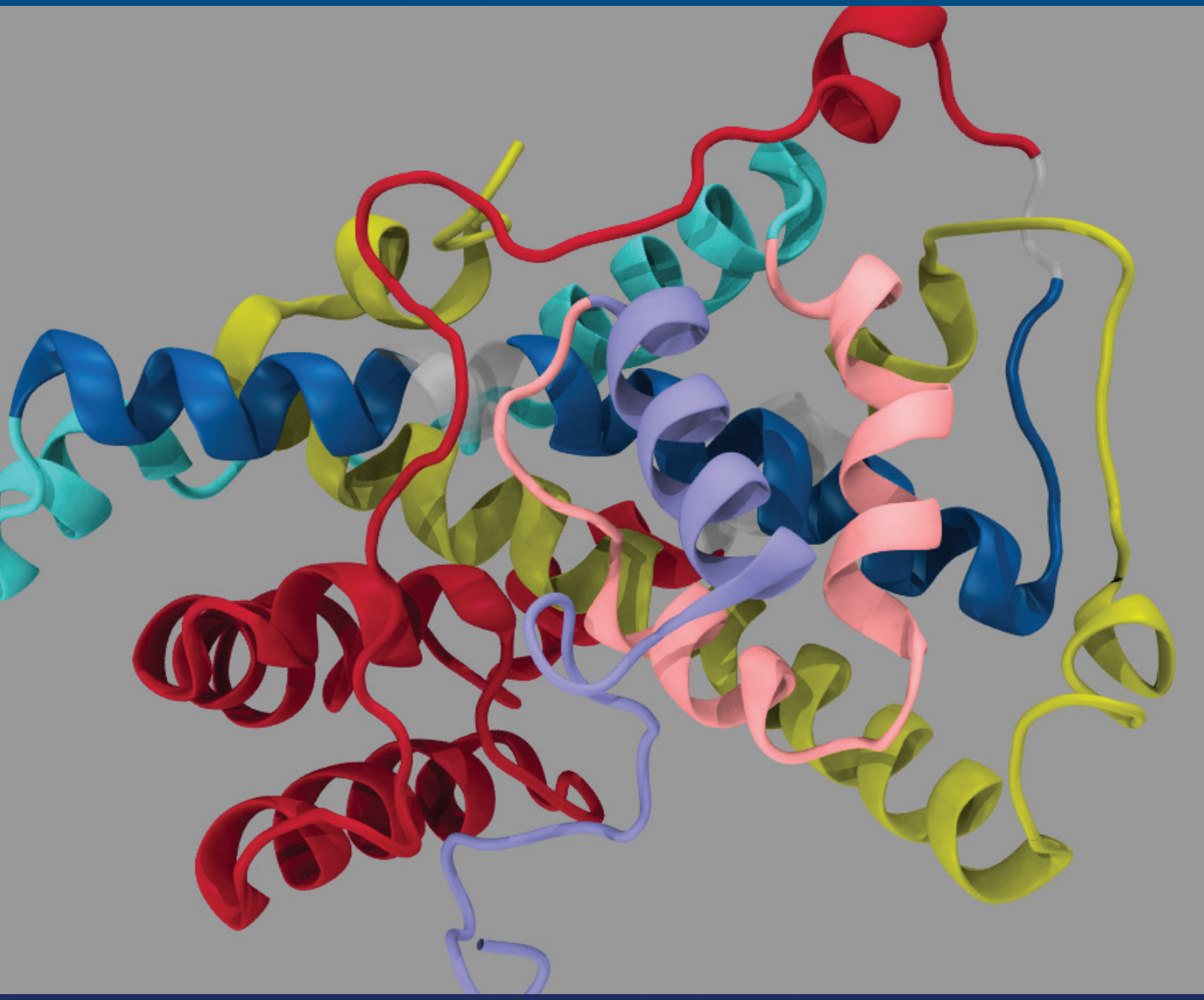
Significance of Knotted Structures for Function of Proteins and Nucleic Acids

SEPTEMBER 17–21, 2014 | WARSAW, POLAND



Biophysical Society

UNIVERSITY OF WARSAW

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September 2014

Dear Colleagues,

We would like to welcome you to the conference on the "*Significance of Knotted Structures for Function of Proteins and Nucleic Acids*". During this meeting we will focus on recent progress in identifying a biological role of knots in proteins and nucleic acids. This is a new, interdisciplinary field, which has recently been extensively explored by many groups. One important motivation to study these topics is an increasing number of proteins with knots being discovered. Knots are surprising from the biological point of view: for a long time it was believed that such structures would complicate folding and unfolding processes, and thus should be eliminated during evolution by the hosting organism. Today, we know that knots exist in all kingdoms of life and they are gradually being recognized as significant structural motifs. The above finding has challenged our preconceptions about the complexity of biological objects and inspired research into how these tangling properties affect the functions of proteins. However, we still do not understand what the biological role of knots is, what their advantage for hosting organisms is, how they can be degraded, or how complex the knots formed in proteins can be. We believe that this conference will shed light on these questions and will inspire new and interesting directions of research.

The other important foci of the conference are pseudo-knots in RNA, as well as knots in DNA and complex structures such as viruses. Pseudo-knots are widespread in non-coding RNAs, where they serve essential biological purposes. There is recent evidence that topological isomers in RNA are more feasible than first thought, raising the possibility of the existence of true knots or more complex pseudoknotted structures. On the other hand, non-trivial topologies of DNA play critical role in transcription and other genetic processes.

Mathematics, and knot theory in particular, play a very important role in the analysis of the above problems. An important part of the conference is devoted to the analysis of mathematical problems inspired by knotting in biomolecules. It is clear that an interdisciplinary approach, which involves biology, physics, chemistry, and mathematics, will be most successful in this field in the years to come.

This meeting will provide a forum for analysis and discussion of entanglement in proteins and nucleic acids. The conference offers a full program with more than 40 lectures and over 50 posters, and brings together around 120 renowned researchers from different fields related to these topics: biology, chemistry, physics, and mathematics. We hope that we can create an inspiring and scientifically challenging atmosphere during the conference.

Sincerely yours,

Wilma Olson, Jose Onuchic, Matthias Rief, Joanna Sulkowska, Sarah Woodson
Programming Committee

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General Information

Registration

The registration and information desk is located in the lobby of the Auditorium in the Old Library of the University of Warsaw. Registration hours are as follows:

Wednesday, September 17	6:00 PM – 8:00 PM
Thursday, September 18	8:00 AM – 5:00 PM
Friday, September 19	8:00 AM – 5:00 PM
Saturday, September 20	8:00 AM – 5:00 PM
Sunday, September 21	8:00 AM – 12:00 PM

Instructions for Presentations

Presentation Facilities

A data projector will be made available in the Auditorium. Speakers are required to bring their laptops. Speakers are advised to preview their final presentations before the start of each session.

Poster Sessions

1) All poster sessions will be held in the Auditorium Lobby. Posters in each poster session will be on display from 8:00 AM – 9:30 PM on the day of the assigned poster session.

   Poster Session I
   All posters scheduled for Poster Session I should be set up in the morning on the September 18 and MUST be removed by 9:30 PM the same day.

   Poster Session II
   All posters scheduled for Poster Session II should be set up in the morning on the September 19 and MUST be removed by 9:30 PM the same day.

2) During the poster presentation sessions, presenters are requested to remain in front of their posters to meet with attendees.

3) A display board measuring 881 mm (width) by 1189 mm (height) (33.1in x 46.8in) will be provided for each poster. Poster boards are numbered according to the same numbering scheme as in the program book.

4) All posters left uncollected at the end of the meeting will be discarded.

Coffee Break
Coffee breaks will be held in the Auditorium Lobby where tea and coffee will be provided free of charge to all participants.

Internet
Internet access is available in the Auditorium of the Old Library building.

Smoking
Smoking is not permitted inside the buildings on The University of Warsaw campus.

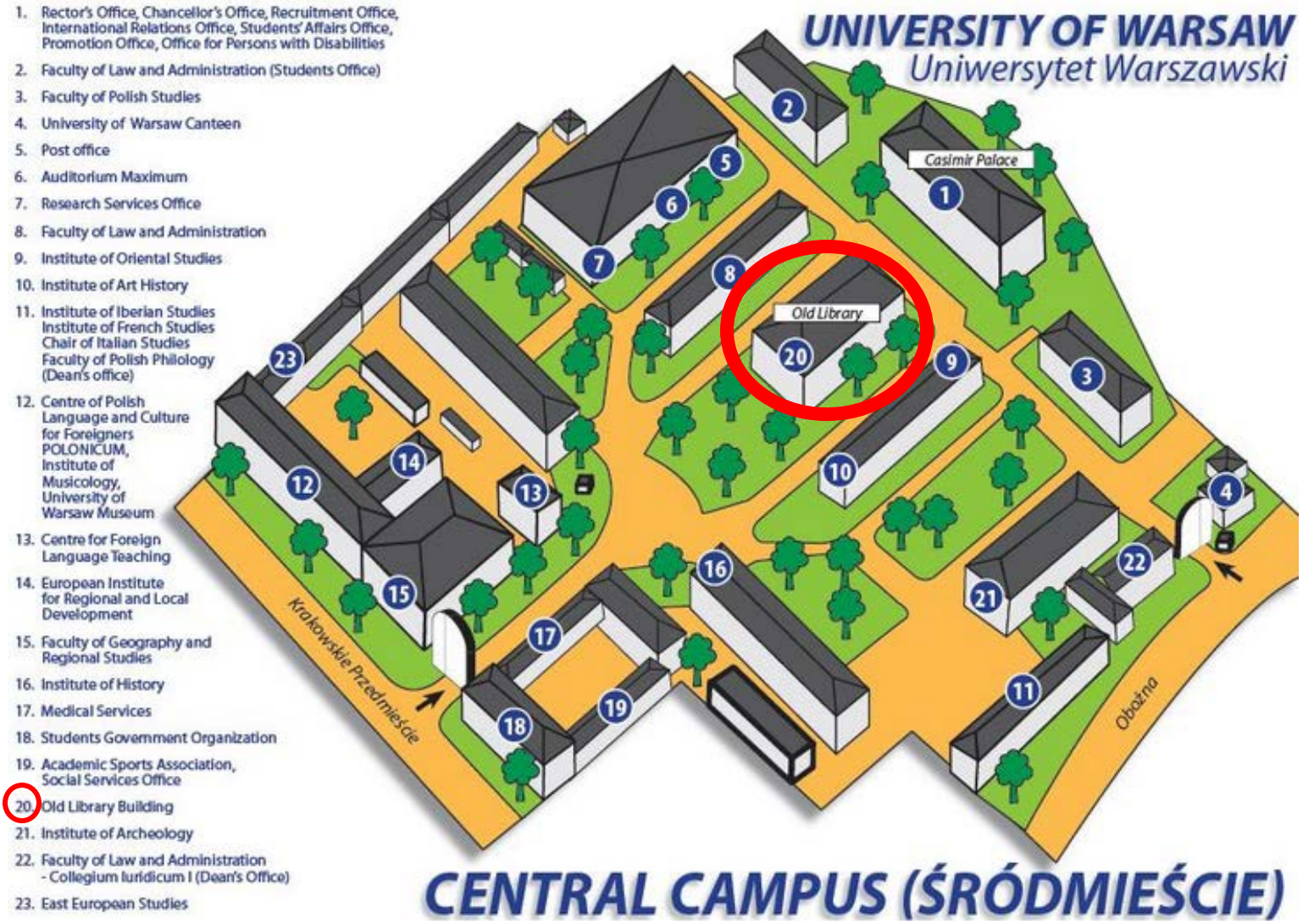
Meals
The welcome reception, coffee breaks, and two lunches September 18 and 19 are included in the registration fee. Meals will be held in the Old Library Building.

Social Events
Welcome Reception with light hors d'oeuvres will be held in the Auditorium Lobby on Wednesday, September 17.

Name Badges
Name badges are required to enter all scientific sessions and poster sessions. Please wear your badge throughout the conference.

Map of the University of Warsaw
Please reference the map on page 7 of the program book.

Contact
If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from September 17 - 21 during registration hours. You may also contact Dorothy Chaconas at DChaconas@biophysics.org or call the Sofitel Hotel at 48 22 657 80 11 and ask for Dorothy's room.


