocratic Potocki family in 1694. In 1866 Bolesław Potocki built a neo-gothic palace and service buildings, and founded an almost 9 hectare land park. The complex remained with the Potocki family until 1907. Afterwards it passed to other aristocratic families. After World War II, the complex, partially reconstructed, served from 1945 as an agricultural school until 1975 when it was donated to the Polish Academy of Sciences.

Overwiev of the abstracts

Invited Lectures

Poster Contributions

Abstracts of Invited Lectures

I-1: Macromolecules, Languages, and Automata

David B. Searls

Independent Consultant, Philadelphia, PA, USA

Molecular biology is replete with linguistic metaphors, from the language of DNA [1] to the genome as "book of life"[2]. Certainly the organization of genes and other functional modules along the DNA sequence invites a syntactic view, which can be adopted for purposes of pattern-matching search via parsing [3]. This in turn has led to the development of novel grammar formalisms specially adapted to the biological domain [4]. It has also been shown that folding of RNA structures is neatly expressed by grammars that require expressive power beyond context-free on the Chomsky hierarchy [5], an approach that has been conceptually extended with other grammar formalisms to the much more complex structures of proteins [6]. Grammars and their cognate automata have even been adopted to describe evolutionary processes and algorithms for their reconstruction via sequence alignment [7], and indeed the analogy between the evolution of species and of languages (first noted by Darwin) has been exploited by applying bioinformatics tools to human languages as well [8]. Processive enzymes and other "molecular machines" can also be cast in terms of automata, and thus of grammars, opening up new possibilities for the formal specification, modeling, and simulation of biological processes, and perhaps tools useful in the fields of DNA computing and nanotechnology. This talk will review linguistic approaches to molecular biology, and perspectives on potential future applications of grammars and automata in this field.

[1] D. B. Searls, *American Scientist* **80**, 579 (1992)
[2] D. B. Searls, *Nature* **420**, 211 (2002)
[3] S. Dong and D. B. Searls, *Genomics* **23**, 540 (1994)
[4] D. B. Searls, *J. Logic Prog.* **24**, 73 (1995)
[5] D. B. Searls, in *Mathematical Support for Molecular Biology*, American Mathematical Society, 117 (1999)
[6] D. Chiang, A. Joshi, and D. B. Searls, *J. Comp. Biol.* **13**, 1077 (2006)
[7] D. B. Searls, *Trends Genet.* **12**, 35 (1996)
[8] D. B. Searls, *Nature* **426**, 391 (2003)

I-2: Charges in the Hydrophobic Interior of Proteins

Bertrand Garcia-Moreno[1], Daniel G. Isom[1], Jamie L. Schlessman[2], Michael J. Harms[1], Michael S. Chimenti[1], Aaron Robinson[1] and Victor Khangulov[1]

[1] Johns Hopkins University, 3400 N. Charles St., Baltimore Maryland, USA
[2] US Naval Academy, 572 Holloway Road, Annapolis Maryland, USA

Charges are not compatible with hydrophobic environments. They destabilize the folded state and are usually excluded from the interior of proteins. However, they are essential for key biochemical processes: they are the recurrent functional motif used for biological energy transduction (H^+-coupled e^- transfer, catalysis, ion homeostasis, etc). Despite their high importance, their properties are poorly understood. To examine properties of internal ionizable groups we engineered a family of 100 variants of staphylococcal nuclease with Lys, Arg, Asp or Glu at each of 25 internal positions [1]. p*K*a values of the internal groups were measured with equilibrium thermodynamic methods [2,3]. Most of the p*K*a values are shifted in the direction that favors the neutral state (elevated for Asp and Glu and depressed for Lys), some by more than 5 p*K*a units. Most of the internal ionizable residues are neutral under physiological conditions of pH. Arg is very different from Lys, Asp and Glu: we have never detected a shifted p*K*a in an Arg residue. Crystal structures of over 30 variants show that the internal ionizable groups really are buried, some of them deeply, either in strictly hydrophobic or in slightly polar microenvironments. Some internal ionizable groups are buried in a semi-hydrated state. The structural consequence of ionization were examined with Trp fluorescence, CD and NMR spectroscopy. Remarkably, the majority of proteins withstand the ionization of the internal group without any detectable structural changes. The proteins remain folded and comparable to the parent protein. A few cases have been found where small regions of the protein experience increased dynamics in the intermediate exchange regime. Few cases have been found where the ionization unfolds the protein. A variety of excited states in which the protein populates a partially or subglobally unfolded state in which the internal ionizable group can gain access to water have been found. Our experimental and structural data have been used to benchmark structure-based p*K*a calculations with a variety of algorithms. The data demonstrate irrefutably that the continuum approximation is not valid to study electrostatic effects inside proteins and in other dehydrated environments such as interfaces between proteins. Overall our studies demonstrate that specialized structural adaptations are not necessary for proteins to tolerate internal ionizable groups of proteins. The p*K*a values of these groups shift naturally into the range required for H^+ just by virtue of being internal. Specialized dipolar cages or dynamics are not require to have stable internal charges; the ability to tolerate ionizable groups in their hydrophobic interior is an inherent property of stable proteins, not a specialized functional adaptation. Our demonstration that internal ionizable groups, even pairs of them, can be engineered in stable proteins suggest that strategies for enzyme design should focus on the interplay between the destabilizing consequences of internal charges and the global stability of the protein.

[1] D. G. Isom, B. R. Cannon, C. A. Castaneda, A. Robinson and B. Garcia-Moreno E, *Proc. Natl. Acad. Sci. USA* **105**, 17784 (2008).
[2] D. G. Isom, C. A. Castaneda, B. R. Cannon, P. D. Velu and B. Garcia-Moreno E, *Proc. Natl. Acad. Sci. USA* **107**, 16096 (2010).
[3] D. G. Isom, C. A. Castaneda, B. R. Cannon and B. Garcia-Moreno E, *Proc. Natl. Acad. Sci. USA* **108**, 5260 (2011).

I-3: Structure and Function of Chitooligosaccharide *N*-Methylase: a Small Step Towards Understanding Plant-Bacterium Symbiosis

Mariusz Jaskólski[1,2], Ozgur Cakici[2], Michal Sikorski[2], Tomasz Stepkowski[2] and Grzegorz Bujacz[2]

[1]Department of Crystallography, Faculty of Chemistry, A. Mickiewicz University, Poznan, Poland
[2]Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland
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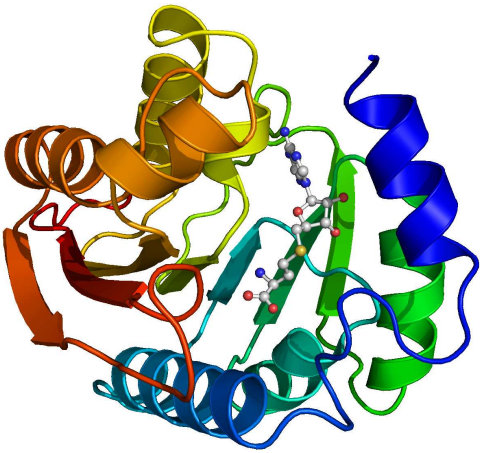
NodS is an *S*-adenosyl-*L*-methionine- or SAM-dependent *N*-methyltransferase involved in the biosynthesis of Nod Factor (NF) in nitrogen-assimilating bacteria known as rhizobia. NF is a modified lipochitooligosaccharide signal molecule that must be received and recognized by the legume plant host in order to establish productive plant-bacterium symbiosis for atmospheric nitrogen fixation. So far, there has been no structural information about the NodS enzyme from any rhizobium. We have undertaken X-ray crystallographic studies of a recombinant NodS protein from *Bradyrhizobium japonicum*, which infects lupine and seradella plants. Two crystal forms, of ligand-free NodS and of NodS in complex with *S*-adenosyl-*L*-homocysteine (SAH), which is a byproduct of the methylation reaction, were obtained and their structures were refined to 2.43 and 1.85 Å resolution, respectively. Although the overall fold, consisting of a seven-stranded open β-sheet flanked on each side by a layer of α-helices, is similar as in other SAM-dependent methyltransferases, NodS also has specific structural features connected with binding of its unique oligosaccharide substrate. In particular, docking of the methyl donor SAM substrate results in ordering of an *N*-terminal α-helix, which tightly seals the methyl-donor cavity and at the same time shapes a long canyon on the molecular surface that is evidently recognized as the binding site for the second substrate, the oligosaccharide methyl-acceptor molecule. By gaining insight about how NodS binds its donor and acceptor substrates, we hope to better understand the mechanism of NodS catalysis and the basis of its functional difference in various rhizobia.



NodS from *Bradyrhizobium japonicum* in cartoon representation, with the SAH ligand shown as a ball-and-stick model.

I-4: Coarse-grained Model for Self-assembling DNA

Ard A. Louis[1], Thomas Ouldridge[1] and Jonathan P. K. Doye [2]

[1] Rudolf Peierls Centre for Theoretical Physics, University of Oxford, 1 Keble Road, Oxford, OX1 3NP, United Kingdom
[2] Physical and Theoretical Chemistry Laboratory, Department of Chemistry, University of Oxford, South Parks Road, Oxford, OX1 3QZ, United Kingdom

We introduce a coarse-grained rigid nucleotide model of DNA that reproduces the basic thermodynamics of short strands: duplex hybridization, single-stranded stacking and hairpin formation, and also captures the essential structural properties of DNA: the helical pitch, persistence length and torsional stiffness of double-stranded molecules, as well as the comparative flexibility of unstacked single strands [1,2]. We apply the model to calculate the detailed free-energy landscape of one full cycle of DNA 'tweezers'[1], a simple machine driven by hybridization and strand displacement. We also study other nanomachines such as DNA walkers, basic processes such as force-induced melting, as well as biologically motivated processes, including cruciform formation and the dynamics of Holliday junctions.

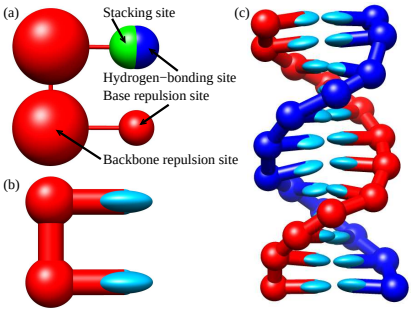


Figure 1: Schematic of the model

[1] DNA nanotweezers studied with a coarse-grained model of DNA, Thomas E. Ouldridge, Ard A. Louis, Jonathan P.K. Doye, *Phys. Rev. Lett.* **104** 178101 (2010)
[2] Structural and thermodynamic properties of a coarse-grained model of DNA, Thomas E. Ouldridge, Ard A. Louis, Jonathan P.K. Doye, *J. Chem. Phys.* **134**, 085101 (2011)

I-5: The Nanomechanics of Neurotoxic Proteins

Mariano Carrión-Vázquez[1,2]

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In our modern societies amyloid-related neurodegenerative diseases have a high impact and are currently untreatable. Although they are caused by specific "neurotoxic proteins", their underlying molecular mechanisms remain elusive. Elucidating them seems a prerequisite for treatment and cure. The "conformational change hypothesis" postulates that these proteins become toxic after a critical unknown conformational change. Using force spectroscopy and a new methodology for unequivocal single-molecule identification, we show that sampling monomeric conformations from four representative neurotoxic proteins unveils a rich polymorphism. Remarkably, this polymorphism was found strongly correlated with amyloidogenesis/neurotoxicity: unchanged by an inhibitor of oligomerization, completely abolished by a fibrillation-less mutant, favoured by familial-disease mutations, and discouraged by a surprisingly promiscuous inhibitor of the toxic monomeric conformational change, neurotoxicity and neurodegeneration. We suggest that formation of certain mechanostable conformers could be the primary cause of these diseases, which may constitute new early-diagnostic/therapeutic targets. Our findings strongly support the conformational change hypothesis at the level of monomer, extend to this level (further upstream in the pathogenic cascade) the view that neurotoxic proteins share many of the molecular mechanisms underlying amyloidogenesis, open the door to understanding the molecular mechanism of early amyloidogenesis, and offer hope for therapeutic advances for preventing and curing many neurodegenerative diseases simultaneously.

I-6: Ion Mobility Separation Coupled with MS Detects Two Structural States of Alzheimer's Disease Aβ1-40 Peptide Oligomers

Michal Dadlez

Institute of Biochemistry and Biophysics, Warsaw, Poland

Mounting evidence points to the soluble oligomers of amyloid β (Aβ) peptide as important neurotoxic species in Alzheimer's disease, causing synaptic dysfunction and neuronal injury, and finally leading to neuronal death. The mechanism of the Aβ peptide self-assembly is still under debate. Using Ion Mobility separation coupled with MS we have measured collisional cross-section values (Ω) of different oligomeric forms of Aβ, from dimers to hexadecamers. For several oligomers, at least two different species of different Ω values were detected, indicating the presence of at least two families of conformers: compact and extended. To rationalize the obtained results we have constructed a set of molecular models of Aβ oligomer structure that provided a very good correlation between the experimental and theoretical Ω values, both for the compact and the extended forms. Thus, using mass spectrometry we could detect oligomeric species that are on-pathway in the process of fibril formation or decay, but also alternative structures which may represent off-pathway oligomers. The detected compact-extended structural transition is a plausible candidate for the bifurcation point leading to the off-fibril pathway of oligomer evolution and formation of neurotoxic species.

I-7: Light Energy Harvesting and Dissipation in *de novo* Designed Bacteriochlorophyll Proteins

Dror Noy

Plant Sciences Department, Weizmann Institute of Science, Rehovot 76100, Israel

Photosynthetic light-harvesting proteins (LHCs) are characterized by a high pigment-protein ratio and hence significant contributions from pigment-pigment and pigment-protein interactions to the structural stability and integrity of the whole complex. The protein environment and pigment organization are also critical for rapid excitation energy transfer with minimal losses due to non-radiative relaxation processes, the hallmarks of LHCs functionality. The actual number of light harvesting pigments, their types, and relative proportions vary significantly in different photosynthetic organisms, and this variability, complexity, and redundancy makes it very difficult to separate the various roles of protein and pigment components by merely exploring samples that survived natural selection. In this context, the construction of minimal functional analogs of natural protein-cofactor complexes emerges as a complementary strategy for understanding the roles of specific elements within the complex natural system. Progress in the field of protein de novo design and the ability to incorporate various chlorophyll (Chl) and bacteriochlorophyll (BChl) derivatives into these proteins make them appealing as functional LHC models.

