Biomolecules and Nanostructures 4
15–19 May 2013, Pultusk, Poland

Organized by
the Institute of Physics, Polish Academy of Sciences (PAS) in Warsaw, Poland
and the Pro Physica Foundation

The Conference is organized under the auspices of the National Multidisciplinary Laboratory of Functional Nanomaterials - NanoFun, financed by the Innovative Economy Operational Programme, Priority Axis 2: R&D Infrastructure, Action 2.2: Support of Formation of Common Research Infrastructure of Scientific Units. The Workshop is also a conference of the Division of Physics in Life Science of the European Physical Society.

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EDITORS:
Marek Cieplak, Anna Niedźwiecka

ABSTRACTS

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PREFACE

The 2013 conference on *Biomolecules and Nanostructures* in Pułtusk, Poland, continues the tradition of three previous meetings under related names (such as "Structure and Function of Biomolecules" or "Biomolecules and Nanostructures") that were held in the village of Bedlewo near Poznan, Poland, in 2004, 2006 and 2011.

In the spirit of adventure, we have now changed venue to the castle in Pułtusk. However, the goals remain similar. The first is to provide an active forum for cross-disciplinary discussions among specialists coming from different fields of biomolecular science and bio-related aspects of nanotechnology. The second is to share the current research findings in these very active and rapidly evolving branches of research. The third is to present both experimental and theoretical approaches used in the bio- and nano-studies. The fourth is to generate an opportunity for developing scientific and personal contacts in a non-distracting, non-urban setting.

The current edition of *Biomolecules and Nanostructures* encompases a broad range of the covered topics on the crossroads of molecular biology, physics and chemistry, including supramolecular assemblies, single-molecule manipulation, protein folding and aggregation, interactions between nucleic acids and proteins, dynamics of biomembranes, nanomachines, hybrid structures, biosensors and other phenomena at bio-nano interfaces.

The *Biomolecules and Nanostructures* conference is managed by the Division of Physics in Life Sciences of the European Physical Society and is organized under the auspices of the National Multidisciplinary Laboratory of Functional Nanomaterials - NanoFun Project, financed by the Innovative Economy Operational Programme, Priority Axis 2: R&D Infrastructure, Action 2.2: Support of Formation of Common Research Infrastructure of Scientific Units.

Previous editions of this conference have gained a good feedback from participants. Now, we bring together about 140 persons from 22 countries, from 4 continents, and 35% of the participants are women. We hope to provide an informal, creative atmosphere and highly interactive nature of this meeting, contributing to integration of the scientific community.

International Advisory Board

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Paweł Pomorski, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

General Information

Lectures
Speakers are asked to provide their presentations to Dr. Michał Wojciechowski in the Conference Room "Manеż" (Riding School) before each session. It is advisable to check the compatibility of the presentation with the projection equipment ahead of time, for instance the day before.

Posters
Presenting authors are requested to place their posters in the Conference Room "Manеż" (Riding School) on Wednesday, May 15th, immediately upon arrival and dismount the posters not earlier than the Saturday evening, May 18th, so that the posters could be viewed during the whole time of the conference.

WiFi
Network access will be available in the conference venue. Information regarding the passwords in different parts of the Castle will be provided by the hotel reception desk. WiFi will be switched-off in the Conference Room "Manеż" during lecture sessions and swiched-on during coffee breaks and poster sessions.

Workshop Office
Registration desk opening hours

May 15th, 2013 (Wednesday)	15:00-22:00
May 16th, 2013 (Thursday)	8:00-20:00
May 17th, 2013 (Friday)	8:00-20:00
May 18th, 2013 (Saturday)	8:00-20:00
May 19th, 2013 (Sunday)	8:00-11:00

Useful Phone Numbers

Hotel reception desk	+48 23 692 90 00
Conference registration desk	+48 532 751 048 or +48 532 751 049

Emergency Calls

From mobile phone	112 for all emergencies
From stationary and mobile phone:	
Ambulance	999
Police	997
Fire department	998

Phone Calls from Poland

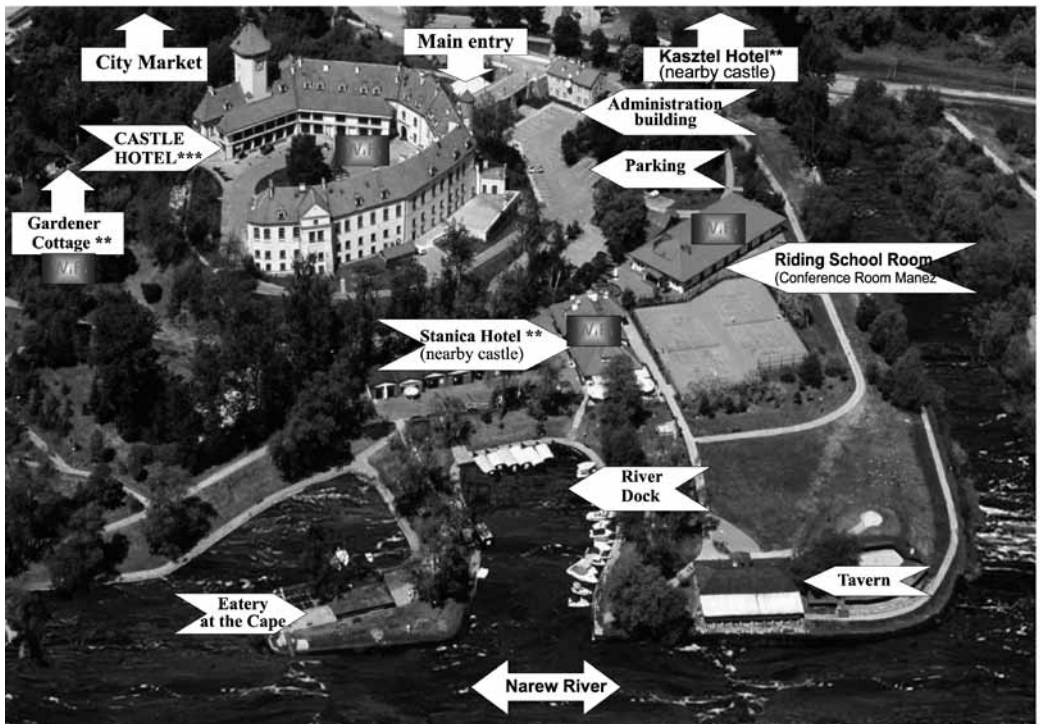
From a stationary phone:	00 Your Country Code The Number Abroad
From a mobile phone:	+ Your Country Code The Number Abroad

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 the Castle in Pułtusk

Pułtusk is one of the oldest Polish towns, located about 60 km north from Warsaw. The conference takes place in a 1319 castle, surrounded by a park and forests and the wild Narew river with several bird sanctuaries. Untill the end of the XVIII century, the castle used to be a residence of the Mazovian bishops.
The castle was a witness to many historic events, such as the wars with the Teutonic Order, the Swedish Deluge, the partitions of Poland and the two World Wars. Napoleon stayed at the castle in 1806 and 1812 to monitor war efforts. The castle was destroyed several times, e.g. in the 17th century when the Swedish invasion (known in Poland as the Deluge) took place. However, it kept being rebuilt. Nowadays, the castle combines the climates of different eras: from the Middle Ages to the Seventies of the twentieth century.
By the government decision of 1974, the castle was given to the Association of Polish Community (Wspolnota Polska) to function as The House of the Polish Diaspora (Dom Polonii). The castle was rebuilt in the renaissance style and its gates were opened in 1989.

TOP VIEW CASTLE



PROGRAMME

Wednesday, 15 May 2013

15:00 - 22:00 REGISTRATION
18:00 - 19:30 WELCOME RECEPTION - GARDEN PARTY

OPENING LECTURE — chairperson: Marek Cieplak

19:30 - 20:15 Jane Clarke, University of Cambridge, Department of Chemistry, UK:
Lessons learned from studying the folding of families of proteins

20:15 - 20:25 Anna Niedzwiecka, Institute of Physics PAS; University of Warsaw, Poland:
National Multidisciplinary Laboratory of Functional Nanomaterials POIG 2.2 - NanoFun

20:30 - 22:00 DINNER AT BONFIRE

Thursday, 16 May 2013

SESSION I: FOLDING — chairperson: Marek Cieplak

9:00 - 9:30 Jasna Brujic, New York University, USA:
Statistical physics plays an important role in single molecule force spectroscopy

9:30 - 10:00 Thomas Kiefhaber, Technical Univeristy Munich, Germany:
The unlocked state: a clue to understand how proteins fold and unfold

10:00 - 10:30 Andrzej Kolinski, University of Warsaw, Poland:
CABS - coarse grained modeling of protein structure assembly, dynamics and interactions

10:30 - 11:00 COFFEE BREAK

SESSION I: FOLDING — chairperson: Jasna Brujic

11:00 - 11:30 George Stan, University of Cincinnati, USA:
Computer simulations of protein unfoldimg and translocation by AAA+ chaperones

11:30 - 12:00 Joan-Emma Shea, University of California Santa Barbara, USA:
Effect of surfaces in modulating protein folding

12:00 - 12:30 Patricia Faisca, University of Lisboa, Portugal:
Intermediate states for protein folding and aggregation

12:30 - 12:45 Roberto Covino, University of Trento, INFN, Italy
Protein folding pathways with realistic atomistic force fields

12:45 - 13:00 Annett Bachmann, Technical Univeristy Munich, Germany
The Mechanism of a Coupled Protein Folding and Binding Reaction: Folding Before Binding or Binding Before Folding?

13:00 - 14:30 LUNCH

SESSION II: COMPLEXES AND RECOGNITION chairperson: Thomas Kiefhaber

14:30 - 15:00 Rebecca C. Wade, Heidelberg Institute for Theoretical Studies; Heidelberg University, Germany:
Protein dynamics and molecular recognition: Insights from simulations

15:00 - 15:30 Joanna Trylska, CeNT, University of Warsaw, Poland:
Ribosomal RNA as a target for sequence-specific inhibition

15:30 - 16:00 Adam Liwo, University of Gdansk, Poland:
Mean field dipole-dipole interactions as essential factors in the formation of biomolecular architecture

16:00 - 16:30 COFFEE BREAK

16:30 - 17:00 Peter Hinterdorfer, Johannes Kepler University Linz, Austria:
Molecular recognition force microscopy/spectroscopy

17:00 - 17:30 Ruxandra Dima, University of Cincinnati, USA:
Multiscale simulations of the mechanics of filamentous proteins

17:30 - 18:00 Pawel Pomorski, Nencki Institute of Experimental Biology PAS, Warsaw, Poland:
Chemistry and mechanics of the calcium signal in the motile cell, the glioma C6 case

18:00 - 18:15 Michal Toborek, University of Miami School of Medicine, USA:
Autophagy is involved in nanoalumina-induced cerebrovascular toxicity

18:15 - 18:30 Juergen Schluetter, LOT-Quantum Design, Germany:
Studying of protein aggregation onto surfaces using QCM-D

18:30 - 19:45 DINNER

20:00 - 22:00 POSTER SESSION I *(Posters A0 vertical)*

Friday, 17 May 2013

SESSION III: MOLECULAR COMPLEXES IN GENE EXPRESSION chaiperson: Anna Niedzwiecka

9:00 - 9:30 Marc Fabian, McGill University, Montreal, Canada:
Structural basis for the recruitment of the CCR4-NOT deadenylase complex by Tristetraprolin

9:30 - 10:00 Marek Tchorzewski, Maria Curie-Sklodowska University, Lublin, Poland:
The ribosome action as transitions between different functional/structural states

10:00 - 10:30 Piotr Zielenkiewicz, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland:
Time-resolved, genome-wide model of translation

10:30 - 11:00 COFFEE BREAK

SESSION IV: BIO-NANO — chairperson: Giovanni Dietler

11:00 - 11:30 Andrzej Sienkiewicz, EPFL, Lausanne, Switzerland:
Multifunctional magnetic-photoluminescent-photocatalytic nanostructures for biomedical applications

11:30 - 12:00 Damien Thompson, Tyndall Institute, Cork, Ireland:
Nanoscale computer-aided design of self-assembling materials for electronics and health applications

12:00 - 12:30 Piotr Garstecki, Institute of Physical Chemistry PAS, Warsaw, Poland:
Automated droplet microfluidics for studies of emergence of drug resistance in bacteria

12:30 - 13:00 Robert Leheny, Johns Hopkins Univeristy, Baltimore, USA:
Interfacial Microrheology of Protein Layers During Formation at Fluid Interfaces

13:00 - 16:30 LUNCH & FREE TIME

16:30 - 17:00 COFFEE BREAK

SESSION IV: BIO-NANO — chairperson: Joan Emma Shea

17:00 - 17:30 Robert Holyst, Institute of Physical Chemistry PAS, Warsaw, Poland:
Biologistics: mobility of ligands, proteins and plasmids in cytoplasm of the eukaryotic and prokaryotic cells

17:30 - 18:00 Jaroslaw Stolarski, Institute of Paleobiology PAS, Warsaw, Poland:
How meaningful is prefix "bio" for minerals formed by organisms: the nanoscale perspective

18:00 - 18:30 Boguslaw Baginski, University of Warsaw, Poland:
Zircon - small but great

18:30 - 19:45 DINNER

20:00 - 22:00 POSTER SESSION II (Posters *A0* vertical)

Saturday, 18 May 2013

SESSION V: PROTEIN STRUCTURE — chairperson: Mariusz Jaskólski

9:00 - 9:30 Catherine Royer, Centre de Biochemie Structurale, Montpelier, France:
Pressure Effects on Protein Folding: Why and for What?

9:30 - 10:00 Wladek Minor, University of Virginia, Charlottesville, USA:
Experiment and modeling: competitive or complementary approaches to structural biology?

10:00 - 10:30 Andrzej Kloczkowski, Nationwide Children's Hospital, Columbus, OH, USA:
New methods to improve protein structure prediction and refinement

10:30 - 10:45 Agnieszka J. Pietrzyk, Institute of Bioorganic Chemistry, PAS, Poznan, Poland:
Structural studies on 30-kDa lipoproteins from mulberry silkworm

10:45 - 11:15 COFFEE BREAK

SESSION VI: VIRUSES — chairperson: Catherine Royer

11:15 - 11:45 Gijs Wuite, Vrije Universiteit, Amsterdam, Holland:
The nanomechanics of viruses

11:45 - 12:15 Neil Ferguson, University College Dublin, Ireland:
Molecular mimicry in hepatitis B virus: How a viral intrinsically disordered protein helps hijacks the host membrane-trafficking machinery

12:15 - 12:45 Wouter Roos, Vrije Universiteit, Amsterdam, Holland:
Dual structural role of the viral RNA: capsid stabilization vs. genome uncoating

12:45 - 13: 15 Cristian Micheletti, SISSA, Trieste, Italy:
DNA knotting inside viral capsids: a computational approach

13:15 - 15:15 LUNCH

SESSION VII: DNA — chairperson: Cristian Micheletti

15:15 - 15:45 Giovanni Dietler, EPFL, Lausanne, Switzerland:
Knots: from the sailing boat down to the cell's nucleus illustrated by means of simple experiments

15:45 - 16:15 Andrew Travers, LMB, Cambridge, UK:
Chromosomes as topological machines

16:15 - 16:45 Joerg Rottler, University of British Columbia, Vancouver, Canada:
Predictions of a systematically coarse grained model for DNA

16:45 - 17:15 COFFEE BREAK

SESSION VIII: FINALE — chairperson: Chris Johnson

17:15 - 17:45 Amos Maritan, University of Padua, Italy:
Emergence of criticality in living systems

17:45 - 18:15 Annalisa Pastore, National Institute for Medical Research, MRC, London, UK:
Kaleidoscopic protein self-assembly: The AXH domain of ataxin-1 undergoes a complex multiple equilibrium of species in solution

18:15 - 18:45 Mariusz Jaskolski, Institute of Bioorganic Chemistry, PAS;
Adam Mickiewicz University, Poznan, Poland:
Modulated macromolecular crystal structure with 28 protein molecules in the asymmetric unit

CLOSING LECTURE

18:45 - 19:30 Gerhard Hummer, NIH Bethesda, USA:
Order and disorder in biomolecular assemblies

20:00 CONFERENCE BANQUET

Sunday, 19 May 2013

7:30 - 10:00	BREAKFAST
8:30	DEPARTURE I
10:00 or 11:00	DEPARTURE II

OVERWIEV OF THE ABSTRACTS

Lectures (IL - Invited Lectures, ST - Short Talk)

Poster Contributions

Abstracts of Lectures

IL - Invited Lectures

ST - Short Talk

IL-1: Lessons learned from studying the folding of families of proteins

Jane Clarke

University of Cambridge, Chemistry Department, UK

Inspired by SCOP we have been studying the folding of families of proteins, making comparisons both within and between families. We have now studied the folding of about 40 different domains and about 1000 variants of these proteins. What lessons have we learned?
"The family is one of nature's masterpieces." ~George Santayana, The Life of Reason

IL-2: Statistical physics plays an important role in single molecule force spectroscopy

Jasna Brujic

New York University, Center for Soft Matter Research, Imaging and Biophysics Lab, New York, USA

Protein unfolding and refolding trajectories under a constant stretching force are manifestations of the underlying molecular processes in the end-to-end length. In the case of ubiquitin, I27 and NuG2 protein, the distribution of unfolding times at a given force is best fit with a stretched exponential function, where the stretching exponent depends on the protein. This complexity may be a result of slow processes in the hidden degrees of freedom that affect the dynamics. Indeed, we present a toy model that explains our observations in terms of anomalous diffusion. On the other hand, the statistics of the non-equilibrium folding trajectories from a highly extended state to the folded length is well captured by simple diffusion along the free energy of the end-to-end length. Nevertheless, the estimated diffusion coefficient of ~100 nm^2 s^{-1} is significantly slower than expected from viscous effects alone, possibly because of the internal degrees of freedom of the protein. The free energy profiles give validity to a physical model in which the multiple protein domains collapse all at once and the role of the force is approximately captured by the Bell model.

IL-3: The unlocked state: a clue to understand how proteins fold and unfold

Thomas Kiefhaber

Technische Universität München, Chemistry Department, Germany

We found a reversible structural unlocking reaction in the native state of villin headpiece subdomain (HP35) by triplet-triplet-energy transfer measurements [1]. Both the locked state (80%) and the unlocked native state (20%) are populated to detectable amounts in equilibrium. Both states have native secondary structure and topology and have a solvent shielded core. The locked state is restricted to low amplitude conformational dynamics, whereas the unlocked state shows increased conformational flexibility. From the unlocked state partial unfolding of individual helices can occur (Fig. 1). Unlocking is associated with an unfavorable enthalpy change (ΔH^0=35±4 kJ/mol) which is nearly compensated for in free energy by a favorable entropy change (ΔS^0=112±20 $J \cdot mol^{-1} \cdot K^{-1}$). The unlocking reaction has a time constant of 1 ms at 5°C and is enthalpy-limited with an activation energy of 32±1 kJ/mol and a large Arrhenius pre-exponential factor of A=7.5 x 10^{11} s^{-1}. On a much slower time scale global unfolding occurs from the unlocked state. These results suggest that native protein structures are locked into conformations with low amplitude motions. Large scale motions and global unfolding require an initial structural unlocking step, which may play an important role in protein function.

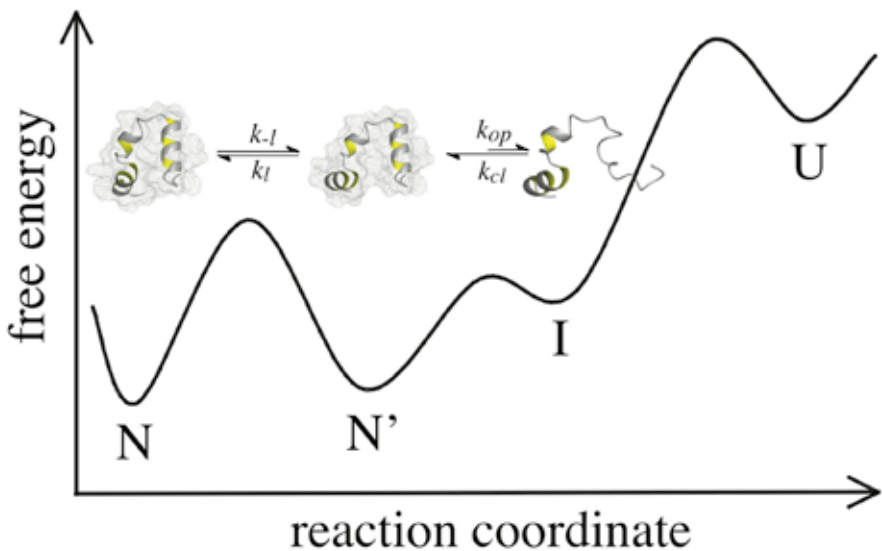


Figure 1: Schematic free energy diagram for folding of HP35.

References:
[1] Reiner A, Henklein P, Kiefhaber T (2010) *Proc Natl Acad Sci USA* **107**: 4955-4960.

IL-4: CABS — coarse grained modeling of protein structure assembly, dynamics and interactions

Andrzej Koliński

Laboratory of Theory of Biopolymers, Department of Chemistry, University of Warsaw, Warsaw, Poland

It is widely recognized that atomistic Molecular Dynamics (MD), a classical simulation method, captures the essential physics of protein dynamics. That idea is supported by a theoretical study showing that various MD force-fields provide a consensus picture of protein fluctuations in aqueous solution. However, atomistic MD cannot be applied to most biologically relevant processes due to its limitation to relatively short time scales. Much longer time scales can be accessed by properly designed coarse-grained models. We demonstrate [1] that the aforementioned consensus view of protein dynamics from short (nanosecond) time scale MD simulations is fairly consistent with the dynamics of the coarse-grained protein model — the CABS model. The CABS model employs stochastic dynamics (a Monte Carlo method) and a knowledge-based force-field, which is not biased toward the native structure of a simulated protein. Since CABS-based dynamics allows for the simulation of entire folding (or multiple folding events) in a single run, integration of the CABS approach with all-atom MD promises a convenient (and computationally feasible) means for the long-time multiscale molecular modeling of protein systems with atomistic resolution. Combination of coarse grained simulations with MD allows also for modeling of entire protein folding processes [2].

References:

[1] Jamroz M, Orozco M, Kolinski A, Kmiecik S (2013) A Consistent View of Protein Fluctuations from All-atom Molecular Dynamics and Coarse-Grained Dynamics with Knowledge-based Force-field. *J Chem Theory Comput* **9: 119-125**.

[2] Kmiecik S, Gront D, Kouza M, Kolinski A (2012) From Coarse-Grained to Atomic-Level Characterization of Protein Dynamics: Transition State for the Folding of B Domain of Protein A. *J Phys Chem B* **116**: 7026-7032.

[3] Kmiecik S, Kolinski A (2011) Simulation of chaperonin effect on protein folding: a shift from nucleation-condensation to framework mechanism. *J Am Chem Soc* **133**: 10283-10289.

IL-5: Computer simulations of protein unfolding and translocation by AAA+ chaperones

George Stan

University of Cincinnati, Department of Chemistry, United States of America

Ring-shaped AAA+ ATPases perform protein quality control by unfolding and translocating substrate proteins (SPs) through narrow central channels. During ATP-driven cycles, mechanical forces that mediate SP remodeling are imparted by flexible loops protruding into the channel. We use coarse-grained and implicit solvent models to study the protein remodeling actions of two hexameric AAA+ nanomachines, the single-ring ClpY and the double-ring p97. We find that conserved central channel loops of ClpY initiate SP unravelling near the tagged C-terminus and mediate SP translocation [1]. Timescales associated with these mechanisms are dependent on the protein fold. Translocation of the SP through the narrow central pore of ClpY involves sharp stepped transitions corresponding to passage of secondary structure elements. An ordered sequential allosteric mechanism of ClpY is found to be more effective than random or concerted allostery. To elucidate the unfolding and translocation of peptides with different secondary structures (random coil, helical, or beta-hairpin) by the ClpY ATPase, we perform molecular dynamics simulations using an implicit solvent model. We find that translocation of the unstructured peptide proceeds on a fast timescale, while unfolding of peptides with helical and hairpin structure imposes a rate-determining step.