1. Rector's Office, Chancellor's Office, Recruitment Office, International Relations Office, Students' Affairs Office, Promotion Office, Office for Persons with Disabilities
2. Faculty of Law and Administration (Students Office)
3. Faculty of Polish Studies
4. University of Warsaw Canteen
5. Post office
6. Auditorium Maximum
7. Research Services Office
8. Faculty of Law and Administration
9. Institute of Oriental Studies
10. Institute of Art History
11. Institute of Iberian Studies Institute of French Studies Chair of Italian Studies Faculty of Polish Philology (Dean's office)
12. Centre of Polish Language and Culture for Foreigners POLONICUM, Institute of Musicology, University of Warsaw Museum
13. Centre for Foreign Language Teaching
14. European Institute for Regional and Local Development
15. Faculty of Geography and Regional Studies
16. Institute of History
17. Medical Services
18. Students Government Organization
19. Academic Sports Association, Social Services Office
20. Old Library Building
21. Institute of Archeology
22. Faculty of Law and Administration - Collegium Iuridicum I (Dean's Office)
23. East European Studies
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Significance of Knotted Structures for Function of Proteins and Nucleic Acids
University of Warsaw
Warsaw, Poland
September 17-21, 2014

PROGRAM

All scientific sessions and poster presentations will be held in the Auditorium of the Old Library at the University of Warsaw unless otherwise noted.

Wednesday, September 17, 2014

6:00 – 8:00 PM	Registration/Information	**Auditorium Lobby**
6:50 – 8:05 PM	**Opening Talks** Jane Clarke, University of Cambridge, United Kingdom *Unfolded, but not Knotted! Mechanistic Studies of IDP Folding Upon Binding* Ada Yonath, Weizmann Institute of Science, Israel *Ribosomes: RNA Machines for Protein Production that Withstand Evolution Pressures*	
8:10 – 9:30 PM	Welcome Reception	**Auditorium Lobby**

Thursday, September 18, 2014

8:00 AM – 5:00 PM	Registration/Information	**Auditorium Lobby**
	Session: Structure of Proteins - Theory Chair: Jose Onuchic, Rice University, USA	
8:30 – 9:00 AM	Alexey Murzin, Medical Research Council Center, United Kingdom *Geometrical and Topological Aspects of Protein Structures*	
9:00 – 9:30 AM	Wladek Minor, University of Virginia, USA *Experiment and Modeling: Competitive or Complementary Approaches to Structural Biology?*	
9:30 – 10:00 AM	Kenneth C. Millett, University of California, Santa Barbara, USA *Knots and Slipknots in Proteins*	
10:00 – 10:30 AM	Robert Jernigan, Iowa State University, USA *Extracting Protein Dynamics from Experimental Structures*	
10:30 – 10:45 AM	Coffee Break	**Auditorium Lobby**

Session: Proteins – Experiment
Chair: Jane Clarke, University of Cambridge, United Kingdom

10:45 – 11:15 AM — Sophie Jackson, University of Cambridge, United Kingdom
Folding of Nascent Chains of Knotted Proteins

11:15 – 11:45 AM — Patricia Jennings, University of California, San Diego, USA
Exploring the Coordinated Functional and Folding Landscapes of Knotted Proteins

11:45 AM – 12:15 PM — Ya-Ming Hou, Thomas Jefferson University, USA
Methyl Transfer from AdoMet by a Knotted Protein Fold

12:15 – 1:30 PM — Lunch

Session: Energy Landscape of Biomolecules, Part 1
Chair: Dave Thirumalai, University of Maryland, USA

1:30 – 2:00 PM — Peter Wolynes, Rice University, USA
Harvesting the Fruits of the Energy Landscape Theory of Protein Folding

2:00 – 2:30 PM — Shoji Takada, Kyoto University, Japan
Knotted Structures in Refolding and Cotranslational Folding of Multi-domain Protein

2:30 – 3:00 PM — Andrzej Kolinski, University of Warsaw, Poland
Coarse Grained Modeling of Protein Structure, Dynamics and Interactions

3:00 – 3:15 PM — Jeffrey Noel, Rice University, USA
Connecting Simplified Models with Explicit-Solvent Forcefields: Slipknotting during the Folding of the Smallest Knotted Protein

3:15 – 3:40 PM — Coffee Break **Auditorium Lobby**

Session: Energy Landscape of Biomolecules, Part 2
Chair: Alexey Murzin, Medical Research Council Center, United Kingdom

3:40 – 4:10 PM — Alexander Grosberg, New York University, USA
Significance, Complexity, and Beauty of Knot-avoiding Structures

4:10 – 4:25 PM — Sebastian Kmiecik, University of Warsaw, Poland*
Multiscale Modeling of Protein Flexibility

**Short talks selected from among submitted abstracts*

4:25 – 4:55 PM	Janusz Bujnicki, International Institute of Molecular and Cell Biology, Poland *Simulations of Folding and Unfolding of Pseudoknots in RNA*	
4:40 – 8:00 PM	Free Time	
8:00 – 9:30 PM	Poster Session I	**Auditorium Lobby**

Friday, September 19, 2014

8:00 AM – 5:00 PM	Registration/Information	**Auditorium Lobby**
	Session: Mathematical Perspectives on Knotting Chair: Kenneth C. Millett, University of California, Santa Barbara, USA	
8:30 – 9:00 AM	Stuart Whittington, University of Toronto, Canada *Defining and Identifying Knots in Linear Polymers*	
9:00 – 9:30 AM	Chris Soteros, University of Saskatchewan, Canada *The Knot Complexity of Compressed Polygons in a Lattice Tube*	
9:30 – 10:00 AM	Eric Rawdon, University of St. Thomas, USA *Knotting in Subchains of Proteins and Other Entangled Chains*	
10:00 – 10:30 AM	Coffee Break	**Auditorium Lobby**
	Session: Puling Knots and Slipknots Chair: Matthias Rief, Technical University of Munich, Germany	
10:30 – 11:00 AM	Michael Woodside, University of Alberta, Canada *Mechanical Unfolding of Single RNA Pseudoknots Reveals that Conformational Plasticity, Not Resistance to Unfolding, is a Determinant of Programmed −1 Frameshifting*	
11:00 – 11:30 AM	Hongbin Li, University of British Columbia, Canada *Mechanically Tightening a Protein Slipknot into a Trefoil Knot*	
11:30 AM – 12:00 PM	Piotr Szymczak, University of Warsaw, Poland *Untying a Protein Knot – Translocation of Knotted Proteins Through a Pore*	
12:00 – 12:15 PM	Katrina Forest, University of Wisconsin-Madison, USA* *Why are Phytochroms Knotted?*	
12:30 – 1:30 PM	Lunch	

**Short talks selected from among submitted abstracts*

Session: RNA Dynamics
Chair: Sarah Woodson, Johns Hopkins University, USA

1:30 – 2:00 PM — Dave Thirumalai, University of Maryland, USA
Crowing Promotes the Switch from Hairpin to Pseudoknot Conformation in Human Telomerase RNA

2:00 – 2:30 PM — Paul Whitford, Northeastern University, USA
Parallels between Protein Folding and Ribosome Dynamics

2:30 – 3:00 PM — Joanna Trylska, Center of New Technologies, Poland
Conformational Dynamics of RNA Functional Motifs: Ribosomal A-site and Thermosensing Hairpin

3:00 – 3:20 PM — Coffee Break **Auditorium Lobby**

Session: DNA-Protein Interaction, Knotted DNA
Chair: Mariel Vazquez, University of California, Davis, USA

3:20 – 3:50 PM — Tamar Schlick, New York University, USA
Chromatin Looping and Interdigitation Mechanisms: Insights from Mesoscale Simulations

3:50 – 4:20 PM — Nicolas Clauvelin, Rutgers University, USA
Protein-induced Entanglement of DNA: Connecting and Organizing Chromosomes via Multiple Loops

4:20 – 4:50 PM — Dorothy Buck, Imperial College of London, United Kingdom
Knotted DNA: Mathematical Models and Biological Consequences

4:50 – 5:10 PM — **Open Discussion**
Wilma Olson, Rutgers University, USA
Jose Onuchic, Rice University, USA
Matthias Rief, Technical University of Munich, Germany
Joanna Sulkowska, University of Warsaw, Poland
Sarah Woodson, Johns Hopkins University, USA

5:10 – 8:00 PM — Free Time

8:00 – 9:30 PM — Poster Session II **Auditorium Lobby**

**Short talks selected from among submitted abstracts*

Saturday, September 20, 2014

8:00 AM – 5:00 PM	Registration/Information	**Auditorium Lobby**
	Session: DNA Topology and Topoisomerase Chair: Lynn Zechiedrich, Baylor College of Medicine, USA	
8:30 – 9:00 AM	Tony Maxwell, John Innes Center, Norwich Research Park, United Kingdom *DNA Topology, DNA Topoisomerases, and Small DNA Circles*	
9:00 – 9:30 AM	Stephen Levene, University of Texas, Dallas, USA *Conformational Free-Energy Calculations for Complex Biopolymer Structures*	
9:30 – 10:00 AM	Phoebe Rice, University of Chicago, USA *Structural Basis for Regulation of Site-specific DNA Recombinases by DNA Topology*	
10:00 – 10:30 AM	Anjum Ansari, University of Illinois at Chicago, USA *Unveiling the Molecular Trajectory during Binding Site Recognition by DNA-bending Proteins*	
10:30 – 10:45 AM	Coffee Break	**Auditorium Lobby**
	Session: DNA/RNA, Nanorobots, Origami – Theory/Experiment, Part 1 Chair: Remus Dame, Leiden Institute of Chemistry, The Netherlands	
10:45 – 11:15 AM	Giovanni Dietler, Swiss Federal Institute of Technology (EPFL), Switzerland *Sedimentation of Macroscopic Rigid Knots and its Relation to Gel Electrophoretic Mobility of DNA Knots*	
11:15 – 11:45 AM	Julie Feigon, University of California, Los Angeles, USA *RNA Pseudoknots in Telomerase and Riboswitches*	
11:45 AM – 12:15 PM	Ebbe Andersen, Aarhus University, Denmark *Single-stranded Architecture for Cotranscriptional Folding of RNA Nanostructures*	
12:15 – 12:30 PM	Zbyszek Otwinowski, UT Southwestern Medical Center, USA* *Single-stranded DNA Topology in Eukaryotes*	
12:30 – 3:30 PM	Lunch and Free Time	

**Short talks selected from among submitted abstracts*

Session: DNA/RNA, Nanorobots, Origami – Theory/Experiment, Part 2
Chair: Giovanni Dietler, Swiss Federal Institute of Technology (EFPL), Switzerland

3:30 – 4:00 PM — Lynn Zechiedrich, Baylor College of Medicine, USA
How Positive or Negative Supercoiling Affects the Structure of Reactivity of DNA

4:00 – 4:15 PM — Pawel Zawadzki, Oxford University, United Kingdom*
Escherichia coli Live Cell Super-resolution Analysis of Topoisomerase IV Action

4:15 – 4:45 PM — Remus Dame, Leiden Institute of Chemistry, The Netherlands
Fine-tuning the Activity of DNA Bridging Proteins

4:45 – 5:00 PM — Coffee Break **Auditorium Lobby**

5:00 – 5:30 PM — Sarah Harris, University of Leeds, United Kingdom
Closing the Loop: Comparing the Results of Experiment and Computer Simulations in the Study of DNA Minicircles

Session: Energy Landscape Proteins
Chair: Joanna Sulkowska, University of Warsaw, Poland

5:30 – 6:00 PM — Jose Onuchic, Rice University, USA
Knotting a Protein in the Computer – From Simple Models to Explicit Solvent Simulations

Sunday, September 21, 2014

8:00 AM – 12:00 PM — Registration/Information **Auditorium Lobby**

Session: DNA Knots and Viral Topology, Part I
Chair: Alexander Grosberg, New York University, USA

8:30 – 9:00 AM — Todd Yeates, University of California, Los Angeles, USA
Discoveries, Implications and Utilities of Proteins with Barriers, Knots, Slipknots, and Links

9:00 – 9:30 AM — Marek Cieplak, Polish Academy of Sciences, Poland
Knotted Proteins under Tension

9:30 – 10:00 AM — Peter Virnau, Johannes Gutenberg University, Germany
Molecular Simulations of Knotted Proteins and DNA

10:00 – 10:30 AM — Tetsuo Deguchi, Ochanomizu University, Japan
Probability of DNA Knots and the Effective Diameter of DNA Double Helix

**Short talks selected from among submitted abstracts*

10:30 – 10:45 AM	Coffee Break	**Auditorium Lobby**
	Session: DNA Knots and Viral Topology, Part II Chair: Wilma Olson, Rutgers University, USA	
10:45 – 11:15 AM	John Maddocks, Swiss Federal Institute of Technology (EPFL), Switzerland *Coarse-grain Models of DNA Free Energy and Sequence-dependent Minicircle Shapes*	
11:15 – 11:45 AM	Mariel Vazquez, University of California, Davis, USA *DNA Unlinking in Bacteria*	
11:45 AM – 12:15 PM	**Public Talk** De Witt Sumners, Florida State University, USA *DNA Knots Reveal Enzyme Mechanism and Viral Capsid Packing Geometry*	
12:15 PM	End of Meeting	

SPEAKER ABSTRACTS

Unfolded, but Not Knotted! Mechanistic Studies of IDP Folding upon Binding

Jane Clarke.
University of Cambridge, Cambridge, United Kingdom.