Here, I will present strategies for designing de novo proteins that fold and incorporate multiple (B)Chl derivatives. The challenges in accounting for specific protein-(B)Chl interactions such as central metal-coordination, and steric perturbations will be discussed as well as means to overcome them by combining automated computational design techniques with empirical and bioinformatics methods. Focusing on the design of water-soluble proteins that assemble specifically with water-soluble (B)Chl derivatives offers additional advantages over working with the natural hydrophobic pigments and proteins, particularly in easier design, self-assembly, and use of recombinant DNA techniques for protein preparation.

The structural and spectral characteristics of two recently successful protein designs will be discussed in detail. These include:

1. A water soluble analog of a transmembranal Chl binding motif from photosystems I and II that binds up to six (B)Chl derivatives.
2. De novo designed four-helix bundle proteins capable of binding up to three (B)Chl derivatives [1].

The new designs provide simple model systems comprising a small number of (B)Chl derivatives embedded in minimal protein scaffolds. As such they can serve as useful benchmarks for elucidating the mechanisms by which the protein environment and pigment organization affect excitation energy transfer and non-radiative relaxation pathways in crowded (B)Chl-protein complexes.

[1] I. Cohen-Ofri, M. van Gastel, J. Grzyb, A. Brandis, I. Pinkas, W. Lubitz, D. Noy, *J. Am. Chem. Soc.* in press (20011).

I-8: Regulation of Excitation Density at the Level of Single Photosynthetic Antenna Complex LHCII

Wiesław I. Gruszecki[1], Monika Zubik [1], Rafał Luchowski [1], Wojciech Grudziński [1], Zygmunt Gryczyński [2,3] and Ignacy Gryczyński [2]

[1] Department of Biophysics, Institute of Physics, Maria Curie-Skłodowska University, Lublin, Poland
[2] Center for Commercialization of Fluorescence Technologies, University of North Texas Health Science Center, Fort Worth TX, USA
[3] Department of Physics, Texas Christian University, Fort Worth TX, USA

LHCII (light-harvesting pigment-protein complex of Photosystem II) is the most abundant membrane protein in the biosphere, comprising approximately half of the total chlorophyll pool. The main physiological role of this protein is to absorb light energy and transfer it towards the photosynthetic reaction centers, where electric charge separation takes place. LHCII can absorb light owing to the presence of cofactors, photosynthetic pigments, including chlorophyll *a*, chlorophyll *b* and xanthophylls. Excitation of the photosynthetic apparatus to a level higher than can be utilized by photochemical reactions results in formation of chlorophyll triplet states and photo-sensitized oxidative destruction of an entire photosynthesizing organism. Therefore, regulation of excitation density at the level of photosynthetic antenna complexes is a process important from the physiological standpoint, vital to plant productivity but also to protect life of photosynthesizing organisms.

We have studied physical mechanisms responsible for singlet excitation quenching in the photosynthetic pigment network of LHCII. Illumination of the complex results in quenching of singlet chlorophyll a excitations, manifested by decrease in fluorescence level and shortening of fluorescence lifetimes . Specific activity of blue light, absorbed by both chlorophyll and xanthophyll pigments, as compared to red light, absorbed exclusively by chlorophylls, indicates that xanthophylls can play a role of photoreceptors involved in triggering light-driven excitation quenching in LHCII. Resonance Raman spectroscopic analysis of the LHCII-bound xanthophylls revealed light-driven molecular configuration changes of neoxanthin and violaxanthin . Analysis of the infrared absorption spectra of LHCII shows that photo-physical effects are associated with reorganization of the antenna protein at the supramolecular level . The model will be presented, based on the experimental results, linking overexcitation of the pigment-protein complex with protective excitation quenching.

[1] W. I. Gruszecki, R. Luchowski, M. Zubik, W. Grudzinski, E. Janik, M. Gospodarek, J. Goc, Z. Gryczynski, I. Gryczynski, *J Plant Physiol* **167**, 69 (2010).
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I-9: Probing Sub-Cellular Force Dynamics with Arrays of Magnetic Microposts

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The biological response of cells to mechanical forces is integral to both normal cell function and the progression of many diseases, such as hypertensive vascular wall thickening. This likely results from the fact that mechanical stresses can directly affect many cellular processes, including signal transduction, gene expression, growth, differentiation, and survival. The need to understand the relationship between applied forces and the mechanical response of cells as a critical step towards understanding mechanotransduction calls for tools that can apply forces to cells while measuring their contractile response. This talk will describe approaches that combine the use of magnetic microparticles and/or nanoparticles in conjunction with deformable micropatterned substrated that simultaneously allow local mechanical stimulation of the adherent surface of single cells or groups of cells and spatially resolved measurements of the local forces and strain fields generated throughout the cells in response to this stimulation. To achieve sub-cellular resolution cells are cultured on the top surfaces of arrays of micrometer-scale posts made from a flexible elastomer (PDMS), and the contractile forces generated by an adherent cell bend the posts. Measurements of the displacement of each post allow the contractile force field of the cell to be mapped out with micrometer-scale precision. To apply forces to cells, rod-shaped magnetic nanoparticles are embedded in some of the posts so that externally applied magnetic fields selectively deform these "magnetic posts", thereby exerting tunable local, mechanical stresses to the adherent surface of attached cells. Alternatively, magnetic particles bound to or internalized by the cell may be employed to apply forces and torques to the cell. With either approach, measuring the deflection of the surrounding non-magnetic posts probes the full mechanical response of the cell to these stresses. To probe the collective response of model tissue constructs, larger versions of this device may be employed. Results that illustrate the temporal dynamics and spatial distribution of the response of fibroblasts and smooth muscle cells and cell constructs to local stresses will be described.

I-10: Optical Control of Cellular Signaling

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A major challenge in biology is to understand how cells sense and process signals from the environment. To understand cellular signaling we require technologies that generate well-controlled temporal and local stimulation. Our past work focused on ionotropic glutamate receptors (GluRs), which are the primary mediators of excitatory synaptic transmission in the mammalian central nervous system. In order to remote control neuronal signaling, we designed a novel GluR that is K^+-selective and light-gated [*Nature Neuroscience* (2010) 18: 1027-1032.]. This hyperpolarizing ion channel termed HyLighter is activated by millisecond light pulses and allows manipulating neuronal activity with unprecedented spatio-temporal resolution. In optogenetic experiments, HyLighter reversibly inhibits action potential firing in neuronal cultures and behavior in zebrafish. Inspired by the surprising compatibility of a K^+-selective pore with a GluR revealed in HyLighter, we discovered a new family of invertebrate glutamate receptors that combine a K^+ selectivity filter with glutamate sensing [*Nature Communications* (2011) 2: 232]. These receptors connect todays GluRs to their ancestral, prokaryotic ion channels and represent missing links in GluR evolution. The goal of our future work is to remote control signaling cascades with light to understand how cells orchestrate local and temporal signals into physiological responses.

I-11: A Tangled Problem: the Structure, Function and Folding of Knotted Proteins

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Since 2000, when they were first identified by Willie Taylor, the number of knotted proteins within the pdb has increased and there are now nearly 300 such structures. The polypeptide chain of these proteins forms a topologically knotted structure. There are now examples of proteins which form simple 3_1 trefoil knots, 4_1, 5_2 Gordian knots and 6_1 Stevedore knots. Knotted proteins represent a significant challenge to both the experimental and computational protein folding communities. When and how the polypeptide chain knots during the folding of the protein poses an additional complexity to the folding landscape.

We have been studying the structure, folding and function of two types of knotted proteins the 3_1-trefoil knotted methyltransferases and 5_2-knotted ubiquitin C-terminal hydrolases. The first part of the talk will focus on our folding studies on knotted trefoil methyltransferases and will include our work on (i) equilibrium unfolding experiments in chemical denaturants, (ii) kinetic analysis of unfolding/folding pathways, (iii) protein engineering on both the small scale (single point mutants) and large scale (creating N- and C-terminal fusions with a stable beta-grasp domain which are the deepest knotted structures known), (iv) circularisation experiments which establish that the polypeptide chain remains knotted even in the chemically denatured state, and (v) recent *in vitro* translation work which shows that knotting is rate limiting and also shows how GroEL/GroES play a role in the folding of these proteins *in vivo*.

The second part of the talk will focus on our studies of knotted ubiquitin C-terminal hydrolases UCH-L1 and UCH-L3. This will include equilibrium and kinetic unfolding and folding studies as well as recent work on the effect of point mutants associated with Parkinsons Disease on the structure, folding and dynamics of UCH-L1. Recent work on the effect of oxidative damage on the structure of UCH-L1 will also be described and evidence that this protein adopts a partially unfolded form (PUF) on modification with the reactive aldehyde and by-product of cellular oxidative stress, HNE, will be presented. The possible cellular effects of this PUF will be discussed.

I-12: Folding and Unfolding of Proteins with Knots

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Proteins with nontrivial topology, containing knots and slipknots, have the ability to fold to their native states without any additional external forces invoked. A mechanism is suggested for folding of these proteins, such as YibK and YbeA, that involves an intermediate configuration with a slipknot. It elucidates the role of topological barriers and backtracking during the folding event. It also illustrates that native contacts are sufficient to guarantee folding in approximate to 1-2simulations, and how slipknot intermediates are needed to reduce the topological bottlenecks. We then discuss folding of a protein fragment of 92 amino acids which contains a knot with six crossings (a so called Stevedore knot).

Unfolding of knotted proteins through mechanical pulling provides interesting information about the dynamics of topological transformations in the proteins. Attempting to untie a native knot in a protein can succeed or fail depending on where one pulls – in similarity to shoelaces which can be either tightened or untied. However, thermal fluctuations induced by the water that surrounds the protein affect conformations stochastically and may add to the uncertainty of the outcome. When the protein is pulled by the termini, the knot can only get tightened, and any attempt at untying results in failure. We show that, by pulling specific amino acids, one may easily retract a terminal segment of the backbone from the knotting loop and untangle the knot. At still other amino acids, the outcome of pulling can go either way. We study the dependence of the untying probability on the way the protein is grasped, the pulling speed, and the temperature. Elucidation of the mechanisms underlying this dependence is critical for a successful experimental realization of protein knot untying.

I-13: Thermodynamics, Kinetics and Mechanics of Structured and Unstructured Polypeptides

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The possibility of monitoring the conformation of single proteins and finely perturb their energy landscape made possible by the development of highly sensitive mechanical probes has opened a new window onto the properties of these complex molecules. I will present some recent results, obtained by a combination of experiments and simulations, which confirm theoretical predictions of "anomalous" unfolding kinetics and discuss the danger of excessively simple projections when representing protein free-energy landscapes. I will also present some recent results, which show how a thorough analysis of rapid intra-chain kinetics of disordered or marginally structured peptides reveals complex and sequence-specific properties of their free-energy landscapes. Both sets of results have some important consequences on the function and disfunction of proteins in the cell.

I-14: Enhanced Sampling of Proteins' Conformational Space beyond the PDB

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Bias Exchange Metadynamics is a sophisticated computational approach that permits exploring complex multidimensional free energy landscapes. It allows predicting the folded state and the folding kinetics of small peptides (shorter than 40 residues) with a relatively moderate compuational effort (a few hundred ns). We recently applied the same approach to perform an exhaustive exploration of the conformational space of a 60-residue polyvaline. We generated a database of around 30,000 compact folds with at least 30Even if the simulated system is an homopolymer, these structures correspond to local minima of the potential energy. This ensemble plausibly represents the universe of protein folds of similar length: indeed, all the known folds are represented in the set with good accuracy. However, we discover that the known folds form a rather small subset, which *cannot* be reproduced by choosing random structures in the database. Rather, *natural* and *possible* folds differ by the contact order, on average significantly smaller in the former. Beside their conceptual relevance, the new structures open a range of practical applications such as the development of accurate structure prediction strategies, the optimization of force fields, and the identification and design of novel folds.

I-15: Enzyme - Containing Nanoreactors

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Similar to conventional lipids suitable amphiphilic block copolymers are able to self-assemble in aqueous media into supramolecular structures those membrane mimic the biological membranes. The properties of these membranes can be controlled to a large degree via the chemical constitution, the molecular weight and the hydrophilic-to-hydrophobic block length ratio of the polymers. Compared to conventional low molar mass building blocks (e.g. lipids), membranes based on macromolecular self-assembly, not only have the advantage of superior stability and toughness, but in addition, allow tailoring physical, chemical and biological properties since multifunctionality can be chemically implemented in one single macromolecule. Other well-defined functions, such as molecular recognition, cooperativity, and catalytic activity can be introduced by combining these polymeric superstructures with suitable biological entities, e.g., by incorporation of integral membrane proteins or by encapsulation of enzyme(s).

We used the concept of bio-synthetic combination to develop polymer nanoreactors by encapsulating water-soluble enzymes inside the aqueous cavity of vesicles generated by self-assembly of amphiphilic copolymers. Channel proteins inserted into the polymer membrane selectively controlled the exchange of substrates and products with the environment, supporting the *in situ* activity of the enzymes.

For example, enzymatic cascade reactions inside polymeric nanocontainers have been used as an effective means to detect and combat superoxide radicals, involved in oxdative stress and related pathologies. By simultaneously encapsulating a set of enzymes that act in tandem inside the cavities of polymeric nanovesicles and by inserting channel proteins in their membranes, an efficient catalytic system was formed, as demonstrated with *in situ* activity tests inside THP-1 cells. Nanoreactors avoid the inherent drawbacks of other enzyme delivery approaches, such as possible inactivation of the enzyme by extensive modification, significant leakage from liposomes due to structural defects, and mechanical instability or short circulation lifetime, and poor control of release from the polymer microspheres.

By synthesis of appropriate functionalised polymers (e.g. biotin, antibody) we successfully created specifically decorated nanoreactors for targeting approaches to predefined cells. After cellular uptake the nanoreactors retained their function over extended periods of time, thus acting as artificial organelles that continuously exchange molecular information with the host cell. This opens new ways in protein therapy, and intracellular sensing approaches.