Reference:
[1] Kravats A, Jayasinghe M, Stan G (2011) *Proc Natl Acad Sci USA* **108:** 2234-2239.

IL-6: Effect of surfaces in modulating protein folding

Emanuel Peter[1], Bongkeun Kim[1], **Joan-Emma Shea**[1,2]

[1]UC Santa Barbara, Department of Chemistry and Biochemistry, USA;
[2]UC Santa Barbara, Department of Physics, USA

Proteins fold in a dense cellular environment that can significantly affect their folding mechanisms relative to bulk folding. Surfaces such as membranes, or those presented by chaperones, are ubiquitous in the cell. In this talk, I will present molecular dynamics simulations of the folding of the Trp-Zip2 peptide in the presence of biologically-inspired surfaces.

IL-7: Intermediate states for protein folding and aggregation

Patrícia F. N. Faísca[1], Sílvia G. Estácio[1], H. Krobath[1], Diogo V. Vicosa[2], Miguel Machuqueiro[2], E. I. Shakhnovich[3]

[1]Centre for Condensed Matter Physics, Department of Physics, University of Lisbon, Portugal; [2]Center of Chemistry and Biochemistry, Department of Chemistry and Biochemistry, University of Lisbon, Portugal; [3]Department of Chemistry and Chemical Biology, Harvard University, USA

The identification of intermediate states for folding and aggregation is an important challenge not only from a fundamental standpoint but also for the design of novel therapeutic strategies targeted at the so-called conformational disorders. A well-known example is dialysis related amyloidosis (DRA), affecting individuals with kidney impairment undergoing dialysis. In DRA, protein beta-2-microglubulin (β2m) aggregates into amyloid fibrils eventually leading to erosion and destruction of oeteoarticular tissues.

In this talk I will present and discuss two recent computational studies in which we identified intermediate states for folding and aggregation in the Spc-SH3 folding domain and in β2m. In particular, I will show how structure-based Molecular Dynamics (MD) folding simulations combined with MD simulations at constant pH and 'ensemble' docking simulations can be successfully used to explore the conformational space of these proteins leading to the identification of intermediate states that dimerize in a pH-dependent manner. I will show that the mechanistically structurally-resolved picture of intermolecular association we obtain provides a rationalization for experimental results and leads to testable experimental predictions.

ST-8: Protein Folding Pathways with Realistic Atomistic Force Fields

Roberto Covino[1,2], Silvio a Beccara[3], Tatjana Skrbic[4], Cristian Micheletti[5], Pietro Faccioli[1,2]

[1]University of Trento, Department of Physics, Italy; [2]INFN, Gruppo Collegato di Trento, Italy; [3]Bruno Kessler Foundation, LISC, Italy; [4]Bruno Kessler Foundation, ECT*, Italy; [5]SISSA, Statistical and Biological Physics Sector, Italy

Although protein folding has been studied for decades, many open issues still exist. In particular, it is still under debate whether proteins fold through few well-defined pathways or through a large multitude of independent ways. Answering these questions is made difficult by the fact that standard molecular dynamics (MD) simulations are very computationally expensive and often impracticable. The Dominant Reaction Pathway (DRP) is an approach that permits to efficiently study the thermally activated conformational dynamics of bio-molecules in atomistic detail [1]. In particular, it can be used to characterize and portray the folding pathways of a protein once the unfolded and folded configurations are given. We first apply the DRP to a realistic protein studying the folding pathways of the Fip35 WW Domain, a 35 amino-acids long protein [2]. Performing all atom simulations, we can show that this small protein folds following only two pathways, defined by the order of formation of secondary structures. Notably, our results are compatible with ultra long MD simulations and consistent with the analysis of the experimental available data on the folding kinetics of the same system. Exploiting the efficiency of the DRP formalism, computing a folding trajectory of this protein only requires about one hour on 48 CPU's.We apply then our simulation scheme to a much more challenging task: performing an all-atom folding simulation of a 82 amino-acids long protein displaying a topological knot in its native conformation [3]. We can portray the folding mechanism and identify the essential key contacts leading to the proper formation of this knot. Interestingly, we show that non native contacts, i.e. transient contacts formed during the folding of the protein but absent in its native state, can sensibly enhance the probability of correctly forming the knot.

References:
[1] Mazzola G *et al*. (2011) *J Chem Phys* **134**: 164109.
[2] a Beccara S *et al*. (2012) *PNAS* **109**: 2330-2335.
[3] a Beccara S *et al*. (2013) *PLoS Comput Biol* accepted for pubblication.

ST-9: The Mechanism of a Coupled Protein Folding and Binding Reaction: Folding Before Binding or Binding Before Folding?

Annett Bachmann, Thomas Kiefhaber

TU München, Department of Biophysical Chemistry, Germany

The 20 amino-acid S-peptide interacts with the folded S-protein to form ribonuclease S. Upon association the S-peptide adopts α-helical structure and a tightly packed interface between S-protein and S-peptide is formed. We mapped the structural properties of the transition state for this coupled folding and binding reaction by φ-value analysis using backbone-thioxylation and side-chain variations in the S-peptide. Thioxylation of individual amide bonds of the S-peptide, which generally destabilizes helical structures [1], destabilizes the S-protein/S-peptide complex but does not affect the association kinetics. In contrast, association becomes faster when the hydrophobicity at positions 8/9 or 12/13 of the S-peptide is increased [2]. Inverting the net charge of the S-peptide also leads to faster association [3]. In the electrostatically-driven variants increased hydrophobicity does not lead to a further acceleration of association. These results show that helical structure in the S-peptide is not important for association and recognition between S-peptide and S-protein. Recognition rather occurs by hydrophobic contacts between defined positions on the unfolded S-peptide and the folded S-protein. The mechanism of recognition can be switched from purely hydrophobicity-driven to purely electrostatically-driven.

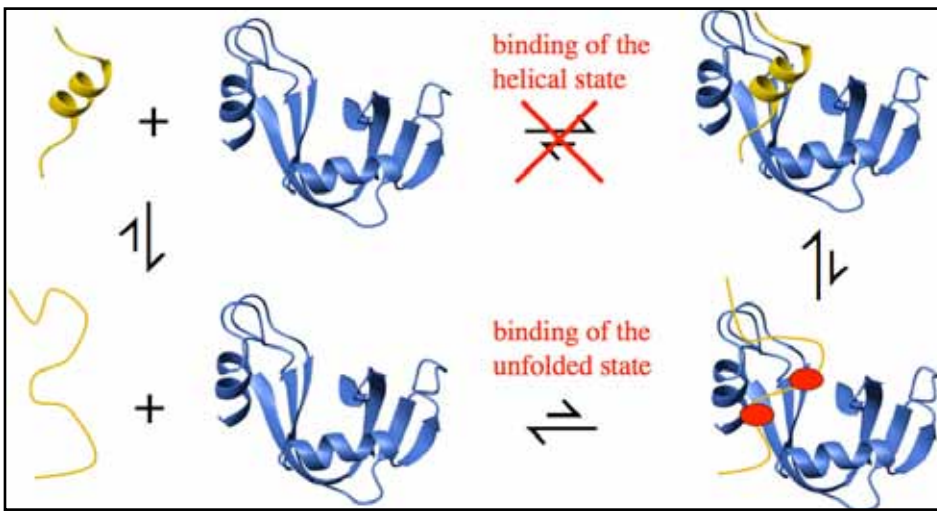


Figure 1: Possible mechanisms for the coupled folding and binding reaction of the S-peptide upon interaction with the S-protein. Our experiments reveal a folding-after-binding mechanism.

References:
[1] Reiner *et al*. (2008) *J Am Chem Soc* **130**: 8079-8084.
[2] Bachmann *et al*. (2011) *Proc Natl Acad Sci USA* **108**: 3952-3957.
[3] Goldberg, Baldwin (1999) *Proc Natl Acad Sci USA* **96**: 2019-2024.

IL-10: Protein Dynamics and Molecular Recognition: Insights from Simulations

Rebecca C. Wade

Molecular and Cellular Modeling Group, Heidelberg Institute for Theoretical Studies (HITS), Schloss-Wolfsbrunnenweg 35, 69118 Heidelberg, Germany, and Center for Molecular Biology (ZMBH), Heidelberg University, Germany

The dynamic character of proteins is a crucial determinant of their binding properties and, hence, their function. In a biological organism, proteins are not only constantly undergoing conformational changes but they are also diffusing in a crowded environment. I will describe some of our recent developments of molecular dynamics and Brownian dynamics simulation methods to study protein binding properties. I will then discuss selected applications such as the simulation of enzyme tunnel dynamics to investigate substrate specificity profiles and allosteric regulation [1-3], the simulation of protein-surface interactions [4, 5], the modeling of large macromolecular complexes [6], and simulations of crowded environments with many diffusing proteins [7-9].

References:
[1] Cojocaru V, Balali-Mood K, Sansom MSP, Wade RC (2011) Structure and dynamics of the membrane-bound cytochrome P450 2C9. *PLoS Comput Biol* **7**:e1002152.
[2] Feldman-Salit A, Wirtz M, Lenherr ED, Throm C, Hothorn M, Scheffzek K, Hell R, Wade RC (2012) *Structure* **20**: 292-302.
[3] Biedermannová L, Prokop Z, Gora A, Chovancová E, Kovács M, Damborský J, Wade RC (2012) *J Biol Chem* **287**: 29062-29074.
[4] Mereghetti P, Wade RC (2011) *BMC Biophys* **4**: 9.
[5] Brancolini G, Kokh DB, Calzolai L, Wade RC, Corni S (2012) *ACS Nano* 021/3: 9863-9878.
[6] Oguchi Y, Kummer E, Seyffer F, Berynskyy M, Anstett B, Zahn R, Wade RC, Mogk A, Bukau B (2012) *Nat Struct Mol Biol* **19**: 1338-1346.
[7] Mereghetti P, Wade RC (2012) *J Phys Chem B* **116**: 8523-8533.
[8] Mereghetti P, Wade RC (2013) *AIP Conf Proc* **1518**: 511-516.
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IL-11: Ribosomal RNA as a target for sequence-specific inhibition

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Many antibiotics block protein synthesis (translation) in pathogenic bacteria by interacting with bacterial ribosomal RNA and inhibiting the ribosome function [1]. Current antibiotics have side effects because in many cases they lack specificity toward the ribosomes of pathogenic bacteria. Also, bacteria have developed various resistance mechanisms against known antibiotics so there is a need to look for new antibacterials. Designing new scaffolds or modifying the known compounds has been of moderate success and most importantly will not eliminate the cross-resistance of related antibiotics. To design effective inhibitors of bacterial translation one has to explore new sites and possibly new inhibition mechanisms.
The atomic-resolution structural data of ribosomes and ribosome-antibiotic complexes enables structure-based computational design of both the ligands and targeted RNA sites [2]. Using a variety of molecular modelling and bioinformatics techniques we search for putative binding pockets in ribosomal RNA and investigate their dynamics and physicochemical properties. We test these ribosomal sites by designing short modified oligonucleotides to invade and hybridize with the ribosomal RNA sequences and physically block their functions. Oligomers are designed to bind to selected ribosomal functional sites in a sequence-specific manner. We test different DNA analogues such as 2'O-methyl-RNA, peptide nucleic acid (PNA), and locked nucleic acid (LNA) which hybridize well with RNA and are resistant to cellular enzymes. PNA is also known to have good strand invasion properties. Next, we check the ability of such oligomer-based compounds to inhibit protein synthesis in vitro in a cell-free transcription/translation system. To confirm the mode of binding to isolated ribosomal RNA fragments we use spectroscopic and calorimetric techniques.

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IL-12: Mean field dipole-dipole interactions as essential factors in the formation of biomolecular architectures

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Proteins, nucleic acids, and polysaccharides are key biopolymers that determine the shape and motility of the living cells and perform the functions of life. These biopolymers exhibit very characteristic structural patterns that consist of alpha-helices, beta-sheets, double helices, and triple helices. These building blocks are further arranged into a specific tertiary and quaternary structure. One successful attempt at the explanation of the origin of secondary structure was made by Banavar and coworkers [1], who developed a tube model of biopolymer chains. In this model, different secondary structures result from varying chain thickness and propensity to bend along the sequence, as well as from excluded-volume interactions. In this communication, an alternative approach is proposed, which is based on the fact that the repeated units of biopolymers (peptide groups, nucleic-acid bases, sugar rings) are highly polar and their charge distributions can be represented crudely as point dipoles. Reduction of the representation to one center per polar-interaction site leads to the representation of average site-site interactions as mean-field dipole-dipole interactions. Further expansion of the potentials of mean force of biopolymer chains into Kubo's cluster-cumulant series [2], as in the coarse-grained UNRES force field for proteins [3] developed in our laboratory, leads to the appearance of mean-field dipole-dipole interactions, averaged in the context of local interactions within a biopolymer unit. By appropriate tuning the dipole-electrostatic and local components in the cluster-cumulant terms and of the excluded-volume potentials, all secondary-structure elements observed in biomolecules, such as alpha-helices and beta-sheets in proteins and double helices in nucleic acids, appear in the simulated structures. Results of these simulations, together an analysis of the respective cluster-cumulant terms, which explains the stability of the most common architectures, will be presented. The results obtained strongly suggest that mean-field dipole-dipole interactions can be considered as the simplest formers of biomolecular architectures.

Acknowledgements:
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IL-13: Molecular Recognition Force Microscopy/Spectroscopy

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In molecular recognition force microscopy (MRFM), ligands are covalently attached to atomic force microscopy tips for the molecular recognition of their cognitive receptors on probe surfaces. A ligand-containing tip is approached towards the receptors on the probe surface, which possibly leads to formation of a receptor-ligand bond. The tip is subsequently retracted until the bond breaks at a certain force (unbinding force). In force spectroscopy (FS), the dynamics of the experiment is varied, which reveals a characteristic dependence of the unbinding force from the loading rate. These studies give insight into the molecular dynamics of the receptor-ligand recognition process and yield information about the binding pocket, binding energy barriers, and kinetic reaction rates. Applications on isolated proteins, native membranes, viruses, and cells will be presented. We have also developed a method for the localization of specific binding sites and epitopes with nm positional accuracy. A magnetically driven AFM tip containing a ligand covalently bound via a tether molecule is oscillated at a few nm amplitude while scanning along the surface. In this way, topography and recognition images on membranes and cell surfaces are obtained simultaneously. Finally, applications of high speed bio-AFM in filming the dynamics of molecular recognition are shown.

IL-14: Multiscale simulations of the mechanics of filamentous proteins

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Large-size biomolecular systems that assemble, disassemble, and self-repair by controlled inputs play fundamental roles in biology. Microtubules (Mts) are important in cytoskeletal support and cell motility. Their biomechanical functions depend on dynamic instability — a stochastic switching between periods of rapid growing and shrinking. This process is controlled by many cellular factors such as molecular motors and severing proteins so that growth and shrinkage periods are correlated with the life cycle of a cell. Resolving the molecular basis for the action of these factors is of paramount importance for understanding the diverse functions of MTs. Fibrinogen, upon enzymatic conversion to monomeric fibrin, provides the scaffold of blood clots. The force-induced unfolding of fibrin(ogen) is the foundation for the mechanical and rheological properties of fibrin, which are essential for hemostasis.

We focus on deciphering the microscopic origin of the physico-chemical properties of such biological assemblies and the molecular mechanisms of their response to controlled mechanical inputs. Because assemblies have modular architecture and strong inter- and intra-molecular coupling that modulate their properties, any approach has to model them on multiple spatial scales. We developed a multi-scale approach, combining coarse-graining [1-3] with atomic details [3, 4], to map out the mechanical properties of large size biological systems on experimental timescales. I will present our results for the micromechanics of microtubule protofilaments [4], with implications for the mechanism of protein co-factors, and our findings regarding the mechanisms of the elongation of fibrinogen protofilaments [5, 6], in direct correspondence with dynamic force spectroscopy experiments.

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IL-15: Chemistry and mechanics of the calcium signal in the motile cell, the glioma C6 case

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Cell motility is the phenomenon in which mechanics crosstalk with chemical signaling. Movement of the cell mass is facilitated by two basic motors: actin microfilament polymerization and actomyosin contraction. Motor activity however needs cell substratum adhesion to convert force generation into cell movement. These three factors are regulated by two groups of chemical regulation pathways: small GTP-ase dependent pathways of RhoA and Rac and calcium signal.

In our studies of cell response to external nucleotides we have found that calcium pathway induction by G protein coupled receptors may compensate pharmacological inhibition of RhoA pathway. As the experimental system glioma C6 cell line was used. Cells were induced by extracellular UTP, agonist of $P2Y_2$ nucleotide receptor. Canonically, this receptor is coupled to $G_{\alpha q}$ subunit of G-protein coupled to the GPCR, and activates phospholipase β, which in turn produces IP_3, opening IP_3 dependent channels in endoplasmic reticulum and releases calcium into cytoplasm, leading to complex calcium signal known as capacitative store operated calcium signal. From mechanical point of view calcium signal leads to the actomyosin contraction and acts synergistically to RhoA pathway activation.

We will present here results showing how complicated in details may be such seemingly simple mechanism. We will show and try to explain why observed calcium signal is temporarily polarized in motile cells and why this polarity disappears after RhoA pathway inhibition? Why calcium compensation of RhoA inhibition is accompanied in regulatory effects on the actin polymerization even if this phenomenon is not controlled by calcium signaling? Why compensation strongly depends on the presence of extracellular calcium, even if the main source of GPCR driven calcium signal are calcium stores of the endoplasmic reticulum? We will show, that all those effects are result of interplay between chemical signaling, cell microstructure and cell adhesion.

IL-16: Autophagy is involved in nanoalumina-induced cerebrovascular toxicity

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The current study focused on blood-brain barrier (BBB) disruption and neurovascular damage induced by engineered nanomaterials. Exposure to nanoalumina, but not to nanocarbon, induced a dose-dependent mitochondrial potential collapse, increased autophagy of brain endothelial cells, and decreased expression of tight junction proteins, occludin and claudin-5. Inhibition of autophagy by pretreatment with Wortmannin attenuated the effects of nanoalumina on decreased claudin-5 expression; however, it did not affect the disruption of occludin. These finding were confirmed in mice by administration of nanoalumina into the cerebral circulation. Systemic treatment with nanoalumina elevated autophagy-related genes and autophagic activity in the brain, decreased tight junction protein expression, and elevated BBB permeability. Finally, exposure to nanoalumina, but not to nanocarbon, increased brain infarct volume in mice subjected to a focal ischemic stroke model. Overall, our study reveals that autophagy constitutes an important mechanism involved in nanoalumina-induced neurovascular toxicity in the central nervous system.

IL-17: Studying of protein aggregation onto surfaces using QCM-D

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Protein drug aggregation can occur during manufacturing or formulation stages. Examples of surfaces where proteins aggregate include glass, oils, plastic polymers and metals in concentrators, tubings, containers or bags. Understanding proteins' aggregation propensity is crucial to minimize loss of often expensive therapeutic proteins during manufacturing and to ensure the development of a fully functional drug component. Quartz Crystal Microbalance with Dissipation monitoring, QCM-D, has been successfully used to study such aggregation processes. The QCM-D technique enables monitoring of the adsorption and conformational changes of biomolecules at a solid-liquid interface and can be used to provide information such as mass, thickness, viscosity and shear modulus of the sensor surface adhering film.

Biolin Scientific has studied such aggregation processes in collaboration with the two pharmaceutical companies Eli Lilly and Bristol-Meyers Squibb. In the collaboration with Eli Lilly, two monoclonal antibodies (mAb1 and mAb2) with significant differences in hydrophobicity and self-oligomerization behavior in solution were studied. It was concluded that mAb2 adsorbed stronger than mAb1 on all types of surfaces used. Addition of the surfactant PS-80 did significantly reduce the adsorption for both proteins.

In the Bristol-Meyer-Squibb collaboration the protein drug Abatacept and its interaction with the silicone oil/water interface was analyzed. The drug is administrated through a silicone oil lubricated syringe which is known to cause severe protein aggregation. SiO_2 sensors were spin-coated with silicone oil to form a homogeneous film. The adsorption of Abatacept was monitored in the presence and absence of two surfactants (PS-80 and Poloaxamer 188). It was concluded that the surfactant PS-80 reduced the amount of adsorbed Abatacept whereas Poloxamer 188 did not alter the binding significantly.

To summarize; QCM-D is shown to be a valuable tool in monitoring aggregation of proteins and antibodies onto different surfaces used in production or manufacturing in the pharmaceutical industry. By gaining better understanding of the aggregation processes of therapeutic protein drugs, both minimization of product loss and a more accurate end-products can be attained.

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IL-18: Structural basis for the recruitment of the CCR4-NOT deadenylase complex by Tristetraprolin

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Tristetraprolin (TTP) is an RNA binding protein that controls the inflammatory response by limiting the expression of several proinflammatory cytokines. TTP post-transcriptionally represses gene expression by interacting with AU-rich elements (AREs) in 3'UTRs of target mRNAs and subsequently engenders their deadenylation and decay. TTP accomplishes these tasks, at least in part, by recruiting the multi subunit CCR4-NOT deadenylase complex to the mRNA. Here we identify an evolutionarily conserved motif in human TTP that directly binds to CNOT1, a core subunit of the CCR4-NOT complex. A high-resolution crystal structure of the TTP-CNOT1 complex was determined, providing the first structural insight into an ARE-binding protein bound to the CCR4-NOT complex. Mutations at the CNOT1-TTP interface impair TTP-mediated deadenylation, demonstrating the significance of this interaction in TTP-mediated gene silencing.

IL-19: The ribosome action as transitions between different functional/structural states

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Ribosome is a multisubunit massive (megadaltons) molecular machine, which translates genetic information into protein. This large complex is a processive apparatus responsible for two major tasks in protein synthesis: decoding of the genetic information and formation of peptide bound, using messenger RNA as a template and aminoacyl-transfer RNAs as substrates [1]. The ribosome functions as a Brownian machine that harnesses thermal structural fluctuations into directed motion [2], however the main source of energy to advance all steps of protein synthesis is gained from GTP hydrolysis catalyzed by translational GTPases (tGTPases) which confer unidirectional trajectory for the translational apparatus [3]. The landing platform for tGTPases is located on the large ribosomal subunit, and it is called GTPase-associated center (GAC) [4]. The most prominent structure recognized in the GAC is called ribosomal stalk. It is thought, that the common functional denominator of the ribosomal stalks from all domains of life is recruitment of tGTPases and stimulation of factor-dependent GTP hydrolysis during translation [5]. A unique characteristic of the stalk is that its activity depends mainly on ribosomal proteins, unlike other ribosomal processes which are, in general, RNA-dependent. The stalk occurs as an oligomeric complex with a protein composition dependent on the taxonomic position of the organism. In prokaryotes, this complex has two structural configurations; pentameric complex L10-(L12)4 is found in mesophiles, while heptameric organization L10-(L12)6 has been reported for thermophiles. A similar diversity was found in archaeal/eukaryotic ribosomes, where a heptamer L10-(L12)6 was observed in archaea, and pentamer P0-(P1-P2)2 in eukaryotes [6]. The principle of ribosome action has been described by numerous elegant works, but interestingly the ribosomal stalk remains one of the few ribosomal elements with unknown structure and with only general understanding of its function [1]. Our team is focused on deciphering the function of the stalk, especially with an eye toward structural architecture and assembly of this oligomeric complex in eukaryotic cells.