Many intrinsically disordered proteins function by folding upon binding to a target protein. It is often said that IDPs provide high specificity with low affinity, but kinetic analysis of a number of systems suggests that this is not universally correct. Why then are disordered proteins so ubiquitous? Is disorder in the IDP important for the function? I will discuss some of our recent kinetic and mechanistic studies of a number of IDPs that fold upon binding.

Ribosomes: RNA Machines for Protein Production that Withstand Evolution Pressures

Ada Yonath.
Department of Structural Biology, Weizmann Institute, Rehovot, Israel.

Ribosomes, the universal cellular machines for translation of the genetic code into proteins, possess spectacular architecture accompanied by inherent mobility, allowing for their smooth performance as polymerases that translate the genetic code into proteins. The site for peptide bond formation is located within a universal internal semi-symmetrical region. The high conservation of this region implies its existence irrespective of environmental conditions and indicates that it may represent an ancient RNA machine. Hence, it could be the kernel around which life originated. The mechanistic and genetic applications of this finding will be discussed.

Geometrical and Topological Aspects of Protein Structures

Alexey G. Murzin.
MRC Laboratory of Molecular Biology, Cambridge, United Kingdom.

Proteins are biological objects subjected to natural selection. Protein structures are also governed by the laws of physics and chemistry and, in general, combine both singular and regular features. The former usually contribute to protein function and therefore tend to be evolutionarily conserved, the latter result from the general principles of protein folding and contribute to overall structural stability. The capability of protein structures to accommodate potentially unfavourable sigularities greatly increases the number of possible folds suitable for functional selection. It also allows the formation of peculiar architectures and topologies occasionally found in the protein folds.

I will illustrate my overview of protein structure principles and evolution with the examples of rare and unusual structural and topological features discovered since the creation of our Structural Classification of Proteins (SCOP) database twenty years ago.

Experiment and Modeling: Competitive or Complementary Approaches to Structural Biology?

Wladek Minor.
University of Virginia, Charlottesville, USA.

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) increases 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 where 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 identity between the model and an experimentally derived structure drops below 30%.

A combined approach of multidisciplinary experimental and computational methods will lead to a dramatic increase in accuracy of structural predictions and computational screening. The process will be discussed in the context of knotted proteins.

Knots and Slipknots in Proteins

Kenneth Millett.
University of California, Santa Barbara, Santa Barbara, USA.

The strict conservation of knotted and slipknotted features within protein families despite large sequence divergence suggests the possibility of an important physiological role and the utility of a deeper understanding of their spatial character vis-à-vis the entire structure in order to identify contributing evidence that can clarify their role. The "knotting fingerprint" has provided a foundational method by which to encode and assess these structures. Its generation and application to protein structures provide the principal foci of the presentation.

What Forces Drive Conformational Changes?

Robert L. Jernigan, Jie Liu, Yuan Wang, Kejue Jia, Kannan Sankar.
Iowa State University, Ames, USA.

There are now many conformational transitions known for proteins. In many cases the transition directions appear to be an intrinsic property of the structure itself, and this has been observed in many cases by the use of elastic network models. This approach is successful for transitions from open to closed forms in enzymes. However, the elastic models cannot describe the opposite transitions from closed to open forms. In such cases forces may be required to open a protein structure. If the protein is an enzyme and its chemical reaction is exothermic, then this could be the origin of the forces. Wherever ATP hydrolysis is involved, this seems likely.

Meaningful dynamics information can be extracted from multiple experimental structures of the same, or closely related, proteins or RNAs. Usually only a few principal components dominate the motions of the structures, and these usually relate to the functional dynamics. This dynamics information provides strong evidence for the plasticity of protein and RNA structures, and also suggests that these structures almost always have a highly limited repertoire of motions. The variabilities of the internal distances among such a set of structures can be used to construct elastic models that represent well these variabilities.

We are computing pathways for transitions from closed to open forms, by applying forces to elastic models, by generating structures with a Metropolis Monte Carlo method, using free energies for structural intermediates computed using our 4-body potentials and entropies from elastic network models.

Folding of Nascent Chains of Knotted Proteins

Nicole Lim, Anna Mallam, Elin Sivertsson, Joe Rogers, Danny Hsu, Laura Itzhaki, **Sophie Jackson**.
University of Cambridge, Cambridge, United Kingdom.

Since 2000, when they were first identified by Willie Taylor, the number of knotted proteins within the pdb has increased and there are now nearly 300 such structures. The polypeptide chain of these proteins forms a topologically knotted structure. There are now examples of proteins which form simple 31 trefoil knots, 41, 52 Gordian knots and 61 Stevedore knots. Knotted proteins represent a significant challenge to both the experimental and computational protein folding communities. When and how the polypeptide chain knots during the folding of the protein poses an additional complexity to the folding landscape.

We have been studying the structure, folding and function of two types of knotted proteins – the 31-trefoil knotted methyltransferases and 52-knotted ubiquitin C-terminal hydrolases. The talk will focus on our folding studies on knotted trefoil methyltransferases and will include our recent work using cell free in vitro translation systems to probe the folding of nascent chains of knotted proteins. This approach has also been used to show that GroEL/GroES play a role in the folding of these proteins in vivo. New results on the degradation of knotted proteins by the bacterial ClpXClpP system will be presented.

Exploring the Coordinated Functional and Folding Landscapes of Knotted Proteins

Patricia Jennings.
UCSD, La Jolla, USA.

Flexibility and conformational changes allow proteins to perform the biological processes, such as ligand binding, oligomerization, conformational rearrangements and catalysis. Modulation of the dynamic states within the folded ensemble of a protein connect folded (or unfolded) states with biological functional states. Therefore, the interplay between a protein's structure, fold, and function add complexity to the already delicate heterogeneous energy balance between functional states. Clusters of frustrated interactions within various conformational states are beginning to be identified as regions within protein scaffolds that may correlate with functional regions. In our work we explore the hypothesis that regions with competing geometric restraints that result in frustrated regions on the energy landscape of a given protein have both folding and functional relevance. Of the structurally unique, yet significant knotted conformations available in nature, the SPOUT and cysteine knot classes, both demonstrate these coordinated, yet complex interactions and are the subject of our current explorations.

Methyl Transfer from AdoMet by a Knotted Protein Fold

Ya-Ming Hou.
Thomas Jefferson University, Philadelphia, USA.

TrmD is a bacteria-specific tRNA methyl transferase that transfers the methyl group from AdoMet to the N1 of G37-tRNA. The reaction product of TrmD, m1G37-tRNA, is essential for growth and it maintains the reading frame accuracy during tRNA translation on the ribosome. TrmD binds AdoMet using a rare trefoil-knot protein fold, whereas Trm5, the eukaryotic counterpart of TrmD, binds AdoMet using a common Rossmann-fold. While TrmD and Trm5 are fundamentally distinct in the AdoMet domain, we ask the question whether they are distinct in the catalytic mechanism. This is important for understanding the relationship of these two enzymes. Using pre-steady-state kinetic assays, we show that these two enzymes are distinct and unrelated in all aspects of the reaction mechanism. The striking distinction between these two enzymes supports the notion that TrmD is an attractive target for antibacterial discovery.

Harvesting the Fruits of the Energy Landscape Theory of Protein Folding

Peter G. Wolynes
Rice University, Houston, Texas

Protein folding can be understood as a biased search on a funneled but rugged energy landscape. This picture can be made quantitative using the statistical mechanics of glasses and first order transitions in mesoscopic systems. The funneled nature of the protein energy landscape is a consequence of natural selection. I will discuss how this rather simple picture quantitatively predicts folding mechanism from native structure and sequence. I will also discuss recent advances using energy landscape ideas to create algorithms capable of predicting protein tertiary structure from sequence, protein binding sites and the nature of structurally specific protein misfolding relevant to disease.

Knotted Structures in Refolding and Cotranslational Folding of Multi-domain Protein

Shoji Takada.
Kyoto, Japan.

Co-translational folding (CTF) is known to facilitate correct folding in vivo, but its precise mechanism remains elusive. For the CTF of a three-domain protein SufI, it was reported that the translational attenuation is obligatory to acquire the functional state. Here, for SufI, we performed comparative molecular simulations that mimic CTF as well as refolding schemes, addressing how the translational attenuation affects the folding. First, a CTF scheme that relied on a codon-based prediction of translational rates exhibited folding probability markedly higher than that by the refolding scheme. When the CTF schedule is speeded up, the success rate dropped to a probability similar to that by the refolding scheme. Whereas, the CTF that has a uniformly slow rate led to essentially the same result as the codon-based CTF scheme. Most notably, misfolding of the middle domain was much more frequent in the refolding scheme than that in the codon-based CTF scheme. The middle domain is less stable and can fold only when it is stabilized via interactions with the N-terminal domain. In a kinetic trap, while a segment of the middle domain entangled with the C-terminal domain, domain-domain interfaces were formed to lock these interfaces. Thus obtained knotted misfolds could not be escaped in the simulations. Folding pathway networks showed that the refolding scheme sampled diverse states with no clear pathways, while the codon-based CTF showed a clear and narrower pathways to the native state. The degree of folding acquisition was shown to modestly correlate with the elongation time.

Coarse Grained Modeling of Protein Structure, Dynamics and Interactions

Andrzej Kolinski.
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).

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Connecting Simplified Models with Explicit-Solvent Forcefields: Slipknotting during the Folding of the Smallest Knotted Protein

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Recently, experiments have confirmed that trefoil knotted proteins can fold spontaneously, consistent with predictions from simulations of simplified protein models. These simulations suggest folding the knot involves threading the protein terminal across a twisted loop via a slipknot configuration. Here, we report unbiased all-atom explicit-solvent simulations of the knotting dynamics of a protein. In simulations totaling 40 μs, we find that 5 out of 15 simulations reach the knotted native state when initiated from unknotted, slipknotted, post-transition-state (post-TS) intermediates. The completed threading events had durations of $0.1-2$ μs. On the μs timescale, post-TS structures rarely backtracked and pre-TS structures often backtracked and never completed. Comparison of explicit-solvent to structure-based simulations shows that similar native contacts are responsible for threading the slipknot through the loop; however, competition between native and non-native salt bridges during threading results in increased energetic roughness. Overall, these simulations support a slipknotting mechanism for proteins with complex topology, and help verify that simplified models are useful tools for studying knotted proteins.

Significance, Complexity, and Beauty of Knot-avoiding Structures

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If a long polymer is forced to collapse while remaining unknotted, it adopts a peculiar structure which is very different from regular conformations where the amount of knots is consistent with thermodynamic equilibrium. These structures are hypothesized to play an important role in genome folding across biological realms. Their importance for proteins is an interesting subject of discussion. Present understanding of these unknotted structures is incomplete, despite significant efforts in both computer simulations and theoretical estimates. In present work, the scaling properties of unknotted globules will be discussed, with focus on finite size corrections to scaling, including new results on contact and surface roughbness exponents. The conclusion of this work is that forced lack of knots is at least equally important to the actual presence of knots.

Multiscale Modeling of Protein Flexibility

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Conformational flexibility plays an important role in protein function. Structural characterization of protein flexibility is a challenge for both, experimental and simulation techniques.
Recently, we have developed a multiscale modeling procedure for the efficient simulation of flexibility of globular proteins. The method have been made available as CABS-flex server (http://biocomp.chem.uw.edu.pl/CABSflex) [1]. The CABS-flex method was shown to be a computationally efficient alternative to all-atom molecular dynamics - a classical simulation approach [2]. We also demonstrated that the relative fluctuations of protein residues obtained from CABS-flex are well correlated to those of NMR ensembles [3].
Since CABS-flex requires an input in the form of a complete (without breaks) protein chain, the CABS-flex input for the proteins with missing structure data needs to be additionally prepared. In such cases, the modeling can be supported with our other tool for the prediction of protein structure: the CABS-fold server (http://biocomp.chem.uw.edu.pl/CABSfold) [4].