I-16: Structural Studies of DNA Repair

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DNA in the cell constantly incurs chemical damage which must be repaired. One of the primary mechanisms to achieve this is Nucleotide Excision Repair (NER) pathway. Its main feature is the ability to recognize and repair a wide spectrum of different DNA lesions. In bacteria the first protein in this pathway is a dimeric ABC ATPase called UvrA. Its role is to locate the site of the DNA lesion. The DNA is then handed over to UvrB which verifies the presence of the damage. UvrC nuclease next excises the DNA fragment containing the lesion In order to elucidate the mechanism of DNA damage detection, we solved a crystal structure of *T. maritima* UvrA protein in complex with a modified DNA [1]. In the structure, the DNA is bound in a cleft running across the UvrA dimer. The protein binds the DNA duplex in its terminal regions on both sides of the modification site. The DNA is deformed - bent, stretched and unwound. Only this deformed conformation is complementary with the protein surface. Since these types of deformation are often observed in various modified DNAs, we propose, that UvrA uses them for indirect readout of the presence of the damage. The protein not only senses the deformations of the DNA caused by the lesion but it may also adjust them, so that the duplex fits to the protein surface. It is facilitated by the fact that modified DNA duplexes are more flexible.

Binding of the damaged DNA activates the ATPase activity of UvrA which is thought to be the signal that the DNA lesion was found and downstream proteins can be recruited. Based on a comparison of our UvrA-DNA structure with the previously determined structures of UvrA proteins without the DNA bound we proposed the mechanism for the ATPase activation.

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I-17: Biochemical and Structural Analysis of Protein Complexes Involved in RNA Metabolism

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Although numerous proteins are able to work alone, many if not most of them form a large repertoire of macromolecular complexes to perform their tasks in the cell as a team. The major interests of my laboratory concern the analysis of macromolecular assemblies involved in RNA metabolism. We combine biochemical and functional studies with structural biology approaches in order to achieve deep insight into analyzed complexes.

For the last few years, our primary research target has been the major eukaryotic ribonuclease, the exosome complex. The Exosome complex is involved in variety of RNA processing and turnover reactions both in the nucleus and cytoplasm. It is composed of 9-subunit ring similar to phosporolytic ribonucleases like PNPase or archaebacterial exosome but devoid of any detectable activity and associated catalytic subunits (Dis3 and Rrp6). The exosome was identified as a 3'-5' exoribonuclease but latter on we have shown that it is also endowed with endoribonuclease activity and that both activities reside in different domains of the Dis3 subunit. In addition in the nucleus, the exosome complex associates with additional distributive 3'-5' exoribonuclease Rrp6. Structural biology data supported by biochemical experiments provided information about position of the Dis3 active sites with the relation to the exosome ring and suggested that RNA riches the exo active site passing through the central channel in the ring. Cryo-electron microscopy structure of both apo and RNA-bound exosome holo-complexes supported this model and provided indication for large domain movement within the Dis3 protein caused by the substrate binding. Interestingly however analysis of carefully designed yeast point mutants suggest presence of alternative RNA pathways toward the exosome active sites.

Another macromolecular assembly we are interested in is the THO/TREX complex. The THO complex is a key factor in the co-transcriptional formation of export-competent messenger ribonucleoprotein particles, yet its structure and mechanism of chromatin recruitment remain unknown. Our recent collaborative work provided the THO complex architecture and structural basis for its chromatin targeting.

I-18: HIV-2 Leader RNA Structure: the Interplay Between Experiment and Modeling

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Retroviral genomes are assembled from two sense-strand RNAs by interactions at their 5' ends. The HIV RNA dimerization - an essential step in the retroviral life cycle is intensively studied in our Laboratory [http://www.ibch.poznan.pl/adamiak/], to design a new antyretroviral therapies. Dimerization and encapsidation signals, closely linked in HIV-2, are located in the leader RNA region. The SL1 RNA motif and nucleocapsid protein, containing two zinc fingers, are considered important for both processes. Recently we have reported the structure of the HIV-2 leader RNA (+1-560 nt) captured as the loose dimer [1]. Two kissing loop interfaces within the loose dimer have been identified: SL1 and additional one in TAR.

Structural properties of the SL1 RNA hairpin motif (*right*, shown in black within PAL-SL1-PSI region) and its complex formation with the recombinant nucleocapsid protein (NCp8) will be presented in a view of the experimental data and modeling based on the simulation of molecular dynamics, docking and RNA model building using our new automatic modeling method, called RNAComposer [2].

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I-19: eIF4E Recognition Specificity for Mono- and Trimethylated Structures of mRNA 5'cap - an Open Question

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Specific binding of the ribonucleic acid 5' termini (mRNA 5' cap) by the eukaryotic translation initiation factor 4E (eIF4E) is a key, rate limiting step during the initiation of protein biosynthesis. Contrary to mammalian and yeast eIF4Es that discriminate in favour of 7-methylguanosine cap (MMG-cap), three out of five eIF4E isoforms from the nematode *Caenorhabditis elegans* as well as eIF4Es from the parasites *Schistosome mansoni* and *Ascaris suum*, exhibit dual specificity for both MMG-cap and N^2,N^2,7-trimethylguanosine cap (TMG-cap). Several methods, including X-ray diffraction, were applied to address differences in the mechanism of the cap recognition by those highly homological proteins [1, 2]. However, even analysis of the crystal structures of *A. suum* eIF4E in two complexes, with MMG- and with TMG-cap [2], left the problem unresolved. Molecular dynamics simulations in water of three *apo* and complexed eIF4Es, two isoforms from *C. elegans*, MMG-cap specific IFE-3, and dual specific IFE-5, and of murine eIF4E, pointed to a dynamical mechanism of discrimination between two types of the cap. The differences in the recognition specificity may be ascribed to variations in the loops mobility at the entrance into the protein cap-binding pockets during the association/dissociation processes, and not to formation and/or disruption of well defined stabilizing contacts. Nonetheless, an exact specification of the role of particular amino acids in the proposed dynamical model needs further investigations.

[1] W. Liu *et al.* Structural insight into parasite eIF4E binding specificity for m^7G and $m^{2,2,7}G$ mRNA cap. *J. Biol. Chem.* **284**:31336-31349, 2009

[2] W. Liu *et al.* Structural Basis for Nematode eIF4E binding an $m^{2,2,7}G$-Cap and its Implications for Translation Initiation, Nucl. Acids Res. 2011, in press

[3] K. Ruszczynska-Bartnik *et al.*: Dynamical insight into *Caenorhabditis elegans* eIF4E recognition specificity for mono- and trimethylated structures of mRNA 5' cap. *J. Mol. Modeling* **17**, 727-737, 2011

* supported by Polish Ministry of Science and Higher Education grant Nos. N N301 035 936, N N301 267 137, and BST-1534/BF

I-20: Exploring the Landscape for Protein Folding: from Function to Molecular Machines.

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Globally the energy landscape of a folding protein resembles a partially rough funnel with reduced energetic frustration. A consequence of minimizing energetic frustration is that the topology of the native fold also plays a major role in the folding mechanism. Some folding motifs are easier to design than others suggesting the possibility that evolution not only selected sequences with sufficiently small energetic frustration but also selected more easily designable native structures. The overall structures of the on-route and off-route (traps) intermediates for the folding of more complex proteins are also strongly influenced by topology.

Going beyond folding, the power of reduced models to study the physics of protein assembly, protein binding and recognition, and larger biomolecular machines has also proven impressive. Since energetic frustration is sufficiently small, native structure-based models, which correspond to perfectly unfrustrated energy landscapes, have shown to be a powerful approach to explore larger proteins and protein complexes, not only folding but also function associated to large conformational motions. Therefore a discussion of how global motions control the mechanistic processes in the ribosome and molecular motors will be presented. For example, this conceptual framework is allowing us to envisage the dynamics of molecular motors from the structural perspective and it provides the means to make several quantitative predictions that can be tested by experiments.

I-21: Fluctuation Correlation Spectroscopy: from Peptides to Molecular Machines

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Analysis of fluctuations in spectroscopic signal through the autocorrelation function can give information about the diffusive properties of biological molecules, which are a function of their shape and size, as well as probing the dynamics of motions and intramolecular contact formation. We are using FCS methods to study a wide range of processes from peptide dynamics, which has a key role in the early events of protein folding, to the dynamics and assembly of larger and more complex biological macromolecules. Any interpretation of these observations is considerably strengthened through using a range of approaches, from single molecule to ensemble measurement, as well as a range of complimentary biophysical methods.

I-22: Molecular Chaperones: Nanomachines in the Protein Folding Assembly Line

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Protein folding is usually a problem in the crowded environment of the cell and nature has devised a group of proteins called molecular chaperones that assist the folding of other proteins [1]. Chaperones mostly function by protecting the aggregation-prone regions of their substrates or by providing them with a proper environment so that they can fold by themselves. Many processes in the living cell are performed as a coordinated action between multiple proteins, often forming multicomponent complexes. The chaperone-assisted protein-folding pathway is a clear example of this, as many different molecular chaperones have been identified to form a network of interactions that is ultimately aimed at the holding of their common substrates. Examples of these interactions are those taking place between Hsp60s and prefoldins, Hsp70s and Hsp40s, Hsp70s and Hsp90s [2-5]. Unfortunately, some of these complexes are of a transient nature and therefore difficult to purify, which precludes their crystallization and therefore its structural characterization at high resolution (NMR cannot be used for these large macrocomplexes). Electron microscopy is a powerful structural tool to deal with this problem because only tiny amounts of complexes are needed to generate medium resolution structural information, which then can be complemented with high-resolution information generated for some of the components of the complexes. The work presented here describes the structural characterization, by electron microscopy and image processing, of some of these complexes and provides clues on their mechanism of action.

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I-23: Discrete Energy Landscapes in Protein Folding and Function

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A central goal in protein science is to characterize the free-energy landscapes of folding and function. High-dimensional continuous energy landscapes of proteins have helped to understand general aspects, but are difficult to apply in practice. Therefore, a current focus is on discrete energy landscapes, i.e. on Markov state models of folding and function. Detailed Markov models can be constructed from molecular dynamics simulations of folding and function, and are important tools for analyzing simulation trajectories without prior assumption of reaction coordinates. These detailed Markov models from simulations can be compared to coarse Markov models obtained from experimental data.

I-24: Lipid Bilayer - the Simplest Biological System

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Biological systems have intrinsic properties underlying their capacity to maintain the organized systems of complex functions which enable evolutionally, to overcome the environmental challenges. These capabilities are possible because of a combination of certain qualities of biological systems, including; the dominant role of "entropic forces" and thermal fluctuations, local molecular specificity, molecular crowding, self-organization and tensegrity. These properties result in the effect known as "the emerging properties" which make the system able to evolve. The complexity of real biological systems make their systematic analysis difficult therefore a suitable model possessing at least some of these qualities is needed. One of the most abundant structures in biological systems, lipid bilayer, seems to serve this purpose well. It is as a permeability barrier enabling compartmentalization of biological space" and the maintenance of the electrochemical gradients. These functions require the lipid bilayer to be stable in time, however the membrane thermal fluctuations results in the local defects in its structure. The lipid bilayer structure is very dynamic with topology dependent on the local specificity of its components. Without external constraints it is mechanically balanced. The molecular density in the lipid bilayer is very high providing a convenient model of a crowded space and the properties of a complete aggregate results from the short range interactions, which are not predictable from the properties of single lipid molecules. All these attributes satisfy the requirements of a system with capacity for emerging properties. The presentation demonstrates the selected experimental results obtained using simple lipid bilayer models providing new perspective on the biological systems.

I-25: Protein Rehabilitation by the Hsp70 Chaperone

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Protein misfolding and aggregation is one of the major causes of neurodegenerative diseases such as Huntington, Alzheimer and Parkinson. One of the first lines of defense of cells against protein misfolding and aggregation is a chaperone network which relies on the combined action of several Heat Shock Proteins (Hsp) such as Hsp70, Hsp60, Hsp100. In this work we focus on the rehabilitating action of Hsp70 that, in conjunction with its co-chaperones DnaJ and GrpE, can rescue misfolded proteins by unfolding them thus giving them a chance to properly refold according to the Anfinsen's rule. We show that the energy price (hydrolyzed ATP molecules) is much smaller than it would be if each misfolded protein had to be degraded and re-synthesized.

I-26: Membrane Protein Structure Determination by High-resolution Microscopy Techniques

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Membrane proteins represent an important class of proteins that comprises about 30 percent of the proteome of most organisms. However, only 281 (status: June 2011) unique membrane protein structures have been deposited so far in the Protein Data Bank compared to the more than 15000 unique structures from soluble proteins. Furthermore, these membrane protein structures originate from bacterial sources and are in the detergent-solubilized form.

The native environment of membrane proteins is the lipid bilayer. Thus, membrane protein structure and function are preferably assessed in this environment. High-resolution imaging techniques such as transmission electron (TEM) and atomic force microscopy (AFM) allow the structure of membrane proteins to be studied while embedded in the lipid bilayer. The high signal-to-noise ratio in AFM topographs makes the visualization of single membrane proteins at sub-nanometer resolution and under near-physiological conditions, i.e. in buffer solution, room temperature and normal pressure, possible. On the other hand, structural information of membrane proteins in lipid bilayers can be obtained by analyzing 2D protein crystals by negative stain TEM and cryo-TEM/electron crystallography.

The examples discussed during this presentation will illustrate the power of TEM and AFM in structure analysis of membrane proteins embedded in lipid membranes.

I-27: Nanopatterning of Soft Surfaces for Organizing Proteins and Cells

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Assembly of protein architectures at the level of single entities as well as manipulation and analysis of single cells are among the ultimate goals of nanobiotechnology. However, in practice, it is critically important that these new tools and devices are based on reliable and cost-efficient biochip and biomaterial platforms. Our experience in this field will be briefly introduced by discussing and comparing our results in fabrication of functional nanopatterns on organic surfaces such as self-assembled monolayers, polymeric hydrogels, lipid membranes and proteins. For this purpose, we typically use a powerful array of so-called unconventional fabrication techniques, including dip-pen nanolithography, inkjet printing and soft lithography. We have demonstrated the advantages of combining these techniques with advanced surface chemistries such as modular self-assembled monolayers, multivalent chelators, enzyme-mediated modifications of nucleic acids or photografting of functionalized hydrogels. For example, we have been able to construct nanoscopic protein domains for quantitative measurements of receptor-ligand interactions. Currently, we are involved in integration of such nanosctructures into clinically-relevant devices such as biosynthetic cornea implants. Opportunities and challenges in developing other nanostructure-based cell programming and control tools will be also addressed.