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IL-20: Time-resolved, genome-wide model of translation

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We have developed a comprehensive and quantitative model [1] of translation, characterising protein synthesis separately for individual genes. The main advantage of the model is that basing it on only a few datasets and general assumptions allows the calculation of many important translational parameters, which are extremely difficult to measure experimentally. In the model, each gene is attributed with a set of translational parameters, namely the absolute number of transcripts, ribosome density, mean codon translation time, total transcript translation time, total time required for translation initiation and elongation, translation initiation rate, mean mRNA lifetime, and absolute number of proteins produced by gene transcripts. Most parameters were calculated based on only one experimental dataset of genome-wide ribosome profiling. The model was implemented in *Saccharomyces cerevisiae*, and its results were compared with available data, yielding reasonably good correlations. The calculated coefficients were used to perform a global analysis of translation in yeast, revealing some interesting aspects of the process. The model is universal and can be applied to any organism, if the necessary input data are available. The model allows us to better integrate transcriptomic and proteomic data. A few other possibilities of the model utilisation are discussed concerning the example of the yeast system. For the first time, this model allows to simulate the translation process in a (real)time-resolved manner. Applications to heterologous expression will be discussed.

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IL-21: Multifunctional magnetic-photoluminescent-photocatalytic nanostructures for biomedical applications

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There is a growing interest in producing composite nano-structured materials made of inorganic nanoparticles entrapped in organic polymer matrices or encapsulated in silica (SiO_2). The controlled fabrication of well-defined nano-sized composite constructs, like nanostructured fibers, has become one of the main topics in materials science oriented towards bio-medical applications. Networks and nonwoven fabrics based on such composite fibrillar structures offer a plethora of exceptional properties, such as ease of functionalization via simple chemistry, high surface area, and multi-functionality, just to name a few of them. These are promising features for applications in tissue engineering, controlled drug delivery and drug release, bio-sensors, nano-micro electromechanical systems, and advanced filtration, etc.

In general, nano-structured composites can be designed either by top-down or bottom-up approaches.

Firstly, we will focus on electro-spinning (ES), a relatively well established top-down approach of shaping the materials directly into the desired fibrillar structures. In particular, we will discuss ES-based encapsulation of up-converting $NaYF_4:Er^{3+},Yb^{3+}$-based nano-phosphors (UCNPs) and gamma-Fe_2O_3 superparamagnetic nanoparticles (SPIONs) into polystyrene (PS) matrices. We will show that these nano-structured fibers have interesting luminescent, magnetic and photocatalytic properties.

Secondly, we will discuss a bottom-up approach to encapsulation of UCNPs and SPIONs in SiO_2 shells, towards obtaining UCNPs&SPIONs@SiO_2 nano-constructs. We will show that such complex nano-constructs reveal similar multi-functionality to the materials obtained by ES-based encapsulation. In particular, in addition to their magnetic and luminescent features, both types of nano-constructs generate electronically excited di-oxygen (singlet oxygen, $^1O_2$) in aqueous media under illumination with near infrared (NIR) light.

Thus, these nano-composites have the potential for numerous biomedical applications, including: NIR-light fluorescence imaging, $^1O_2$ generation for performing deep-tissue bio-oxidations in photodynamic therapy (PDT), cell separation, enhanced contrast in magnetic resonance imaging (MRI), magnetic field-driven drug delivery, and hyperthermia.

IL-22: Nanoscale computer-aided design of self-assembling materials for electronics and health applications

Damien Thompson

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In this talk I will discuss the difficulties in controlling nanoscale physics and describe how computer simulations can aid experiments in the realization of nanostructured functional materials. I will present recent results on molecular modeling and design of nanostructured interfaces for technology applications. I will focus on self-assembled monolayer (SAM) films on noble metals [1-2] and graphene [3], including surface interactions with proteins [4]. I will also describe recent combined experiments and simulations of dendrimer-wrapped gold nanoparticles [5-6]. These (ultra)thin films and single-molecule nanostructures are used in molecular devices and have potential applications in medical diagnostics and therapeutics.

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IL-23: Automated droplet microfluidics for studies of emergence of drug resistance in bacteria

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Automated microfluidic systems that use microdroplets as miniature reactors embody the most acclaimed promises of microfluidics: ultra-miniaturisation and throughput. I will demonstrate systems that are remarkably simple in contrast to the complexity and scaling of the number of processes that they perform. We developed a technology for handling processes in microdroplet reactors that provides the flexibility of standard glassware for chemists. In particular, it is possible to i) produce hundreds of individual microdroplet reactors and address each one individually; ii) manipulate the chemical environments of the microdroplet reactors in time and over prolonged intervals; and iii) monitor the chemical processes in each of the microdroplets in parallel.

We describe the application of this new technology to the study of dynamics of microbial populations over extended time intervals and under fluctuating chemical stresses. The method that we present is attractive in its simplicity: the system consists of an automated microfluidic network in which ~10 input and output channels control the formation and composition of $>10^2$ microdroplets reactors in parallel over several days.

These studies provide a foundational technology for highly parallel, long-term studies of microbial evolution, adaptation, and ecology in response to fluctuating chemical environments (in this case, small molecule antibiotics). Our approach introduces a method for the parallel study of hundreds of individual and fully addressable microreactors and opens vistas for studies that are too complex to efficiently handle with the conventional techniques and instrumentation. These capabilities provide new opportunities for studying the genetic and phenotypic adaptation of bacteria to chemicals and open a new window through which to view the processes of evolution.

IL-24: Interfacial Microrheology of Protein Layers During Formation at Fluid Interfaces

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The tendency for proteins to adsorb at air-water or oil-water interfaces and to create stiff interfacial layers impacts current and developing technologies in the biomedical and pharmaceutical industries. Knowledge about the rheology of protein layers, and particularly the evolution of their viscoelasticity, is also crucial for a thorough understanding of the mechanisms driving layer formation, which can further provide a unique perspective on issues of protein denaturation, protein-protein interactions, and the gel transition. However, characterizing the rheology of such layers is difficult due to their confined geometry, the fragility of the layers, and the possibility of spatial heterogeneity. Interfacial microrheology, which employs colloidal probes to interrogate the mechanical properties of films at fluid interfaces, is emerging as a powerful approach to investigate interfacial protein layers.

This talk will describe interfacial microrheology studies of the time-dependent viscoelastic behavior of layers of the proteins β-lactoglobulin and lysozyme adsorbing at air-water and oil-water interfaces. The experiments combine complementary passive and active microrheology techniques. The passive approach, which employs multiple particle tracking of spherical colloids undergoing Brownian motion at the interface, provides high-sensitivity measurements of the interfacial rheology that shed light on incipient layers with modest interfacial viscosities and that reveal transient mesoscale heterogeneity in the layers. The active microrheology, in which ferromagnetic nanowires at the interface rotate in response to magnetic torques, extends the dynamic range of the measurements to stiffer layers and enables characterization of the nonlinear rheology. Comparisons of layers forming at air-water and at oil-water interfaces demonstrate a strong dependence of the rate and nature of the viscoelastic transition on the hydrophobic phase. Additional measurements on layers formed by spreading protein directly onto the air-water interface illustrate the sensitivity of layer rheology to the manner in which the protein is introduced to the interface.

IL-25: BIOLOGISTICS: mobility of ligands, proteins and plasmids in cytoplasm of the eukaryotic and prokaryotic cells

Robert Hołyst

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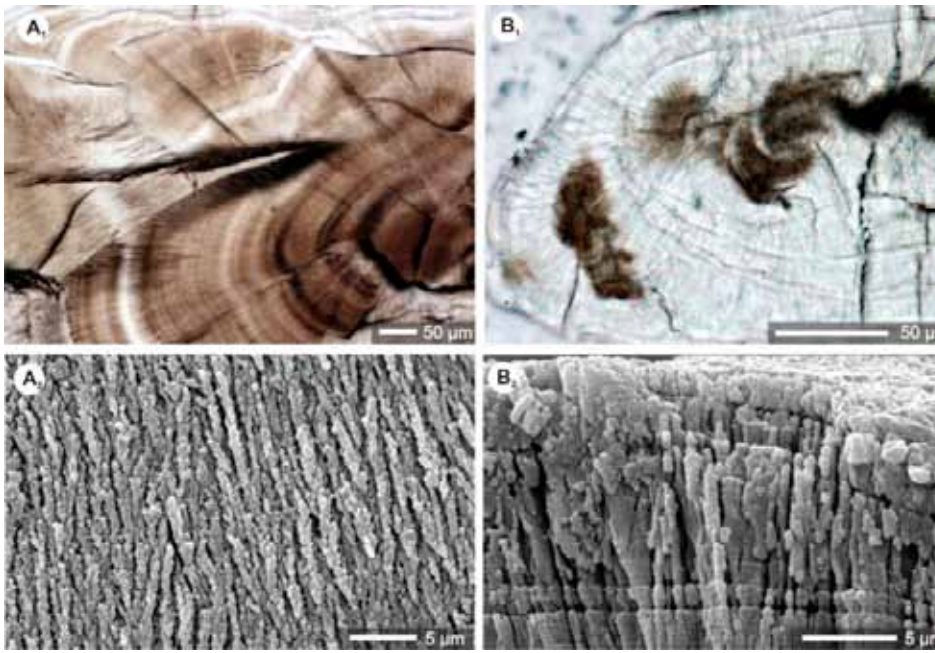
Biologistics and biochemistry in a crowded environment are two emerging interdisciplinary fields of science. They provide quantitative analysis of transport of proteins and their interactions involved in gene expression and regulation. These processes inside living cells strongly depend on the physics of liquids at the nanoscale. As I will try to convince you during my talk the length-scale dependent nanoviscosity [1-4] characterizing motion of proteins at the nanoscale is a key to quantitative analysis of biochemical reactions in living cells. Genes are activated and repressed by proteins referred to as transcription factors (TF). The binding of TFs to the operator region on DNA is diffusion limited. TFs search for operators by performing a



combination of three-dimensional (3D) diffusion in a defined volume and one-dimensional (1D) diffusion along DNA molecule. The diffusion coefficients for 3D diffusion, D, and 1D diffusion, D_1, are inversely proportional to the viscosity. For the model Gram-negative bacterium *Escherichia coli*, the nanoviscosity of the cytoplasm depends on the size of diffusing objects. This scale dependent nanoviscosity changes by a factor of $>10^4$ between 0.001 Pas for water molecules (size 0.14 nm) and 18 Pas for large plasmids (size 300 nm). Accordingly D for biomolecules in *E. coli* varies by a factor of $\sim 10^8$; representative diffusion coefficients for biomolecules in *E. coli* at temperature 310 K, include: D= 474 $\mu m^2/s$ for arginine (radius r_p= 0.34 nm); D=80.4 $\mu m^2/s$ for protein TrpL (r_p=1.1 nm); D=0.23 $\mu m^2/s$ for protein IbpB (oligomer 100 subunits r_p=10.3 nm); and D=0.5×10^{-5} $\mu m^2/s$ for plasmids (r_p=210 nm). Reaction rates of biomolecules are proportional to

D, and therefore they are sensitive to diffusion that arises with substrate size. An understanding of how D, D_1 and the reaction rates for gene expression depend on the length-scale dependent nanoviscosity and non-specific interactions between DNA and proteins are an essential step for understanding metabolic and proteomic networks. The final outcome of the work of my group is a database (6600 records) of diffusion coefficients for all proteins and their complexes from the proteome of *E. coli* [4]. This is the first such database for any organism. Only 10–20 measurements of diffusion coefficients are needed to construct the databases for any cell or its organelles (nucleus, mitochondria). Similar analysis of the scale dependent nanoviscosity has been done in my group for the cytoplasm of cancer HeLa cells and fibroblasts Swiss 3T3 cells [1].

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IL-26: How meanignful is prefix "bio" for minerals formed by organisms: the nanoscale perspective

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Lowenstam's (1961) discovery that magnetite can be biochemically precipitated under ambient temperatures and pressures as a capping material of teeth of some marine molluscs challenged the long-held view that this mineral occurs only in igneous or metamorphic rocks. This discovery and those in consecutive years, were highly inspirational for founding new routes for the synthesis of functional materials, but revealed also our limited understanding about how living organisms influence formation of their biomineral structures at micro- and nanoscale level. Still existing problems will be herein overviewed using two examples of calcium carbonate biominerals: skeleton of reef corals and otoliths, the inner ear structures of fish.

Figure 1. Minute-scale structural features of otolith (A) and scleractinian coral skeleton (B). Optical images show banding pattern of aragonite fiber layers (A_1, B_1), whereas SEM polished and etched sections (A_2, B_2) show fibrous biomineral deposits composed of nanogranular material.

Coral skeletons and otoliths, despite of very different phylogenetic relationships of organisms that produce them, are typically composed of concentrically arranged layers of aragonite biocrystals, that show crystallographic continuity across the layers and have organo-mineral composite structure (nanograins ca. 50–100 nm in diameter; Fig. 1). However, there is no consensus (and knowledge) about the biological control of the mineralization process at low structural level, i.e., about actual interplay between organic macromolecules and inorganic ions.

The biomineralization process is best explained in zebra fish otoliths. Otolith formation starts from precursor particles (probably glycogen) which become tethered to the tips of hair cell knidocilia and is followed by formation of calcium carbonate stabilized by protein and glycoprotein matrix. Radial-concentric structure (or its lack) and selection of calcium carbonate polymorph (aragonitic *vs*. calcitic) is controlled by expression levels of starmaker gene. Starmaker protein acts as a crystal inhibitor, regulating growth in a concentration-dependent manner, and selection of aragonite over calcite (default, in the absence of this protein) is most likely due to specific spacing of the aspartic acid and serine residues that attract calcium cations and make denser (typical of aragonite) packing of ions possible.

On the other hand, the model of coral mineralization, that is still widely used in geosciences refers to "inorganic" calcium carbonate precipitation from extracellular calcifying fluid (ECF) of similar composition to the sea-water. However, observations of frozen-hydrated samples that show a direct contact between the tissue and the skeleton and also geochemical composition of the skeleton differ markedly from the sea-water equilibrium do not support "inorganic" model of coral skeletogenesis.

IL-27: Zircon — small but great

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Zircon ($ZrSiO_4$) is undoubtedly the most valuable accessory mineral in crustal rocks, i.e. those that make up the main volume of the continents on which we live. Although tiny (usually zircon crystals in rocks are below 0.1mm) and hardly visible with the naked eye, the mineral retains very valuable information on the host rock's geological history. We can "read" the main thermal episodes of that history by measuring the ratios of U and Pb isotopes (the oldest rocks on Earth were dated using zircon grains). Hf and O isotopes provide valuable information on magma sources and on such processes as magma fluid interactions. By investigating nano-inclusions in zircon we can gain important insights into the "frozen" temperature-pressure-time paths that the host rocks experienced. Zircon can also precipitate from fluids or fluid-saturated melts at very low temperatures, completely different to those recorded by the methods above. We have found and described low-temperature nano- crystals of zircon in sedimentary rocks affected by hydrothermal alteration. Zircon was dissolved from early-diagenetic concretions, transported in fluids and reprecipitated in voids. This is the first record of precipitation of authigenic zircon in sedimentary rock as a new phase, and not as overgrowths (Bojanowski *et al*., 2012).

Reference:
Bojanowski *et al*. (2012) *Contribution to Mineralogy and Petrology* **164**: 245-259.

IL-28: Pressure Effects on Protein Folding: Why and for What?

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For nearly 100 years since the first report of pressure-induced protein denaturation, a small community of experimental and theoretical biophysicists has tried to determine the physical basis for this effect. We have shown recently that the magnitude of the pressure effect does not correlate with the size of the protein. This demonstrates that unfolding by pressure does not depend on the change in solvation of protein surface exposed to bulk solvent upon unfolding. In contrast, single amino acid changes that create or enlarge internal cavities can more than double its absolute value. Thus, we surmise that internal packing defects play a significant role in pressure effects. Pressure acts due to specific properties of folded protein structures, not because of properties of their unfolded state. Moreover, transition states in the folding reaction have higher volumes than the unfolded state, leading to very slow relaxation kinetics at high pressure. We have used these two unique properties of pressure coupled with NMR and computational approaches to explore in exquisite detail the folding free energy landscapes of model proteins.

IL-29: Experiment and modeling: competitive or complementary approaches to structural biology?

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The three-dimensional structures determined by X-ray crystallography play a central role in understanding protein-small molecule and protein-protein interactions at the molecular level. Each unique structure deposited to the Protein Data Bank (PDB) increase the number of models that can be calculated (predicted) for experimentally unknown structures. The experimental verification of models produced by the CASP competition shows that top experts can accurately predict the overall structure of proteins when there is a similar protein of known structure and in some cases even when a protein is not similar to any protein with a known structure. However, the experimental verification of applicability of automatic methods developed for meta-servers shows that the accuracy of a predicted model significantly drops when the sequence similarity between the model and an experimentally derived structure drops below 30%.
Protein 3-D structures have long been used to search for new drug targets, but only a fraction of new drugs coming to the market were developed with the use of structure-based drug discovery method. The '*in silico*' screening of potential ligands is much less successful than prediction of native protein structures. The combined approach of experimental and computational methods will lead to a dramatic increase of accuracy of computational screening. Thus our understanding of protein-ligand and protein-protein interactions and our understanding of the molecular foundation of human diseases and thus leading to a high-output structure-based drug discovery system.

IL-30: New Methods to Improve Protein Structure Prediction and Refinement

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We have developed and combined several novel methods to improve protein structure prediction from the amino acid sequence, and the structural refinement of protein models. One of the most promising developments in protein structure prediction are many-body potentials that take into account dense packing, and cooperativity of interactions in protein cores. We developed a method that uses whole protein information filtered through machine learners to score protein models based on their likeness to native structures. Testing on CASP8 targets showed that our method is superior to the common DFIRE and its derivatives as well as to the current version of RWPlus, both of which are considered a standard in the field. These results were recently published by us [1], and tested successfully in CASP 9, where our prediction group 4_BODY_POTENTIALS was among top three predictors in the category of template-free modeling for the most difficult targets. Recently we have significantly improved our potentials used for the prediction of protein structure and tested them in CASP 10. Our prediction group Kloczkowski_Lab was ranked as the third one in prediction of structure (based on the single model) for all targets, and ranked also as the third one for template free-modeling (see: http://www.predictioncenter.org/casp10/groups_analysis.cgi). Additionally our group MQAPsingle (Pawlowski-Kloczkowski) was ranked as the third one in CASP10 in Model Quality Assessment, and the second one in MQAPs using single-model methods. By combing statistical contact potentials with entropies from the elastic network models of proteins we can compute free energy and improve coarse-grained modeling of protein structure and dynamics [2]. The consideration of protein flexibility and its fluctuational dynamics improves protein structure prediction, and leads to a better refinement of computational models of proteins [3, 4]. We proposed a novel protein structural refinement procedure based on Anisotropic Network Model (ANM) of protein fluctuational dynamics and Go-like model of energy score. The starting structures were models from past CASP experiments. We changed positions of C-alpha atoms using ANM, creating a new set of 250 structures from the initial model, and computed energies of these structures using Go-like energy score. The top 5 coarse-grained structures were fully rebuilt with BBQ and Scrwl4. To remove bond stretches and the excluded volume clashes, short Molecular Mechanics simulations (up to 10,000 steps) were performed with OPLS-AA force field and implicit solvent GBSA-OBC. The whole structural refinement process was performed iteratively leading to the improvement of average RMSD from 3.8Å to 2.6Å in 50 iterations.

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ST-31: Structural studies on 30-kDa lipoproteins from mulberry silkworm

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Mulberry silkworm hemolymph contains a unique group of proteins, known as 30-kDa lipoprotein family, which could be found only in insects belonging to Lepidoptera order. The accumulation of the proteins occurs in fifth instar larvae [1]. 30-kDa lipoproteins are involved in immune response to fungal infections *via* b-glucan binding [2], have antiapoptotic [3] and cell-penetrating properties [4].

Five crystal structures of two different 30-kDa lipoproteins, *Bombyx mori* lipoprotein 3 (Bmlp3) and 7 (Bmlp7), were determined. Bmlp3 and Bmlp7 are the most abundant proteins in silkworm hemolymph. The overall fold is similar for both proteins which consist of two domains: the N-terminal VHS-domain created by six α-helices, and a C-terminal β-trefoil domain. Both lipoproteins were isolated from hemolymph as unknown proteins and the identification of their amino acid sequences was done according to the electron density maps of a good quality and supported by LC/MS/MS and N-terminal sequencing. Structural analysis of three Bmlp7 crystal structures suggested a potential role of the protein in detoxification mechanisms related to heavy metal pollutions [5]. In the case of Bmlp3 the search for putative ligand-binding cavities was performed and the electrostatic potential of the protein surface at physiological pH 7.4 conditions was calculated [6]. The structural studies of both Bmlp3 and Bmlp7, are a good starting point for the elucidation of the biological role of the 30-kDa lipoproteins.

Acknowledgements:
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IL-32: The Nanomechanics of Viruses

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Protective shells of viruses are regular, self-assembled, nanometer-sized containers which are minimalistic in design, but combine passive and active functions. Bacteriophage capsids are capable of carrying a highly compacted genome under considerable pressure. We used Atomic Force Microscopy (AFM) to image and probe the mechanical properties of various virusses. We found that bacteriophage capsids are tough like hard plastic, while displaying surprising elasticity. In contrast the capsid of the plant virus CCMV is very soft. Interestingly this capsid doesn't store its genome under pressure. We also observe that the elastic response to local indentation is linear over a large force range. This response, however, varies across the surface and is correlated to the shell protein organization. As a result we can resolve the hexameric and pentameric protein organisation on the shell surface. Repeated pushing on viral shells cracks this structure, weakening, but not necessarily changing its elastic response. Characterization of the breakage lines gives information about the arrangement and the local interactions of the protein subunits in the shell.

IL-33: Molecular mimicry in hepatitis B virus: How a viral intrinsically disordered protein helps hijack the host membrane-trafficking machinery

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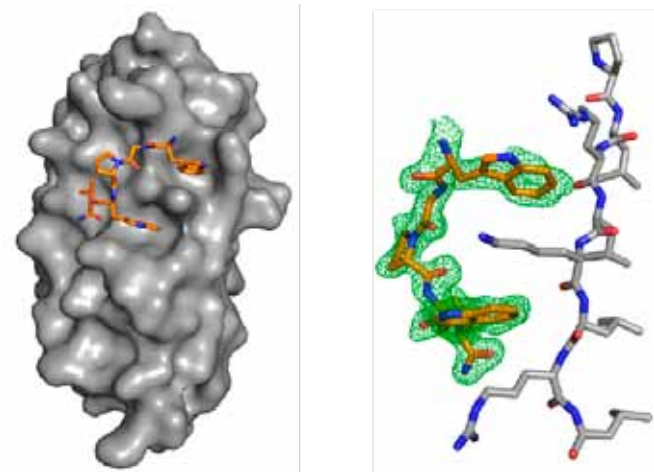
Hepatitis B virus (HBV) is an infectious, potentially lethal human pathogen, yet there are no effective therapies for the chronic HBV infections affecting 350 million people worldwide. Drug-development is hampered by the lack of structure-function insights on protein-protein interactions (PPIs) essential for HBV replication. The PPI between an HBV envelope protein and its cognate human binding-partner is essential for nucleocapsid envelopment. However, very little is known about this PPI, a putative drug-target. I will present atomic-resolution insights into the binding-thermodynamics and structural-biology of this PPI. Heteronuclear NMR spectroscopy demonstrated that the viral domain is an intrinsically disordered protein (IDP). The viral IDP contains multiple motifs that mimic the membrane-trafficking motifs and binding modes of host-proteins, allowing the virus to piggy-back on the host membrane-trafficking machinery. Surprisingly, the IDP binding motifs co-localized to a relatively rigid and functionally important region of the IDP and displayed little disorder-order transition upon binding the host-factor. The conformational restrictions within this region may be important for molecular recognition. This PPI was low-affinity and efficiently outcompeted by a synthetic peptide derived from phage-display experiments. Since efficient peptidomimetic design and optimization requires in-depth knowledge of the structure and binding-thermodynamics of target PPIs, our work represents an important first step in developing new types of HBV antivirals.