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Connecting Simplified Models with Explicit-solvent Forcefields: Slipknotting during the Simulations of Folding and Unfolding of Pseudoknots in RNA

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RNA pseudoknot is an element of RNA architecture comprising at least two helix-loop structures, in which a region in the loop associated with one helix base-pairs with complementary nucleotides outside that helix, thereby forming a second helix. Pseudoknots can be formed by regions of RNA that are very distant in primary sequence and are difficult to predict computationally from RNA sequence because of their non-linear character. Pseudoknots fold into knot-shaped three-dimensional conformations, but are not true topological knots. The pseudoknot architecture is capable of supporting various stable 3D folds that display a diverse range of functions in a variety of biological processes. First recognized in the genomes of plant viruses in 1982, pseudoknots are now established as evolutionarily conserved elements of functionally important RNAs such as RNase P or telomerase RNA.
We used SimRNA, our recently developed method for coarse-grained RNA folding simulations, to model the folding and unfolding of several RNA pseudoknots with experimentally detemrined structures. Our simulations provide insight into the folding trajectories, in particular into the order of helix formation, depending on RNA sequence.

Defining and Identifying Knots in Linear Polymers

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Identifying topological entanglements in ring polymers is straightforward since knotting is well-defined. For linear polymers this is not so simple and a variety of schemes have been proposed to identify entanglements in linear polymers. These schemes all have advantages and disadvantages and several schemes will be described and compared. The difficulty of finding an unambiguous definition of knotting in linear polymers will be illustrated by examples that correspond to an entanglement by one definition but not by another. For very long polymers it will be shown that all the schemes being considered detect knotting even though they may disagree about the particular knot type.

The Knot Complexity of Compressed Polygons in a Lattice Tube

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Towards characterizing the likelihood and the nature of knotted structures in proteins and nucleic acids, a polygon model of ring polymers confined to a lattice tube and under the influence of a tensile force has been developed. This simplified model has the advantage that results about entanglement complexity can be proved and generation of all conformations for small tube sizes is possible. One objective of this work is to obtain exact results about the entanglement complexity of compressed polygons in lattice tubes. The methods used include both theoretical and numerical approaches based on the transfer-matrix method. We prove a pattern theorem for compressed polygons and obtain exact results about the probability of knotting for small tube sizes. A comparison is made to the results for stretched polygons. We conclude that all but exponentially few sufficiently long compressed polygons in an L x M x infinity lattice tube are highly knotted. We also observe from the numerical data that, as expected, polygon entanglement complexity decreases as the stretching force strength is increased.

Knotting in Subchains of Proteins and Other Entangled Chains

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Researchers have discovered interesting knotting and slipknotting patterns in proteins by analyzing the knotting of all subchains.The subchains typically form simpler knot types (which we call "subknots'") than the full chain. By analyzing the knotting within subchains of some energy-minimizing closed knots, we are able to draw certain conclusions about the geometry of knotted proteins. This is joint work with Kenneth Millett, Andrzej Stasiak, and Joanna Sulkowska.

Mechanical Unfolding of Single RNA Pseudoknots Reveals that Conformational Plasticity, Not Resistance to Unfolding, is a Determinant of Programmed −1 Frameshifting

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Programmed −1 frameshifting, whereby a ribosome shifts reading frame on a messenger RNA in order to generate an alternate gene product, is often stimulated by a pseudoknot structure in the mRNA. Viruses in particular use frameshifting to regulate gene expression, making pseudoknots potential targets for anti-viral drugs. The efficiency of the frameshift varies widely for different sites, but the factors that determine frameshifting efficiency are not yet fully understood. Previous work has suggested that frameshifting efficiency is related to the resistance of the pseudoknot against mechanical unfolding. We tested this hypothesis by studying the mechanical properties of a panel of pseudoknots with frameshifting efficiencies ranging from 2% to 30%. Using optical tweezers to apply tension across the mRNA, mimicking the tension applied by the ribosomal helicase when unfolding structure in the mRNA, we measured the distribution of forces needed to unfold each pseudoknot. We found that neither the unfolding force, the unfolding kinetics, nor the properties of the energy landscape for unfolding could be correlated to frameshifting efficiency. Surprisingly however, increased frameshifting efficiency was correlated with an increased tendency to form alternate structures, suggesting a more complex role for the pseudoknot involving conformational dynamics. These results were corroborated by studying the effects of a ligand that reduces frameshifting associated with the SARS pseudoknot: binding of the ligand to the pseudoknot abolished the formation of alternate conformers. In addition to providing a novel framework for future studies aimed at understanding mechanisms regulating −1 PRF efficiency, our work suggests that targeting the conformational dynamics of pseudoknots may be an effective strategy for anti-viral drug design.

Mechanically Tightening a Protein Slipknot into a Trefoil Knot

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Knotted polypeptide chain is one of the most surprising topological features found in some proteins. How knotted proteins overcome the topological difficulty to fold into their native three dimensional structures proteins has become a challenging problem. It was suggested that a structure of slipknot could serve as an important intermediate state during the folding of knotted proteins. Here we use single molecule force spectroscopy (SMFS) as well as steered molecular dynamics (SMD) simulations to investigate the mechanism of transforming a slipknot protein AFV3-109 into a tightened trefoil knot by pulling. Our results show that by pulling the N-terminus and the threaded loop of AFV3-109, the protein can be unfolded via multiple pathways and the slipknot can be transformed into a tightened trefoil knot, which involves ~13 amino acid residues. SMD simulation results, which are consistent with our experimental findings, provide a detailed molecular mechanism of mechanical unfolding and knot tightening of AFV3-109. SMD simulations reveal that interactions between β-strands on the threading loop and knotting loop that are sheared during stretching provide high mechanical resistance in the process of forming the trefoil knot, i.e., pulling threaded loop through knotting loop.

Untying a Protein Knot - Translocation of Knotted Proteins through a Pore

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In less than 1% of proteins the polypeptide chain adopts a knotted configuration. What is it then about the knotted proteins that makes them so rare in living matter? One possibility, proposed in [1-3] is that the presence of a knot may affect the ability of proteins to be degraded in proteasome or translocated through the membranes. The smallest constrictions in the mitochondrial pores or proteasome openings are 12-14 Angstrom in diameter, too narrow to accommodate folded structures, thus translocation must be coupled to protein unfolding. Unfolding and import of proteins into mitochondria or proteasome are facilitated by molecular motors acting with the forces of the order of 30pN. However, as shown in [2,4-5], the protein knots tend to tighten under the action of the force. The radius of gyration of the tight knot is about 7-8 Angstroms for a trefoil, which means that the knot seems to be a shade too large to squeeze through the pore openings. This leaves us with two possibilities: either the knot diffuses towards the end of the chain and slides away or gets tightened and jams the opening [3]. We report the result of molecular dynamics simulations of protein translocation demonstrating 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, transforms chemical energy into directed motions via nucleotide-hydrolysis-mediated conformational changes, which are cyclic in character.

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Why are Phytochromes Knotted?

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Members of the phytochrome family of photoreceptors contain a deep figure-of-eight knot. We have hypothesized that signal transduction by phytochromes is efficient because of this unusual topology. Absorption of a 700 nm photon by the chromophore directs 41 kcal/mol into the protein; the knot may rigidify the photosensory core of phytochrome so that work is done to reposition the effector domain appropriately in the transition from dark to lit state, rather than permitting energy losses to random motions. To test this model, we are studying the biochemistry and structural biology of the signal transduction pathway of the bacterial phytochromes of Deinococcus radiodurans and Ramlibacter tatouinensis. Each contains a histidine kinase effector domain, and each is predicted to be a light-regulated enzyme that phosphorylates across a homodimeric interface and subsequently transfers phosphate to a response regulator protein. We have established a robust in vitro kinase assay and our preliminary results suggest R. tatouinensis bacterial phytochrome is an autokinase that interacts specifically with its bacterial phytochrome response regulator (Brr), with lower activity under red light. We have used recombineering to generate a knotless bacterial phytochrome and will test its kinase activity and the light-dependence of that activity. In addition, we have refined the X-ray crystal structure of the R. tatouinensis Brr protein. In an unusual topology, a C-terminal extension of the canonical response regulator fold wraps around its symmetry mate and returns to the originating monomer, forming an alpha helix that packs against the globular domain. Thus, to our surprise, this single domain response regulator dimerizes to form a light-independent, noncovalent catenane. The coincidence that a knotted photoreceptor is paired with a linked response regulator dimer is an intriguing phenomenon whose biological relevance has yet to be investigated.

Crowding Promotes the Switch from Hairpin to Pseudoknot Conformation in Human Telomerase RNA

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Formation of a pseudoknot (PK) in the conserved RNA core domain in the ribonucleoprotein human telomerase is required for function. In vitro experiments show that the PK is in equilibrium with an extended hairpin (HP) structure. We used molecular simulations of a coarse-grained model, which reproduces most of the salient features of the experimental melting profiles of PK and HP, to show that crowding enhances the stability of PK relative to HP in the wild type and in a mutant associated with dyskeratosis congenita. In monodisperse suspensions, small crowding particles increase the stability of compact structures to a greater extent than larger crowders. If the sizes of crowders in a binary mixture are smaller than that of the unfolded RNA, the increase in melting temperature due to the two components is additive. In a ternary mixture of crowders that are larger than the unfolded RNA, which mimics the composition of ribosome, large enzyme complexes and proteins in Escherichia coli, the marginal increase in stability is entirely determined by the smallest component. We predict that crowding can partially restore telomerase activity with decreased PK stability.

Parallels between Protein Folding and Ribosome Dynamics

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The study of protein folding has provided a rich quantitative and conceptual foundation for describing the physical-chemical properties of biomolecular dynamics. In particular, the folding of complex proteins, such as knotted proteins, has illustrated the strong role that steric interactions have on biological dynamics. By utilizing the foundation provided by theoretical studies of protein folding, we are now able to use molecular simulation to gain deeper insights into the roles that steric factors have during the functional dynamics of large biomolecular assemblies. In our studies of the ribosome, we are finding many common themes between protein folding and conformational dynamics of this assembly. In the case of tRNA accommodation and translocation on the ribosome, we have found that tRNA molecules and elongation factors frequently exploit a delicate balance between entropy enthalpy, similar to the process of protein folding. In addition, through the use of simplified modeling strategies, we are finding that the shape of the ribosome introduces many steric obstacles, which are analogous to the geometric limitations imposed during the folding of knotted proteins. Finally, we are also adopting quantitative tools developed for folding to identify proper coordinates that are capable of precisely capturing the relevant transition states. Together, these studies highlight how an understanding of complex proteins can enable insights into a broad range of biomolecular processes.

Conformational Dynamics of RNA Functional Motifs: Ribosomal A-site and Thermosensing Hairpin

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Internal dynamics of RNA is often crucial for its function. For instance, various steps of mRNA translation are regulated by changes in conformational states of single nucleotides in ribosomal RNA. Inherent dynamics of regulatory elements of mRNA, such as riboswitches, enables response to environmental conditions. These regulatory elements include RNA thermosensing sequences that allow for cell's feedback to heat or cold shock conditions.

I will describe two RNA motifs whose dynamics is important for (i) aminoglycoside antibiotic binding and (ii) switching on translation upon temperature increase. These RNA fragments have special sequence properties that make their structures uniquely flexible. The methodology used were molecular dynamics simulations, fluorescence spectroscopy and thermal melting experiments.

The first example is the aminoacylated-tRNA binding site (A-site) in the small ribosomal subunit whose sequence differs between bacterial, human cytoplasmic and mitochondrial ribosomes. In the crystal structures of the mitochondrial A-site we have identified a specific S-turn conformation of the RNA backbone. This topology is not present in bacterial A-sites (both in models and 30S subunits). Simulations show that mitochondrial and bacterial A-sites show different propensities to form S-turn2. Also, mitochondrial A-site with a single mutation (A1555G) resembles more the bacterial one explaining the ototoxicity of aminoglycosides in patients carrying this mutation.

The second example are RNA thermosensors which are temperature sensing short RNA sequences located in the 5' untranslated region of mRNA. They typically form a hairpin with noncanonical base pairing to allow for unfolding in response to temperature increase. Local melting of RNA exposes the Shine-Dalgarno sequence and allows for its binding to the ribosomal RNA (anti-Shine-Dalgarno region) in the small subunit. We have investigated the mechanism of thermal unwinding of a fourU thermometer and repression of heat shock gene expression element (ROSE).