I-28: Protein Translocation Through a Pore

Piotr Szymczak

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Proteins need to be unfolded when translocated through the pores in mitochondrial and other cellular membranes. Although the details of this process are still not fully understood, it is clear that this process is qualitatively different from AFM-induced unfolding, with differences both in unfolding pathways and in the overall stability of the protein structure.

The results of simulations of this process are reported, using a coarse-grained Go-type protein model combined with a simple cylindrical pore model and a variety of pulling protocols: constant velocity, constant force as well as intermittent loading. A possible role of a Brownian ratchet in the translocation process is discussed. The results are then compared with the experimental data on mitochondrial import

I-29: Mineralogical Processes under Sulphate Reducing Condition in Soils Contaminated by Crude Oil

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The presented study reviews the geomicrobiological role of sulphate reducing bacteria (SRB) in different mineral phases formation in soil contaminated by crude oil. Sulphate-reducing bacteria play the main role in the utilisation of crude oil compounds Most of the isolated bacteria utilizing hydrocarbons belong to the SRB. However microorganisms have been show to be important active and passive promotes of redox reaction influencing geological processes. Environmental condition impact the bacterial community, which in turn alters the environment through its metabolic activities This feedback biotic/abiotic mechanism determines the characteristic mineral products of the particular system.

Dissimilatory reduction of sulphates results in the formation of many secondary sulphides, mainly of iron, such as: pyrrhotite (FeS), pyrite and marcasite (FeS_2), galena (PbS), sphalerite (ZnS), chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S), covellite (CuS), cinnabar (HgS), realgar (AsS), etc.

The results of the experiments indicate that one of the most important microorganism groups responsible for the biodegradation of hydrocarbons under anaerobic conditions.

The formation of mineral phases in anaerobic conditions in the presence of oil-derived products and sulphides as the final electron acceptors is not restricted only to the products of transformations such as sulphates and carbonates, depending on direct SRB activity. In some conditions SRB activity may lead to the formation of other mineral phases such as elemental sulphur or mineral compounds containing iron (II), such as vivianite.

I-30: Signal Transducers and Activators of Transcription in Renal Transplantation and Renal Cell Carcinoma

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Cytokines and growth factors are the main tool of the organism to battle any kind of immune challenge like inflammation or cancer. Cytokines exert their effect on cells by binding to a specific cell surface receptor and activating an intracellular signaling cascade. Progress in our understanding of cytokine signaling pathways has identified important signal transduction and regulatory components, among others members of the Signal Transducer and Activator of Transcription (STAT) family of transcription factors. STATs induce the expression of many different target genes that act on the boarder of innate and adaptive immunity and significantly participate in the pathology and progression of inflammatory diseases and cancer. STATs are tightly regulated by Suppressors Of Cytokine Signaling (SOCS). We study the expression and activation of members of both families: STATs and SOCS in cancer and renal transplantation. We hypothesize that changes in gene expression, protein levels and protein activation of STATs and SOCS (together with its downstream targets) can be correlated with the progression and/or severity of the disease and could be molecular indicators or pathological changes during the course of the disease. Using a multidisciplinary approach and modern genomic technologies we aim to better understand the disease process, to develop diagnostic and/or prognostic assays and to identify novel therapeutic targets that could be implemented in the clinics.

I-31: The Kinetic and Thermodynamic Framework of the Chaperone Protein Hfq Interactions with Bacterial RNAs

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Non-coding regulatory RNAs mediate the response of the bacterial cell to changing environmental conditions [1]. The ring-shaped chaperone protein Hfq is necessary for these RNAs to regulate the translation of selected messenger RNAs (mRNAs). This chaperone protein binds regulatory RNAs and facilitates their interactions with target mRNAs. Besides, Hfq can self-aggregate to form higher-order fibrillar structures [2]. Formation of nanostructures was also reported for one of regulatory RNAs [3]. Overall, elucidating the binding properties of the chaperone protein Hfq is important for understanding its function.

Here, the high-throughput filter retention assay was employed to study the kinetics of association and dissociation of the complexes of the chaperone protein Hfq with several regulatory RNAs. The results showed that all regulatory RNAs bound to the same site on the surface of the protein. The difference between the thermodynamic stability of binding calculated from kinetic rates, and measured directly, suggested that the pathway of RNA association to the chaperone involves intermediate steps. Despite very tight binding affinities regulatory RNAs were rapidly exchanged on Hfq. While the RNAs bound Hfq with similar affinities, the rates of exchange widely differed depending on the competing RNAs.

Overall, the results suggest that the similar binding of different regulatory RNAs to the same site on Hfq may be a necessary requirement for their efficient recycling. This is further modulated by the individual properties of each RNA, thus allowing for the flexible bacterial cell adaptation to changing environmental conditions.

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I-32: Modeling of Protein Folding and Docking Pathways - a Multiscale Approach

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Systematic sequencing of numerous genomes provides enormous library of protein sequences. Only for a small fraction of these proteins their three dimensional structures have been determined. Knowledge of protein structures is necessary for understanding and controlling of protein biological function, from enzymatic activity, through transport and signaling, to mechanisms and thermodynamics of complex macromolecular assemblies. It is also important to know protein folding mechanisms and the dynamic and thermodynamic characteristics of the denatured state. Understanding protein dynamics and folding mechanisms may be even more challenging than theoretical prediction of protein structure. Classical methods of molecular dynamics are applicable only to not too large systems and/or to a relatively narrow time frame. The time-scale of biomacromolecular processes is usually orders of magnitude wider. Therefore, simplified models could be very useful in a large scale molecular modeling. Reduced space CABS (Ca, Cb, Side chain representation) methodology proven to be quite effective in *de novo* protein structure prediction. Here we describe applications of CABS in studies of protein folding and docking mechanisms. It is demonstrated that the stochastic (Monte Carlo) dynamics, combined with all-atom refinement of the coarse-grained structures follows observed in experiments folding pathways of small proteins. The model is also used in model studies of chaperonin-assisted protein folding. It is shown that Iterative Annealing Mechanism of chaperonin action, where periodic distortion of the polypeptide chains by non-specific hydrophobic interactions can promote rapid folding and leads to a decrease in folding temperature. It is also demonstrated how chaperonin action prevents kinetically trapped conformations and modulates the observed folding mechanisms from nucleation-condensation to a more framework-like. Finally, we show that the CABS based coarse grained simulations could be used in studies of protein-peptide and protein-protein docking, allowing for a large scale conformational flexibility of the associating molecules.

I-33: Molecular Dynamics Simulations of Interactions of Amino Acids and Proteins with Gold Surfaces

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The specific adhesion of peptides and proteins on inorganic surfaces is important in biomedical and nanotechnological applications. Of particular importance as surface is gold due to its biocompatibility and ease of handling. However, the mechanism of polypeptide adsorption on gold is not well understood. Yet, only a detailed understanding of this process will yield the possibility of rational design of gold- binding proteins or a better understanding of biohazards potentially caused by gold nanoparticles. Here, based on the recently developed polarizable force field GolP, we describe the absorption of amino acids and proteins onto gold surfaces and calculate the potential of mean force of the non-covalent interaction of all twenty amino acids with a gold (111) surface. We find that the interaction energy is correlated the propensity of amino acids to form a -sheet, hinting at a design principle for gold binding peptides. For understanding the biological impact of gold-nanoparticles, the interaction of gold surfaces with extracellular matrix proteins is of great importance. Extracellular matrices will be among the first proteins encountered by these particles. A major component of the extracellular matrix is fibronectin, an important protein involved in cell adhesion, migration and cancer progression. We investigate the interaction of the two fibronectin domains FNIII(9-10), containing a main cell-binding motif, with a (111) gold surface by long molecular dynamics simulations in explicit water. We find that fibronectin binds fast and strongly to the surface. Within a simulation time of altogether more than a s, no unbinding or unfolding tendency is observed. However, domain re- orientation may occur. Arginine residues are critically involved in forming early protein-gold contacts. The importance of arginine for mediating early protein-surface contacts can be exploited for the rational design of gold-binding patches on protein surfaces. Our results further indicate that in a biological matrix, no bare gold surfaces will be present. Gold surfaces will quickly be covered by proteins and/or bind to the extracellular matrix. Bare gold surfaces will therefore very quickly not be exposed to the biological environment. However, the biological activity of the adsorbed protein may be altered due to structural re-orientations, potentially causing an immunological risk.

I-34: Enhanced Sampling in Protein Simulations

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A key component in the molecular machinery of cells are proteins, "nanomachines" that are responsible for transporting molecules, catalyzing biochemical reactions, or fighting infections. However, despite the remarkable progress in experimental machinery and techniques for producing and characterizing proteins, a detailed understanding of folding and interaction of proteins is still missing. Hence, there is a need for reliable computational tools that allow one to describe these processes from the physical interactions between the atoms within a protein, and between the protein and the surrounding environment. Unfortunately, the complex form of the forces within and between proteins leads to a rough energy landscape with a large number of local minima acting as traps. The resulting difficulties in sampling the energy landscape increase exponentially with the size of the system. Generalized-ensemble and replica exchange techniques, developed by us and others, have alleviated this sampling problem. However, these methods and algorithms need to be advanced further to allow detailed description of fundamental processes of protein folding, aggregation and interaction in a cell. I will describe our recent progress and discuss some applications.

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I-35: Control of Protein Fibril Superstructure by the Conformation of Pendant Domains

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Hierarchical, self-assembling structures formed from protein domains are central to cellular function. Self-assembled structures capable of mediating electron transfer over tens of nanometres are an attractive scientific and technological goal. Therefore, systematic variants of SH3 domain-Cytochrome b562 fusion proteins were designed to make amyloid fibres with pendant electron transfer proteins. TEM and AFM data show that fibre morphology responds systematically to placement of the cytochrome within the fusion proteins. UV-Vis spectroscopy shows that, for the fusion proteins under test, only half the fibre-borne b_{562} binds heme with high affinity. Cofactor binding also improves the AFM imaging properties and changes the fibre morphology through changes in cytochrome conformation. Systematic observations and measurements of fibril geometry suggest that longitudinal registry of subfilaments within the fibre, mediated by the interaction and conformation of the displayed proteins and their interaction with surfaces, gives rise to the observed morphologies. Of most interest is modulation of fibril structure and mechanical stability by heme binding and the observation that steric frustration can lead to length limited nanostructures. What started as a technological project has provided new insight into amyloid fibril superstructure.

Abstracts of Poster Contributions

P-1: Protein Structure Prediction Using CABS a Consensus Approach

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We have designed a new pipeline for protein structure prediction based on the CABS engine [1]. The procedure is fully automated and generates consensus models from a set of templates. Restraints derived from the templates define a region of conformational space, which is then sampled by Replica Exchange Monte Carlo algorithm implemented in CABS. Results from CASP9 show, that for great majority of targets this approach leads to better models than the mean quality of templates (in respect to (GDT_TS). In five cases the obtained models were the best among all predictions submitted to CASP9 as the first models. The pipeline consists of 6 steps:

1. Input structure selection
2. Restraints generation
3. CABS modeling
4. Clustering
5. Reconstruction and refinement
6. Model selection

One of the most challenging point of the procedure is selection of the input structures. We have tested a number of MQAP methods used during CASP experiments. Combination of some of them allows to improve significantly accuracy of final structures.

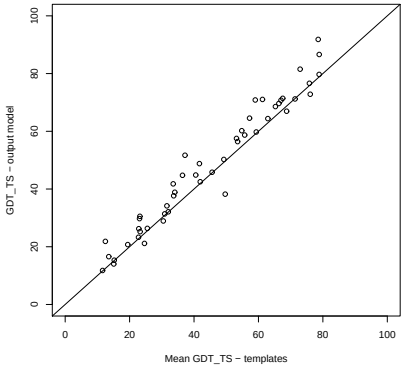

Figure 2: Comparison of accuracy of the input and the output structures

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P-2: The Close-Packed Helix

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Helical structures appear in Nature, molecular examples are the alpha-helices and the double helix of DNA. Packing plays an important role in condensed mater science. We may therefore ask the related question: Which helix has the best packing, i.e. optimizes the volume fraction?

We solve this mathematical problem by showing that for a certain pitch angle, the volume is more efficiently used than for any other value [1]. We call this the close-packed single, double, triple and quadruple helix. The results for optimal pitch angles are compared with values for helical polypeptide backbone structures, such as the single pi-, alpha- and 3.10-helix. The A-, B- and Z- form of DNA is also considered. The alpha-helix and the B-DNA is remarkably near to being perfectly close-packed. The close-packed structure has a central channel. We suggest, that this is relevant for the understanding of the triple helix of collagen [2].

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P-3: Interactions of BAR Proteins with Lipid Membranes

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BAR proteins create big group of membrane biding proteins. Conserve quaternary structure and high affinity towards lipid membranes of these proteins enhance bilayers deformation and tubulation. It was shown recently, that the group of these molecules is important player in endocytosis, filopodia formation and rearranging of the actin skeleton [1,2,3]. Though crystal structure of several BAR proteins was obtained, many aspects of action of these proteins are unknown [4]. The aim of this work is to gain knowledge about lipid bilayer in this context. For this purpose artificial membrane systems are used (Supported Lipid Bilayers SLBs, Giant Unilamellar Vesicles GUVs).

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P-4: Atomistic Calculation of Screened Coulomb Interactions in Semiconductor Nanostructures.

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Dielectric screening of Coulomb interactions affects optical and electronic properties of semiconductor nanostructures including multi-exciton generation rates in colloidal nanocrystals [1], excitonic spectra and self-energy corrections in nanopillar or graphene quantum dots [2,3]. Using tight-binding approach we compare several methods of accounting for dielectric screening in atomistic calculation including semi-classical approximation [4], Thomas-Fermi approach [5] and real-space dielectric matrix inversion [6]. We propose an efficient method in which dielectric screening can be decomposed into contribution from volume and surface-polarization terms, further screened by microscopic Thomas-Fermi like contribution. We illustrate our approach by calculating electronic and optical properties of several different spherical semiconductor nanocrystals differing in size and composition. We compare results obtained with proposed approach and real-space dielectric matrix inversion and study role of different approximation used in calculation. We discuss possibility generalizing our model for a multi-million atom systems like self-assembled quantum dots.