IL-34: Dual structural role of the viral RNA: capsid stabilization *vs*. genome uncoating

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In recent years, two powerful new research tools have evolved to probe the structure and stability of macromolecular complexes, e.g. viral assemblies, based on in liquid Atomic Force Microscopy (AFM) and native Mass Spectrometry (native MS). They fill in a great need, because where more established structural biology techniques suffer, they effectively capture assembly intermediates exposing details of viral assembly and maturation pathways. Using these techniques here in a hybrid fashion, employing both AFM and native MS, I present a study on the topology, mechanical properties and mechanism of genome release in the picorna-like Triatoma Virus (TrV). TrV is a member of the Dicistroviridae family and is closely related to common human pathogens like Polio Virus. Using naturally occurring, infectious particles we reveal that genome release takes place via disassembly of the capsid into pentamers of capsid proteins, and that these pentons subsequently reassemble into empty capsids. We also demonstrate that pH-induced genome release is preceded by a mechanically weakened state of the intact virus. Characterising the force-induced disassembly products of single virions by AFM reveals the presence of pentameric intermediate particles and this observation was confirmed by native MS. Our results provide, in a hybrid fashion, substantial evidence to conclude that these pentons are the fundamental structural units of picornavirus-like viral capsids.

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IL-35: DNA knotting inside viral capsids: a computational approach

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The packing of DNA inside bacteriophages arguably yields the simplest example of genome organisation in living organisms [1, 2]. An indirect indication of how DNA is packaged is provided by the detected spectrum of knots formed by DNA that is circularised inside the P4 viral capsid [3, 4]. The experimental results on the knot spectrum of the P4 DNA are here compared to results of coarse-grained simulation of DNA knotting in confined volumes. We start by considering a standard coarse-grained model for DNA which is known to be capable of reproducing the salient physical aspects of free, unconstrained DNA [5]. Specifically the model accounts for DNA bending rigidity and excluded volume interactions. By subjecting the model DNA molecules to spatial confinement it is found that confinement favours chiral knots over achiral ones, in agreement with P4 experiments. However, no significant bias of torus over twist knots is found, contrary to what found in P4 experiments [6, 7]. A good agreement with experiment is found, instead, upon introducing an additional interaction potential that accounts for tendency of contacting DNA portions to order as in cholesteric liquid crystals. Accounting for this local potential allows us to reproduce the main experimental data on DNA organisation in phages, including the cryo-EM observations and detailed features of the spectrum of DNA knots formed inside viral capsids. The DNA knots we observe are strongly delocalized and, intriguingly, this is shown not to interfere with genome ejection out of the phage [8].

Joint work with: D. Marenduzzo, E. Orlandini, A. Stasiak, D.W. Sumners, L. Tubiana

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IL-36: Knots: from the sailing boat down to the cell's nucleus illustrated by means of simple experiments

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Knots are pervasive in our daily life at the macroscopic level as well as at the microscopic level. With the help of some "experiments", I will present few important features of knots starting with macroscopic knots and then go into the cell's nucleus where knots play a role in the functioning of the DNA. Among the presented examples I will discuss some mathematical properties, the hydrodynamic behavior of knots, their resistance to traction and the unknotting of DNA knots by enzymes

IL-37: Chromosomes as topological machines

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The double-helical structure of DNA confers topological properties not only on limited domains within a genome but also on chromosomes as a whole. Many of the topological trnsitions within DNA are driven by DNA translocases which can not only operate topological devices in the neighbourhood of transcription initiation sites but also, acting through the classic Liu/Wang mechanism. act on whole chromosomes influencing both the expression and orgnisation of genes. Similarly topological devices, such as the eukaryotic 30 nm fibre and the nucleosome core particle, can act as repositories of negative superhelical tension.

IL-38: Predictions of a systematically coarse grained model for DNA

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We introduce a bead-spring model for DNA with bases modeled as rigid-body ellipsoids to capture their anisotropic stereochemistry. Interaction potentials are all physicochemical and generated from all-atom simulations/force fields with minimal phenomenology. Persistence length, degree of base stacking, and twist are studied by molecular dynamics simulations as function of temperature, salt concentration, sequence and interaction potential strength. The model also exhibits chirality with a stable right-handed and metastable left-handed helix.

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IL-39: Emergence of criticality in living systems

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In physical systems, critical phenomena are observed on fine-tuning parameters. For example, at the liquid vapor critical point, one must tune the pressure and temperature to their critical values to observe droplets of water and bubbles of gas of all sizes thoroughly interspersed among each other. Scale free behavior characterized by power laws is found at critical points.
In recent years, evidence has been mounting that biological systems (or parts, aspects or groups of them) seem to operate at the vicinity of critical points. Examples include spontaneous brain activity, gene expression patterns, cell growth, bacterial clustering, and flock dynamics. In some cases, theoretical models have suggested that such critical-like behavior leads to optimal computational capabilities, optimal transmission and storage of information, and maximal sensitivity to sensory stimuli. Nevertheless, a general mathematical framework providing deep theoretical principles behind the common behavior of these diverse systems and explaining the origin of criticality in living adaptive systems is still lacking.
We will present a mathematical framework, rooted in statistical physics and information theory, revealing a link between the emergence of criticality in biological systems and related evolutionary advantages.

IL-40: Kaleidoscopic protein self-assembly: The AXH domain of ataxin-1 undergoes a complex multiple equilibrium of species in solution

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Ataxin-1 is a human protein responsible for spinocerebellar ataxia type 1, a hereditary disease associated with protein aggregation and misfolding. Essential for ataxin-1 aggregation is the anomalous expansion of a polyglutamine tract near the protein N-terminus, but the sequence-wise distant AXH domain modulates and contributes to the process. The AXH domain is also involved in the non-pathologic functions of the protein, including a variety of intermolecular interactions with other cellular partners. The domain forms a globular dimer in solution and displays a dimer of dimers arrangement in the crystal asymmetric unit. Here, we have characterized the domain further by studying its behaviour in the crystal and in solution. We solved two new structures of the domain crystallised under different conditions that confirm an inherent plasticity of the AXH fold. In solution, the domain is present as a complex equilibrium mixture of monomeric, dimeric and higher molecular weight species. This behaviour, together with the tendency of the AXH fold to be trapped in local conformations, and the multiplicity of protomer interfaces, make the AXH domain an unusual example of a chameleon protein whose properties bear potential relevance for the aggregation properties of ataxin-1 and thus for disease.

IL-41: Modulated macromolecular crystal structure with 28 protein molecules in the asymmetric unit

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In modulated crystals, short-range translational order is lost and the atomic structure can no longer be defined by the contents of a single unit cell. The modulation represents systematic disorder, which distinguishes it from random disorder. In modulated structures, the wave of disorder can be described by a modulation function, which is periodic and restores long-range periodicity. If the modulation period divided by the corresponding unit cell translation is a rational number, then the modulation is commensurate, and can be described in an expanded unit cell of the superstructure. Otherwise it is incommensurate, meaning that the modulated structure cannot be described by expanding the number of unit cells. The diffraction pattern of a modulated structure contains strong main reflections from the basic unit cell, surrounded by much weaker satellite reflections from the modulation wave. Modulated structures are well studied in small-molecule crystals but are very rare in macromolecular crystallography.

Stress factors, such as pathogens, induce in plants the expression of so-called Pathogenesis-Related (PR) proteins, which have been divided into seventeen classes. PR proteins of class 10 (PR-10) are mysterious since no unique biological function can be attributed to them despite their abundance and involvement in processes, such as developmental regulation or symbiosis. Recent results strongly implicate PR-10 proteins in phytohormone binding and regulation. PR-10/hormone complexes are studied *inter alia* using fluorescent probes such as ANS (8-anilino-1-naphthalene sulfonate). We crystallized Hyp-1, a PR-10 protein from St John's wort, in complex with ANS. Solution of the apparent $P4_122$ crystal structure was impossible by standard molecular replacement methods because of evident tetartohedral twinning and a bizarre modulation of reflection intensities *hkl* with *l* periodicity of 7, which indicated seven-fold non-crystallographic translation along c. Ultimately, the structure was solved using Phaser after data expansion to *P*1 symmetry, with as many as 56 Hyp-1 molecules in the unit cell. Analysis of their arrangement revealed the true *C*2 space group with 28 independent protein molecules, arranged in 14 dimers around a non-crystallographic (NCS) 21 screw along c with a pitch of 1/7. The seven-fold repetition along c is indicative of a commensurate modulated structure because the seven NCS copies, while similar, are not quite identical. For instance, the Hyp-1 molecules in the stack have a varying number (from 0 to 3) of the ligand molecules bound. The monstrous crystal structure has been successfully refined, after detwinning, using conventional methods (i.e. with the unit cell expanded to encompass the entire commensurate modulation period) to an R-factor of 21%. This is a rare case of a modulated macromolecular crystal structure and it holds the absolute record in the PDB from the point of view of complexity and size of the asymmetric unit contents.

IL-42: Order and disorder in biomolecular assemblies

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The focus of structural biology and molecular biophysics is rapidly shifting towards studies of the dynamic supramolecular assemblies involved in the processing of genetic information, signaling, protein trafficking, and many other key cellular processes. To overcome the emerging challenges associated with large molecular sizes, transient interactions, extensive motions, and partial disorder, we combine simulation and theory with experiment. At the structural level, we developed a coarse-grained simulation approach and ensemble refinement techniques that allow us to integrate data from diverse experiments, including X-ray crystallography, small-angle X-ray scattering (SAXS), spin-label distance measurements (EPR), single-molecule fluorescence energy transfer (FRET), and paramagnetic relaxation enhancement (PRE) experiments as well as conventional solution NMR. At the theoretical level, we found that transient specific and non-specific protein interactions exert evolutionary pressure both on the proteome size and on the topology protein-protein interaction networks.

Abstracts of Poster Contributions

P-1: Continuous and delayed photohemolysis sensitized with photofrin and iron oxide nanoparticles (Fe_3O_4)

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Photofrin is one of the main photosensitizer that clinically admitted as a Photodynamic therapy drug for cancer treatment. On the other hand nanoparticles recently are widely employed in medical applications. Iron oxide (Fe_3O_4) nanoparticles, for example, are used in diagnostic, and in cancer therapy as a drug carrier. In this work, the photosensitivity of combination the photofrin and iron oxide (Fe_3O_4) nanoparticles on red blood cells is investigated. Red blood cells sensitivity were investigated, for post-irradiation (delayed) photohemolysis (DPH) and during irradiation (continuous) photohemolysis (CPH), to the photofrin and iron oxide combinations at different concentrations at room temperature. The photohemolysis rate, relative steepness and power dependant parameter were measured for a range of Iron oxide nanoparticles and photofrin concentrations for CPH and DPH. Resulting sigmoidal curves of CPH and DPH are in good agreement with mathematical modeling using Gompertz function. Analyzing the data using power law led to an approximately square dependence of the photohemolysis rate on DPH irradiation time for Photofrin® and to a power dependent of 1.5 with Iron oxide nanoparticles presence. The power dependence of CPH measurements show about –0.117 for different concentrations of Iron oxide nanoparticles combined with 2 μg/ml Photofrin concentration. Relative steepness of the photohemolysis curves is independent of the light dose for DPH with and without Iron oxide nanoparticles. Furthermore, Iron oxide nanoparticles incorporated with photofrin delay the photohemolysis process and decrease the photohemolysis rate.

P-2: Functionalized ZnO Nanorods for Protein Sensing Applications

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It is well known that sensitive, selective, and cost-effective detection of biomolecules has huge applications in clinical diagnostics, industrial and environmental monitoring. ZnO nanorods based sensors are one of the candidates for various biosensing applications.
In this work, the performance of a ZnO nanorod based sensor has been studied for detecting Immunoglobulin G (IgG)-targeted protein. A simple and novel one-step process of IgG immobilization was developed. It was to apply IgG solution on the surface of ZnO nanorods as substrates by drying method at room temperature. A square pattern of thioctic acid self-assembled ZnO nanorod arrays was grown on a large thermoplastic polyurethane (TPU) flexible substrate using the conventional hydrothermal process at low temperature (50^{o}C). With the addition of dimercaptosuccinic acid (DMSA), the surface chemistry forms a disordered ZnO phase, and the morphology of the ZnO-DMSA nanorods changes with various DMSA addition times.
The fabricated ZnO nanorods based bio-sesnors were examined for IgG detection. Current-voltage (I-V) and Scanning Electron Microscopy (SEM) characterization were used to monitor the change in the conductivity as well as morphology. By comparing with the reference sample, the specific binding event between anti-IgG and IgG antibodies was detected. Obtained results indicated a conductivity change by more than 20% after the protein hybridization. SEM images confirm the morphological change from reference samples to reacted samples. Through this work, we have demonstrated to use ZnO nanorods as building blocks to fabricate bio-sensors which can potentially detect many well known proteins. It is a cost effective process, which can be exploited further by expanding into arrays and integrating with microfluidics. This approach can be easily applied to other protein systems through selection of the proper receptor and functionalization methods for the nanostructures.

P-3: Thermodynamic linkage between protein-folding, allostery and capsid assembly in the hepatitis B virus core protein

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Hepatitis B Virus (HBV) is a highly infectious, human pathogen. There are no effective therapies for the chronic HBV infections that afflict 350 million people worldwide and massively increase the risk of developing liver cancer. The HBV core protein, HBc, plays many essential roles in replication including the formation of an icosahedral capsid that encloses the HBV genome. Structural plasticity in HBc has been proposed to underpin its diverse functionality. Thus, understanding the origins of HBc structural dynamics is central to developing novel antivirals against HBV. However, HBc is notoriously difficult to study as a dimeric protomer since it has evolved to aggressively self-assemble into capsids. We used a combination of high-end biophysical methodologies and extensive protein engineering to characterise the folding thermodynamics for a truncated HBc variant that was trapped as a dimeric, native protomer. The 34 kDa HBc homo-dimer unfolds via a dimeric, highly helical intermediate with thermodynamic coupling evident between different structural domains. Further, the highly plastic native state could be thermodynamically trapped in different conformations by introducing point mutations associated with specific HBV phenotypes. For example, point mutations could completely prevent capsid assembly, even when the mutated residues were distant to the inter-dimer interface that drives capsid assembly. These residues are structural gatekeepers that facilitate the adoption of capsid-assembly competent conformations in the wild-type protein. Thus, point mutations can allosterically regulate HBc structure and attendant functionality. Our findings show that the energy landscape for HBc protein folding and allostery intersects that of capsid-assembly. These processes can be thermodynamically tuned to selectively stabilize capsid-assembly-inactive conformations, paving the way for next generation small-molecule inhibitors of HBV replication.

P-4: Development and application of a force sensor with sub-piconewton resolution

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In this experimental work, we present an elegant approach using radiation pressure to minimize the effect of thermal-mechanical noise of the AFM cantilever. It is a simple method and straightforward. There is little need to modify the atomic force microscopy system (AFM). In addition, commercial cantilevers are used. In liquid environments, the cantilevers are cooled from room temperature to less than 3°K, using laser radiation pressure techniques controlled *via* a PI controller. Therefore, the thermal noise of the cantilevers has been reduced by more than eight-fold. Consequently, the uncertainty in normal-displacement (amplitude) of the cantilevers is minimized to be less than 2Å (peak to peak). This setup allows to the AFM to measure unfolding force of a soft protein that was previously impossible.

P-5: DNA and protein sensors based on ZnO and ZnO/ZnS nanofibers

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One-dimensional (1D) ZnO nanostructures have been widely studied because of their electronic and optoelectronic applications. Devices based on ZnO electrospun nanofibers are very promising biodetectors due to their low toxicity and high sensitivity at room temperature.
We obtain ZnO nanofibers by electrospinning followed by air calcinations. Characterization of these 1D nanostructures was performed by using X-ray diffraction, atomic force microscopy, transmission electron microscopy, energy dispersive X-ray spectroscopy, cathodoluminescence and photoluminescence techniques. The elemental composition and structural characterization revealed polycrystalline nature of the nanofibers. The activation energy for grain-growth was estimated to be 12 kJ/mol and thus the crystals growth required an order of magnitude less energy than attainable for bulk. It implied that the crystal growth was faster and achievable doping level was higher in these nanostructures. The interband emission was increasing with the nanocrystal size, consistent with decreasing of the surface-to-volume ratio.
ZnO nanofibers were very effective materials for a UV-light sensors. The measured current increased by three orders of magnitude when the fibers were exposed to UV light. Moreover, the resistivity decreased when the nanofibers were immersed in water and ethanol, or exposed to nitrogen stream.
In order to fabricate a ZnO based biosensor passivation of the nanofibers was required because of low stability at biological liquids. For these applications, the core/shell ZnO/ZnS nanofibers were synthesized by the hydrogen sulphide treatment. The other tested coating of ZnO nanofibers was silan (GOPS), water-insoluble organic substance. ZnO nanofibers functionalized by silan and following connection of *oligonucleotides* were demonstrated to be very specific and ultrasensitive (at pM level) at DNA detection. The potential of ZnO/ZnS nanofibers for biosensing was demonstrated using biotin and streptavidin as model system.
Summarizing, the ceramic electrospun devices showed great potential in developing an ultrasensitive, fast and real-time sensors and biosensors.

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P-6: Implementation of the physic based side-chain side-chain potential in coarse-grained UNRES force-field

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For several years, we have been developing the coarse-grained UNRES model and force field for simulations of protein structure and dynamics [1]. In the UNRES model, a polypeptide chain is represented by its alpha-carbon trace with united peptide groups (p) located halfway between two consecutive C-alpha atoms and united side chains (SCs) attached to them. In the present UNRES force field, the side-chain – side-chain interaction potentials were assigned Gay-Berne functional forms that take anisotropy of interactions into account, and their parameters were determined by fitting to correlation functions and to side-chain-contact energies determined from the Protein Data Bank (PDB) [2]. The Gay-Berne-type potential assumes that the interacting sites are ellipsoids of revolution, with a single interaction site per side chain. Such potential well describes non-polar amino acids side chains, however most of amino-acids side chains are composed of polar or charged "head" and nonpolar "tail". Therefore we developed an improved SC-SC interaction model in which non-polar side chains are represented by one interacting site, and polar or charged side-chains are represented by two interacting sites (nonpolar and polar/charged). The interaction energy between the nonpolar sites is composed of the Gay-Berne term and the cavity term; the interaction energy between the charged sites consists of the Lennard-Jones term, the Coulombic term, the generalized-Born term, the isotropic cavity term and the quadrupol term, while the interaction energy between the nonpolar and charged sites is composed of the polarization term. The parameters for new SC-SC interaction function were obtained by fitting analytical function to potential of mean force (PMF) derived from all-atom MD simulations for model compounds (see for example ref. [3]). Preliminary results of simulations with new UNRES SC-SC potential are presented. The new potentials reproduce desolvation barriers and are more realistic for sequence regions composed of many polar or charged residues than the previous statistical potentials implemented in UNRES.

Acknowledgements:
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P-7: Theoretical tests of the mechanical protection strategy in protein nanomechanics

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We provide theoretical tests of a novel experimental technique to determine mechanostability of proteins based on stretching a mechanically protected protein by single-molecule spectroscopy. This technique involves stretching a homogeneous or heterogeneous chain of reference proteins (single-molecule markers) in which one of them acts as host to the guest protein under study. The guest protein is grafted into the host through genetic engineering. It is expected that unraveling of the host precedes the unraveling of the guest removing ambiguities in the reading of the force-extension patterns of the guest protein. We study examples of such systems within a coarse-grained structure-based model. We consider systems with various ratios of mechanostability for the host and guest molecules. For a comparison, we also study the force-displacement patterns in proteins which are linked in a serial fashion. We find that the mechanostability of the guest is similar to that of the isolated protein. We also demonstrate that the ideal configuration of this strategy would be one in which the host is much more mechanostable than the single-molecule markers. Finally, we discuss systems in which the linkages involve a highly stable protein with a cystine knot, either monomeric or dimeric, in which the serial linkage gives about 30% reduction in the mechanostability compared to a single protein case. We show that it is troublesome to use the cystine knot proteins as host to graft a guest in stretching studies because this would involve a cleaving procedure.

Reference:
Chwastyk M, Galera-Prat A, Sikora M, Gómez-Sicilia À, Carrión-Vázquez M, Cieplak M (2013) Submitted for publication.

P-8: Temperature-dependent overexpression of large unstructured proteins in *E. coli*

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Many biophysical experimental methods require the use of large samples of highly purified protein. Thus, the process of optimizing protein overexpression and purification becomes essential. Translation of proteins can be slowed down or interrupted due to codon usage issues between the host and the overexpressed protein. Ribosomes can also be stalled at polyproline stretches, owing to conformational constraints. In case of such problems overexpression results in obtaining a mixture of N-terminal fragments of the desired longer polypeptide chain. Applying a C-terminal tag enables purification of a full-length protein. However, it is possible to shift the proportions between full-length protein *versus* N-terminal protein fragments that contaminate the sample. Such a change saves time, energy and materials and can have a much greater effect on protein yield than optimizing the proceeding purification protocol.

Decreasing the overexpression temperature has been known to increase the amount of soluble fraction of proteins, their potential for correct folding and conformation. Lower temperature also renders proteases less active and provides more time for proper tRNA recruitment upon slower protein biosynthesis. Overall these factors can lead to production of increased amounts of full-length protein.

Here we study the effect of temperature on overexpression of an N-terminal GST-tagged and C-terminal FLAG-tagged unstructured 40 kDa protein. Significant lowering of the overexpression temperature results in a much more favorable proportion of full-length protein versus protein fragments, as well as in a much higher yield of the purified protein.

P-9: Tuning the phase diagram and critical current density in superconductor-ferromagnet bilayers

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Ferromagnet-superconductor bilayers consist of a sequence of nanometer-thin superconducting (S) and ferromagnetic (F) layers. While in the bulk the superconductivity and ferromagnetism are mutually exclusive, they coexist on a mesoscopic scale in S/F structures in which the S and F layers are spatially separated. Close proximity of the F and S layers causes strong interactions between these two competing long-distance orders, leading to new surprising phenomena and creating great potential for applications in modern electronics.

In this work we present the results of recent studies of the influence of inhomogeneous magnetic fields, generated by magnetic domains in the F layer, on the basic superconducting properties of the S layer, which are important for applications, that is, the phase transition line to the superconducting phase, and the mechanism of vortex pinning. We use the F/S bilayers in which the S layer is niobium, and the F layers are built from Co/Pt or Co/Pd multilayers with perpendicular magnetic anisotropy. This property allows for the easy and reversible modifications of the magnetic domain patterns *via* various demagnetization or remagnetization protocols. One of the most amazing properties of these structures is the appearance of nonlinear dependence of the phase transition line on the external magnetic field, which is usually not observed in bulk superconductors. In addition, the magnetic domains strongly affect the pinning of vortices, leading to nonmonotonic dependence of the critical current density on the magnetic field and temperature.