Chromatin Looping and Interdigitation Mechanisms: Insights from Mesoscale Simulations

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In all eukaryotic organisms, the chromatin fibers, composed of DNA complexed with core histones and linker histone proteins, store a vast amount of information. The chromatin building blocks, namely nucleosomes -- nanometric beads made of DNA and eight core histones -- connect to one another by DNA linkers and form hierarchical structures whose folds are essential for the nderstanding of basic regulatory processes in the cell. However, the precise organization of chromatin remains elusive. In particular, it is not clear whether chromatin organizes in independent or inter-digitated fibers. To address this question, we have developed and applied a mesoscale computational model in collaboration with experiments to probe structural, energetic, and dynamical questions associated with chromatin as a function of various internal and external factors. We will describe several mechanisms that affect fiber architecture, including the size of the linker DNAs and the presence of linker histones. Depending on the conditions, these molecular mechanisms favor the formation of segregated fibers, inter-digitated fibers, and chromatin loops. The modeling thus helps interpret the variation in chromatin fibers for different cell types and represents a reference framework to investigate local and global mechanisms that regulate chromatin structure in the nucleus.

Protein-induced Entanglement on DNA: Connecting and Organizing Chromosomes via Multiple Loops.

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The control of gene expression sometimes entails the folding of DNA into looped structures mediated by the binding of protein. Although the literature abounds with examples of single DNA loops induced by the attachment of sequentially distant genetic elements on a common protein core, recent studies have demonstrated the occurrence of multiple loops formed by three or more remote, protein-anchored sites. The direct physical connections between these DNA sites stem from the capability of protein, such as the *Escherichia coli* Gal repressor, to form oligomeric structures. Structure-based genetic analyses have shown that dimeric units of the Gal repressor stack one above the other in a V-shaped tetrameric assembly. Repeated dimeric associations of the same type lead to higher-order helical protein pathways that can secure multiple chromosomal connections. We are examining the entanglement of DNA loops that attach to such proteins with the help of a novel energy minimization method. Our method makes it possible to optimize DNA pathways at the base-pair level under various constraints, such as imposed end-to-end displacement and rotation. We focus on the multiple loops that can be induced by oligomeric Gal assemblies and compute the relevant energy landscapes and topological properties as functions of the number of Gal repressors and the chain lengths of the different loops. The binding of the less stacked Lac repressor to a DNA minicircle, which segregates the double helix in two loops, is also investigated. In addition, we take advantage of the fact that our optimization method accounts for the presence along DNA of bound ligands to study how the binding of architectural proteins (*e.g.*, the*Escherichia coli* histone-like HU protein) can ease or suppress the formation of such loops.

Knotted DNA: Mathematical Models and Biological Consequences

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We'll discuss recent work on knotted and linked DNA molecules. Using several case studies as examples, we'll consider the knot theoretic techniques used to model the processes that knot and link DNA. We'll explore the biological ramifications of DNA knotting and linking, and how the results of these topological models can inform experimentalists.

DNA Topology, DNA Topoisomerases and Small DNA Circles

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DNA topology is vitally important to many biological processes and is controlled by DNA topoisomerases. There are two types, I and II, depending on whether their reactions proceed via single- or double-strand breaks. Type IIs cleave DNA in both strands and transport another segment of DNA through the break. This leads to DNA relaxation, decatenation and unknotting, and, in the case of DNA gyrase, supercoiling, in reactions coupled to ATP hydrolysis. It is clear why supercoiling by gyrase requires ATP, but not obvious with other type IIs. One potential role for ATP in the non-supercoiling topoisomerases is in topology simplification: generating steady-state distributions of topoisomers that are simpler than at equilibrium. However, the energetic requirements for topology simplification are very small. Therefore we propose that the ATP free energy is used to disrupt protein-protein interfaces, which are very stable in order to prevent unwanted DNA breaks. Although the biological significance of topology simplification is questionable, its mechanism is the subject of debate. It is accepted that DNA bending by is likely to contribute to this process, but we have shown that this cannot be the sole determinant. Recent work points to a protein interface, known as the ‘exit gate’, to be an important feature of the ability of these enzymes to carry out topology simplification. We have used small DNA circles as probes in this work. This has led to research towards understanding how topology controls gene expression: investigating how DNA recognition is influenced by supercoiling, using a combined molecular dynamics and biochemical/biophysical approach. Using small circles of varying linking differences, we are analysing the binding of two probes: a triplex-forming oligonucleotide and a repressor. Coupled with atomistic simulations, this work is giving insight into topology-dependent DNA recognition.

Conformational Free-Energy Calculations for Complex Biopolymer Structures

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Quantitative knowledge of free-energy changes are central to understanding protein and RNA folding, motion and energy transduction in molecular machines, macromolecule-ligand interactions, genome organization, and many other biological phenomena. We focus here on problems related to DNA tertiary structure and topology, especially loop-mediated interactions involving protein molecules bound to sites separated by large linear distances along DNA. Computing the free-energy cost of forming DNA or chromatin loops entails a delicate and length-scale-dependent balance of enthalpic and entropic contributions and is a challenging problem in statistical mechanics. Moreover, the effects of chromatin organization on such interactions are poorly understood. However, new insights can come from novel experimental approaches and computational models of DNA flexibility and folding under geometric and/or topological constraints. Experimental studies of DNA looping in systems such as Cre-loxP recombination and lac-repressor-mediated gene regulation will be discussed in conjunction with a new method for computing the free energies of looped nucleoprotein assemblies.

Structural Basis for Regulation of Site-specific DNA Recombinases by DNA Topology

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DNA resolvases are a group of site-specific recombinases that are regulated by DNA topology, and that act to "resolve" a circular DNA molecule into two smaller ones. We study two related resolvases: 1) Sin, which is encoded by many staphylococcal plasmids and presumably aids in their stability by resolving dimeric forms into monomeric ones and 2) Tn3, which is encoded by the Tn3 transposon and resolves the fused-circle cointegrate product of the replicative transposition process into two product circles.

These resolvases are only catalytically active when incorporated into a larger complex (a "synaptosome") that brings together the two DNA partners and traps 3 supercoiling nodes (dsDNA over dsDNA crossings) between them. Rendering activity dependent on synaptosome formation serves several purposes. 1st, it favors intra- over inter-plasmid recombination because trapping 3 crossings is easier within one supercoiled circle than between two separate ones. 2nd, it ensures correct alignment of the two crossover sites (parallel vs. antiparallel). 3rd, when the strand exchange process is carried out within the synaptosome it relaxes supercoiling strain and is thus energetically favorable.

We used a combination of crystallography, SAXS and molecular biology to construct 3D models for the both the Sin and Tn3 resolvase synaptosomes. For both systems, individual structures of all the sub-complexes can be docked together in silico in a manner that agrees with activity data on a large array of mutant and chimeric proteins with altered DNA- and protein-protein interactions. Our results show that even though protein sequence and mechanistic similarities show these recombinases must have diverged from a common ancestor, they probably later converged upon the use of a synaptic complex that traps 3 supercoiling nodes because it is so well-suited to channeling the reaction to produce only resolution products.

Unveiling the Molecular Trajectory during Binding Site Recognition by DNA-bending Proteins

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Many proteins that bind to specific sites on DNA locate these sites by first binding to nonspecific sites on DNA and then scanning nearby sites for their target binding site. Characterizing the rates and mechanism by which proteins switch from nonspecific to specific binding has been a challenge in the field. Here we demonstrate how the enhanced sensitivity and time-resolution of laser temperature-jump (T-jump) allows us to capture the kinetics of molecular rearrangements during binding site recognition by two classes of proteins: (1) Integration Host Factor (IHF), a eubacterial architectural protein involved in chromosomal compaction and DNA recombination, which recognizes specific DNA sites and bends them into sharp U-turns, and (2) XPC/Rad4, a DNA damage repair protein that initiates nucleotide excision repair by recognizing diverse DNA lesions caused by environmental insults. We use T-jump to perturb the protein-DNA complex and time-resolved fluorescence measurements to monitor the dynamics of DNA bending, unwinding, and nucleotide-flipping upon protein binding.

In the two systems: IHF bound to one of its cognate sites on DNA and XPC bound to DNA containing a model (3-bp mismatch) lesion, we resolve relaxation kinetics occurring over two distinct timescales. A rapid (~100 microseconds) phase, which is found to be independent of the DNA sequence, is attributed to nonspecific “interrogation” of the DNA binding sites by the protein, while a slower (~10 milliseconds) phase is attributed to the ultimate recognition step to form the specific complex. These results represent the first observation of an apparent two-step (interrogation then recognition) process that bridges the gap between relatively slow (> ms) recognition of target sites and relatively rapid (< ms) one-dimensional diffusion of proteins scanning the DNA.

Sedimentation of Macroscopic Rigid Knots and its Relation to Gelelectrophoretic Mobility of DNA Knots

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We address the general question of the extent to which the hydrodynamic behaviour of microscopic freely fluctuating objects can be reproduced by macrosopic rigid objects. In particular, we compare the sedimentation speeds of knotted DNA molecules undergoing gel electrophoresis to the sedimentation speeds of rigid stereolithographic models of ideal knots in both water and silicon oil. We find that the sedimentation speeds grow roughly linearly with the average crossing number of the ideal knot configurations, and that the correlation is stronger within classes of knots. This is consistent with previous observations with DNA knots in gel electrophoresis.

RNA Pseudoknots in Telomerase and Riboswitches

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RNA pseudoknots are a common element in structured RNAs, and can play both structural and functional roles. We have investigated the pseudoknot structures and dynamics in telomerase and preQ1 class I and II riboswitches. Telomerase is an RNP composed of a unique telomerase reverse transcriptase, a telomerase RNA (TER), and accessory proteins. While TER varies in length from ~150 nts in ciliates to >2000 in yeasts, all contain a pseudoknot. We found that human TER pseudoknot contains a triple helix that is critical for function. Similar triple helices were predicted for yeasts, and we found that in K. lactis the overall pseudoknot fold is remarkably similar to human despite differences in sequence. A series of bulge bases, which were not predicted, allow an extended triple helix to form. Riboswitches are RNA regulatory elements, often found in the 5' untranslated region of bacterial genes or operons, that change conformation upon binding to a ligand, generally a metabolite related to the following genes. This conformational change affects transcription or translation, depending on the riboswitch, by exposing or sequestering a transcriptional terminator or the Shine-Delgarno sequence, respectively. Two classes of riboswitches that bind preQ1, a precursor to the modified nucleotide queosine almost universally found in the anticodon of some tRNAs, have been identified. Structures of both classes of riboswitch in complex with preQ1 have been determined by us and others. Both classes of preQ1 riboswitch form a pseudoknot, but the overall folds, cation interactions, and mechanism of ligand recognition are different.

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Single-Stranded Architecture for Cotranscriptional Folding of RNA Nanostructures

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RNA nanotechnology uses the diversity of structural and functional modules from natural RNA molecules to engineer novel nanoscale devices. An important goal is to be able to design large and well-defined RNA structures that can assemble during the transcription process, since such structures can be genetically encoded and expressed in cells with diverse applications in synthetic biology. Here, we introduce a general architecture for designing artificial RNA structures that can fold from a single strand: Arrays of antiparallel RNA helices are precisely organized by RNA pseudoknot motifs and a new type of crossover pattern that can fold without topological problems. To validate our architecture we construct RNA tiles that assemble into hexagonal lattices, and demonstrate that lattices can be made by annealing and/or cotranscriptional folding. We further show that the tiles can be scaled up to 660 nucleotides in length, reaching a size comparable to that of large natural ribozymes. We conclude that the folding path of complex RNA nanostructures can be rationally designed and will discuss the geometric principles, structure prediction algorithms, and kinetic folding models that are required to extend this design paradigm further.

Single-stranded DNA Topology in Eukaryotes

Zbyszek Otwinowski, Dominika Borek.
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In a single chromosome, both template strands are wrapped around each other and become replicated at hundreds of sites in parallel to form new dsDNA molecules, which are subsequently individualized into sister chromatids in prophase and segregated to daughter cells later in anaphase. How the entanglement is avoided during individualization remains unclear. The replication of holocentric and ring chromosomes provides much insight into this process. The products of replication do not become entangled during mitosis for either chromosome type, and this indicates that during replication the template strands are differentiated in a coordinated manner. Additionally, patterns of labeling in diplochromosomes that are created during endoreduplication show that this mechanism relies on the memory of DNA strands formation, i.e. information about the order in which two strands have been replicated is propagated across generations. Because information that differentiates strands in dsDNA, is passed on from one generation to the next one, the mechanism of this process is by definition epigenetic.
We propose a mechanism of coordinated strand recognition that relies on formation of single-stranded topological structures, which are generated during DNA synthesis. Structures of such type, called hemicatenanes, have been observed in plasmids, viruses and Crenarchaeota – the prokaryotes with the replication mechanism most closely related to that of eukaryotes. Topological structures based on single-stranded DNA are likely to be a missing element that hinders our understanding of: (1) the differentiation of the sister chromatids during their individualization, (2) the formation of higher-order structures in mitotic chromosomes coupled to individualization, (3) ORI definition, (4) cis-regulation in epigenetic processes and (5) asymmetric cell division, (6) spatial, temporal, and genomic co-linearity of transcriptional programs that control developmental processes.