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P-5: Functional Insight into the Role of GW182 Proteins in microRNA-mediated Repression

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Many biomolecular complexes composed of proteins and nucleic acids are systems of nanometer-scale dimensions and regulate gene expression programs in living cells. MicroRNAs (miRNAs) are short ($\sim$ 21 nucleotides) non-coding RNAs that silence gene expression of target mRNAs at the post-transcriptional level. MiRNAs shut down protein synthesis by repressing translation and/or initiating RNA deadenylation and subsequent degradation of target mRNAs. To elicit this repression, miRNAs recruit a ribonuclear protein complex to target mRNAs, referred to as the miRNA induced silencing complex, or miRISC. At its core, the miRISC consists of a miRNA-loaded Argonaute protein, and the Argonaute-interacting GW182 family of proteins. GW182 proteins are essential for miRNA-mediated silencing and help effect both translational repression and deadenylation of target RNAs. We set out to gain mechanistic insight into how GW182 proteins facilitate miRNA-mediated repression. Here we present data generated in a mammalian cell-free extract that recapitulates miRNA repression (Mathonnet et al., 2007; Fabian et al., 2009; Jinek et al., 2010) and has allowed us to biochemically dissect how GW182 facilitate deadenylation of miRNA-targeted mRNAs.

*Supported by International PhD studies in Nano and Bio Science at the Faculty of Physics University of Warsaw and Polish Ministry of Science and Higher Education grant No. N N301 035 936

P-6: Combined Theoretical and Experimental Study of Amikacin and Its Ribosomal Binding Site

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Amikacin is an aminoglycoside antibiotic, commonly used in the treatment of sepsis and various hospital infections. It influences bacterial translation process by binding to the decoding region of the 30S small ribosomal subunit, called the A-site. Understanding the molecular basis of its action is the key to design novel drugs with similarly high activity but less side effects. Herein, we present an insight into the dynamics of amikacin, both free and bound to ribosomal RNA.

The starting model of the drug-RNA complex was taken from the Protein Data Bank (code 2G5Q). We performed 100 ns long molecular dynamics simulations for: 1) the complex, 2) free A-site RNA model and 3) amikacin, all in aqueous solutions and physiological concentrations of NaCl. Trajectories were analyzed to obtain the dynamical properties of theses molecules, and of the hydrogen bond network between amikacin and the A-site model.

We observed significant changes in the root mean square deviations of free amikacin, which suggest that this drug switches between several major conformations. These major conformations were also detected from the clustering analysis. The analysis of amikacin atomic fluctuations showed notable variety in the mobility of its different rings. The orientation of the rings and distances between certain hydrogen atoms corroborated with the NMR data for a similar aminoglycoside, kanamycin [1].

We also compared the flexibility of the free amikacin and in the complex with the A-site RNA. In the simulation of the free RNA, the most flexible residues, according to *E. coli* enumeration [2], were the bases A1492 and A1493. The mobility of these bases was reduced when amikacin was bound, which correlates with high effectiveness of amikacin [3].

We have also determined the thermodynamics and energetics of amikacin binding to the Asite with isothermal titration calorymetry (ITC), obtaining the dissociation constant K_d in the micromolar range, and compared the results with our molecular dynamics data.

Acknowledgements: Calculations were performed at ICM supercomputing facility and supported by the Foundation for Polish Science Team project (TEAM/2009-3/8) co-financed by European Regional Development Fund operated within Innovative Economy Operational Programme.

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P-7: Sensitivity of Go Model to Fine Details of the Native Structure

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Go models are exceedingly popular in computer simulations of protein folding. These models are native-centric, i.e., they are constructed from the protein's native structure. Therefore, it is important to understand up to which extent the fine details of the native structure dictate the folding behavior exhibited by a Go model. We address this challenge by carrying out Discrete Molecular Dynamics simulations of a Go model that explicitly considers a full atomistic representation of the protein. In particular, we compare the folding thermodynamics of the N47G mu tant form of the spc-SH3 folding domain (pdb code 1qkw) with that of a decoy structure that was constructed from the wild-type protein (1shg) by molecular replacement of amino acid Asn at position 47 by Gln followed by energetic relaxation via the AMBER force field.

P-8: Simulating Fluctuations Of Neurotoxic Proteins Using Monte Carlo

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Neurodegenerative diseases are currently incurable. We do not even know the details of the molecular mechanism involved, although it is established that they are caused by the so-called neurotoxic proteins. Their primary cause is thought to involve conformational changes in the monomeric species. One of the proposed models postulates that polypeptide fluctuations would result in an acquisition of β-structure. These fluctuations are assumed to happen most likely on large timescales (seconds), and hence molecular dynamics simulations have not yet been able to follow this process. One alternative strategy to tackle this problem consists in using Monte Carlo simulations. Here, we present a model based on this type of simulations where the formation of hydrogen bonds within the polypeptide are favoured, which increases the likelihood of establishing stable structures. We have applied this approach to three protein structures (a model fold based on the I27 module of human cardiac titin as a control, and two polyglutamine models with 20 and 60 residues). The resulting structures were classified before applying coarse-grained molecular dynamics in order to compute their mechanical stability.

P-9: Protein Structure Modeling Guided by Fragmentary NMR Data and SAXS Scattering Profiles.

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Conventional methods for protein structure determination require collecting huge amounts of high-quality experimental data. In many cases the data on itself cannot discriminate between alternative conformations; a unique structure therefore cannot be determined. Moreover, a number of experimental techniques, such as Nucleic Magnetic Resonance can deliver only fragmentary and/or ambiguous restraints. In many cases the measured observables relate atoms separated by only a few covalent bonds (e.g. Chemical Shift Tensors, J-couplings) and their information content is relatively low. Small Angle Xray Scattering (SAXS) is another example of such a "weak" experiment. Informational entropy analysis suggests that a scattering profile may be used to determine only several independent degrees of freedom. To the contrary of NMR, a scattering curve is a function of all atomic coordinates for a given system and encodes global description of its geometry in a very synthetic way.

In this contribution we utilized both local information obtained from NMR measurements and global description of a macromolecule defined by a SAXS profile with a knowledge-based bimolecular force field to determine tertiary and quaternary structure of model protein systems. Various kinds of local NMR data such as isotropic Chemical Shifts (CS) and their tensors (CST), J-couplings, RDC, backbone NOE, REDOR from NMR in solid phase are parsed with the "experimental" module of BioShell modeling suite[1]. BioShell prepares a scoring method used by Rosetta fragment picking tool[2] to find among known protein structures such short molecular fragments that comply with the data. The fragments are subsequently used in protein modeling simulations with the Rosetta package[3]. Results show that local NMR experimental data bears enough information to uniquely determine structures of a small globular proteins without any guidance of long range restraints such as NOE. SAXS spectra have been introduced into a modeling procedure as a χ^2 scoring term that assesses the fit between the experimental and an actual (theoretical) spectra. It has been successfully applied to guide docking of smaller units into quaternary structure. Combining local NMR restraints with global description obtained from SAXS in a single modeling protocol may lead to a very efficient method for structure determination of large biomolecules and their complexes.

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P-10: A Redox-chain Maquette - a New Protein to Study Electron Transfer

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Electron transfer between different redox cofactors are very common events in nature. The multi-cofactors pathways include for example iron-sulfur clusters and heme molecules, as in fumarate reductase in mitochondrion or cytochrome b6f complex in chloroplasts. Simple inorganic prosthetic groups, iron-sulfur clusters, consist of iron and sulfur atoms, commonly organized in cubane (4Fe4S) or diamond (2Fe2S) form. Within the protein scaffold, the FeS cofactors serve as electron donors or acceptors in redox reactions. Specific holoprotein roles depend on redox potential, modulated by protein microenvironment. Full understanding of these processes is necessary not only for understanding how natural redox proteins function, but also for biotechnological applications.

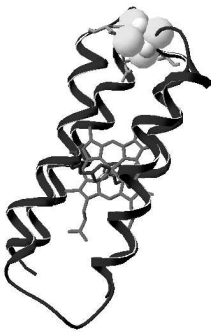

Fig.1. Model of RCM with cofactors. Heme is assembled by histidinyl residues and FeS cluster is coordinated by cysteines.

To study electron transfer in such complexes, we designed and expressed a Redox-chain maquette (RCM), It is a four-helix bundle assembling heme or other porphyrins inside its hydrophobic core, and iron-sulfur clusters (FeS) in the loops (Fig.1). The protein was overexpressed in a bacterial system and reconstituted with cofactors. Based on first characterisation, mutations were proposed, which should improve protein properties. Here, we are comparing the affinity of RCM and its mutant to various porphyrin ligands (heme, Zn-mesoporphyrin), the possibility and efficiency to FeS incorporation, as well as secondary structure and oligomerization properties.

Our results suggest that mutations increased the stability of RCM. Future perspectives will be discussed.

Acknowledgements: Project supported by the European Regional Development Fund under the Operational Programme Innovative Economy NanoFun POIG.02.02.00-00-025/09 and Foundation for Polish Science, Homing Plus Programme.

P-11: The Method for Estimation the Exchange of Amhiphiles Between Lipid Aggregates

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We propose a novel method for determining the thermodynamic parameters for the reactions where the molecules bind to the model biological membranes. It is a combination of two most typical experimental set-ups namely binding and release protocols. Our method is based on the amphiphilic molecules (free fatty acids) exchange between two lipid aggregates. After two lipid aggregate populations are mixed, equilibrium is reached and thermodynamic parameters of the reaction can be estimated.

P-12: From Discrete Multi-exponential Model to Lifetime Distribution Model and Power Law Fluorescence Decay Function

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Experimental and theoretical studies of the fluorescence intensity decays in biomacromolecular systems showed that under constraints of typical experiment fluorescence lifetime distribution is given by gamma function [1], which led to a power-like decay function $I(t) = [(2-q)/\tau_0][1-(1-q)t/\tau_0]^{(1/(1-q))}$. The factor $(2-q)/\tau_0$ results from normalization, and the mean decay time $< t_p >$ is given by $< t_p >= t_0/(3-2q)$. Decays are described by mean value of lifetime distribution (τ_0) and one new parameter of heterogeneity (q) related to the relative variance of fluctuations of $\gamma = 1/\tau$ around the $< \gamma >= 1/\tau_0$ value: $q - 1 =< (\gamma - < \gamma >)^2 > / < \gamma >^2$. Taking into account decay with N decay channels, the total decay rate $\gamma$is expected to be a sum $\sum \gamma_i$ of a number N of partial rates γ_i. In that case, q=2/N+1, i.e. N=2/(q-1). It is worth noticing that the normalization of power-like decay function leads to constraint on the q values (q¡2), which, through N=2/(q-1) implies constraint on N (N¿2). Furthermore, requirement of existence of the mean value of decay time $< t_p >= \tau_0/(3-2q)$ implies that 1¡q¡3/2, hence N¿4. Figure 1 shows the gamma distribution for several values of q and N, including the fact that in the classical limit, when the number of decay channels goes to infinity, i.e. $q \rightarrow 1$, the gamma distribution becomes the Dirac delta function, and decay function converges from power-like form to the single-exponential form.

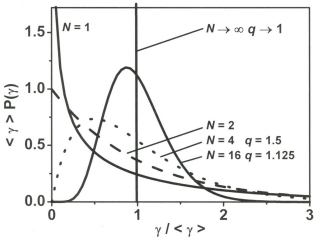

Figure 1:
Gamma distribution function at N=1, 2, 4(q=1.5), 16 (q=1.125), and $N \rightarrow \infty (q \rightarrow 1)$.

When the heterogeneity parameter value increases stepwise from 1 to 3/2, deviation from single-exponential form increases, and it is more pronounced for the tail part of each decay. The power-like function well fits complex (heterogeneous) as well as simple mono-exponential decays, and describes fluorescence decay kinetics by the parameter of heterogeneity objectively reflecting physical heterogeneity of the system, and the mean lifetime value from distribution characterizing the average rate of the excited-state decay. Numerous examples illustrate applications of a new model to rational analysis of complex fluorescence decays of biomacromolecules [1-4], e.g. protein-ligand complexes [2], which led to identification of tautomeric forms selectively bound by the enzyme [2, 3, 5]. The latter is of great importance for the studies of the mechanism of protein (enzyme) action as well as for more rational drug design.

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P-13: Identification of Aggregation-prone Intermediate States in the Folding Pathways of spc-SH3 Amyloidogenic Variants via Discrete Molecular Dynamics Simulations

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The ability to form amyloid is not restricted to a few specific proteins associated with disease. Indeed, there is accumulating evidence that under appropriate physicochemical conditions (e.g, temperature, pH and protein concentration) many different protein sequences can form amyloid. Here we use a continuum, off-lattice model that combines a full atomistic protein representation with the Go interaction potential to investigate in detail the folding pathways of selected variants of the spc-SH3 protein domain. The wildtype and the N47G mutant do not form amyloid, but the N47A and the D48G(2Y) variants are both amyloidogenic, with the triple mutant being clearly more prone to aggregate. Our model predicts the formation of an aggregation-prone intermediate species in which more than half of the major hydrophobic core residues are highly solvent exposed. In agreement with the experimental results for the amyloidogenic propensities of the spc-SH3 variants we found that the intermediate is not present in the folding pathway of the WT protein, is only transiently populated by the N47G mutant, and is low populated in the case of the N47A and D48G(2Y) mutants. However, it is clearly more ubiquitous in the folding pathway of more aggressive D48G(2Y) mutant. Interestingly, the triple mutant significantly populates an additional intermediate state that is less native-like and even more aggregation prone than the common intermediate species. These results suggest that the formation of aggregation prone conformations is an intrinsic property of the native topology that can be modulated by specific amino acid interactions, whose strength depends in turn on specific environmental conditions.

P-14: eIF4E Family Members from *Arabidopsis thaliana*: Binding Affinities for Cap Analogs

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Eukaryotic initiation factor (eIF) 4E plays an important role during initiation of protein biosynthesis. It is able to specifically recognize the 7-methylG(5')ppp(5')N cap structure which is present at the 5'-end of majority of eukaryotic mRNAs. The binding of eIF4E to mRNA cap commences the recruitment of other translation factors, including ribosomal subunits. eIF4E genes are highly evolutionally conserved but surprisingly many eukaryotes express multiple eIF4E family members. It is believed that one of these eIF4E isoforms is involved in translation initiation, while the other are supposed to have different functions, for example translational repression of specific mRNAs.