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P-10: Optimization of dynamic fragment assembly for protein structure prediction with conformational space annealing and UNRES force field

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The prediction of protein structure when only the amino-acid sequence is known has been a problem of major interest of biomolecular sciences for many years. Currently, the fragment based prediction approaches that assemble fragments of known structures into a candidate structure have consistently outperformed alternative methods. Recently, the dynamic fragment assembly (DFA) method was proposed by Sasaki *et al*. (2008) in which knowledge-based information corresponding to local structure of a given protein is incorporated into a target function as a set of additional energy terms. These terms include two-body potential, pseudo-dihedral angle potential, neighboring-number potential which are based on fragment library constructed for a given protein sequence and β-sheet potential based on the neural-network estimation of the probability of the β-sheet pairing. De novo prediction requires both an accurate force field and an efficient conformational sampling method for successful protein modeling. We have combined the physics-based united-residue (UNRES, Liwo *et al*. 2007) force field developed in our laboratory with the dynamic-fragment-assembly (DFA) technique and conformational space annealing (CSA, Lee *et al*. 1997) which is one of the most effective procedures for the global optimization of protein structures. We have applied our UNRES+DFA/CSA protocol to a set of 30 small single-domain proteins and in blind prediction of several target proteins within the CASP10 experiment. In this communication we report further optimization of the weights of the DFA energy terms by the maximum likelihood principle using a set of four proteins with different folds: 1r69 (folded leaf of 4 helices), d1mlaa2 (α+β sandwich with antiparallel β-sheet), d1tfia (metal-bound fold with antiparallel β-sheet), d1gpta (α+β disulfide-bound fold). The results are compared to earlier optimization of DFA for CHARMM+DFA/CSA simulations using all atom CHARMM force field (Lee *et al*. 2011).

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P-11: Automated multistep purification of recombinant proteins with SUMO-tag

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In recent years the increasing importance of high-throuput experiments has stimulated the development of many automated systems for protein production and purification. Most of them use affinity chromatography as an easy-to-automate first step of purification, because of its high selectivity and scalability.

One of the commercially available systems, the AktaXpress from GE Healthcare, offers several multistep protocols for automated purification of recombinant proteins with optional on-column tag removal. This system has been tested and optimized for two different proteases: AcTEV and PreScission for purification of HIS- and GST-tagged proteins, respectively. Unfortunately, according to our tests, the AktaXpress system is not compatible with SUMO protease, an enzyme that recognizes the conformation of SUMO (small ubiquitin-like modifier) protein — a large tag that strongly improves both the solubility and expression level of fusion protein.

In our lab we have developed an optimized pipeline for protein production and purification, from cloning and constructs' preparation using SLIC method, to overexpression of proteins in autoinduction media and final purification on automated multistep AktaXpress system. Here, the novel AktaXpress protocol is presented, that allows the usage of SUMO tag system along with the automated multistep purification. For this purpose, the on-column protease treatment is held in high imidasole concentration. In these conditions the protease is still active, but the one-step nickel column purification doesn't separate the cleaved protein from the SUMO tag and the protease. Instead, we have used combined desalting and nickel columns as a second step of purification. An optional third step of purification can be either gel filtration or ion exchange. In addition, our AktaXpress protocol collects protein samples from each step of purification. That ensures better control over the whole purification process and facilitates the optimization in case of insufficient final amount or purity of recombinant protein.

We believe that our protocol for AktaXpress along with the whole protein production pipeline will be useful for many small- and medium-sized laboratories, where fast and efficient purification of large amounts of different proteins is required, especially in the field of structural biology.

P-12: Multiple assay strategy for revealing molecular mechanism of 5' mRNA cap degradation by Decapping Scavenger enzyme

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The 5' terminal mRNA cap structure is required for pre-mRNA splicing, export, nucleolytic stability and cap-dependent translation. The abundance and lifetime of mRNA is controlled by two major degradation pathways, one of which entails enzymatic hydrolysis of the cap by Decapping Scavenger. The enzyme functions as a homodimer, and is not expected to exist in a stable monomeric form due to large domain-swapping. In addition to the wild-type protein we expressed and purified two heterodimeric mutants with one of the monomers modified at the binding site for loss of its enzymatic activity. The first mutation, H277N, disables cleavage activity, yet the protomer is capable of binding a ligand. The second mutation, N110A / W175A, results in compromised binding capability. Kinetic studies of the cap degradation by the wild-type protein as well as the mutants gave further insight into the enzymatic process, product release and inhibition. Moreover, the cap-binding mechanism was followed using a broad class of non-susceptible, synthetic, cap analogues. Steady-state fluorescence titration provided the protein-ligand equilibrium association constants. Influence of various chemical modifications within the cap structure gave further insight into the details of the enzyme active site that are not visible form the X-ray structure. Fluorescence time-resolved experiments showed changes in intrinsic protein fluorescence life-times upon the cap binding. Pioneer application of dye-labelled cap analogues enabled a 'reversed' observation concept, focusing on the ligand emission. Furthermore, novel method of circular dichroism measurements in near UV was applied, in which each aromatic amino-acid type exhibited signal change in a different wavelength. Such effect enabled observations of tertiary structure rearrangements. The overall picture was completed with calorimetric measurements. The latter imply energy effect of the electrostatic contacts, especially H-bonds. Comparison of the results with those previously obtained for the initiation factor eIF4E gave initial data for a broader view of the protein-mRNA interactions.

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P-13: Conformational transitions of evolutionarily related serpin proteins, from atomistic simulations in with a realistic force field

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Serpins are a unique group of proteins that are trapped in a metastable structure when they fold and undergo a massive structural change to a stable structure when they inhibit their target proteases. Serpin linked diseases result when serpins polymerize, not through conversion to beta-sheet rich amyloids, but through novel domain swaps in which the stable structure is achieved through intermolecular linkage rather than intramolecular structural change.
Understanding the physical mechanisms underlying serpin conformational transitions is the first crucial step towards designing strategies to prevent their polymerization. Unfortunately, the large size of these systems (consisting of almost 400 amino-acids) and the very low rate of their conformation transition (spanning from hours to days, for different mutants) make it unfeasible to simulate this reaction in atomistic detail using MD, even on a special-purpose machine. However, the simulation of these processes has now become possible using the Dominant Reaction Pathways (DRP) approach, a variational method based on the path integral representation of the stochastic Langevin dynamics [1].
The main advantage of the DRP approach is that it does not waste time in simulating the thermal oscillations in the reactant and that the computational time scales extremely slowly with the rate (approximatively like log[log (k)], where k is the reaction rate).
The method was recently tested on a smaller protein (FIP35 WW domain) and it shown that it gives the same result of ms-long MD simulations performed with the ANTON machine using the same force field [2]. More recently, the DRP approach was successfully applied to study in atomistic detail the folding and knotting of a 83-amino acid protein [3].
The serpin transitions to be presented in this talk arguably represent the largest and most complex protein conformational reactions ever simulated using a realsitic force field (amber). We show that our DRP predictions compare well with the available kinetic experiments and deuteron substitution experiments for different mutants.

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P-14: Properties of Gold Nanoparticles Synthesized Using Streptosporangium spp. 94A and Thermoactinomyces spp. 44Th

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Two bacterial strains of alcalophilic actinomycetes the Streptosporangium spp. 94A and Thermoactinomyces spp. 44Th (isolated from black soil of Shiraky in Georgia) were used for microbial synthesis of gold nanoparticles and its properties were studied. The bacterial cells were grown at pH 7-11 in suitable nutrient medium, harvested and then suspended in aqueous solutions of 10^{-3}M $HAuCl_4$ to produce of gold nanoparticles. The variation in time of reaction of bacterial cells with aqueous solutions from 40 hours to 8.5 days allows to determine the optimal conditions for metal nanoparticle formation. A complex of analytical and spectral methods, specifically, UV-visual spectrometry, X-ray diffraction (XRD), scanning electron microscopy (SEM), and X-ray photoelectron spectroscopy (XPS) were used to characterize the obtained nanoparticles.

In the UV-vis absorption spectra the band related to surface plasmon resonance (SPR) of gold nanoparticles were observed at ~ 530 nm both in the biomass and supernatant, though in the letter the SPR peak was lower almost by an order of magnitude. The intensity of absorption bands in suspension grows in dependence as the reaction time increases indicating the gain of the number of nanoparticles.

The XRD data for gold nanoparticles confirm the presence of fcc structure. The efficiency of gold nanoparticle formation was estimated on the intensity of reflection peak from (111) plane. The relative concentration of gold nanoparticles in Streptosporangium spp. 94A based on the intensity of XRD (111) line does not depend on the reaction time although it is 3-4 times lower to compare with that in Thermoactinomyces spp. 44Th. The ratio between intensities of (200) and (111) diffraction peaks is varied from 0.2 to 0.3 in dependence on the reaction time and is lower that the conventional bulk intensity ratio (0.53) suggesting that the (111) plane is the predominant orientation. The distance between (111) planes in nanocrystals is 0.2365 nm, but the tabulated data gives 0.2350 nm. The width of (111) reflection peak is used to calculate the size of nanoparticles. The average size of gold nanoparticles in Thermoactinomyces spp. 44Th is 17±2 nm, in Streptosporangium spp. 94A it equals to 9.8±0.5 nm.

In the XPS spectra the doublet Au 4f was registered in the sediment of Streptosporangium spp. 94A and Thermoactinomyces spp. 44Th. By sputtering with Ar^+ ions the gold nonoparticles were disclosed and two electronic states of gold atoms were detected. The SEM images illustrate that the extracellular production of gold nanoparticles in both biomatrices takes place, the most of the formed nanoparticles are spherical and do not create large agglomerates.

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P-15: A new software library for coarse-grained modeling of biomolecules

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A new software library for biomolecular modeling has been carefully designed and implemented in Java programming language. One of the most important concepts the design has been based on is its modularity. A few groups of components were defined such as energy functions, neighborhood detection, sampling schemes and Monte Carlo moves. Different groups hardly depends on each other, therefore particular components can be used interchangeably. This single modeling library therefore may be applied to a wide range of molecular modeling problems; from bilayers, simple polymer models and coarse-grained proteins to biomolecules in atomistic representation. CHARMM, MARTINI and a custom knowledge-based force fields are available, implementation of other energy functions is straightforward.

P-16: Computational studies of self-assembly of chiral polymers with various twist

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We would like to present a Langevin simulation study of self-assembling semi-elastic, chiral polymers. Described on a very coarse-grained level, these polymers serve as a very simple and general model of protein fibrils. In this work we study the influence of the extent of the initial twist of single polymers on the resulting small clusters. We observe that the clusters inherit the chirality. i.e. they are also uniformly twisted, however the handedness of the aggregates depends on the twist — it is the same for (initially) less twisted polymers and inverse for highly twisted polymers with a threshold initial twist region for unordered polymers.

P-17: Interaction of ferredoxin:NADP$^+$ oxidoreductase with model membranes

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Ferredoxin:NADP+ oxidoreductase (FNR) is an enzyme, involved in photosynthetic electron transfer [1]. It is present both in all plants and cyanobacteria. FNR interacts with photosystem I in linear electron transfer chain, and with cytochrome b_6f or directly with thylakoid membrane in cyclic electron flow [2, 3]. FNR isoforms are present in non-photosynthetic tissues and are involved in biosynthetic pathways [4]. We have proved already [5] that FNR isolated from spinach binds directly to artificial membranes, as evidenced by Langmuir-Blodgett monolayer techniques (LB). Here we present continuation of that research using FNR enzyme from cyanobacterium, *Synechocystis* sp. PCC 6803. Although amino acid sequence is homologous only in 5% with spinach enzyme, the secondary and tertiary structure and the enzymatic characteristics are almost identical.

We again applied LB technique to characterize FNR binding to selected lipids (dipalmitoylphosphatidylcholine-DPPC, monogalactosyldiacylglycerol-MGDG, digalactosyldiacylglicerol-DGDG), in two different pH conditions (pH 5 and pH 8). MGDG and DGDG are natural thylakoid galactolipids, different in their packing parameter, and forming reversed hexagonal phase (HII) or bilayer, respectively. DPPC is not present in thylakoids, but is used as a control for protein binding preferences and membrane recognition — it forms bilayer, but in contrary to DGDG, it has its polar head group charged. Different pH conditions simulate changes observed in chloroplast during illumination. As we shown previously, pH change induce also conformational changes of FNR. To complete the picture, we also compared FNR binding to the membrane in presence of ferredoxin, a small protein with iron-sulfur cluster, natural reaction partner of FNR in linear electron transfer.

We found mainly that FNR binds to all tested lipids, however binding is stronger in pH 5. Binding to DPPC was less pH-dependent. Presence of ferredoxin increased strongly the rate of FNR attachment into lipid monolayers. Preliminary identification of FNR regions, involved in binding, were done by analysis of digestion pattern with trypsin and proteinase K.

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P-18: Development of a novel method for the identification of mouse gastric stem cells Using Raman Spectroscopy

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The stomach is lined by different types of epithelial cells which are generated continuously by gastric stem (GS) cells. These cells are rare and difficult to identify. Previous reports demonstrated the use of gold nanoparticle-based surface-enhanced Raman scattering (SERS) for probing the differentiation of embryonic stem cells (1). As a first step to use SERS as a tool to identify and characterize GS cells we tested the effect of gold nanoparticles (GNP) on growth and viability of GS cells. Trypsinized mouse GS cells were incubated with GNP either directly or after forming embryoid bodies using the hanging drop method. Transmission electron microscopy (TEM) was used to localize GNP inside cells, whereas PicoGreen assay were used to measure cell proliferation. Neutral red uptake (NRU) assay was used to test GNP cytotoxicity. TEM confirmed the intracellular localization of GNP. PicoGreen assay showed that the number of GS cells treated with GNP increased by 32.8% within 3 days in comparison to untreated cells. Additionally, NRU assay showed that GNPs have no toxic effect on GS cells.

Reference:
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P-19: Quantitative measurement of DNA labelling efficiency by fluorescence correlation spectroscopy

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Partial labeling of single-stranded DNA with efficiency of labeling smaller than 100% results in a mixture of the annealed double-stranded DNA molecules with two, one or without a dye. It is even difficult to distinguish the doubly-labeled DNA component from the singly-labeled component when using the same dye. Existing methods are insufficient to measure the percentage of the doubly-labeled dsDNA component in the fluorescent DNA sample. Here we show how to measure the percentage of doubly-labeled DNA in the total fluorescent pool of DNA. This percentage is defined here as DNA doubly labeling efficiency. In the method we add known amount of unlabeled and partially labeled ssDNA and perform annealing of such DNA samples to obtain dsDNA. We further use fluorescence correlation spectroscopy (FCS) to obtain the average number of fluorescent molecules in the focal volume. We also determine percentage of molecules in the triplet state. In this way we determined the amplitude of the auto-correlation function at short times. We apply the result to quantitative analysis of kinetics of DNA cleavage by restriction enzyme Hind III. Our method needs only micro-liter volume of dsDNA samples at nano-molar level of concentrations.

P-20: Adsorption of cytochrome *c* on montmorillonite nanoplates

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pH-dependent properties of cytochrome *c* (Cyt *c*) are theoretically studied with aim to find optimal conditions for Cyt *c* adsorption onto monmorilonite (MM) nanoplates. The main calculated characteristics for free Cyt *c* in solution are: Net-charge titration [Z(pH)], Electrostatic term and total free energy (stability) [ΔG_{el}(pH) and ΔG_{tot}(pH)], Proton affinities of the individual ionic groups [$pK_{a,i}$(pH)], Coulomb interaction of each site [E_{el}(pH)] and Electric moments (vectors and scalars) [μ_e(pH) and μ_s(pH)]. In agreement with the experiments the reduced protein is more stable with 7.5 kcal/mol than its oxidized form and has isoelectric point (pI) about pH 10. In pH interval 5–8 all pH-dependent properties are practically non-changed; the electric moments are parallel orientated to the hem plane in wide pH interval. We have researched the electro-optical behavior of montmorillonite particles with adsorbed protein at different pH-values; the isoelectric point of Cyt *c* has been determined. The electrical and optical properties of the protein-particle complexes are examined by the dependence of the degree of orientation on the strength and frequency of the applied ac field. Electro-statically docked negative charge that simulate MM binding along the dipole vector (pH 9.3) shows relative vector stability and distance-dependent perturbation of protein intermolecular interactions. The application of the electro-optical techniques to Cyt *c*-montmorillonite nanoparticles traces a new approach to the protein electrostatics.

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P-21: Electrochemical biosensors for environmental monitoring and medical diagnostics

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In recent years, intensive research has been observed in the development of biosensors for a wide range of application in medical, food, agricultural and environmental areas.The methods for early, fast and low cost detection of many dangerous diseases are highly desired. The electrochemical biosensors fulfill all of these requirements.
The crucial parameter deciding about the sensitivity and selectivity of biosensors, containing the receptor molecules applied as recognition elements, is their oriented immobilization on the electrodes surface and the maintance of their biological activity.
Our work considers the development of the electrochemical genosensors and immunosensors which can be divided into the biosensors based on ion channel mechanism and the biosensors based on redox active monolayer deposited on the electrodes surface.
We proposed several biosensors successfully applied for :
Early detection of Avian Influenza Virus (AIV) H5N1 genotype, which is a common infectious disease spreading among wild and domestic birds but also constitute a threat to humans including mammals and can cause death. Early detection of Plum Pox Virus (PPV), which is considered as the most destructive viral disease of cultivated fruit plants such as plums, apricots and peaches
Determination of interaction between selected histagged domains of Receptor for Advanced Glycation Endproducts (RAGE), immobilized on the redox active monolayer, and amyloid beta (Aβ) peptide which could serve as biomarker for early detection of Alzheimer disease.

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P-22: Interactions of modified oligonucleotides with RNA of the prokaryotic and eukaryotic decoding site

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Many known antibiotics that hinder protein synthesis in bacteria, target various functional regions of ribosomal RNA (rRNA). In principle, rRNA function can also be inhibited in a sequence-specific manner by using short oligonucleotides hybridizing with functional rRNA motifs. Indeed, somemodified oligonucleotides were shown to hybridize with the RNA of bacterial ribosomes and inhibit the translation process [1]. Typically, the oligonucleotides are designed based solely on the sequence of rRNA, and the secondary and tertiary structures of the targeted rRNA motif are not taken into account. However, to increase the binding affinities of such oligonucleotides their interactions with RNA need to be fully elucidated, taking into account thermodynamic stability, structural and dynamical properties of the RNA target.

We have studied the binding of three S-DNA and 2'O-methyl-RNA decamers complementary to the models of prokaryotic and eukaryotic rRNA decoding sites (A-sites). We have used a structural model of the bacterial ribosomal target: the HX RNA construct [2] (PDB: 3LOA), which contains a fragment of the helix h44 of 16S rRNA.

The thermodynamics of binding was determined by the UV-monitored thermal denaturations and isothermal titration calorimetry (ITC) experiments. We have studied the specificity of the oligonucleotides, by testing the effect of one, two or three mismatches on the binding process. We have also performed 300 ns explicit solvent molecular dynamics (MD) simulations of both rRNA models to characterize the flexibility of the targeted rRNA structures. The fluctuations of the nucleotides indicate which fragments of the target are more susceptible to strand invasion by oligonucleotides. We compare the solution binding experimental studies with the computational predictions derived from the analysis of the MD trajectories. We focus on the relationship between the flexibility of the target fragment and the binding energy between the oligonucleotide and the rRNA. In addition, we have compared the results obtained for model structures with in vitro studies employing whole ribosomal subunits in a cell-free transcription/translation system. We are looking for correlations between the physical parameters of binding of the oligonucleotides with their inhibitory efficiency on bacterial translation. Detailed study of the interaction between modified oligonucleotides and rRNA model structures is crucial for understanding the mechanisms of action of the therapeutic antibacterial oligonucleotide-containing compounds.

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P-23: Biophysics meets Virology: New Insights into HBV Protein — Cellular Protein Interactions

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Currently, there are no effective cures to treat the chronic HBV infections that afflict 350 million people worldwide. Viruses often mimic human interaction motifs in order to hijack cellular factors to their own ends, frequently with pathogenic consequences. HBV membrane protein(s) must interact with viral nucleocapsids at precisely the right time to ensure timely envelopment, thereby yielding mature virions that exit infected host liver cells by the cellular exocytosis pathways. In this work, an essential HBV-host protein-protein interaction — previously only described *in vivo* — was successfully recapitulated *in vitro*. This interaction was investigated at atomic resolution using a combination of nuclear magnetic resonance spectroscopy, extensive protein engineering, isothermal titration calorimetry, multi angle laser light scattering and X-ray crystallography. We demonstrated that synthetic peptides can completely ablate this protein-protein interaction, which is essential to HBV replication. Our work provides important new insights into structural biology and thermodynamics of binding of HBV-host protein-protein interactions. Importantly, the knowledge we have gained will be used in our drug development programme, with the long term view of generating much needed, novel HBV antivirals.

P-24: Activation energy for diffusion of proteins in crowded environment

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Diffusion is one of the most important mechanisms of mass transport in living cells. It is responsible for signaling and metabolic processes occurring in living cell. The cytoplasm of living cells is an extremely crowded environment consists of proteins and other macromolecules. In our recent papers [Kalwarczyk 2011; Kalwarczyk 2012] we have shown that the mobility of small (macro)molecules, in the cytoplasm of eukaryotic and of prokaryotic cells, is affected by the length-scale dependent viscosity. The length-scale dependent viscosity is the one effectively experienced by the molecule diffusing in the crowded environment; in particular the cytoplasm. It is dependent on the scale of flow around moving molecule and increase exponentially with size of the molecule. The concept of the length-scale dependent viscosity rely on geometrical properties of the system such as distance between crowding agents and their size. In terms of length-scale dependent viscosity the diffusion coefficient D is proportional to $\exp[-b(R_{eff}/\xi)a]$, where b is a constant, R_{eff} is a function of hydrodynamic radius of the diffusing molecule and hydrodynamic radius of the crowding agents, ξ is a correlation length, and a is the exponent of the order of 1. On the other hand diffusion in crowded environment is thermally activated and can be expressed by Arrhenius like equation with D proportional to $\exp(E_a/RT)$ where E_a is an activation energy for diffusion [Masaro 1999], R is the gas constant and T is absolute temperature. In this work we combine those two models. We use aqueous solutions of polymers as a model crowded system and we measure $D(T)$ for probes varied in size in different concentrations of polymer. We propose that parameter b in our equation is equal to E_a/RT. We show that E_a depends on hydrodynamic radius of the diffusing protein, and approaches constant value when hydrodynamic radius of the diffusing object tends to infinity.

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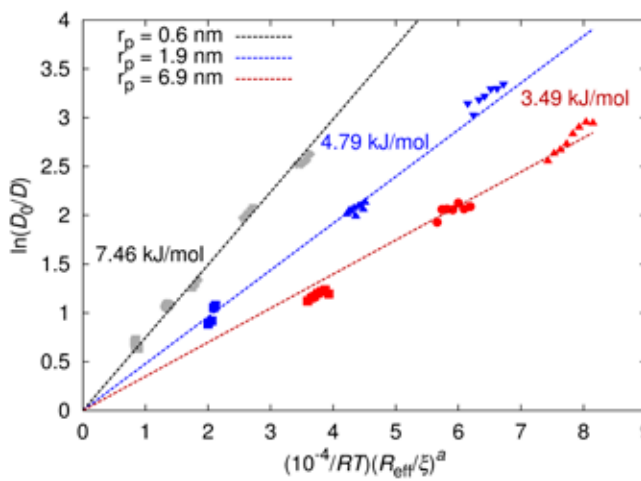

P-25: Upconverting Gd_2O_3, $Gd_3Al_5O_{12}$, $Y_3Al_5O_{12}$ nanoparticles for *in vitro* fluorescent imaging

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Upconverting (UC) materials have attracted attention as potential solid state lasers and elements for medical diagnostics and therapeutics. Previous attention has been focus on halides and sulfides because of their high upconversion efficiency. However, their application has been greatly restricted due to relatively high production cost and poor chemical stability in aqueous solutions. Recently, oxide ceramics or nanopowders with high chemical stability and low phonon energy have been adopted as the host candidates for upconversion materials.