How Linking Number Affects the Structure and Reactivity of DNA

Jonathan M. Fogg, Rossitza N. Irobalieva, Daniel J. Catanese, Anna K. Barker, Michael F. Schmid, Wah Chiu, **Lynn Zechiedrich**.
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The negatively charged sugar-phosphate backbone contains no genetic information yet forms the accessible exterior of the DNA double helix. Hydrophobic bases, the readout of the genetic code, are buried within the interior of the helix. We hypothesized that the seemingly contradictory requirements of DNA stability and readout are accomplished via a tightly-regulated switch whereby torsional strain causes localized structural alterations, including base-flipping, denaturation, and other non-B-DNA structures. Molecular dynamic simulations had indicated that our hypothesis was correct (Randall, G.L., Zechiedrich, L., and Pettitt, B.M. (2009) Nucleic Acids Res 37, 5568), but it had never been tested directly. Using tiny, closed circles of DNA of defined linking number, we demonstrate, using a combination of gel electrophoresis, chemical probing, and cryo-electron tomography, that the structural alterations brought about by torsional stress, base-flipping, and denaturation in the underwound, negatively supercoiled direction, and, likely, inside-out Pauling-like DNA in the overwound, positively supercoiled direction, facilitate access to the genetic code to initiate DNA readout. At very low amounts (~3%) of underwinding or overwinding, the torsional stress in these tiny circles is relieved nearly exclusively with writhe. At higher degrees of underwinding (~6%), base-flipping and denaturation alters twist and the circles are not additionally writhed until even more underwinding (~9-20%). In the positive direction, the circles continue to writhe. Funded by NIH T90DK070121 (R.N.I.), NIH P41RR02250 (W.C.), and NIH R01AI054830 and the Human Frontier Science Program (L.Z.).

Escherichia coli Live Cell Super-resolution Analysis of Topoisomerase IV Action

Pawel Zawadzki[1,2], David Sherratt[1].
[1]Oxford University, Oxford, United Kingdom, [2]Oxford University, Oxford, United Kingdom.

~440, 000 links between duplex strands need to be removed every E. coli cell generation. Type II topoisomerses perform the majority of this task. Gyrase relaxes (+)ve supercoils in front of replication fork, while TopoIV plays an essential function in decatenation of newly replicated DNA and can additionally relax both (+)ve and (-)ve supercoils. We counted and followed the in vivo behaviour of single molecules of the two TopoIV subunits, ParC and ParE by using live-cell super-resolution PALM imaging. Approximately 40% of both subunits are present as an immobile population that forms discrete foci suggesting that action of Topo IV is precisely localised in slowly growing cells. Analysis of colocalisation with DNA regions and protein complexes showed that TopoIV is enriched at ori, where it co-localizes with MukBEF, and at ter in cell approaching replication termination and division. The localisation and diffusional profiles of both subunits were independent of replication. Removal of functional MukBEF, or interference of the interaction between MukBEF and TopoIV led to the loss of most of the immobile TopoIV molecules associated with ori (and MukBEF), thereby demonstrating that the immobile TopoIV molecules are physically associated with MukBEF molecules within foci. Additionally we show that the TopoIV molecules associated with MukBEF foci are catalytically active, by showing that after covalent attachment to DNA during inhibition of catalysis with norfloxacin, that their association with MukBEF foci is retained. Taken together our results show where, when and how TopoIV performs its function in living cell.

Fine-tuning the Activity of DNA Bridging Proteins

Remus Dame.
Leiden University, Leiden, Netherlands.

Loop formation is key to the global organization of genomes in organisms from all three domains of life. Genomes are organized dynamically and their re-modelling is implied in translating external signals into specific gene products. The bacterial chromatin proteins H-NS and the archaeal chromatin protein Alba are capable of forming bridges between DNA segments *in vitro* and thus candidates for genomic loop formation *in vivo*. H-NS is a global regulator of transcription and a similar role has been suggested for Alba. We have investigated how the DNA bridging activity of these proteins is fine-tuned by physico-chemical conditions and interaction with other proteins (1,2). We demonstrate that for both proteins there is a delicate balance between two binding modes (stiffening and bridging), which can be shifted when conditions are changed or interaction partners are present. Our observations yield models for fine-tuning of genome organization and for the translation of changes in genome organization into transcriptional activity.

1) Laurens *et al*., Nature comm. (2012)
2) Van der Valk *et al.* Submitted.

Closing the Loop: Comparing the Results of Experiment and Computer Simulations in the Study of DNA Minicircles

Sarah Harris.
University of Leeds, Leeds, United Kingdom.

Small DNA circles offer a controllable model system for the systematic exploration of the dependence of DNA structure on supercoiling. We use computer simulation to explore the supercoiling-dependent conformation of small DNA circles and how this is affected by supercoiling, salt concentration, DNA sequence and the size of the circles [1]. The calculations use atomistic molecular dynamics simulation, and employ both implicit and explicit solvent models.

However, even given the most powerful supercomputers currently available, and the current interest in using state of the art experimental biophysical and biochemical techniques to study very small DNA loops, it can be challenging to identify situations in which the results of simulation and experiment can be directly compared. I will present a critical comparison of our computer models with results from cryoEM [2], AFM, gel electrophoresis [3] and biochemical measurements [4] on DNA minicircles, and will then invite discussion of the successes and caveats associated with both the theory and the experimental data.

[1] Mitchell J. S., Laughton C. A. & Harris S. A., Atomistic simulations reveal bubbles, kinks and wrinkles in supercoiled DNA. Nucleic Acids Res. 2011. 39: p. 3928-3938.
[2] Lionberger T. A. et al, Co-operative kinking at distant sites in mechanically stressed DNA Nucleic Acids Res., 2011, 39, 9820-9832.
[3] Fogg et al, Exploring writhe in minicircle DNA J. Phys Condensed. Matter, 2006, 18, S145-S159.
[4] Du, Q., A. Kotlyar, and A. Vologodskii, Kinking the double helix by bending deformation. Nucleic Acids Res., 2008. 36: p. 1120-1128.

Knotting a Protein in the Computer - From Simple Models to Explicit Solvent Simulations

Jose N. Onuchic.
Rice University, Houston, USA.

Recently, experiments have confirmed that trefoil knotted proteins can fold spontaneously, consistent with predictions from simulations of simplified protein models. These simulations suggest folding the knot involves threading the protein terminal across a twisted loop via a slipknot configuration. To further support these conclusions, we have performed unbiased all-atom explicit-solvent simulations of the knotting dynamics of a protein. In simulations totaling 40 μs, we find that 5 out of 15 simulations reach the knotted native state when initiated from unknotted, slipknotted intermediates. The completed threading events had durations of 0.1–2 μs. Comparison of explicit-solvent to structure-based simulations shows that similar native contacts are responsible for threading the slipknot through the loop; however, competition between native and non-native salt bridges during threading results in increased energetic roughness. Overall, these simulations support a slipknotting mechanism for proteins with complex topology, and help verify that simplified models are useful tools for studying knotted proteins.

* Supported by the NSF

Discoveries, Implications and Utilities of Proteins with Barriers, Knots, Slipknots, and Links

Todd Yeates
University of California, Los Angeles, USA

Proteins have notoriously complex structures, and the routes by which they fold into such complex shapes remains an important and largely outstanding problem in biology. Proteins whose backbones have unusually complex topologies provide valuable model systems for exploring ideas related to folding mechanisms and landscapes, while also providing potentially valuable building blocks for creating interesting materials. Previous discoveries and new ideas will be discussed for proteins with diverse topological features.

Knotted Proteins under Tension

Marek Cieplak.
Institute of Physics, PAS, Warsaw, Poland.

We highlight the diversity of mechanical clamps, some of them topological in nature that have been found by making surveys of mechanostability of just under 20 000 proteins within structure-based models. The existence of superstable proteins (with the characteristic unfolding force in the range of 1000 pN) is predicted. In these studies, mechanostability has been assessed by stretching at constant speed. Here, we focus on stretching of knotted proteins at constant tension - the case which is more relevant biologically. In particular, we find that proteins with knots unravel in a way similar to those without knots: there is a crossover between the inverse Gaussian distribution of unfolding times at high forces to the exponential distribution at low forces. However, we observe that sudden jumps in the extension of a protein do not necessarily lead to jumps in the location of the ends of the knot and the knot can get fully tightened before the protein is stretched. We then propose a model to study the proteasome-induced protein translocation. It involves constant-force pulling through a funnel-shaped potential. We find that a) the process of translocation unfolds proteins bound for degradation efficiently, b) the tension along the protein backbone is non-uniform, and c) the stalling force is smaller than the force of puling by the proteasomal motor. Our results provide insights into the mechanisms of unfolding used by biological unfoldases and indicate that the experimental paradigm used for measuring the traction power of the proteasome Finally we discuss some aspects of folding of proteins with native knots.

Molecular Simulations of Knotted Proteins and DNA

Benjamin Trefz[1], Thomas Wüst[2], Florian Rieger[1], **Peter Virnau**[1].
[1]Uni Mainz, Mainz, Germany, [2]WSL, Birmensdorf, Switzerland.

After providing a short introduction to knotted proteins, I will present simulations of a coarse-grained heteropolymer model and argue that the addition of sequence may facilitate evolution towards unknotted proteins. I will also discuss implications of knots in technological applications such as nanopore sequencing, and present a mechanism which allows two knots on a polymer chain to pass through each other and swap positions along the strand. Associated "topological" free energy barriers only amount to a few kT, which may enable the interchange of knots on a single DNA molecule.

Probability of DNA Knots and the Effective Diameter of DNA Double Helix

Tetsuo Deguchi, Erica Uehara.
Ochanomizu University, Tokyo, Japan.

We evaluate the probability for self-avoiding polygons (SAP) being equivalent to a given knot type, which we call the knot probability of the given knot, through simulation making use of knot invariants. We consider SAP consisting of cylindrical segments whose radius parametrizes the excluded volume. We show that a scaling formula of the knot probability as a function of the number of segments and the radius of cylindrical segments give good fitting curves to the numerical data of the knot probability for various knots not only prime knots but also a wide variety of composite knots [1]. Extending the cylindrical SAP to the worm-like ring-chain model we produce theoretical estimates of the knot probability which can be compared with experimental results of DNA knots, where the diameter of cylindrical segments of the worm-like chain corresponds to the effective diameter of DNA double helix. Here we recall that the distribution of knot types produced by random cyclization of phage P4 DNA via its long cohesive ends was investigated experimentally in solution with different concentrations of counter ions [2,3].

[1] E. Uehara and T. Deguchi, in preparation.
[2] S.Y. Shaw and J.C. Wang, Science Vol. 260, 533 (1993).
[3] V. V. Rybenkoov et al., PNAS Vol. 90, 5307 (1993).

Coarse-grain Models of DNA Free Energy and Sequence-dependent Minicircle Shapes

John H. Maddocks.
Section of Mathematics, Swiss Federal Institute of Technology, Lausanne (EPFL)

cgDNA is a sequence-dependent coarse grain model of the free energy of DNA at the level of rigid bases (http:lcvmwww.epfl.ch/cgDNA). I will briefly describe this model, and then show how it can be combined with the theory of birods to compute shapes of equilibria (and in particular associated values of the free energy) of DNA minicircles, ie closed loops of DNA of arbitrary length and sequence (including for this conference some knotted cases).

DNA Unlinking in Bacteria

Koya Shimokawa[1], Kai Ishihara[2], Ian Grainge[3], David Sherratt[4], **Mariel Vazquez**[5].
[5]University of California, Davis, USA.[1]Saitama University, Saitama, Japan, [2]Yamaguchi University, Yamaguchi, Japan, [3]University of Newcastle, Callaghan, Australia, [4]University of Oxford, Oxford, United Kingdom,

Chromosomes are long, rod-shaped,DNA molecules encoding the genetic code of an organism. The genome of bacterium Escherichia coli (E. Coli) is encoded in one single circular chromosome. Multiple cellular processes such as DNA replication and recombination change the topology of circular DNA. In particular, newly replicated circular chromosomes are topologically linked. Controlling these topological changes, and returning the chromosomes to an unlinked monomeric state is essential to cell survival. The cell uses enzymes to simplify the topology of DNA. We use mathematical techniques from knot theory, aided by computational tools, to study the action of these enzymes.