Plants differ substantially from animals because they utilize two eIF4E isoforms, named eIF4E and eIF(iso)4E, as canonical translation initiation factors. The reason why plants need two cap-binding translation initiation factors remains elusive. Apart from eIF4E and eIF(iso)4E genes, plants conserve gene for another protein from eIF4E family, termed nCBP. The function of nCBP is still unknown.

In our studies we investigate cap-binding properties of eIF4E family members from *Arabidopsis thaliana* so as to get insights into their potential roles. We use the method of fluorescence titration to determine binding affinities of these proteins for a set of synthetic cap analogs. We found that eIF4E binds cap stronger than eIF(iso)4E, while binding affinities of nCBP and eIF(iso)4E are comparable. In *A. thaliana* there are two another genes encoding eIF4E isoforms. These proteins, termed eIF4E-2 and eIF4E-3, are very highly homologous to eIF4E and in our ongoing studies we are examining their binding specificities to cap analogs.

P-15: Structural and Functional Features of *Xenopus* eIF4E1b Protein

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The biological role of the fundamental eukaryotic initiation factor 4E, eIF4E1a, is linked to the presence on this factor two binding sites for the mRNA cap and for several proteins like eIF4G, 4E-BP or 4E-T. Among many different isoform eIF4E1a present in vertebrates, eIF4E1b homologue characterize 71% of identity to eIF4E1a at the level of primary structure with all residues required to bind the cap and protein partners conserved. However, m^7GTP Sepharose assay and quantitative fluorescence measurements show weaker affinity of *Xenopus* eIF4E1b to the cap analogues. In contrast to eIF4E1a, eIF4E1b does not interact with eIF4G and therefore cannot carry out the same initiation function in translation. For the ovary specific eIF4E1b protein, which was identified as a component of the CPEB complex along with the eIF4Ebinding protein 4E-T (eIF4E-Transporter), the Xp54 RNA helicase and other RNA-binding proteins, is proposed mediating function in silencing of translation during *Xenopus* oogenesis. Interestingly, eIF4E1b can bind 4E-T in a manner independent of the consensus eIF4E1a-binding site, YSKEELL. With reference to this knowledge the cap binding and 4E-T specificity to Xenopus eIF4E1b are under investigation. Structural features of Xenopus eIF4E1b, as a conclusion from sequence analysis, model structure analysis and fluorescent assays, which influence on its functional aspects will be presented.

P-16: A Coarse-grained Model of Nucleic Acids Based on Interaction between Base Multipoles

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We propose a new model of nucleic acids, which is based on the UNRES model of polypeptide chains developed in our laboratory. A nucleotide residue is represented by a sugar+nucleic acid base (SB; each of which is located at the center of the respective base) and a phosphate (P) group. The effective base-base interaction energy is derived as a potential of mean force expressed as a function of the distance-base centers and the orientation of the C5'...SB vectors. The potentials of mean force (PMFs) of base-base interactions were calculated by direct Boltzmann summation from the AMBER energy surfaces of base pairs, the summation being over the angles of rotation of the bases about their long axes. Analytical expressions for the electrostatic part of the PMF were obtained by cluster-cumulant expansion of the free energy of interactions of base dipoles and quadrupoles; these expressions correctly reproduce the dependence of base pairing and stacking on orientation. The other base-base interactions were modeled by Gay-Berne anisotropic potentials. Multibody terms were also derived based on the cumulant expansion. The analytical expressions for base-base interactions were fitted to the PMF surfaces calculated from AMBER energy surfaces. The P...P interactions were modeled by a Coulombic and a Lennard-Jones term, while the P...SB interactions were modeled by the Gay-Berne potential. These potentials and the local (virtual-bond-stretching, virtual-bond-angle bending, virtual-bond-dihedral-angle, and nucleic-base rotamer) were tentatively determined as statistical potentials from a database of nucleic-acid structures. The model was implemented in our coarse-grained dynamics UNRES package. Results of ab initio folding simulations of small DNA and RNA molecules will be presented.

Supported by grants from the Polish Ministry of Science and Higher Education (N N204 049035) and the National Institutes of Health (GM-14312).

P-17: Structural and Electronic Properties of Functionalized Carbon Nanotubes and Graphene Layers

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We present results of extensive theoretical studies of functionalized carbon nanotubes (CNTs) and graphene layers (GLs). Our studies are based on *ab initio* calculations in the framework of the density functional theory (DFT). We have performed calculations for various metallic and semiconductor single- and multi-wall CNTs and epitaxial graphene layers, functionalized with simple organic molecules such as OH, COOH, NHn, CHn and metals, such as Al, Fe, Ni, Cu, Zn, and Pd. We determine the stability of the functionalized systems and resulting changes in the electronic structure. These studies provide valuable quantitative predictions that are of importance for design of novel composite materials and functional devices such as electric sensors of chemical and biological substances.

The functionalization of CNTs and GLs is an important problem of nanotechnology, which has recently attracted a lot of research activities, mostly because of the huge potential of applications for new materials and functional devices. The functionalization is a prerequisite for effective dispersion and binding of the systems in polymer or epoxy matrices. It is also necessary to facilitate attachment of biological substances to channels made out of CNTs and/or GLs in electrical devices. The functionalization changes also the electronic structure of the systems studied, causing, for example, opening of the electronic band gap. This in turn opens new perspectives for design of field effect transistors (FETs) based on single and bilayer graphene.

We discuss the dependence of the cohesive properties of functionalized carbon systems on the density of the adsorbed molecules. We calculate binding energies, heat of formation, resulting deformations of CNTs and GLs, and changes in the electronic structure induced by functionalization. We determine the critical density of molecules and metallic atoms that could be adsorbed on the surface of CNTs and GLs. All these factors influence the electrical and mechanical properties of the functionalized systems and are important for reliable description of the sensor electrical characteristics and also for modeling composite materials. Our studies shed light on physical mechanisms governing the binding of the adsorbed molecules and allow for comparison of the role played by two hosts.

Generally, the stability of the functionalized CNTs is weakly dependent on the diameter of the CNTs and its metallic or semiconducting character, whereas the stability strongly decreases with the density of the adsorbed groups. In particular, we find that the NH_n and CH_n groups with n larger than two do not bind to the CNTs. It turns out that practically only CH_2 groups make reasonably strong bonds to the CNTs (CH_3 groups bind, but extremely weakly with bond length of 1.56 Å). In the case of CH_4 groups, we observe their dissociation into CH_2 and H_2 dimer placed in the surrounding of CNT; CH_2 binds to the CNTs, whereas H_2 remains unbound. The functionalization of CNTs with NHn causes practically no deformation of the CNTs, whereas the CHn groups attached to the CNTs cause reconstruction of the CNTs.

We predict that the functionalization of even a single GL by OH, NH, NH_2, and COOH opens its energy gap by 0.11, 0.12, 0.25, and 0.24 eV, respectively. We observe that the band gap increases with the concentration of the attached molecules.

The very interesting and intriguing physical picture emerges from studies of CNTs and GLs covered with metallic atoms, particularly in the context of spintronic applications.

P-18: The Impact of PAMAM Dendrimers on Catalase Activity and Structure

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Dendrimers are a relatively novel highly branched, globular molecules which possess many interesting and unique properties. They consist of a central core and several layers of branched monomers what results in a large number of reactive end groups on the surface [1]. A high concentration of end group on the surface and empty internal cavities make them good candidates as capture and delivery systems for small molecules e.g. drugs. Hydrophobic drugs can be complexed within hydrophobic dendrimer interior to make them water-soluble or drugs can be covalently coupled onto the surface of the dendrimer. To use dendrimers injected into blood and tissues for drug delivery and gene therapy, knowledge of their potential interactions with biological components at the cellular and molecular levels is required. It was found that the dendrimers can change the conformation, intramolecular dynamics and binding properties of proteins and change enzyme activities [2].

The aim of this study was to examine the effect of PAMAM dendrimers generation 3 and 3.5 on catalase activity and structure. The catalase activity with/without dendrimers were measured in human erythrocytes and in pure catalase. The obtained results showed changes both in the pure catalase activity and in erythrocytes catalase after incubation with dendrimers. For both dendrimers a slight increase in enzymatic activity of catalase was observed.

Circular dichroism was used to determine the secondary structure of catalase alone and in the presence of PAMAM dendrimers. The CD spectrum of non-incubated catalase was typical for α-structure. Incubation of this catalase with dendrimers resulted in shape changes to the CD spectrum, indicating changes in the secondary structure of the protein. It was found that used dendrimers reduce the amount of α-helix.

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P-19: Amino Acids at ZnO-water Interfaces in Molecular Dynamics Simulations

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We determine the effective potentials for interactions of amino acids with the (0001)-O, $(000\bar{1})$-Zn, $(10\bar{1}0)$ and $(11\bar{2}0)$ surfaces of ZnO by using molecular dynamics simulations with and without water. The proposed scheme is one in which the amino acid is embedded in a chain of glycines at a prescribed distance from the surface. The surface induced layered structure of water introduces ondulations in some effective potentials. The binding energies are found to exhibit large specificity.

P-20: Searching for DcpS Enzyme Inhibitors Among Cap Analogs Modified In The Phosphate Chain

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Cap (m^7GpppN) is a structure which naturally occurs in cells, at the 5' mRNA end. It protects mRNA from enzymatic degradation and has an efficient impact on processes connected with protein expression, such as initiation of translation. It performs also a stimulating role in mRNA splicing. Caps remaining after 3'-5' mRNA decay are degraded by DcpS (Decapping Scaveger) enzyme. DcpS tears bonds between caps $\beta - \gamma$ phosphates. As a modulator of methylated nucleotides levels, function of the DcpS enzyme is not limited only to the mRNA decay, but it also impacts mRNA transcription and translation courses. The permanent interplay between DcpS and the cap binding proteins, such as CBP20 and eIF4E, points necessity of finding DcpS inhibitors, in order to alter either DcpS or the cap binding proteins activities. For example, there is an idea to exploit eIF4E-cap binding to prevent production of overexpressed proteins in cancer cells using competitive modified cap analogs to inhibit eIF4E factor. The therapeutic effect of the reduction of the eIF4E active pool may be obtained on condition that at the same time we inhibit DcpS enzyme, in order to assure the cap analog stability.

Our hypothesis was that DcpS inhibition will be dependent on the insertion of a chemical group between $\beta - \gamma$ phosphates of a cap structure. We used HPLC to identify products of enzymatic reaction in order to check resistance of modified caps on degradation by *C. elegans* DcpS enzyme. To calculate association constants (K_{AS}) we used fluorescent titration. It showed us a force of interaction between modified cap and DcpS, which should be stronger than for a natural cap.

The analysis confirmed our suppositions that m^7GpNHppG and m^7GpCH_2ppG cap analogs are resistant to enzymatic hydrolysis. Neverthelater, their low K_{AS} values indicate that they cannot be considered as efficient *C. elegans* DcpS inhibitors. By contrast, cap analogs with substitution between $\alpha - \beta$ phosphates (for example: m^7GppNHpG or m^7GppCH_2pG) were degraded by the enzyme. We checked not only bridging modifications of the triphosphate bridge, but also some groups substituted instead of an oxygen atom, for instance BH_3. We found that m^7Gp_{BH3}ppG D1 cap analog is not surrendered of hydrolysis and has the highest K_AS value (40.04 ± 1.92) among all examined cap analogs. Furthermore, its D2 counterpart which differs only in the BH_3 orientation, is efficiently hydrolysed by DcpS enzyme, which points stereoselectivity of the enzymes active site.

P-21: Cold Denaturation of Lennard-Jones Helices with Applications to α-Helical Polypeptides

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We investigate biological helical molecules modelled as continuous helical lines with Lennard-Jones-like self-interactions. It is shown that such helices exhibit a low-temperature, non-entropic, first order phase transition between two states: with high and low curvature. This is consistent with the cold denaturation phenomenon often observed for proteins with α-helical backbone [1]. Estimating the Lennard-Jones parameters for the peptide bond, we found that the first order phase transition takes place at about 280 K. This is in remarkable agreement with what is seen for proteins where only a fraction of all proteins cold-denature at temperatures higher than the freezing temperature of the aqueous media. The phase diagram of the idealized Lennard-Jones helix is constructed, which shows a critical point at pitch angle 33.1° and temperature 160 K. The curvature of the helix is shown to change abruptly with the radius, i.e. it can be considered as the order parameter of the phase transition. Since no entropic factor is involved, the cold denaturation of α-helices can be explained from an energetic consideration, based on the interplay between attractive and repulsive forces.

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P-22: Mutations in the Bacterial Ribosomal Protein S12: Structural Implications for Decoding and Aminoglycoside Binding Studied by Molecular Dynamics

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The aminoacyl-tRNA binding site (A-site), a part of the 30S ribosomal subunit, modulates translational fidelity, with two flexible adenines 1492 and 1493. Their dynamics is crucial for correct codon-anticodon recognition and is perturbed by binding of aminoglycosides, which underlies the mechanism of antibacterial action of these antibiotics. Interestingly, the adenines are located at the base of helix 44 (h44), near an interface between h44 and ribosomal protein 'S12'. Experimental studies of S12 suggest that it plays an important structural role in the decoding centre. To explore the dynamical properties of the decoding site, and specifically the role of S12, we performed molecular dynamics (MD) simulations of an A-site fragment from the Escherichia coli ribosome. Guided by experimental studies, we simulated the system with native and mutated (K42A, K43Q, K43E, R53A) S12 variants in the presence and absence of paromomycin (PAR).

We found that K43 and R53 relax to conformations that differ from the crystallographic starting structures. The primary amine of K43 forms salt bridges with the phosphate oxygens of A1492, thereby coupling the motion of these two moieties. Interestingly, the presence of the antibiotic in the A-site modulates the predominant interactions between K43 and A1492, in terms of strength and type of interatomic contacts. We also observed that in all the mutated systems without PAR, apart from K42A, the adenines 1492 and 1493 were less often stacked than in the wild type variant. On the other hand, the K42A mutation made the base pair of U1406 and U1495 (in the PAR binding site) less stable. Furthermore, we noticed that in the wild type, the motions of S12 and the A-site rRNA are anticorrelated, due to a conformational change of h44. However, when S12 mutations are introduced, the structural change does not occur and the correlations are weaker.