We tested Gd_2O_3 nanoparticles, combinations of Gd_2O_3 and Al_2O_3, such as GAG ($Gd_3Al_5O_{12}$) and YAG ($Y_3Al_5O_{12}$) doped Yb and Er, Gd, Pr, Tb, Tm. They were prepared by two independent methods: combustion synthesis (with/without NaCl) and solution aerosol method. The nanoparticles characterization was performed by using: XRD, TEM, SEM, EDX and PL.

The nanoparticles were applied as luminescent markers in biological materials. They were incubated with HeLa cells. Their presence in the cells was confirmed using a confocal microscope. As a result of endocytosis the nanoparticles were visualized in the cellular cytosol. Applying infrared illumination (980nm) autofluorescence was minimized and no cellular damage was observed under our experimental conditions.

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P-26: CABS-flex: server for fast simulation of protein structure fluctuations

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The CABS-flex server [1] implements CABS-model based protocol for the fast simulations of near-native dynamics of globular proteins. In this application, the CABS model was shown to be a computationally efficient alternative to all-atom molecular dynamics (MD) [2]. The simulation method has been validated on a large set of MD simulation and NMR ensemble data. Using a single input (user-provided file in PDB format), the server outputs an ensemble of protein models (in all-atom PDB format) reflecting the flexibility of the input structure, together with the accompanying analysis (residue mean-square-fluctuation profile and others). The ensemble of predicted models can be used in structure-based studies of protein functions and interactions. The CABS-flex method is freely accessible at http://biocomp.chem.uw.edu.pl/CABSflex

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P-27: Stochastic transitions in a bistable reaction system on the membrane

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Transitions between steady states of a multi-stable stochastic system in the perfectly mixed chemical reactor are possible only because of stochastic switching. In realistic cellular conditions, where diffusion is limited, transitions between steady states can also follow from the propagation of travelling waves. Here, we study the interplay between the two modes of transition for a prototype bistable system of kinase-phosphatase interactions on the plasma membrane. Within microscopic kinetic Monte Carlo simulations on the hexagonal lattice, we observed that for finite diffusion the behaviour of the spatially extended system differs qualitatively from the behaviour of the same system in the well-mixed regime. Even when a small isolated subcompartment remains mostly inactive, the chemical travelling wave may propagate, leading to the activation of a larger compartment. The activating wave can be induced after a small subdomain is activated as a result of a stochastic fluctuation. Such a spontaneous onset of activity is radically more probable in subdomains characterized by slower diffusion. Our results show that a local immobilization of substrates can lead to the global activation of membrane proteins by the mechanism that involves stochastic fluctuations followed by the propagation of a semi-deterministic travelling wave.

P-28: Fibril formation rates and irreversibility

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It is well known that the protein aggregation is a slow and irreversible process. If the size of the preformed template exceeds the nucleus critical size, then its thermal fluctuations are expected to be small and the fibril elongation follows the seeding mechanism where a nascent monomer is added to the template one-by-one. The effect of the template fluctuations and irreversibility on fibril formation rates has not been explored either experimentally or theoretically so far. In this paper we make the first attempt to consider this problem by performing the simulations where monomers of the preformed template are kept fixed. The kinetics of addition of a new peptide onto the template is explored using all-atom simulations with explicit water and the GROMOS96 force field 43a1. It was shown that the docking of a nascent monomer onto the fixed template may proceed with intermediates. The immobility of template greatly increases time for incorporating a new peptide into the preformed template, τ_{inc}, compared to the case of the fluctuating template.

P-29: Structural and energetic determinants of co-translational folding

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We performed extensive lattice Monte Carlo simulations of ribosome-bound stalled nascent chains (RNCs) to explore the relative roles of native topology and non-native interactions in co-translational folding of small proteins. We found that the formation of a substantial part of the native structure generally occurs towards the end of protein synthesis. A detailed assessment of the conformational ensembles populated by RNCs with different lengths reveals that the directionality of protein synthesis has a fine-tuning effect on the probability to populate low-energy conformations. In particular, if the participation of non-native interactions in folding energetics is mild, the formation of native-like conformations is majorly determined by the properties of the contact map around the tethering terminus. Likewise, a pair of RNCs differing by only 1-2 residues can populate structurally well-resolved low energy conformations with significantly different probabilities. An interesting structural feature of these low-energy conformations is that, irrespective of native structure, their non-native interactions are always long-ranged and only marginally stabilizing. If non-native interactions participate strongly in protein energetics, they completely overcome the topological features of the native structure and drive the formation of transiently populated conformations, which leads to energetically broad and structurally heterogeneous ensembles of conformations across different chain lengths. In the limit of completely synthesized chains we find that the excluded volume effect imposed by the ribosome together with abundant non-native interactions can drive a local topology into misfolding, whereas a native topology dominated by long-range interactions still folds correctly, although not necessarily *via* a strict two-state transition. Thus, the empirical correlation between folding rate and contact order may not apply to surface-tethered protein folding. A comparison between the conformational spectra of RNCs and chain fragments folding freely in the bulk reveals drastic changes amongst the two set-ups depending on the native structure. Furthermore, they also show that the ribosome may enhance (up to 20%) the population of low energy conformations for chains folding to native structures dominated by local interactions.

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P-30: Clindamycin ribosome interactions: a molecular dynamics study

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Antibiotics are drugs that treat the diseases of bacterial and fungal origin. Secondaryuses include strengthening the immune system in cases of lowered immunity. Clindamycin isone of the antibiotics from the lincosamide class which are used to treat diseases causedmostly by *Gram-positive* bacteria and against some protozoal diseases. Lincosamidesinteract with the bacterial large (50S) ribosomal subunit and inhibit the process of proteinsynthesis leading to bacterial cell death. The increase of resistance of many bacterial strains against known antibiotics is caused by the expanded use of antibiotics in medical practice and veterinary. This is a very important reason for continuous work to find new, better and more effective antibacterial drugs.
Mutations of the antibiotic target are one of the common modifications that lead to bacterial resistance because such alterations typically prevent proper binding of the antibiotic in the targeted site. Currently, there are three structures of clindamycin in the complex with the 50S [1-3] ribosomal subunit available in the Protein Data Bank coming from different organisms. Interestingly, two of the structures show significantly different conformations of the drug. The aim of this study was to compare the dynamic properties of the clindamycin binding site in the 50S subunit with and without the A2058G mutation to understand why this nucleotide substitution blocks the binding of lincosamides. To achieve this goal we applied full-atom molecular dynamics. Using the NAMD [4] package we performed four types of simulations: (1) the complex of clindamycin with the fragment of the 50S subunit of the ribosome as well as (2) the unbound ribosome fragment, (3) the complex of clindamycin with the mutated ribosome fragment and (4) the unbound mutated ribosome fragment.
To prepare the starting systems for the simulations we chose the 3OFZ 50S subunit structure from *Escherichia coli*, which consists of ribosomal RNA, ribosomal proteins, one clindamycin molecule, magnesium and zinc ions and crystal waters. For our simulations, we cut a sphere with the radius of about 20Å around clindamycin to account for the long-range interactions of the antibiotics in the 50S subunit. We added 228 K^+ ions to neutralize the charge and approximately 27000 TIP3P water molecules to solvate the system shaped in a truncated octahedron around the complex. The effect of the mutation on clindamycin positioning in the binding cleft resulting from these molecular dynamics simulations will be discussed.

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P-31: Plasmon control chirality in photosynthetic complexes

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Plasmon excitations in metallic nanoparticles are effective means for controlling the optical properties of naturally evolved and artificial emitters [1-8]. Recent work has demonstrated that the emission of light-harvesting complex, peridinin-chlorophyll-protein can be enhanced by coupling to inhomogeneous distribution of silver islands [2, 5], while fluorescence and absorption of the Photosystems can increase as well by attaching gold nanoparticles [3, 4].
Due to strong interactions between the pigments embedded in light-harvesting complexes they exhibit strong chirality, also known as circular dichroism. In this work we describe experimental results obtained for several geometries involving light-harvesting complexes and metallic nanostructures. In these structures we observe not only strong enhancement of the fluorescence intensity due to interaction between the pigments and plasmon excitations in metallic nanoparticles. Importantly, we find that the chirality of the light-harvesting complexes can be enhanced via plasmonic interactions.
Our findings demonstrate that metallic nanostructures are efficient means for controlling the optical properties of functional biomolecules.

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P-32: Conformational analysis of original fragment of the IGD protein and its derivatives: the influence of charged amino-acid residues on the β-hairpin structure

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Chain reversals are often nucleation sites in protein folding. In this study we continued our investigation of the possibility of the induction of a chain reversal by the presence of charged residues at the end of a loop, which screen loop's nonpolar residues from solvent. For this purpose, we studied the solution conformations of the following four peptides with sequences taken from immunoglobulin binding protein G (PDB code: 1IGD) derived from Streptococcus: Ac-DDATKT-NH2 (1IGD 51-56) (hereafter referred to as Dag1) and its mutants: Ac-DVATKT-NH2 (Dag2), Ac-OVATKT-NH2 (Dag3) and Ac-KVATKT-NH2 (Dag4) by circular dichroism (CD) and nuclear magnetic resonance spectroscopy (NMR). Our previous potentiometric titration studies (determination of the pK_a values of charged groups) for the all four peptides showed that in the original sequences there are interactions between the charged Asp2 group and Lys5 group meaning that their presence is probably necessary to keep the bent structure. For the Dag2 peptide such strong tendency is not observed. In Dag3 and Dag4 chain reversal seems to be caused by screening of the nonpolar core from the solvent by the hydrated charged residues. Results of the NMR and MD studies presented here support these conclusions.

P-33: Amino Acids and Proteins at ZnO-water Interfaces in Molecular Dynamics Simulations

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We determine potentials of the mean force for interactions of amino acids with four common surfaces of ZnO in aqueous solutions. The method involves all-atom molecular dynamics simulations combined with the umbrella sampling technique. The profiled nature of the density of water affects the approach of an amino acid to the surface and makes the optimal binding energies to be of order 4 kJ mol^{-1} which is comparable to those of the hydrogen bonds in a protein. In vacuum, they are nearly 40 times stronger. The precise manner in which the density of water molecules is profiled influences the binding energies in a crucial way and makes them different than binding to, for instance, gold surfaces. For a given surface, the energies vary between amino acids with the dispersion of 15% of the mean. The means vary with the dispersion of 6% between the four surfaces. A small protein is shown to adsorb to ZnO only intermittently and with only a small deformation. Various binding events lead to different patterns in mobilities of amino acids.

P-34: The effect of depletion layer on diffusion of nanoparticles in polymer solutions

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Depletion layer is an exclusion zone that forms around a particle immersed in a solution of non-adsorbing polymer. The effect occurs due to the changes of configurational entropy of polymer chains: Close to the particle surface, the entropy strongly decreases because of chain deformation, so that the centres of masses of polymer chains are excluded from that region. This results in a polymer concentration gradient across the depletion layer boundary.
We use the "walking confined diffusion" model to describe the motion of nanoparticles in the presence of depletion layer. The depletion layer is approximated by a shell with reflecting boundary, which diffuses itself but hinders the diffusion of the particle enclosed in it. The presence of depletion layer leads to nonlinear dependence of the mean square displacement (MSD) on time: fast diffusion in a short time scale and slow diffusion in a long time scale. This two-scale diffusion may be an important factor affecting e.g. chemical reaction rates in crowded environments.
We compared with experiments two variants of the model: The single-shell model very well reproduces the measurement results for solutions of monodisperse and rigid polymers (fd-viruses and F-actin). The double-shell model shows a very good agreement with the experimental data for solutions of flexible and polydisperse polymers (PEG).

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P-35: Characterization of curli A production on living bacterial surfaces by scanning probe microscopy

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Curli are adhesive surface fibers produced by many Enterobacteriaceae, such as *Escherichia coli* and *Salmonella enterica*. They are implicated in bacterial attachment and invasion to epithelial cells. In this study, atomic force microscopy (AFM) was used to determine the effects of curli on topology and mechanical properties of live *E. coli* cells. Young's moduli of both curli-deficient and curli-overproducing mutants were significantly lower than that of their wild-type strain, while decay lengths of the former strains were higher than that of the latter strain. Surprisingly, topological images showed that, unlike the wild-type and curli-overproducing mutant, the curli-deficient mutant produced a large number of flagella-like fibers, which may explain why the strain had a lower Young's modulus than the wild-type. These results suggest that the mechanical properties of bacterial surfaces are greatly affected by the presence of filamentous structures such as curli and flagella.

P-36: Thermal unfolding of trp-cage miprotein variants. NMR and SAXS study

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The Trp-cage is a 20 amino acid residue peptide/miniprotein which folds into a well defined three-dimensional structure, [1], with folding temperature estimated to be in range 311-317 K (depending on the experimental method used). Recently, using NMR spectroscopy we showed [2] that, with temperature increase up to 313K, the trp-cage miniprotein undergoes structural rearrangements which could be summarized as follows: i) conformational phase transition (melting) that could be described as a cooperative breaking of the Trp6-Pro12 long-range hydrophobic interactions and the melting of the N-terminal α-helix; ii) up to 313K indole ring of the Trp6 residue is strongly shielded from interactions with the solvent; iii) many ROE signals corresponding to local or short-range interactions vanishing rapidly with temperature increase, however long-range interaction such as Trp6-Arg16 remains until 313K. Moreover, because of the presence of the native long-range interaction at 313K, the conformational ensemble resembles a very diffuse native state but not the mixture of the folded and unfolded states, as it should be expected based on the common two-state theory. We will present the results of our investigation using a combination of NMR spectroscopy and SAXS measurements on several variants of the trp-cage protein characterized by a wide range of folding temperatures (from 264 to 336 K). Preliminary results suggest that, regardless of the sequence studied, the mechanism of thermal unfolding remains unchanged (melting of the N-terminal alpha-helix coupled with a decrease of the number of the long-range interactions), as found for the wild type trp-cage sequence studied ealier [2]. The results of SAXS measurements indicate that, for all trp-cage variants studied, the chain remains pretty compact, even at the temperatures well above the melting temperature determined earlier based on result from Ramman spectroscopy measurements [3].

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P-37: Protein structures from Monte Carlo simulations with NMR restraints

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In this contribution we describe how the amount of experimental data such as NOE restraints and chemical shifts affects the resolution of calculated model protein structures. The models were obtained by Biosimulations package of BioShell suite- a new protein modeling tool which uses both knowledge based and molecular mechanics force fields. Conformational space of a system is explored by means of Monte Carlo sampling. NOE experimental data (obtained from the BMRB database) has been introduced into simulations as distance restraints. Chemical shift measurements were converted to main chain dihedral angles with TALOS+ software and imposed as dihedral restraints.

P-38: Motional characteristics of cholesterol in pure cholesterol domains that form in lipid bilayers a computer modelling study

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Experimental studies using Electron Paramagnetic Resonance (EPR) spectroscopy [1] show that pure cholesterol domains, called the cholesterol bilayer domains, are formed in the phosphatidylcholine (PC) bilayer containing cholesterol (Chol) when the Chol:PC molar ratio exceeds 1. The organization of these domains is different than that of the cholesterol crystal. To get atomic scale characteristics of Chol in the Chol domain, we have carried out a comparative molecular dynamics (MD) simulation study of the organization and dynamics of Chol molecules in three different environments: (1) a hydrated pure Chol bilayer that models the Chol bilayer domain, which is a pure Chol domain embedded in the bulk membrane; (2) a PC bilayer saturated with cholesterol where the Chol:PC molar ratio is 1 (PC-Chol50) that models the bulk membrane; and (3) a Chol crystal. Each bilayer was hydrated with 30 water molecules per lipid. Our MD simulations indicate that structural features and motional behaviour of Chol molecules are qualitatively the same in the PC-Chol50 and the Chol bilayers, although diffusional motion of Chol molecules in the Chol bilayer is slower. They also show that water accessibility to Chol-OH groups in the Chol bilayer is not limited. On average, each Chol molecule makes 2.3 hydrogen bonds with water in the Chol bilayer, as compared with 1.7 hydrogen bonds in the POPC-Col50 bilayer. These characteristics of Chol are entirely different from those in monohydrate [2] or anhydrous [3] cholesterol crystals where Chol molecules are immobile and de- or low-hydrated. The results of this MD simulation study strongly support the conclusions of experimental EPR studies, however, are at variance with calorimetric [4] and X-ray diffraction [4] studies which suggest that Chol domains in the bilayer possess the same properties as those of Chol crystals our studies imply that these crystals most likely form outside the lipid bilayer and cannot be identified with pure Chol bilayer domains within the lipid bilayer.

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P-39: Self-assembly of nanoparticles into clusters and stripes.

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Most of biologically relevant macromolecules, or particles in soft-matter systems are charged and repel each other with screened electrostatic forces. On the other hand, complex solvents in biological systems may induce effective attraction between the macromolecules. I will present generic lattice model for systems containing particles interacting with short-range attraction and long-range repulsion (SALR) potential, and describe results obtained in one dimension and in two dimensions on a triangular lattice. In both cases the ground state was found analytically, and phase diagram was determined in Mean Field approximation. Moreover, in one dimension the equation of state and the correlation function were calculated exactly, and the two dimensional model was studied by means of Monte Carlo simulations Metropolis algorithm). The phase behaviour of the model is very rich. When the repulsion is sufficiently strong, periodic ordering of self-assembled clusters or stripes is obtained for some range of thermodynamic states. We find coexistence of ordered phases with the same symmetry but different degree of order, and reentrant melting upon decreasing temperature or increasing density.

P-40: Homodimerization of disordered N-terminal domain of Ultraspiracle protein — a member of the nuclear receptor family

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The yellow fever mosquito Aedes aegypti is a mosquito that can spread many serious diseases such as the dengue fever. In this insect, vitellogenesis is stimulated by ecdysteroids. The functional receptor for ecdysteroids is a transcription factor comprised of two nuclear receptors: the 20-hydroxyecdysone receptor (EcR) and the Ultraspiracle protein (Usp). Usp is an insect homolog of the vertebrate retinoid X receptor (RXR). RXR plays an important role in many fundamental biological processes such as reproduction, cell proliferation and differentiation and embryonic patterning. Nuclear receptors share a common structural organization with functional regions: a highly variable in sequence and length N-terminal domain (NTD), evolutionally conserved DNA binding domain (DBD) and a ligand binding domain (LBD). In fact, the architecture of Usp N-terminal domain is poorly conserved among species. Thus, its biochemical and structural characterization of NTD is crucial for understanding molecular function of Usp and its role in the control of the ecdysteroid signaling pathway. In our studies, we characterized biophysically and biochemically the full length N-terminal domain of Ultraspiracle (Usp) protein isoform B from Aedes aegypti (aaUsp-NTD). We established efficient two-step purification procedure and conducted preliminary bioinformatics analyses. Obtained results suggested that aaUsp-NTD exhibited properties of Intrinsically Disordered Protein (IDP) molecule. IDPs are a specific group of proteins that are characterized by a lack of stable unique 3D structure in a native state, either entirely or in part. Using size-exclusion chromatography (SEC), we investigated atypical hydrodynamic properties such as ca. 2 times larger hydrodynamic volume and ca. 1.5 times larger Stokes radius in comparison with a globular protein of the same molecular mass. Moreover, we examined GdmCl effects on the secondary structure, which confirmed structure-forming potential of aaUsp-NTD in the presence of refolding agent. Interestingly, using three independent experiments such as SEC, sedimentation velocity analytical ultracentrifugation (SV-AUC), and chemical cross-linking, we observed that the aaUsp-NTD exhibited tendency to homooligomerization and probably coexists in solution as monomeric and dimeric species. The results show that aaUsp-NTD is an IDP possessing residual secondary structures. Features of IDPs were reflected in an anomalous behavior of aaUsp-NTD in SDS-PAGE electrophoresis, in secondary structure analyses determined by the far-UV CD spectroscopy and SEC experiments.

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P-41: Linking multi-scale properties of biomolecules and machine learning prediction of their biological function

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Modern molecular biology provides a vast amount of experimentally verified information, mostly coming from high-throughput studies, such as the 1000 Genomes Project initiative [1], where large-scale sequencing is taking place. In typical drug design procedure pharmaceutical companies perform high-throughput screening studies, where biological activity on the selected protein target is performed for hundreds of thousands small chemical molecules [2]. Typical microarray experiments store relative expression profiles for thousands of genes in selected tens of time points [3]. Collecting and verifying currently available experimental data is therefore an important goal of bioinformatics, a rapidly developing computational discipline, which focuses on completing the functional annotation of proteins, inhibitors, DNA/RNA molecules, genes, or more generally all types of biomolecules that can be found in living cells [4].

I will present the evaluation of various computational and mathematical algorithms used for prediction of biological function. I will provide multi-scale, holistic view of biomolecules, therefore combining different single-scale techniques and simulation tools. I will focus here on proteins, metabolites, and small chemical molecules such as drugs. Protein-protein interactions are important for the majority of biological processes including signal transduction, hormone receptor binding or antigen-antibody interactions and many others [5]. A significant number of computational methods have been developed to predict protein-protein interactions. Recent advances in high-throughput experimental techniques lead to the increase of the number of known bimolecular interactions that are available in public databases. Yet, this information is stored in different data formats, or presented in various ways, therefore researchers tackle with serious obstacles, when willing to perform 'omics' analyses.

First, I will present data fusion techniques used together with manual data curation to prepare high-quality training datasets [4]. Later, various machine learning and clustering methods are evaluated in order to facilitate the functional annotation of biomolecules based only on partial biological information describing them (such as sequences in the case of proteins) [6]. In the case of proteins, the subset of known proteins with crystallized three-dimensional structures, where both protein sequence and structure is directly linked to performed protein biological function [7]. The computational approaches include various machine learning techniques, dynamic programming methods, clustering techniques, or similarity searchers. Moreover, classical physico-chemical methods, such as protein-ligand docking and scoring functions, can be combined with pattern recognition algorithms.

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P-42: NA2Dsearch: fast and easy tool for secondary structure searches through large datasets in parallel

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We present here a new tool for searching in RNA secondary structures. The program performs a search for a motif depicted by a descriptor in a database of secondary structures. Sequence databases can be also searched with exploitation of external folding programs. We introduced a comfortable graphical user interface as the program aims to be usable for a wide constituency of users lacking extensive computer skills. The motif descriptor composed of single- and double-stranded regions can be constructed in a visual way. Each region allows to define minimal, maximal and optimal length, optional sequence and score weight that can express regions of significance. An easy way of defining bulges or inner-loops that are tolerated within a given double stranded region is offered as well. Internally, a tree representation for the secondary structure is used and each single- or double- stranded region is represented as a single node. The algorithm traverses a structure tree and in utilization of NDFA (non-deterministic finite automaton) looks for matching pattern in target structure. The program is multi-threaded Java application fully utilizing current multi-core processors. Search speed is around 10^4 structures per second (2.8GHz, 4GB RAM) and is primarily influenced by complexity of the query descriptor. NA2Dsearch extends a family of RNA secondary structure search programs and brings the comfortable user interface as well as high search speed and ability to deal with vast datasets.

P-43: Biopolymer elastic substrates — a novel tool in cell migration research

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Cell migration is one of the most important biological processes. It is connected to several biological phenomena like embryonic development, neural development, wound healing and immunological processes. Moreover, cell migration is also involved in pathological processes like arthritis, osteoporosis and cancer metastasis. For these reasons studies of cell migration regulatory processes are of particular interest for science.

In this work we present the development of acrylamide elastic substrate as a relatively novel research tool in studies of cell migration. It is known that adherent and migrating cells respond to the mechanical properties of their environment by changing their regulatory pathways. Most studies of cell migration were done on stiff substrates, mostly on glass or plastic. The main goal in development of elastic substrates is to imitate the natural cellular environment in soft tissues. We adapted known methods to prepare polyacrylamide substrates with well defined elasticity for cell migration studies. Fish scale keratocytes were tested as model, fast migrating cells on these substrates. Our results show that migration parameters like average velocity, directional persistence and even cell shape are correlated with the elasticity of the substrate. Moreover, it is possible to determine cell tractions during migration by measuring elastic substrate deformations caused by cells migrating on that elastic substrate.