DNA Knots Reveal Enzyme Mechanism and Viral Capsid Packing Geometry

De Witt Sumners.
Florida State University, Tallahassee, USA.

Cellular DNA is a long, thread-like molecule with remarkably complex topology. Enzymes that manipulate the geometry and topology of cellular DNA perform many vital cellular processes (including segregation of daughter chromosomes, gene regulation, DNA repair, and generation of antibody diversity). Some enzymes pass DNA through itself via enzyme-bridged transient breaks in the DNA; other enzymes break the DNA apart and reconnect it to different ends. In the topological approach to enzymology, circular DNA is incubated with an enzyme, producing an enzyme signature in the form of DNA knots and links. By observing the changes in DNA geometry (supercoiling) and topology (knotting and linking) due to enzyme action, the enzyme binding and mechanism can often be characterized. This talk will discuss topological models for DNA strand passage and exchange, including the analysis of site-specific recombination experiments on circular DNA and the analysis of packing geometry of DNA in viral capsids.

POSTER ABSTRACTS

POSTER SESSION I
Thursday, September 18
8:00 PM – 9:30 PM
Auditorium Lobby

All posters being presented in Poster Session I should be set up on the morning of September 18 and must be removed by 9:30 PM on September 18.

Baez, Mauricio	1-POS	Board 1
Beldowski, Piotr	2-POS	Board 2
Belvin, Benjamin	3-POS	Board 3
Blaszczyk, Maciej	4-POS	Board 4
Borek, Dominika	5-POS	Board 5
Budek, Agnieszka	6-POS	Board 6
Chwastyk, Mateusz	7-POS	Board 7
Dąbrowski-Tumański**,** Pawel	8-POS	Board 8
Dawid, Aleksandra	9-POS	Board 9
Gómez-Sicilia, Àngel	10-POS	Board 10
Goyal, Sachin	11-POS	Board 11
Gruziel, Magdalena	12-POS	Board 12
Gupta, Asmita	13-POS	Board 13
Hsu, Shang-Te Danny	14-POS	Board 14
Hsu, Shang-Te Danny	15-POS	Board 15
Japaridze, Aleksandre	16-POS	Board 16
Jarmolińska, Aleksandra	17-POS	Board 17
Jasinski, Maciej	18-POS	Board 18
Kochanczyk, Marek	19-POS	Board 19
Kulczycka-Mierzejewska, Katarzyna	20-POS	Board 20
Kulik, Marta	21-POS	Board 21
Kurcinski, Mateusz	22-POS	Board 22
Lewis, Janina	23-POS	Board 23
Li, Thomas	24-POS	Board 24
Li, Mai Suan	25-POS	Board 25
Lim, Nicole	26-POS	Board 26
Link, A. James	27-POS	Board 27
Medalion, Shlomi	28A-POS	Board 28
Przybyl, Sylwester	28B-POS	Board 29

1 – POS Board 1

The Energetic Cost to Knot the Unfolded State of a Protein Determined by Optical Tweezers

Andrés Bustamante[1], Javiera Reyes[1], Christian A.M. Wilson[1], Daniel Guerra[2].Carlos J. Bustamante[3,4,5].**Mauricio Baez**[1],
[1]Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]QB3 California Institute for Quantitative Biosciences, Berkeley, CA, USA, [4]Jason L. Choy Laboratory of Single-Molecule Biophysics, Berkeley, CA, USA, [5]Howard Hughes Medical Institute, Berkeley, CA, USA.

The unfolded state of a protein is considered as a highly fluctuating state of the polypeptide chain, but its tendency to form entangled structures like knots has not been quantified. In this work, we determined the energetic cost of threading a designed knotted protein. The bacteriophage P22 ARC repressor is a homodimer, which can be converted into a single-chain monomer (mARC) adding a 15-residue glycine-rich linker to connect both subunits. Structural models show that flexible glycine-rich linker have the potential to create a knotted or not knotted chain by movements around the protein structure. Using optical tweezers, we explored the folding mechanism of mARC pulling their C and N-terminal extremes at the single molecule level. Analysis of 537 unfolding events obtained from eleven molecules of mARC show two populations of proteins characterized by contours lengths of 37 (7 molecules) and 43 nm (4 molecules). These values agree with the expected size of the polypeptide being stretching from a knotted (37 nm) or unknotted (43 nm) conformation. Furthermore, thermodynamic analysis obtained from the knotted or unknotted conformation of mARC indicates that the unfolded knotted conformation is destabilized by 6 kcal/mol with respect to the unknotted fully unfolded conformation. This situation contrasts with respect to naturally-occurring knotted proteins where knots prevail in the unfolded state. Fondecyt 11110534.

2 – POS Board 2

Hyaluronan Crosslinking in Synovial Fluid as Controlled by Comprehensive Biopolymer Elastic Diffusion

Piotr Beldowski[1], Adam Gadomski[1], Satnislaw Jung[3], Wayne Augé[2].
[1]University of Technology and Life Sciences, Bydgoszcz, Poland, [2]University of Massachusetts Dartmouth, Fall River, MA, USA, [3]University of Technology and Life Sciences, Bydgoszcz, Poland.

Hyaluronic acid (HA) is one of the most ubiquitous biopolymers in the human body, displaying many biologic functions reflective of its wide-ranging molecular weight. Due to its anomalous viscoelastic and network formation properties, HA is considered an important synovial fluid (SF) component and has been implicated in both joint organ inflammatory modulation and lubrication functions at articular cartilage surfaces. Despite varied clinical success, intra-articular HA injections have been an osteoarthritis viscosupplement since the 1970s, with therapeutic efficacy being related to preparation features such as molecular weight, concentration, and dosing schedule. The aim of this work is to establish what manner HA crosslinking affects SF properties in order to optimize the viscoelastic aspects of HA supplementation deemed important for OA treatment. Because molecular weight influences the viscoelastic properties of SF as governed by the Mark-Houwink-Sakurada law, we describe three viscosity regimes undergoing Rouse and/or Zimm biopolymer chain dynamics to evaluate the ideal molecular weight range that optimizes HA crosslinking and network formation. The results indicate that a molecular weight range between 0.1 and 1 MDa equates to the most suitable viscosity regime for viscosupplementation. This viscosity regime formally displays Zimm chain dynamics in Θ solvent conditions such that when the HA biopolymer is sufficiently coiled, it is prone to form a viscoelastic network. The intrinsic network formation mechanism appears based upon the longitudinal diffusion coefficient being twice the transversal counterpart resulting in the same network spring constant ratios with an appreciable HA molecular length dependent on the rotational diffusion coefficient. These results begin to describe biopolymer molecular weight influences on the biological functions of SF hyaluronan necessary for both joint organ inflammatory modulation and lubrication functions at articular cartilage surfaces.

3 – POS Board 3

HcpR of *Porphyromonas gingivalis* Utilizes Heme to Bind Nitric Oxide

Benjamin Belvin, Janina P. Lewis.
Virginia Commonwealth University, Richmond, USA.

The obligate anaerobe *Porphyromonas gingivalis* is the etiological agent responsible for periodontal disease. It must withstand high levels of reactive nitrogen species in the oral cavity generated by the host and other members of the oral flora. HcpR is a putative FNR-CRP family regulator that has been implicated in regulation of the nitrosative stress response in *P. gingivalis*. **Objective**: Characterizing the structural and biochemical properties HcpR will garner a better understanding of the mechanisms HcpR utilizes in its role as a regulator of the nitrosative stress resposne *P. gingivalis*. **Methods**: To characterize the biochemical and structural properties of HcpR small angle X-ray scattering, analytical ultracentrifugation, circular dichroism, bioinformatics, UV-Vis spectroscopy, and resonance raman spectroscopy were utilized. **Results**: HcpR is a homo-dimer that is composed of three domains – a heme-binding domain, dimerization helix, and a DNA-binding domain. Our studies show that HcpR binds the heme cofactor. Bound heme is capable of binding the diatomic gas molecule NO. As shown by UV-Vis and Raman specrtroscopy, binding of NO causes a change in the oxidation state of the iron. Finally, our SAXS data shows that the protein bears a structural resemblance to the crystal structure of Dnr, an FNR-CRP regulator of nitrosaitve stress that binds heme in *Pseudomonas aeruginosa*. **Conclusion**: HcpR exists as a dimer in solution and bears a structural resemblance to the FNR-CRP family of bacterial regulators. Binding of heme to HcpR allows the protein to specifically bind NO that in turn reduces Fe(3+) to Fe(2+) thus leading to structural rearangment of the protein.

4 – POS Board 4

Modeling of Protein Structure and Flexibility using CABS-fold and CABS-flex Web Servers

Maciej Blaszczyk, Michal Jamroz, Sebastian Kmiecik, Andrzej Kolinski.
University of Warsaw, Warsaw, Poland.

Recently, we developed automated modeling methods for efficient prediction of protein structure - CABS-fold - and protein flexibility - CABS-flex. Both methods have been made available as web servers (see http://biocomp.chem.uw.edu.pl/tools) and can be easily used in various protein modeling tasks.

The CABS-fold [1] server provides tools for protein structure prediction from sequence only (*de novo* modeling) and also using alternative templates (consensus modeling). The web server is based on the CABS [2] modeling procedures ranked in previous Critical Assessment of techniques for protein Structure Prediction competitions as one of the leading approaches for de novo and template-based modeling. Except for template data, fragmentary distance restraints can also be incorporated into the modeling process.

The CABS-flex [3] server provides an efficient modeling protocol for the fast simulations of near-native dynamics of globular proteins. The CABS-flex was shown to be a computationally efficient alternative to all-atom molecular dynamics - a classical simulation approach. Moreover, we demonstrated that the relative fluctuations of protein residues obtained from CABS-flex are well correlated to those of NMR ensembles [4].

References:
1. Blaszczyk, M., Jamroz, M., Kmiecik, S. and Kolinski, A. (2013) CABS-fold: server for the de novo and consensus-based prediction of protein structure. Nucleic Acids Research, W406-11.
2. Kolinski, A. (2004) Protein modeling and structure prediction with a reduced representation. Acta biochimica Polonica, 51, 349-371.
3. Jamroz, M., Kolinski, A. and Kmiecik S. (2013) CABS-flex: Server for fast simulation of protein structure fluctuations. Nucleic Acids Research, W427-31.
4. Jamroz, M., Kolinski, A., and Kmiecik, S. (2014) CABS-flex predictions of protein flexibility compared with NMR ensembles.
Bioinformatics, doi: 10.1093/bioinformatics/btu184.

5 – POS Board 5

Topology of Eukaryotic Chromatin

Dominika Borek, Zbyszek Otwinowski.
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How nucleosomes are arranged into higher-order structures in vivo is not understood, even though the efficiency, precision, and microscopic images of metaphase chromosomes imply a high level of structural organization. The current views of higher-order eukaryotic chromatin organization involve either nucleosomal particles self-organizing by association and in a hierarchical manner into higher-order structures or postulate the presence of polymer-melt with ill-defined properties. Neither model agrees with the observed mechanical properties of chromatin, nor with microscopic in situ and in vitro observations of chromatin in its native state, nor with the distributive character of chromatids' individualization.
We have re-analyzed published observations and experimental data from the last 50 years of work on eukaryotic chromatin, looking for consistency with expanded biological knowledge. We propose a new model of eukaryotic chromatin organization, where the higher-order structure of chromatin is organized not only by proteins but also by DNA-based topological restraints that are formed in kinetically controlled processes. Modifications of nucleosomal particles play an important role in this model, serving as memory markers for remodeling complexes. The proposed organization of eukaryotic chromatin agrees well with experimental data and explains the specificity of distant cis-acting elements in transcription.

6 – POS Board 6

Self-entanglement of Bovine Serum Albumin in Shear Flow

Agnieszka Budek[1], Marek Cieplak[2], Piotr Szymczak[1].
[1]University of Warsaw, Warszawa, Poland, [2]Polish Academy of Science, Warszawa, Poland.