Our simulations indicate that S12 mutations can modulate the dynamics of structural elements in the decoding site, and suggest the atomic basis for this coupling. Our determination of a dynamical coupling between (i) the S12 amino acids, (ii) paromomycin and (iii) the A-site rRNA may illuminate the function of the S12 protein as a translational fidelity-controlling signal transducer. Longer term, such knowledge could facilitate the design of more potent antibiotics, acting at both structural and dynamical levels.

bf Acknowledgements: The authors acknowledge support from ICM University of Warsaw (BST and G31-4), Polish Ministry of Science and Higher Education (N N301 245236), and the Foundation for Polish Science (Focus program and Team project TEAM/2009-3/8 co-financed by European Regional Development Fund operated within Innovative Economy Operational Programme). MD simulations were performed using the resources of University of Warsaw (Biomolecular Machines Laboratory and grant no G31-4, ICM) and University of Virginia.

P-23: Photocatalytic and Phototoxic Properties of TiO_2based Nanofilaments: ESR and AFM Assays

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Nanosized-TiO_2 (nano-TiO_2) has been widely used in photocatalysis, coating, plastics, pharmacy, food and cosmetics [1]. Nanosized TiO_2 (nano-TiO_2) is one of the most widely used nanoscale materials to date [2]. In spite of massive use, the biological activity and toxicity of nano-TiO_2 remains the subject of intense debate. There is uncertainty in understanding of the relationship between physico-chemical parameters of nano-TiO_2 and its toxicity when brought into contact with living cells [3]. Cells contain a variety of biomolecules that are potential targets for oxidation by various types of ROS. This study provides a multidisciplinary experimental insight into to the toxicity and phototoxicity of the custom-made TiO_2 nanowires (TiO_2-NWs). The human melanoma cell line was treated with low concentrations of TiO_2-NWs ($\sim 2.5\mu g/mL$). High-resolution surface topography and cell elasticity measurements using atomic force microscopy (AFM) revealed toxic effects both in cells incubated with TiO_2-NWs in the dark and exposed to the photo-oxidative stress under UVA radiation [Fig.1]. In contrast to ROS generation efficacy in the absence of cells in vitro, no direct correlation was found between the physical parameters of nano-TiO_2 and cell toxicity.

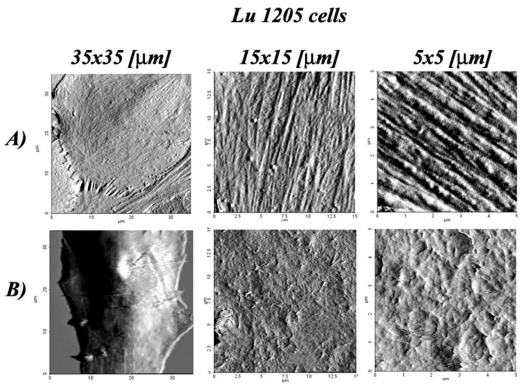

Figure 1: The AFM images of the metastatic melanoma 1205Lu cells. (A) Non-treated cells (control); (B) Cells after 0.5 h incubation with TiO_2 nanowires, which was then followed by 230 sec exposure to UVA light (1 mW/ cm^2)

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P-24: Cytotoxic Activity of the Al_2O_3-Ag Nanoparticles on Mammalian Cells

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Nanotechnologyone of the fastest growing areas of advanced technologyhas become a source of hope for many branches of modern industry, as well as for medicine and pharmacy. It is believed that in the near future, properly designed nanoparticles will be used to impregnate clothing and medical instruments, to combat viruses and bacteria, in new drug delivery systems or cancer therapy, as well as for cell labelling and in biosensors [1, 2].

The Ag nanoparticles have the ability to kill a great variety of gram-positive and gram-negative bacteria strains and fungi strains. It is very important, that bacteria cannot create immunity against them as they can with many antibiotics [3]. As has been discovered, they have also anti-inflammatory properties and the ability to destroy viruses [4]. There is a lot of possibilities in application of Ag nanoparticles: as an additive to wound-dressing materials [5], protective coatings on medical implants [6], filtration membranes and anti-fouling coatings [7] However, it is important to evaluate the toxicity of these nanoparticles in order to realize their widespread applications in biomaterials.

The aim of this study was to investigate the cytotoxic effect of the Al_2O_3-Ag (2,84% w/w) nanoparticles on the viability of selected mammalian cells in vitro. The Al_2O_3-Ag nanopowders were produced by the thermal decomposition-reduction method and were characterised using SEM and BET analyses. Cell viability were measured by MTT assay and cofirmed by EZ4U assay. Al_2O_3-Ag nanoparticles showed no significant cytotoxicity on three selected cell lines: L929, BJ, HeLa, after 24 hours of exposure to nanoparticles in the tested range of concentrations. The cells remained more than 75% viability, measured by MTT assay and more than 102% viability, obtained by EZ4U assay, relative to control at the concentration as high as 200 μg/ml.

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P-25: Computational Studies of Aminoglycoside Binding Sites in Ribosomal RNA and Bacterial Enzymes

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Aminoglycosides are antibiotics used in hospitals worldwide to treat serious bacterial infections. They target bacterial protein synthesis by binding to ribosomal RNA (rRNA) and disabling proper discrimination of cognate and non-cognate tRNAs. Although these drugs are very potent, they need to be taken in large doses, which results from their relatively low specificity and is one of the causes of their toxicity. Moreover, bacteria become resistant to aminoglycosides — either by mutating their primary binding site on the rRNA (called the A-site) or by producing specific enzymes that modify aminoglycosides. Therefore, there is a need to improve aminoglycosides, which requires detailed knowledge of their recognition and bacterial resistance mechanisms.

We study the two mentioned resistance methods employed by bacteria against aminoglycosides. We performed multiple MD simulations of the model A-site RNA fragment introducing mutations that were experimentally shown to increase resistance against paromomycin, an aminoglycoside representative. We found that the specific substitutions affect the shape and dynamics of the binding cleft as well as significantly alter its electrostatic properties. We showed that the physicochemical reasons for bacterial resistance could be different for each mutation[1,2].

There are numerous aminoglycoside modifying enzymes (AME), which vary in sequence and structure. The aminoglycosides are, however, a highly homogenous group. Therefore, we ask what makes these different enzymes bind a group of very similar drugs? To investigate this we performed MD simulations of the representative structures of AME. We reproduced the hydrogen bond network between the enzymes and the antibiotic. We also found differences in global movements of various enzymes as well as in the conformations adopted by the drug. The energetical analysis pointed to the residues that contributed the most while forming the complex with an antibiotic and helped explain why aminoglycosides are such indiscriminate binders.

Acknowledgements. This work was supported by University of Warsaw, Polish Ministry of Science and Higher Education, Fogarty International Center, and Foundation for Polish Science (Focus program and Team project co-financed by European Regional Development Fund operated within Innovative Economy Operational Programme); co-financed by technical assistance funds of the EEA Financial Mechanism and the Norwegian Financial Mechanism within the framework of the Scholarship and Training Fund. Work at UCSD is supported in part by National Science Foundation (NSF), National Institutes of Health, Howard Hughes Medical Institute, Center for Theoretical Biological Physics, National Biomedical Computation Resource, and the NSF Supercomputer Centers.

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P-26: Coarse-grained Treatment for cis-trans Isomerization of Peptide Groups

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Many bioactive peptides and proteins contain cis peptide groups, which are crucial for maintaining their structure. The cis-trans isomerization of peptide groups also often is a rate-limiting step of protein folding [1]. Therefore in this work, we extended the coarse-grained UNRES force field [2] developed in our laboratory to treat peptide-group cis-trans isomerization.

The potentials of mean force (PMFs) of the peptide groups as functions of the $C_i^\alpha \ldots C_{i+1}^\alpha$ distance (d) were calculated. N-methylacetamide and N-pyrolidylacetamide were used as models of regular and proline peptide-group type, respectively. Energy was evaluated on grid of improper angle H-N-C-C(O) (α)and virtual $C^\alpha \ldots C^\alpha$ bond length (d) by using MP2/6-31G** ab initio method. The energy was minimized with respect to all other degrees of freedom. Statistical sum were calculated by Boltzmann summation.

Furthermore the $C_{i-1}^\alpha \ldots C_i^\alpha \ldots C_{i+1}^\alpha$ virtual-bond-valence bending (γ) and $C_{i-1}^\alpha \ldots C_i^\alpha \ldots C_{i+1}^\alpha \ldots C_{i+2}^\alpha$ virtual-bond-dihedral-angle (θ) PMFs involving cis and trans peptide groups were calculated from energy surfaces of terminally-blocked proline, glycine and alanine (where alanine modeled all residue types except proline and glycine). The energy surfaces were calculated by using ab initio molecular quantum mechanics in the MP2/6-31G** scheme. The energy was calculated on the grid of the angles of rotation of the peptide groups about the $C^\alpha \ldots C^\alpha$ axes [$\lambda^{(1)}$)and $\lambda^{(2)}$] and the energy was minimized with respect to all other degrees of freedom. The PMF's were determined by using the factor-decomposition procedure described in our earlier work [2-4]. The potentials were implemented in the UNRES force field and the results of test simulations of peptides and proteins where cis-trans isomerization occurs are reported.

Supported by grants from the Polish Ministry of Science and Higher Education (N N204 049035) and National Institutes of Health (GM-14312).

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P-27: Sol-gel Synthesis of ZnO and ZnO/MgO Core/Shell Nanocrystals for Biological Application

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Neurodegenerative diseases are a group of congenital or acquired diseases of the nervous system, where the primary pathological phenomenon is the loss of nerve cells. The process leading to the symptoms of neurodegenerative disease starts much earlier and runs for a long time (often for years) without any symptoms. The first symptoms appear when a significant number of neurons are damaged. To find an effective therapy, ways for diagnosis of early stage disease are required.

Our aim was to synthesize nanoparticle probes with potential diagnostic capability. We have elaborated three strategies for designing biosensors. The first is based on the Fluorescent Resonance Energy Transfer (FRET) between nanoparticle and a dye on its surface, the second one on opto-magnetic properties, and the third one on the FRET between nanoparticle and a protein to which it is attached.

The nanoparticles have a number of advantages (small size, lack of photobleaching) but they have a fundamental disadvantage, a relatively low sensitivity to the external environment. The biomolecule attached to the surface of such nanoparticles will be responsible for specificity.

We synthesized the ZnO/MgO core/shell nanoparticles of about 6 nm. In addition to the characterization of the synthesized nanoparticles, we also passivated them with various types of organic layers. In the first phase, we covered the ZnO/MgO nanoparticles by carboxymethyl-beta-cyclodextrin (CMCD). The interior of cyclodextrin is hydrophobic so that it can form inclusion complexes with hydrophobic molecules. The energy transfer between ZnO/MgO coated beta-cyclodextrin nanoparticles and organic dye Nile Red built into the holes of cyclodextrin was examined. The effect of temperature on the FRET system was observed, what can be of importance for the disease diagnostics.

Acknowledgements: The research was partially supported by the European Union within European Regional Development Fund, through grant Innovative Economy (POIG.01.01.02-00-008/08) and was partially supported by the Ministry of Science and Higher Education (Poland) through Grant No. N N518 424036.

P-28: Linking Gold Nanoparticles Using Molecular Interconnects - a Theoretical Study

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Recent improvements in the synthesis of citrate-protected gold nanoparticles (Au NP) mean that it is now relatively easy and inexpensive to produce highly monodisperse NPs with well defined diameter and shape. Surface plasmon resonance allows for detection of Au NPs, provided the NP-NP distance is short [1], and it has also been shown that Au NPs can be detected on microelectrodes, by affinity electrophoresis. There is thus much current interest in using Au NPs in the development of ultra- sensitive point of care medical devices. For example, Au NPs can be used to assess the risk of a cardiac arrest by monitoring C-reactive protein levels in blood plasma. The method hinges however on self-assembly of Au NPs into larger, detectable clusters. This can be done by introducing molecules with two "anchor" groups [2] that can covalently link NPs; the number of linkers per NP determines the size and shape of the clusters formed, and depends on many factors, including the citrate versus linker binding energies, the NP size (surface curvature) and NP chemical composition (for example, the presence of surface defect and/or adatom sites). So while individual NPs can be produced with exquisite control over size and shape, the complex aggregation processes makes it difficult to achieve a similar level of control over the multi-NP clusters. Better understanding of the assembly process may provide for rational design of new NP-linker combinations and so here we present a theoretical description of linker mediated self assembly of Au NPs. Using mesoscale simulations with a coarse- grain model for the Au NPs and linker molecules, we investigate the conditions under which large clusters can grow and construct a phase diagram that identifies favourable growth conditions in terms of floating and bound linker concentrations. The findings can be considered as generic, as we expect other NP-linker systems to behave in a qualitatively similar way. We extract some general rules for NP linking that may aid the production of size- and shape-specific NP clusters for technology applications. Our model is used primarily for systems with the rheno-organic linkers. It is then applied to systems in which the gold NPs are covered by the C-reactive protein (CRP) antibodies and the linkers are the CRP molecules.

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Acknowledgements. This work has been supported by the EC FUNMOL project under FP7-NMP-2007-SMALL-1, and by the European Union within European Regional Development Fund, through grant Innovative Economy (POIG.01.01.02-00-008/08).

P-29: Mechanostability of Proteins with Cystine Slipknots and Multidomain Proteins

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Single molecule techniques have provided novel means of determining the mechanostability of biomolecules. The experimental studies have been accomplished just for a small set of proteins, and a general guidance is needed for selection of new experimental targets. We have performed a theoretical survey of 17143 proteins that are stretched by the termini. The assessment makes use of a simplified structure-based model. The results of the survey are available at www.ifpan.edu.pl/BSDB.

Among them is the identification of a group of exceptionally strong proteins. These proteins contain cystine knots and form a cystine slipknot conformation on stretching. Their mechanostability is predicted to exceed 1000 pN. All-atom simulations of this group of proteins confirm the existence of the cystine slipknot force clamp.

The survey was further extended to include multidomain proteins. We have discovered new, robust resistance mechanisms involving inter-domain contacts. A survey identified also an exceptionally strong mechanostability of aggregation-prone 3D domain swapped cystatin C. We predict that this system may lead to a characteristic force of about 800 pN which would be the largest shear-based mechanostability found.

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Acknowledgements. This work has been supported by the EC FUNMOL project under FP7-NMP-2007-SMALL-1, and by the European Union within European Regional Development Fund, through grant Innovative Economy (POIG.01.01.02-00-008/08).