In conclusion, development of elastic susbtrate for migrating cells can provide new important tool to study mechanotransduction processes in migrating cells.

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P-44: Protein adsorption and deformations on solid surfaces

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Protein adsorption on solid surfaces is a fundamental problem in biophysics with many important applications, including biosensing, biomaterial design, drug delivery and industrial chemistry. Numerical modeling should help predict the details of interactions between proteins and solid surfaces but choosing the proper model is not obvious. All-atom models are restricted to simulations of peptides and small proteins on nanosecond time scales. Therefore, we have devised a coarse-grained model to simulate the processes of adsorption and desorption of large proteins. The latter model is a structure-based model with amino-acid dependent parameters adapted from all-atom simulations. Preliminary results show that the adsorption of various proteins on ZnO surfaces typically does not lead to any noticeable protein deformations. We therefore suggest that many proteins on ZnO surfaces should maintain their functionality.

P-45: Langevin dynamics simulation of a homopolymer chain translocation process through a nano-pore: translocation parameters study

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Langevin dynamics simulation is used to study the dynamics of a homopolymer chain translocation through a nanopore. The system is modeled as a flexible homopolymer chain that translocates through a hole in a solid. The effects of the force applied on the homopolymer chain, the homopolymer chain length and the nano-pore length on the translocation time are investigated.

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P-46: Introduction of new Cα... Cα...Cα virtual valence angle potentials to treat D and L amino acid residues into UNRES force field

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The newly introduced D-amino acid torsional, double torsional, side chain potentials into United Residue (UNRES) force field are in this work extended by new virtual valence angle potentials. The new virtual bond angles potentials were determined by integrating the ab initio energy surfaces of terminally-blocked glycine, alanine and proline calculated in our earlier work at the MP2/6-31G(d,p) level (Sieradzan *et al*., 2012, *J Chem Theory Comput* **8**: 4746), where alanine represents all types of amino-acids residues except for glycine and proline. This resulted in 126 (63 for each terminally blocking system) different free-energy surfaces. Obtained potentials allowed to fully describe energy function in UNRES force field for D-amino acids without previously used pseudo-symmetry.The obtained potentials were tested on the helix stability of model KLALKLALxxLKLALKLA peptide. Verifying the accuracy of newly added potentials. Additionally, newly introduced were tested on natural occurring peptide Thurincin H from *Bacillus thuringiensis* which has D-amino acids in sequence. We present that sulfur-carbon constrains were not mandatory for the folding process of this protein.

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Reference:
Sieradzan *et al*. (2012) *J Chem Theory Comput* **8**: 4746.

P-47: Up-converted $NaYF_4$: Er, Yb nanoparticles for biomedical applications

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For biological applications, it is recommended to use fluorescent markers that can be excited in the near-infrared (NIR) region. We synthesized Er, Yb, Gd doped $NaYF_4$ nanoparticles, with up-conversion of infrared light capable to emit in the visible and the ultraviolet ranges. They could be potentially applied for diagnostic imaging and targeted cancer therapy, through light up-conversion to the ultraviolet radiation.

We synthesized $NaYF_4$ nanoparticles, doped with different amounts of rare earth ions, providing an efficient energy up-conversion. After functionalization by PVP, we were able to introduce these particles into HeLa cancer cells, where we examined their localization in the function of both, incubation time and particles concentration. For short incubation times and in the absence of Lipofectamine 2000 (transfection agent), the nanoparticles were not internalized. However, for short incubation times in the presence of the Lipofectamine vesicles, nanoparticles were endocytosed. Incubation with the liposomes caused enhanced cellular incorporation of the nanoparticles. We performed preliminary attempts to bio-functionalize nanoparticles for specific locations in the cancer cells.

Acknowledgements:
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P-48: Mechanical properties of TGF-beta family proteins

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Recent advances in experimental techniques such as Atomic Force Microscopy (AFM) and optical tweezers have allowed for manipulation of single biomolecules. This in turn enables one to determine elastic properties of proteins, including their general sturdiness and resistance to unfolding. These properties are crucial for structural proteins, such as the muscle-building protein titin, which was among the first proteins studied in this way, giving maximum resistance to stretching of 204 pN. Mechanical properties of proteins are also of great interest in the emerging field of nanotechnology, enabling one to use proteins in modern materials.

Transforming growth factors beta (TGF-beta) form a family of extracellularly secreted proteins abundant in multicellular organisms, responsible for many processes, from embryo development to epithelium growth. However, these proteins are extremely interesting not only because of their biological significance, as they possess two features which make them an appealing objects of mechanistic studies. One of them is one the most remarkable structural motifs found in nature — cystine knot, which stabilizes a mature version of protein. It comprises a piece of a backbone closed by two disulfide bonds, forming a ring, and another disulfide bond connecting another two pieces of the backbone through the ring. Additionally, TGF-beta proteins were recently found to be force-activated. Mechanical tension is required to peal TGF-beta from the propeptide, with which they are expressed.

We carried out molecular dynamics studies using a simplified model to study TGF-beta proteins. They were predicted [1] to withstand forces of unprecedented scale of 1 nN. Their stability is based on cystine slipknot motif, in which the maximal resistance to stretching is due to dragging of a backbone piece through the ring incystine knot. Our recent study of dimeric proteins [2] revealed novel mechanisms in which cystine slipknots play a crucial role. Presence of four possible pulling directions, as opposed to simple pulling by two termini in monomeric proteins, indeed creates a richer spectrum of behaviours. In one of them, cystine slipknot mechanism is enriched by the presence of an additional loop, also closed by a disulfide bond. Undertension, this loop is forced through the cystine ring building a steric hindrance, which results in record-breaking force of 1.5 nN. We analyze this and similar systems and propose novel materials based on unprecedented energy-absorbing properties of the system, for which possible uses range from surgical threads to bio-compatible shock absorbers.

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[2] Sikora M, Cieplak M (2012) *PRL* **109**: 208101.

P-49: Proteins in the electric field near the surface of mica

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We elucidate the nature of the electric field produced by a model mica surfaceand show that above some 0.4 nm it is nearly uniform and of order 12 V/nm. The presence of ions in the solvent above the surface, up to the concentration of about 300 mM, does not modify the nature of the field much. We study the conformational changes of a small protein, the tryptophan cage, as induced by a) uniform electric field and b) the electric field near mica. We use all-atom molecular dynamics simulations and provide evidence forthe existence of unfolded and deformed conformations in each of these cases. The two behaviors are characterized by distinct properties of the radius of gyration and of the distortion parameter that distinguishes between elongated and globular shapes. The nature of the conformations shifts with the strengths of the uniform field in a manner that depends on the nature of the simulation box — whetherit is bounded by neutral walls or not — and on the ionic concentration. Near the mica surface, on the other hand, the fraction of unfolded conformationis found to be smaller at 350 mM than at 20 mM but to be still substantial.

P-50: Synthesis of cap analogues with double thiophosphate modification intended for anti-cancer immunization with mRNA

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Cap analogues are chemically modified derivatives of the unique cap structure present at the 5′ end of all eukaryotic mRNAs. One of the most exciting applications of cap analogues is mRNA-based cancer immunotherapy. In this promiscuous therapy, mRNA coding specific antigen is introduced to dendritic cells to elicit an immune response. One obstacle that has to be overcome for successful treatment is insufficient stability and translation efficiency of mRNA in cellular conditions.

Here we present the new cap analogues with double thiophosphate modification — a simple synthetic tools for amendment of aforementioned mRNA defects after their incorporation into 5′-end of mRNA. This unique double thiophosphate modification is designed for assure the resistance of pyrophosphate bond to decapping enzyme complex Dcp1/Dcp2 and to improve cap binding to eukaryotic translation initiation factor eIF4E. We introduce the novel synthetic method for the preparation of nucleotides possessing such modification (using microwave chemistry), synthesis of exemplary cap analogues (Fig. 1) and some insight into biological properties of these new compounds.

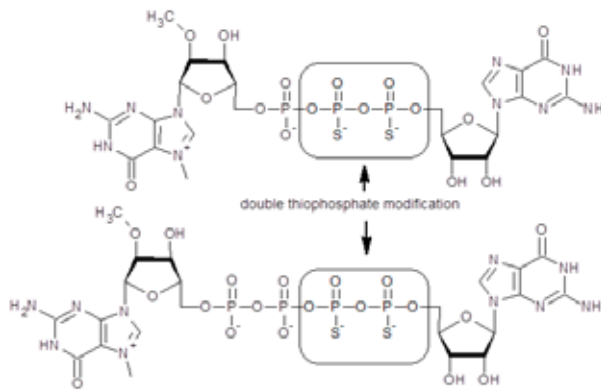

Figure 1: Synthesized cap analogues possessing double thiophosphate modification.

P-51: Genomics-aided structure prediction

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We introduce a theoretical framework that exploits the everincreasinggenomic sequence information for protein structure prediction. Structure-based models are modified to incorporate constraints by a large number of non-local contacts estimated from direct coupling analysis (DCA) of co-evolving genomic sequences. A simple hybrid method, called DCA-fold, integrating DCA contacts with an accurate knowledge of local information (e.g., the local secondary structure) is sufficient to fold proteins in the range of 1–3 Å resolution.

Reference:
Sułkowska JI *et al*. (2102) *PNAS* **109**: 10340-10345.

P-52: The analysis of electrostatic interaction energy during unbinding of nonspecific lac repressor-DNA complex

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The searching of transcription factors for their target sites is accelerated *via* converting their three-dimensional diffusion in cytoplasm into one-dimensional diffusion along DNA. The key in the conversion between the one-dimensional diffusion and three-dimensional diffusion lies in the unbinding of transcription factors from their nonspecific DNA chain. In this study, we presented a computational simulation on the electrostatic interaction energy in this unbinding process. We used the DNA binding domain (DBD) of a dimeric lac repressor (amino acids 1-62) as an example for transcription factor. We gradually increased the distance between lac DBD and DNA to mimic the unbinding process. We used 20 available structures of nonspecific complexes of lac DBD and DNA to calculate the electrostatic interaction energy. We calculated that when the lac DBD dissociates from the DNA chain, the electrostatic interaction energy increases and then decreases, with a maximum value at a distance of 0.12 nm away from the original position. This phenomenon indicates that the unbinding of lac DBD from DNA into the 0.12 nm region is not favored by electrostatic interaction energy. But when the lac DBD succeeds to bypass the barrier of 0.12 nm, the escape of lac DBD from DNA into environment solution is a favorable process by electrostatic interaction energy. We calculated that in water solution lac DBD needs 15.40 kT of electrostatic interaction energy on average to bypass the energy barrier of 0.12 nm. When the salt concentration increases from 0 M to 0.2 M, this energy barrier decreases to 14.14 kT on average, but the maximum value of electrostatic interaction energy is still at a distance of 0.12 nm away. The structured region (amino acids 1-50) is the region that directly contacts to the surface of DNA in nonspecific complex of lac DBD-DNA. We calculated that the structured region only contributes part of the electrostatic interaction energy of the entire lac DBD. The electrostatic interaction energy barrier of structured domain is 12.93 kT on average in water solution and is smaller in salt solutions. The salt dependence of the electrostatic interaction energy ($\partial(\Delta G_{el})/\partial(\log C)$) for structured domain is 4.240 kT/M on average, but for the entire lac DBD is 10.996 kT/M on average. We also calculated that 5.43 ions are released into solution when lac DBD binds with DNA, and 2.09 ions are released into solution when structured lac DBD binds with DNA. The meaning of the energy barrier is also discussed.

P-53: Import of knotted proteins into mitochondria

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In recent years a surge of interest has arisen in properties and function of knotted proteins. As more and more knotted structures are discovered in the Protein Data Bank, it becomes increasingly important to understand how, if at all, the non-trivial topology affects the protein's function in the cell. In particular, it has been hypothesized that the presence of a knot in the polypeptide backbone may affect the ability of knotted proteins to be degraded in proteosome or translocated through the intercellular membranes, e.g. during import into mitochondria. In these processes, the translocating proteins typically have to pass through constrictions that are too narrow to accommodate folded structures, thus translocation must be coupled to protein unfolding. However, as shown in a number of theoretical and experimental studies the protein knots get tightened under the tension. The radius of gyration of the tight knot is about 7-8 Angstrom, whereas the diameters of the narrowest constriction of the mitochondrial pores are in the 12-15 Angstrom range, making it possible for the knots to get stuck during the translocation process. In this communication, we report the result of molecular dynamics simulations of knotted protein translocation which show how such topological traps might be prevented by using a pulling protocol of a repetitive, on-off character. Such a repetitive pulling is biologically relevant, since the mitochondrial import motor, like other ATPases transform chemical energy into directed motions via nucleotide-hydrolysis-mediated conformational changes, which are cyclic in character.

P-54: Biologistics: kinetics of gene regulatory proteins in *Escherichia coli*

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Genome regulatory proteins, e.g. transcription factors (TF) and RNA polymerases (RNAP), use non-energy consuming strategies when searching for specific sites on DNA such as operators or promoters. According to the theory of facilitated diffusion proteins speed up the searching by means of one-dimensional diffusion along DNA chain, a process known as sliding. We will show that macromolecular crowding, present in the cytoplasm of *Escherichia coli*, causes that 1D diffusion constant is 3 orders of magnitude smaller than 3D diffusion making the sliding to be extremely slow and inefficient. Instead transcription factors take advantage of oligomerization and DNA looping. Moreover, *in vivo* concentrations of nucleoid associated proteins (NAP), which are responsible for structure and compactness of chromosome, match optimal conditions for the searching by transcription factors and RNA polymerases.

P-55: Metal-Enhanced Fluorescence of Chlorophylls in Light-Harvesting Complexes Coupled to Silver Nanowires

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Collective oscilation of free electrons in metalic nanoparticles, called localized surface plasmon resonance, can enhance the emission intensity of a fluorophore. The existence of an additional electromagnetic field from plasmons can cause either enhancement of absorption rate or enhancement of the radiative rate of fluorophore, or both of them [1].
In our experiment we examine the influence of plasmon excitations in silver nanowires (AgNWs), synthesized by the polyol method, on the fluorescence of the peridinin-chlorophyll-protein (PCP) complex, isolated from algae *Amphidinium carterae*. The absorption spectrum of AgNWs, with a strong peak at about 400nm, corresponds well to the absorption of the PCP complex. Therefore, we expect to observe fluorescence enhancement of the PCP emission in such a hybrid nanostructure. Moreover, the ability to image the nanowires using confocal or wide-field microscopy enables direct correlation between the morphology of the sample and the corresponding fluorescence pattern.
Samples were prepared in two ways: in the first way we mixed PCP solution with nanowires and then spin-coated it on a coverslide. In the second way we placed a layer of AgNWs and then spin-coated a layer of protein solution on it. Fluorescence maps were measured by inverted wide-field microscope Nikon Ti-U equipped with iXon EMCCD detector for two excitation wavelenghts: 405nm and 480nm. Spectrally and time-resolved measurements were performed using home-built scanning confocal/wide-field microscope. In this way we were able to fully characterize the spectral properties of AgNW-PCP assemblies.
Irrespective of the sample preparation method, we observed increase of the fluorescence emission of the PCP complexes in the vicinity of the silver nanowires. We also observed extremely bright hot-spots at the ends of the AgNWs. Fluorescence spectra are identical both in terms of overall shape and maximum wavelength for PCP complexes coupled and isolated from the AgNWs. Therefore, we conclude that interaction with plasmon excitations leaves no effect upon the biological functionality of the light-harvesting complexes. Fluorescence transients measured for the PCP complexes coupled to the AgNW indicate shortening of the fluorescence lifetime pointing towards modifications of radiative rate due to plasmonic interactions [2-3].
The results confirm that metallic nanoparticles can be applied for controlling the optical properties of biomolecules via plasmon excitation.

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P-56: Antimicrobial Photodynamic Therapy with Radachlorin® Blue O on *Streptococcus* mutans: an *in vitro* study

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SBMU

Introduction: *Streptococcus* mutans is the primary odontopathogen present in supragingival plaque which causes the oral disease known as dental caries. The main purpose of this *in vitro* study was to evaluate the antimicrobial effect of toluidine blue O (TBO) and Radachlorin®, in combination with a diode laser on the viability of *Streptococcus* mutans.

Material and Methods: Bacterial suspensions of *Streptococcus* mutans were exposed to either 0/1 mg/ml TBO associated with (20 mw, 3 j/cm^2, 633 nm diode laser, continous mode, 150 s) or 0.1% Radachlorin® and light irradiation (100 mw, 12 j/cm^2 , 662 nm diode laser, continous mode, 120 s). Those in control groups were subjected to laser irradiation alone or TBO/ Radachlorin® alone or received neither TBO/Radachlorin® nor light exposure. The suspensions were then spread over specific agar plates and incubated aerobically at 37°C. Finally, the bactericidal effects were evaluated based on colony formation.

Results: An approximately 99.99% of bacterial cell killing was only observed following photosensitization with TBO (p0001), whereas Radachlorin® showed significant reduction in dark condition compared to light exposure ($p<0.001$).

Conclusion: In conclusion, TBO-mediated photodynamic therapy seems to be more efficient than Radachlorin® in significantly reducing of the viability of *Streptococcus* mutans *in vitro*.

P-57: The devil is in the details: Atomic level characterisation of a potentially druggable target essential for human hepatitis B virus replication

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Despite the availability of a good prophylactic vaccine, over 2 billion people have been infected by human hepatitis B virus (HBV). Approximately 600,000 people die each year due to the consequences of chronic HBV infections; liver cirrhosis and hepatocellular carcinoma. Current therapies target the viral polymerase and have low efficacy, side effects and lead to the emergence of resistant strains. Hence, a better understanding of HBV life cycle at a molecular level is essential in order to develop novel antivirals against alternative, yet unexploited druggable targets. Here we present detailed, atomic level characterisation of a key protein-protein interaction central to HBV envelopment and export from infected hepatocytes. An intrinsically disordered protein (IDP) from HBV mimics motifs in host proteins in order to hijack the cellular transporting machinery. NMR chemical shift perturbation, extensive protein engineering and ITC helped us to identify the key residues of the viral IDP facilitating viral release through interaction with a specific host factor. Curiously, the recognition motifs in the IDP mapped to a region with fairly rigid backbone dynamics, yet this region was unstructured before and after binding the cognate host protein. Ion mobility spectrometry-mass spectrometry experiments indicated that the viral IDP has multiple conformations including highly compact (but unstructured) conformers, where each conformer could bind the host protein, suggesting that there was no conformational selection bias during binding. The affinity of the interaction was weak and was efficiently outcompeted with a synthetic peptide. Our results provide new insights into an important protein-protein interaction essential for HBV replication and proof-of-principle for developing peptidomimetic inhibitors of this interaction.

P-58: BioShell structure template database

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The Bioshell Threader web server has been recently published as avery accurate tool for protein homologs detection [1]. The searching algorithm (known as 1D threading) is based on a profile-to-profile alignment for both sequence and secondary structure. In order to further improve its accuracy and to speed up the search process a custom protein structure database has been designed. All deposits found in the Worldwide Protein Data Bank were divided into separate domains and split into non-redundant groups. The latter task was achieved by all-vs-all pairwise structural alignments; the former one by structure based heuristic annotation by SCOP data. The database is updated weekly, when new solved structures are released. Database server also allows browsing precomputed structural neighborhoods or perform structure comparison with uploaded model. The user is able to select templates using various restrictions and download them.

Reference:
[1] Gront D *et al.* (2012) *Nucleid Acids Res* **40** (Web server issue): W257-W262.

P-59: The helical hairpin structure of influenza fusion peptide and a hydrophobic moment map. Anything in common?

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Viruses having lipid bilayer envelopes enter into cells through fusion of viral and cellular membranes. This process is facilitated by virial fusion proteins which are embedded in the viral lipid envelope, while the other short fragment of the same molecule (fusion peptide, fp) anchors in the endosomal membrane of a host cell. In the case of influenza virus, membrane fusion is mediated by hemagglutinin (HA) fusion peptide (HAfp) and serves as one of the best characterized examples of enveloped virus cellular entry mechanisms. However, recent NMR structures of the complete HAfp (i. e. HAfp1-23) pointed out its tight helical structure, not observed previously. HAfp, as it is common for membrane-anchoring peptides, shows amphiphilic properties, which can be characterized by the so-called hydrophobic moment (μ_H) values. In this work, μ_H were calculated for the HAfp sequence, assuming structures differed by the helical turn angle. From such analysis, μ_H could be plotted in a form of a hydrophobic moment map against two variables: the center of amino acid window, used for μ_H calculation, and the helical turn angle. Interestingly, the maxima for HAfp corresponded to the both fragments of a helical hairpin with a turn angle close to 100°, as for a perfect α-helix. Surprisingly, such structure-corresponding μ_H maps were pronounced the strongest for Wimley and White whole-residue octanol hydrophobicity scale, however could not be observed in the case of some other commonly used scales. For comparison, a hydrophobic moment map was also calculated for a HIV fusion peptide (gp41), adopting a single helix structure, and a corresponding pattern was obtained. Therefore this simple and not demanding computationally method shows a promising applicatory use, also on larger scales of viral protein sequences.

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P-60: Molecular dynamics and replica exchange simulations of protein folding with dynamic fragment assembly and UNRES force field

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Recently we have combined the physics-based united-residue (UNRES, Liwo *et al*. 2007) force field developed in our laboratory with the dynamic-fragment-assembly (DFA) technique developed by Sasaki *et al*. (2008) for protein structure prediction using global optimization with conformational space annealing (CSA, Lee *et al*. 1997). In this work we carried out protein folding simulations with canonical molecular dynamics (MD) and replica exchange molecular dynamics (REMD) using the same target energy function UNRES+DFA. In UNRES model each amino acid is represented by only two centers of interactions: a united side chain and a united peptide group. The interactions of this simplified model are derived from the generalized cluster-cumulant expansion of a restricted free energy (RFE) function of polypeptide chains. DFA energy terms incorporate knowledge-based information corresponding to structure of a given protein. These terms include two-body potential and pseudo-dihedral angle potential which represent local structure of 9-residue long fragments and neighboring-number potential which shows the environment of central residue of each fragment. All these three terms are based on fragment library constructed for a given protein sequence using profile alignment. The last DFA term, β-sheet potential, is based on the neural network estimation of the probability of the β-sheet pairing (Cheng *et al*. 2005). The results of protein folding simulations using UNRES+DFA energy are compared to pure UNRES protein folding simulations.

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Liwo A *et al*. (2007) *J Phys Chem B* **111**: 260-285.
Lee J *et al*. (1997) *J Comput Chem* **18**: 1222-1232.
Cheng J, Baldi P (2005) *Bioinformatics* **21** (suppl1) i75-i84.

P-61: Analysis of the novel eIF4E-Interacting Protein in Leishmania

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Leishamanias are the trypanosomatid protozoa that are responsible for the diseases called leishamanisis. During their digenetic life cycle they migrate from sandflies alimentary canal to mammalian macrophages. It is associated with temperature increase and decrease of pH of their environment. During the switch they transform from flagellated promastigotes to non-flagellated amastigotes. Trypanosomatid parasites are known from their unique mechanism of mRNA maturation which consists of polycistronical transcription followed by trans-splicing and polyadenylation. At the 5'end of Leishmania's mRNA an atypical cap structure is located. It is called cap-4 and consists of 2'-O-methylations on the first four nucleotides and unique base methylations on the first and fourth nucleotide of the mRNA. Consequently eIF4F complex in Leishmania differs from the one in other eukaryotes. All four homologs of eIF4E fail to complement missing function of the yeast eIF4E. Two of the paralogs, LeishIF4E-1 and LeishIF4E-4, bind strongly to m^7GTP and cap-4. In promastigotes LeishIF4E-4 is a part of conserved eIF4F complex but after the temperature increase the complex disassociates and the expression of LeishIF4E-4 is reduced. LeishIF4E-1 is the only one of isoforms which expression level during heat shock is maintained. Its mode of function is yet unknown as it fails to interact with any of the parasites' MIF4G proteins. A novel 4E-Interacting Protein (Leish4E-IP, 83 kDa) was recently shown to bind LeishIF4E-1 both *in vivo* and *in vitro*. It has no homology with 4E-BPs of higher eukaryotes, neither in sequence nor in size (10 kDa). The expression level of Leish4E-IP remains unchanged before and after heat shock as well as during stage differentiation, yet it could be subject to post-translational modifications. The role of Leish4E-IP is not known. To learn more about this protein we analysed its expression in Leishmania cells. Using a biophysical approach we examined whether the interaction between LeishIF4E-1 and Leish4E-IP is influenced by the cap structure. This could indicate whether the two proteins interact on the 5' end or whether Leish4E-IP serves to sequester LeishIF4E-1 in the cytoplasm and prevents its binding to the mRNA. Preliminary data suggest that the presence of a cap analogue (m^7GTP) stabilizes the interaction between the two proteins. Potential models will be discussed.

P-62: Electrostatics of globular proteins adsorbed on discretely charged solid surface

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The report represents our theoretical and experimental studies of the interactions between globular protein macromolecules and oppositely charged solid surface with discrete distribution of the charges. We lead investigations into two directions. The first is theoretical; its aim is to calculate the electrostatic field in the protein globule and to determine pH-dependant parameters as: total charge, electric moments, total free energy and its electrostatic component. The protein model is built in the fixed atom approximation, protein-solvent boundary determined by solvent accessibility and two types of charges: permanent partial charges (representing the dipole moments of the covalent bonds) and pH-dependent charges of the dissociable groups. The local field intensity is calculated as a sum of all charges of the polypeptide chain and the associated water molecules (atom coordinates are taken from Protein data bank); the result reflects charge-charge, charge-dipole and dipole-dipole interactions. The solid surface is presented by limited-size plate with a single fixed charge; the last assumption is supported by the small charge density of the oxide surfaces: the mean distance between two charged centers is in an order higher than the protein globule size.

Our theoretical research of the protein-surface interactions is divided into three steps depending on the distance d between the globule and the solid surface: (i) $d = \infty$ (isolated protein globule); (ii) $d > 0.6$ nm (only electrostatic interactions are taken into account); and (iii) $d \leq 0.6$ nm (both electrostatic and van-der-Waals interactions are calculated). The results (calculated at different pH) show that the electric moments (including the dipole moment in the isoelectric point) alter their directions and magnitudes at decreasing of d; the both vector and scalar electrostatic parameters depend on the globule orientation.

The second direction of our investigations is experimental: studying of the protein adsorption on colloid particles; its aim is to compare the calculated parameters as the isoelectric point with the measured ones. For that purpose the methods of electric light scattering (particles orientation in sinusoidal electric field) and electrophoresis (mobility in direct field) have been used. The comparison shows out good agreement between the theoretical and experimental results.

Acknowledgement:
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P-63: Sparse NMR data improves protein homology detection method

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Data from NMR experiments has been widely used to obtain information about structure and dynamics of proteins. However, not all NMR experiments provide fully complete data for proteins and not always three-dimensional structure can be resolved quickly and accurately. Here we present a combination of one-dimensional threading method with NMR experimental measurements: chemical shifts, NOE and S2. These data has been combined with evolutionary information in a single dynamic programming calculations. In cases where the full template structure is not available, matching fragments extracted from known structures may be used in Rosetta fragment recombination calculations.

P-64: Particle aggregation dynamics in presence of hydrodynamic interactions and shear flow

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A number of experimental and theoretical studies show that the aggregation processes are significantly influenced by the presence of a shear flow, however a full understanding of this phenomenon is still elusive. We present the results of Brownian dynamics simulations of aggregating spheres in the shear flow, with and without hydrodynamic interactions using the Rotne-Prager-Yamakawa approximation. The dependence of the aggregation rate on the shear flow is determined in a wide range of flow velocities and the results are compared with those of other studies.

P-65: Synthesis of Trimethylguanosine snRNA 5′ Cap Analogs Modified in 5′,5′-Triphosphate Bridge and their Interactions with a Human snRNP-specific Nuclear Import Receptor, Snurportin 1

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Nucleus, the heart of eukaryotic cells, is surrendered by a double membrane. Small molecules (50 kDa) can be transported by free diffusion whereas transport of macromolecules is energy-ad signal-dependent. Import of macromolecules requires a nuclear localization signal (NLS). In the case of spliceosomal small nuclear rybonucleoproteins (snRNPs) the role of the NLS sequence is played out by the structure of a trimethylguanosine (TMG) cap, $m_3^{2,2,7}G$, localized at the 5′ end of the snRNA. This structure is recognized by an adaptor protein — snurportin 1, which in turn binds to importin β — a protein responsible for the interaction with and translocation through the nuclear pore complex. Formation of the complex between above elements (Snurportin-1, $m_3^{2,2,7}G$-cap and Importin β) is essential for nuclear import of snRNPs [1].

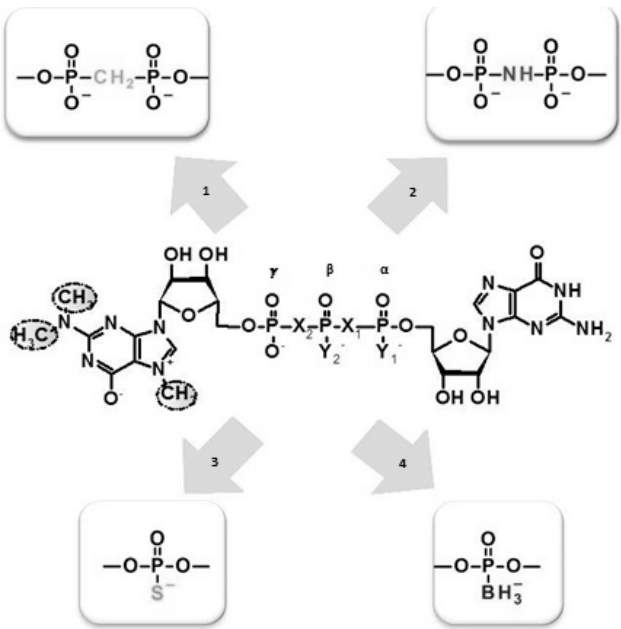

Figure 1: Modifications in 5′,5′-triphosphate bridge of the $m_3^{2,2,7}G$ cap structure (1- methylene-bisphosphonate, 2- imidodiphosphate, 3-thioposphate, 4-boranophosphate).

On the other hand, low efficiency of import of therapeutic molecules into the nucleus is a problem addressed by therapies of diseases caused by gene expression or incorrect mRNA splicing (such as e.g. Duchenne Muscular Dystrophy DMD). Oligonucleotide treatment that affects

splicing proved to be highly promising for the DMD therapy [2]. Previous studies showed that the conjugation of the TMG cap structure to a biotine-avidine construct or to an antisense oligonucleotide increased the efficiency of their nuclear import [3]. Combination of these facts [2, 3] may have clinical importance, hence the goal of our study is to find the optimal TMG cap structure for such applications.

Our previous studies showed that modifications of the 5',5'-triphosphate bridge of the mRNA 5' 7-methylguanosine (m^7G) cap resulted in improved resistance against enzymatic degradation. Moreover, due to these modifications, the m^7G cap analogs had increased affinity to the eIF4E protein factor yielding more efficient translation [4]. The aim of our research is to obtain $m_3^{2,2,7}G$ cap analogs modified in 5',5'-triphosphate bridge which are stable in vivo due to the resistance against deccapping enzymes and enable to be imported efficiently into the nucleus due to the increased affinity to snurportin 1.

In this report we present chemical syntheses of several $m_3^{2,2,7}$-trimethyl guanosine cap analogs modified in the 5',5'-triphosphate bridge (Fig. 1). These compounds contain bridging modifications such as methylenebisphosphonate (1), imidodiphosphate (2) at α-β or β-γ positions, non-bridging modifications such as oxygen atom replaced by sulfur atom (3) or borane moiety (5) at the α or β position of phosphate bridge, and elongated phosphate bridge. Their affinity to snurportin 1 was determined using titration assay based on the intrinsic protein fluorescence quenching by cap analogs [5]. The binding studies have been performed for both full length (41.1 kDa) and shorter form (33.7 kDa) of human snurportin 1, and yielded the equilibrium association constant values in the order of 10^{-6} M^{-1}.

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P-66: Structure of Protein Interaction Networks and Their Implications on Drug Design

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Genome-wide data on interactions between proteins are now available, and networks of protein interactions are the keys to understanding diseases and finding accurate drug targets. This study revealed the architectural properties of the backbones of protein interaction networks (PINs), which is scale-free composed of three layer structure. It is different from conventional view of simple scale-free network. Further analysis revealed that there are extensive interconnections among middle-degree nodes that form the backbone of the networks. Degree distributions of essential genes, synthetic lethal genes, synthetic sick genes, and human drug-target genes indicate that there are advantageous drug targets among nodes with middle- to low-degree nodes. Such network properties provide the rationale for combinatorial drugs that target less prominent nodes to increase synergetic efficacy and create fewer side effects.

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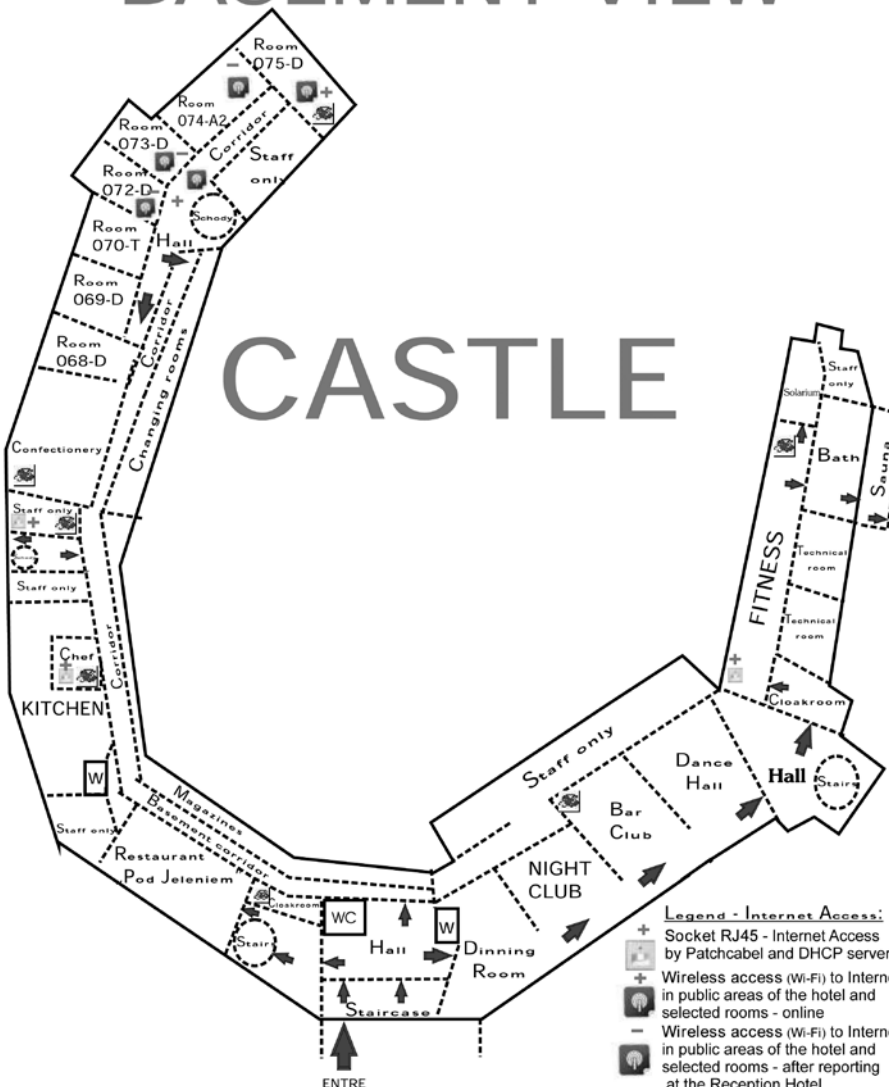
BASEMENT VIEW



Legend:

W - lift
WC - toilet
W1, W2 - towers on the Bridge Acade

Legend - Internet Access:

Socket RJ45 - Internet Access by Patchcabel and DHCP server

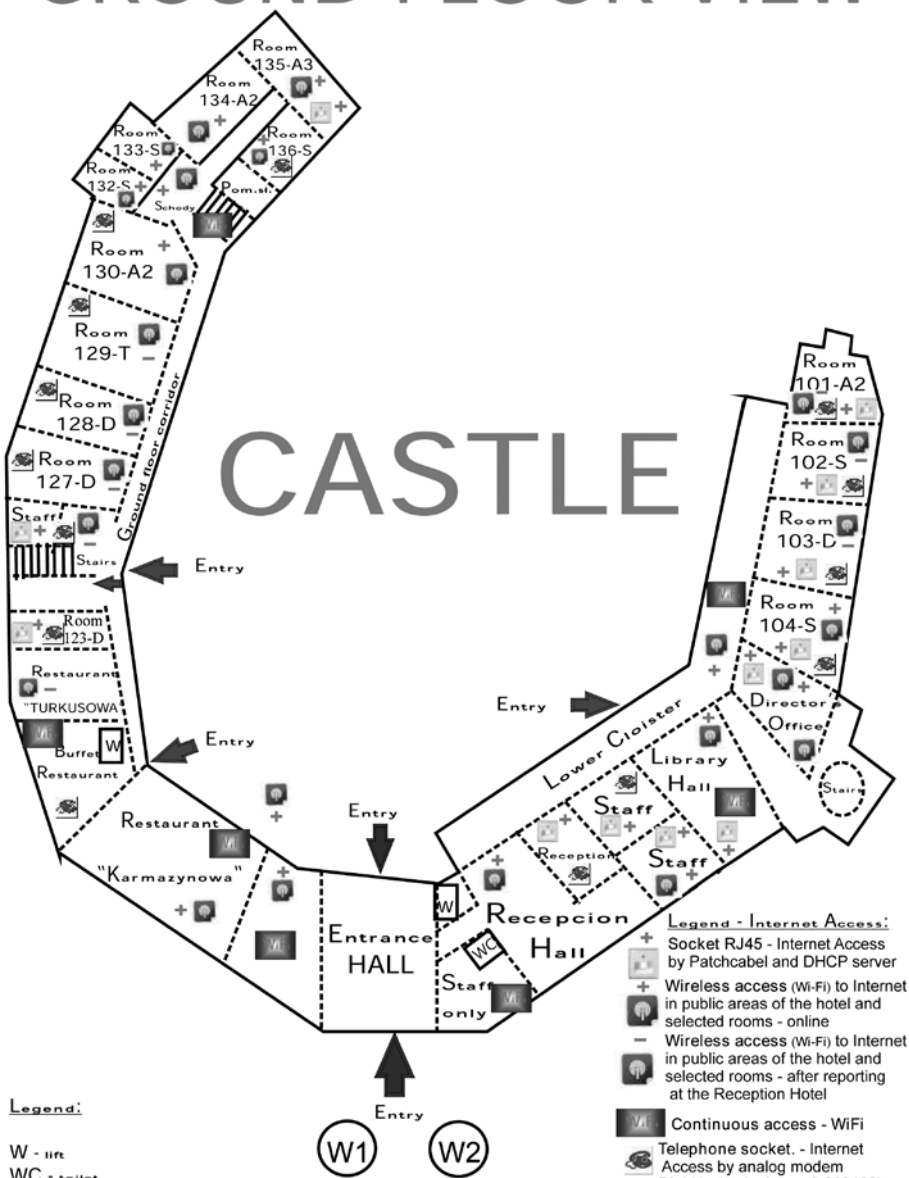
Wireless access (Wi-Fi) to Internet in public areas of the hotel and selected rooms - online

Wireless access (Wi-Fi) to Internet in public areas of the hotel and selected rooms - after reporting at the Reception Hotel

Continuous access - WiFi

Telephone socket. - Internet Access by analog modem Dial Up (telephone: 0-202122) - after reporting at the Reception

GROUND FLOOR VIEW



Legend:

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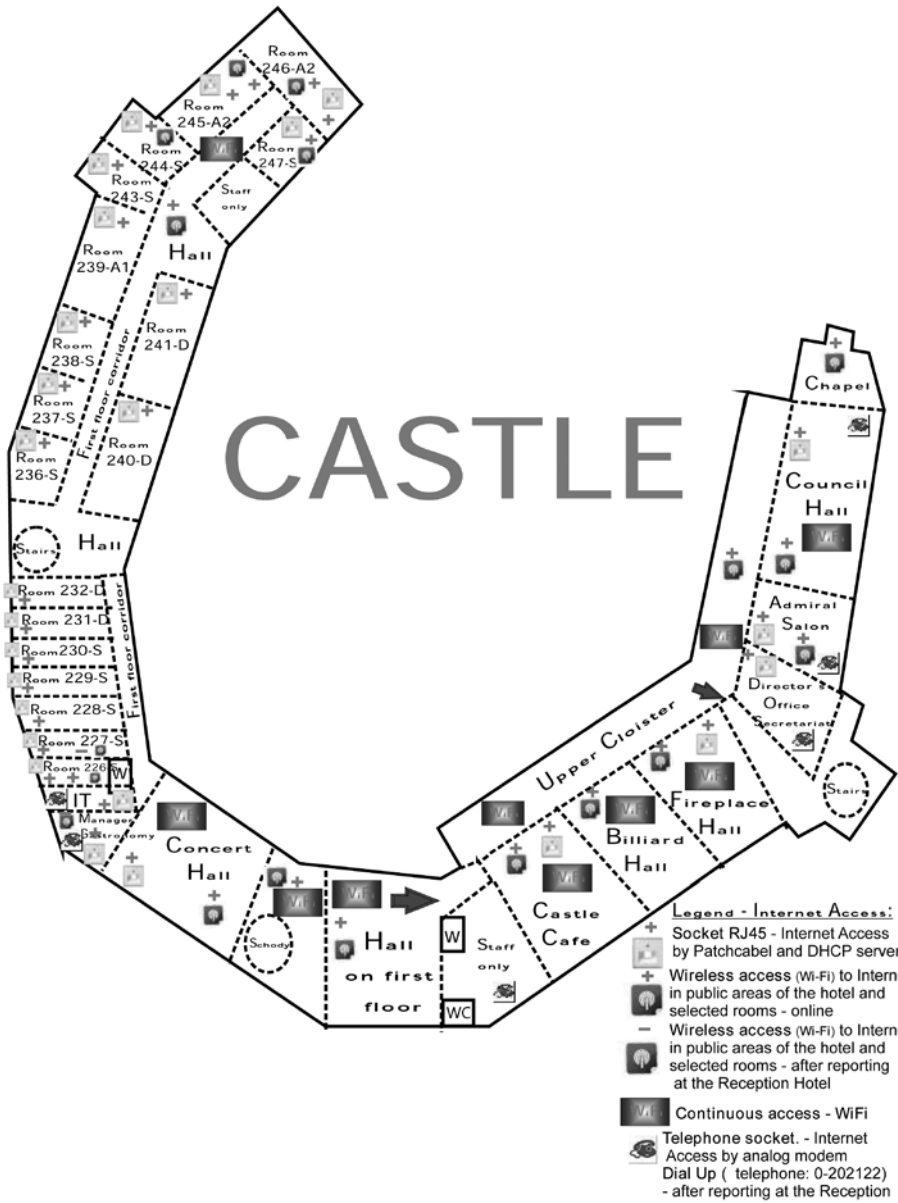
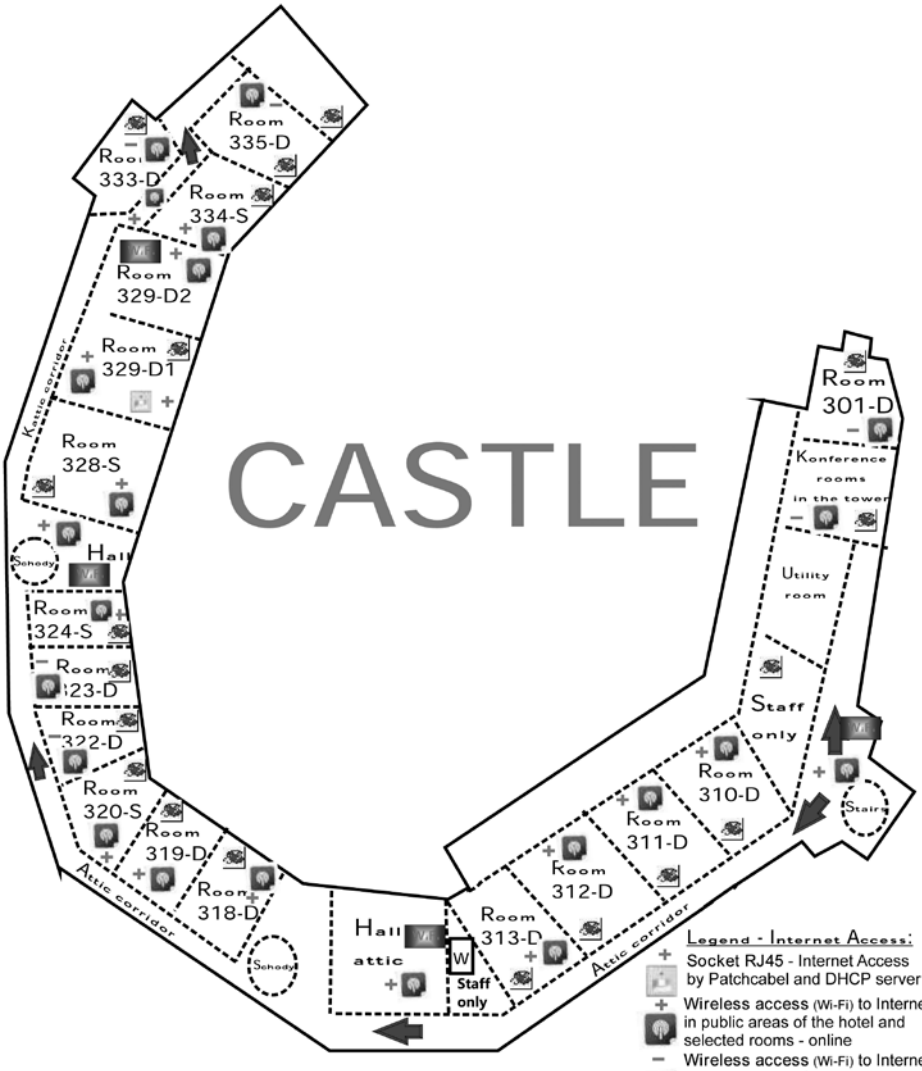
Legend - Internet Access:

- Socket RJ45 - Internet Access by Patchcabel and DHCP server
- Wireless access (Wi-Fi) to Internet in public areas of the hotel and selected rooms - online
- Wireless access (Wi-Fi) to Internet in public areas of the hotel and selected rooms - after reporting at the Reception Hotel
- Continuous access - WiFi
- Telephone socket. - Internet Access by analog modem Dial Up (telephone: 0-202122) - after reporting at the Reception

1ST FLOOR VIEW



ATTIC VIEW



Legend - Internet Access:

+ Socket RJ45 - Internet Access by Patchcabel and DHCP server

+ Wireless access (Wi-Fi) to Internet in public areas of the hotel and selected rooms - online

− Wireless access (Wi-Fi) to Internet in public areas of the hotel and selected rooms - after reporting at the Reception Hotel

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*Nanoscale computer-aided design of self-assembling materials
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