P-30: Denaturation of Proteins Near Polar Surfaces

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All-atom molecular dynamics symulations for two small proteins indicate existance two types of proteins structures in a mica vicinity. One type corresponds with unfolding and the other with a deformation. The two behaviors are characterized by distinct properties of the radius of gyration and a novel distortion parameter that distinguishes between elongated, globular and planar shapes. They also differ in the nature of their single site diffusion and two-site distance fluctuations.

P-31: An Ensemble View of GPCR Structure: CCR5 Case

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G protein-coupled receptors (GPCRs) are intrinsic membrane proteins with seven transmembrane helices. Due to their mediation of numerous critical physiological functions, GPCR are involved in all major disease areas, including cardiovascular, metabolic, neurodegenerative, psychiatric, cancer, and infectious diseases. However, progress in developing new drugs with reduced toxicity and side effects has been hampered by the small number of 3-dimensional structures for human GPCRs.

Despite the compact structure of the core, stablized by a large number of interhelical hydrogen bonds and electrostatic interactions, GPCRs undergo large conformational changes during their activation. The comparison of crystal structure of bovine rhodopsin (inactive) and opsin (active) shows that helices 3 and 6 have their hydrogen bond networks rearranged, while helix 5 becomes elongated and changes its position together with helix 6 to make the structure of the protein more open. There is also evidence that agonist binding may stabilize the inactive state of GPCRs [1]. This experimental evidence show that despite their high intrinsic rigidity GPCR may be subject to conformational changes upon ligand binding/release and activation.

One of the most commonly used methods in investigation of GPCR-ligand binding are binding assays performed for a series of point mutants of the studied protein. It is, however, important to notice that even single point mutation may have a large effect on the structure of the protein even before the binding experiments take place. Recently it has been shown, however, that single-point mutation can completely switch a protein conformation, without unfolding/destabilization of the native system in the switching process [2]. While there is no direct experimental evidence that such large changes of conformations imposed by single-point mutations may take place in GPCRs, other data concerning conformational changes dictated by ligand binding and activation as well as stabilization of GPCRs by various mutations make it a plausible assumption.

In this work we studied the structure of CCR5 protein, a member of chemokine receptors family and an important co-receptor for macrophage-tropic virus, including HIV, to enter host cells. We decided to perform a combined experimental - computational study to investigate the binding site and binding mode of Maraviroc, and PF-232798, two CCR5 inhibitors with similar chemical structure. We also investigated the binding site and binding modes of two other CCR5 antagonists coming from different chemical scaffolds, Aplaviroc and TAK-779. To fully understand this problem we have decided to use our GenSeMBLE protocols, that allows us to go beyond the simple homology modelling and provides an ensemble of low-energy structures of transmembrane proteins. We postulate that CCR5 may adopt different conformations of helices for the wild type and for systems bound to different ligands. We also postulate that CCR5 may adopt different conformations upon single-point mutations.

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P-32: Replica Exchange Molecular Dynamics of the β-hairpin Using Structures from the Coarse-Grained Monte Carlo Dynamics

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Replica Exchange Molecular Dynamics (REMD) of peptides is a well known method of sampling many conformations. There is an attempt to improve it by utilising the output structures from the Coarse-Grained Monte Carlo Dynamics as the input for the all-atom REMD. This approach gives very effective sampling of the conformations and can be helpful for elucidating in the new mechanisms of β-hairpin folding.

Simulations are conducted using implicit and explicit solvent. Thermodynamics of these systems is compared focusing on the analysis of the number of native contacts during simulations. The energy landscape of the simulation and other diagrams are produced by the Histogram Analysis Method.[1] Conformations are also sampled using Principal Component Analysis, RMSD Cα and other properties.

Representative intermediates of 2GB1 β-hairpin folding process are analysed and process of formation of secondary structures is described in detail.

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P-33: Chiral Discrimination of Surfaces Formed via 'Click Chemistry' by Chemical Force Spectroscopy

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Chirality, familiar to all chemists, is usually applied to molecules or assemblies of molecules and plays an important role in both animate and inanimate systems. The ability to control the chemical and structural properties of surfaces is very important for advancements in fields like selective catalysis, chemical sensing and electronics.

Self assembled monolayers provide a way to produce surfaces with desired properties not only by their own tail groups but also by their ability for further reaction. SAMs equipped with special tail groups can be used for introducing different functionality onto surfaces[1]. This opens a way for production of surfaces of desired chirality.

In this study we investigated chiral discrimination of surfaces formed *via* Cu(I) catalysed 1,3-dipolar cycloaddition[2], commonly known as 'click chemistry', by chemical force spectroscopy (CFC)[3].

Purpose-designed SAMs were used to chirally feature AFM tip and gold surface and the adhesion force between them was measured during an approach- retract cycle. Specially designed aminoacids and dipeptides were used for tip and surface functionalization in order to investigate specifically the influence of hydrogen bonding on tip-surface chiral recognition. Also the influence of the presence of additional chiral centre was subjected to analysis.

The studies showed that adhesion forces between tip and probed surfaces possessing one chiral centre repeat the trend presented previously for mandelic acid[4] and Pirkle[5] resin. However, surfaces equipped with two neighbouring chiral centres are adopting a conformation in which the inner chiral centre is hidden. For all studied surfaces the trend of adhesion forces repeats the same pattern and it is thought to be influenced by the inner hydrogen bonding in the studied molecules.

This work was supported by CHEXTAN- Marie Curie Research Training Network.

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P-34: The Test of Site-directed Spin Labeling (SDSL) Technique in the Investigation of EGFP Folding and Unfolding

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Recent developments in expression of recombinant proteins in different systems urge for better understanding of protein folding and unfolding processes. Therefore, besides the fundamental research, the study of protein folding and unfolding has become a central issue in the development of protein engineering, biotechnology, pharmacy, and medicine.

Enhanced green fluorescent protein (EGFP) belongs to the green fluorescent protein (GFP) family. Due to the improved fluorescent properties in comparison to the wild GFP, EGFP is commonly used as a fluorescent intracellular marker in bio-imaging in vitro and in vivo. EGFP has a complex beta-sheet topology and consists of 238 amino acids (including one tryptophan and two cysteines). The specific chromophore is formed upon cyclization of residues Ser65-Tyr66-Gly67 and occurs after protein folding. In EGFP, the fluorescence enhancement is related to the additional mutation at position 64 (change of phenylalanine to lysine). Both the chromophore and the tryptophan residue have fluorescent properties, and can be used as naturally-occurring reporters of the protein conformation changes during folding or unfolding.

Site-directed spin labeling (SDSL) in combination with electron spin resonance (ESR) has become a powerful tool for determining secondary structure and dynamics of both soluble and membrane proteins. SDSL derives information on the local environment within the protein structure via ESR spectroscopy of a site-specifically attached stable nitroxide radical (spin label).

In this preliminary study, we tested the usefulness of the combination of SDSL and ESR to monitor the chemically-induced unfolding process of EGFP. There are two cysteines in EGFP, Cys 48 and Cys70, which do not form a disulfide bond. These both cysteine residues, located in the regions distant from the central part of beta-barrel containing chromophore, can bind the spin label.

The protein was labeled with three spin labels, MTSSL ([(1-Oxyl-2,2,5,5-tetramethyl pyrroline-3-methyl) methanethiosulfonate]) MSL (4-Maleimido-TEMPO) and 5-MSL (3-Maleimido-PROXYL), as markers of EGFP unfolding.

The results were compared with unfolding monitored by tryptophan and chromophore fluorerscence. We are presented our results together of conclusion according to usefulness of spin-probe in the monitoring of GFP unfolding.

P-35: Peptide Nucleic Acid – A-site RNA Interactions

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Peptide nucleic acids (PNA) are synthetic molecules mimicking natural nucleic acids. PNA backbone is composed of N-(2-aminoethyl)-glycine units, which lack the negative phosphate groups. PNA oligomers are unstructured but hybridize easily with different nucleic acids. PNA-DNA or PNA-RNA complexes are stronger than the natural DNA-DNA or RNA-RNA duplexes. Due to the modified backbone PNAs are also resistant against cellular enzymes that cleave nucleic acids or peptides.

Therefore, the usage of PNA shows promise in gene and antisense therapy [1]. In antisense applications, PNAs are designed to hybridize in a sequence-specific manner with various kinds of RNA what results in blocking RNA processing, its transport into cytoplasm or translation. Here, we investigate the interactions of PNA with ribosomal RNA sequences with the long-term aim of inhibiting bacterial translation. We investigate the interactions of PNA with various RNA motifs. To check the binding properties we apply various spectroscopic techniques (including absorbance, fluorescence and circular dichroism) and isothermal titration calorimetry (ITC).

Our target is the ribosomal A-site RNA that is responsible for accommodating the cognate tRNA during protein synthesis. We have designed bis-PNA that targets the A-site RNA fragment with high affinity. The binding constant Ka at pH 5.5 was equal to $2 \cdot 10^6 \pm 5 \cdot 10^5 \mathrm{M}^{-1}$. This confirms that bis-PNA can easily invade into purine-rich region of the double stranded RNA.

We have also determined the thermodynamics of binding of a single stranded 10-mer PNA to the RNA-RNA duplex of a sequence similar as in the A-site RNA. The ssPNA was found to invade into dsRNA oligomer and create a stable PNA-RNA:RNA complex (where ":"represents the Hoogsteen and "-" Watson-Crick base pairing type). The ssPNA displaced one of the RNA strands creating the RNA-PNA duplex, the second RNA strand bound to the RNA via Hoogsteen pairing. The presence of the stable PNA-RNA:RNA triplex was confirmed by measuring the melting temperature of the triplex which was about 20°C higher than of the respective PNA-RNA (51°C) duplex and approximately 33°C higher than that of RNA-RNA. Circular dichroism spectra and the measurements of the hyperchromic effect (absorbance at 295nm) provided further evidence for a new product.

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P-36: Conformational Selection and Induced Fit Mechanism Underlie Specificity in Noncovalent Interactions with Ubiquitin

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Noncovalent binding interactions between proteins are the central physicochemical phenomenon underlying biological signaling and functional control on the molecular level. Here, we perform an extensive structural analysis of a large set of bound and unbound ubiquitin conformers and study the level of residual induced fit after conformational selection in the binding process [1]. We show that the region surrounding the binding site in ubiquitin undergoes conformational changes that are significantly more pronounced compared with the whole molecule on average. We demonstrate that these induced-fit structural adjustments are comparable in magnitude to conformational selection. Our final model of ubiquitin binding blends conformational selection with the subsequent induced fit and provides a quantitative measure of their respective contributions.

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P-37: Diffusion and Dimerization of Single-Pass Receptors Studied by Fluorescence Correlation Spectroscopy

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The mobility of membrane receptors is a crucial determinant of their interaction capabilities and function. To address the mobility and oligomerization properties of single-span receptors, we applied different implementations of fluorescence correlation spectroscopy (FCS) in live cells and giant plasma unilamellar vesicles (GPMVs). GPMVs are cell-derived membrane systems with almost complete protein and lipid repertoire, yet lacking the cytoskeleton and cellular organelles. We studied the diffusion of a truncated and eGFP tagged interleukin-4 (IL-4) receptor alpha chain (IL-4Ra-eGFP) and characterized transmembrane helix-driven dimerization of several receptor pairs in the cytokine class I family. By means of a novel experimental approach based on scanning and dual-color FCS we could confirm that erythropoietin (Epo) receptor functions as a pre-dimer, in contrast to homo- and hetero combinations of IL-4 and IL-13 receptor chains which are present in the membrane as monomers. As a proof of principle, by swapping the transmembrane domains, the interaction potential of Epo receptor was partially transferred to IL-4 receptor and *vice versa*.

How the plasma membrane lateral heterogeneity affects the mobility of lipids and proteins is a subject of current debate. To address this issue we carried out the diffusion measurements in cells and GPMVs for a protein (IL-4Ra-eGFP) and lipid dye analogues (DiO, DiD). Surprisingly, in GPMVs the diffusion coefficients were $\sim 2\mu m^2 s^{-1}$ for both IL-4 receptor and lipid dye analogues. In live cells, however, the diffusion coefficients were reduced to a different extent: by a factor of ~ 1.5 for the lipid dye and 5-7 for the protein. Such a difference shows that the presence of cytoskeleton and the cellular structures beneath the plasma membrane affects more the diffusion of the receptor spanning both leaflets of the membrane as compared to a smaller lipid dye. Our results are the first steps to characterize the diffusion and dimerizations properties of the IL-4Ra as an example of a single-span protein functioning in its natural membrane environment.

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Notes

Notes

Notes

Conference schedule

Sunday, Sep 4		Monday, Sep 5		Tuesday, Sep 6		Wednesday, Sep 7			
		9:00	B. Garcia-Moreno	9:00	J. Sułkowska	9:00	J. Onuchic		
		9:50	M. Jaskólski	9:40	E. Paci	9:45	C. M. Johnson		
		10:25	*Coffee break*	10:20	P. Cossio	10:25	J. M. Valpuesta		
		10:50	A. A. Louis	10:55	*Coffee break*	11:00	*Coffee break*		
		11:30	M. Carrión-Vázquez	11:15	C. Palivan	11:30	T. Weikl		
		12:10	M. Dadlez	11:55	M. Nowotny	12:10	M. Langner		
		12:45	*Lunch*	12:30	*Lunch*	12:50	P. De los Rios		
15:00	*Registration*	15:00	D. Noy	13:30	*Excursion*	13:25	*Lunch*		
		15:40	W. I. Gruszecki	17:15	*Coffee break*	15:00	D. Fotiadis		
		16:15	*Coffee break*	17:30	A. Dziembowski	15:40	R. Valiokas		
		16:45	D. Reich	18:10	R. Adamiak	16:20	P. Szymczak		
18:00	*Coffee break*	17:25	H. Janovjak	18:50	R. Stolarski	16:55	*Coffee break*		
19:30	D. B. Searls	18:05	S. Jackson	19:30	*Dinner*	17:30	D. Wolicka	17:30	U. Hansmann
20:30	A. Niedźwiecka	18:40	*Dinner*	20:15	Poster Session II	18:10	J. Wesoly	18:00	A. Kolinski
20:30	*Dinner*	20:00	Poster Session I		P21 - P37	18:50	M. Olejniczak	18:30	K. Gottschalk
	at bonfire		P1 - P20					19:00	P. Barker
						19:45	*Conference banquet*		