Shear flow is known to speed up the protein aggregation process. This can occur for a variety of reasons, including partial unfolding of the protein structures induced by the flow. However, the experimental evidence regarding the influence of shear flow on the protein tertiary structure is rather contradictory. Some studies indicate unfolding of protein under relatively low shear rates, other do not show any impact on protein structure even after exposure to shear rates which are a few orders of magnitude higher. Also, some experiments indicate that effects of the shear can be a cumulative nature and should be described in terms of the strain history. To investigate this problem we perform Brownian Dynamics simulations of shear-induced unfolding of bovine serum albumin. This protein was reported to unfold at surprisingly low shear rates [1]. Using a coarse-grained model of a protein, we track the conformational changes induced by the flow and observe that after an extended exposure to shear albumin loses its ability to refold even when the flow has been turned off. Instead, it is trapped in a metastable state characterized by a strong self-entanglement which topologically constrains the molecule to fold into the native state. This state becomes more populated with time, which can explain the cumulative effect of the shear observed in the experiments.

[1] Soft Matter, Bekard et al., 8, 385-389, 2012

7 – POS Board 7

PR10 - Proteins with Cavities

Mateusz Chwastyk[1], Mariusz Jaskólski[2,3], Marek Cieplak[1].
[1]Institute of Physics PAS, Warsaw, Poland, [2]Center for Biocrystallographic Research, Poznań, Poland, [3]Faculty of Chemistry, A. Mickiewicz University, Poznań, Poland.

We provide theoretical comparisons of the physical properties of eighteen proteins with the pathogenesis-related proteins of class 10 (PR-10) fold, which is characterized by a large hydrophobic cavity enclosed between a curved ß-sheet and a variable α-helix. Our novel algorithm to calculate the volume of internal cavities within protein structures is used to demonstrate that, although the sizes of the cavities of the investigated PR-10 proteins vary significantly, their other physical properties, such as thermodynamic and mechanical parameters or parameters related to folding, are very close. The largest variations (in the order of 20%) are predicted for the optimal folding times. We show that, on squeezing, the PR-10 proteins behave differently from typical virus capsids.

8 – POS Board 8

How to Detect Experimentally Knots in Proteins

Paweł Dąbrowski-Tumański[1] and Joanna I. Sułkowska[1,2]
[1]Faculty of Chemistry, University of Warsaw, Warsaw, Poland. [2]Centre of New Technologies, University of Warsaw, Warsaw, Poland

Proteins that contains knots in their native states [1] are related to surprisingly many human diseases, e.g. Parkinson disease. They are also targets for psychostimulants, anti-depressants and other drugs in depression, mood abnormalities, and other neurological disorders. It has been proposed, that the knots provide some sort of stabilization by holding together certain domains of the protein. Nevertheless, in majority of cases we are unable to determine a function of a knot. An important obstacle to find a function of knots in proteins is the lack of experimental tools to detect unfolded and unknotted states of a protein.

Here, based on numerical simulations, we propose how knots in proteins could be detected experimentally, for example using the FRET technique. We present the optimal reaction coordinate (a distance between two distinct aminoacids) to distinguish between the unfolded unknotted and the unfolded knotted states. Moreover we study the way the protein unknots and compare it with the way it knots based on a protein with trefoil knot (pdb code 2efv [2]. In our simulations we determine a knot type using implementation of Alexander or HOMFLY polynomial.

References:
[1] J. I. Sulkowska, E.J. Rawdon, K.C. Millett, J. N. Onuchic, A. Stasiak, Conservation of complex knotting and slipknotting patterns in proteins, PNAS, 2012, 109 (26), pp. E1715–E1723
[2] J.K. Noel, J.N. Onuchic, J.I. Sulkowska, Knotting a protein in explicit solvent, The Journal of Physical Chemistry Letters, 2013, 4 (21), 3570-3573

9 – POS Board 9

Sequence-dependent Potentials of Mean Force and Fragmentary Experimental Data in Modeling the Spatial Structure of Proteins

Aleksandra Dawid, Dominik Gront and Andrzej Kolinski
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Since the first complete crystallization of the protein structure have passed more than half a century. During this time, both experimentalists and theorists, were focused on discovering the secrets of evolution. Their work has provided valuable knowledge, but the heart of the matter remains unexplained. This is a serious problem whose solution would be to drive the development of many areas of life. The process of proteins folding is highly complex and depends on a number of parameters. No universal factor initiating the process and controlling its proper course. A huge number of physico- chemical interactions of short and long-range determines the precise position of atoms in space. Taking into account the numerous technical difficulties, cost and effort associated with the experimental assignment of the structure of proteins and the large scale of the problem, it is necessary to improve automated methods of theoretical modeling.

The growing content of the PDB database encouraged us to once again analyze these spatial distributions. Our analysis, conducted for planar and torsional angles as well as for local distances between the residues may be valuable for deriving the potentials of mean force, which are useful in the prediction of secondary structure and protein folding simulations. Each of them is a potential of mean force (PMF) based on knowledge, because it was obtained as a result of the statistical analysis of the local geometry of the main chain of proteins of known structure. PMF is based on an approximated probability distribution function along a coordinate, which is derived from a Boltzmann weighted average. It is useful to know how the free energy changes as a function of reaction coordinates, such as the distance between two atoms or the torsion angle of a bond in a molecule. There are two groups of new potentials in the form of the kernel density estimation (KDE), intended for simulating models with varying degrees of complexity. For the coarse-grained representation of CABS model there are three potentials: the distance - R15, plane angle - A13, torsion angle - T14. For the coarse-grained model with full backbone there is a potential of torsion angle Phi-Psi.

The specificity of the obtained potentials was assessed as results of isothermal simulation using the new force field and the dynamics of the Monte Carlo. Also, the folding process of proteins, which come from benchmark, was simulated with a de novo method for Replica Exchange Monte Carlo. Our ultimate goal is to create a multiscale protocol to obtain the spatial structure of proteins, so we have both PMFs for full atom and coarse grained.

10 – POS Board 10

Mechanical Analysis of Disordered Proteins: Accessing a Third of the Proteome

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With the advent of single molecule force spectroscopy, many proteins could be examined in order to uncover the distribution of their mechanical stabilities. However proteins with low or polymorphic mechanostability could not be unequivocally studied due to the fact that this technique establishes a hierarchy in which the low-stability structures tend to unfold first, where they can be mixed with unspecific interactions in the so-called proximal region.

Intrinsically disordered proteins (IDPs) belong to this class of proteins, represent more than one third of the eukaryotic proteome and many are heavily involved in a variety of diseases. In order to study the mechanical properties of IDPs, we have developed a mechanical-protection strategy where the protein under study is inserted inside a more resistant one, which will unfold far from the proximal region where signal is no longer masked by non-specific noise. Thanks to this strategy, we have been able to study the rich conformational polymorphism involved in proteins associated with amyloidogenic neurodegenerative diseases such as Alzheimer's or Huntington's.

As a test for this experimental strategy, we also examined *in silico* different possible combinations for the protector (host) and protected (guest) molecules using a structurebased model and compared them to serially connected proteins. Our results show that the mechanical protection technique works for all the cases and the mechanostability of the guest is preserved. Also, this method proves that an optimized strategy would be one in which the single-molecule markers flanking the host molecule have a much lower mechanostability than the host, so that the unfolding order in the recording is marker, host, guest and therefore the signal of interest is never mixed with the makers.

11 – POS Board 11

Mapping Consitutive Law of Biological Filaments from MD Simulations

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Dynamics of bending and twisting deformations of biological filaments such as DNA play a central role in their biological functions. For example, looping of DNA is an important step in gene regulatory mechanisms, which is often mediated by protein binding. Continuum models such as elastic rod have evolved as efficient computational tools to simulate the nonlinear dynamics of large twisting and bending of biological filaments. However, a major roadblock to this approach is the inaccurate modeling of the constitutive law, which captures the restoring effects in the bending and torsional deformations of the filament in question and which depends on the filament's atomistic-level structure. Traditional models assume a linear constitutive law and experimental measurements focus on the estimation of just a handful of stiffness parameters such as bending and torsional stiffness. Only recently the focus has shifted to examining the dependence of the stiffness parameters on the base-pair sequence. We are developing a methodology to map non-linear constitutive laws directly from molecular dynamics (MD) simulations without any a priori assumptions of its functional form. The methodology employs a two-step technique using an inverse rod model. Step one estimates the curvature and twist along with internal restoring moments and forces at every cross-section in the deformed states of the filament obtained from MD simulations. Step two fits a constitutive law through the estimated data using function-fitting. We have validated the approach with proof-of-concept results, and have also analyzed its robustness by adding noise in the data.

12 – POS Board 12

Chirality Transfer between the Helical Flow and Chiral Particles

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Helical structures are abundant in nature: from alpha-helical motifs in proteins, or DNA strands, through coiled-coil protein structures to protein fibrils or microtubules. The latter examples are particularily interesting as these macromolecular aggregates, although built from identical achiral "bricks" (either beta-strands of peptides for protein fibrils or tubulin dimers for microtubules) are nevertheless chiral. What is even more interesting, especially with regard to protein fibrils, is the observation of structures with opposite handedness that aggregated from identical monomers, however at different conditions (such as temperature, or intensity of vortexing) [1]. Other experiments, at micrometer scale, demonstrated that enantiomers of simple epoxy-based particles can be separated by helical flow [2]. Thus, a question arises, whether a flow can influence the handedness of a growing structure? Or, in particular, whether the flow of non-zero helicity could determine the handedness of bistable, chiral particles? We explore the latter question with numerical simulations of simple particles that can assume two different conformarions of equal energy but opposite chiralities.

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[2] M.Aristov, R.Eichhorn, and C.Bechinger, *Separation of chiral colloidal particles in a helical flow field,* Soft Matter, 2013, 9

13 – POS Board 13

The Ability of an RNA Pseudoknot to Induce Programmed -1 Ribosomal Frameshift is Modulated by Factors Present Both in and Around the Structure

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In programmed -1 ribosomal frameshift, an RNA pseudoknot stalls the ribosome at specific sequence and restart translation in a new reading frame. A precise understanding of structural characteristics of these pseudoknots and their PRF inducing ability is not clear till date. To investigate this phenomenon, we have studied various structural aspects of a -1 PRF inducing RNA pseudoknot from BWYV using extensive molecular dynamics simulations. A set of functional and poorly functional forms, for which previous mutational data was available, were chosen for analysis. These structures differ from each other either by single base substitutions or base-pair replacements from the native structure. We have rationalized how certain mutations in RNA pseudoknot affect its function e.g. a specific base substitution in loop 2 stabilises the junction geometry by forming multiple non-canonical hydrogen bonds leading to a highly rigid structure which could effectively resist ribosome induced unfolding, thereby increasing efficiency. While, a CG to AU pair substitution in stem 1 leads to an altered local electrostatic environment, loss of non-canonical hydrogen bonds between stems and loop, resulting in a less stable structure and reduced PRF inducing efficiency. Inversion of a pair in stem 2 alters specific base-pair geometry that might be required in ribosomal recognition of nucleobase groups, negatively affecting pseudoknot functioning. These observations illustrate that the ability of an RNA pseudoknot to induce -1 PRF with an optimal rate depends on several independent factors that contribute either towards the local conformational variability or geometry.

14 – POS Board 14

Random Coil-like Behaviour of Chemically Denatured Topologically Knotted Protein Monitored by Small Angle X-ray Scattering

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Recent studies on the mechanisms by which topologically knotted proteins attain their natively knotted structures have intrigued theoretical and experimental biophysicists in the field of protein folding. Despite the lack of spectral signatures to identify the presence of residual secondary and tertiary structures, cyclization-coupled refolding data provided strong biochemical evidence to indicate that YibK and YbeA, two best-studied knotted proteins, remain knotted in their chemically denatured states. Using small angle X-ray scattering, we ask the question of whether these chemically denatured knotted proteins possess any unique structural features that would set them apart from typical random coils. Radius of gyration (R_g) is used to monitor global conformational changes associated with the chemical denaturation of the knotted proteins and the unfolding transitions are in line with previously reported data extracted by spectroscopic methods. By revisiting the scaling law of R_g as function of polypeptide chain length for chemically denatured proteins, and compiling a new empirical scaling law for the natively folded proteins, we find that the chemically denatured knotted proteins in fact follow the same random coil-like behaviour. The results suggest that the formation of topological protein knots do not necessarily require global compaction while the loosely knotted polypeptide chains are capable of maintaining the correct chirality without defined secondary and/or tertiary structures.

15 – POS Board 15

An Equilibrium-based Model for -1 Programmed Ribosome Frame-shift Stimulator

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The functional determinant of RNA pseudoknot for -1 programmed ribosome frame-shift (PRF) stimulation has been inconclusive. Recently, NMR analysis of the MLV readthrough pseudoknot revealed a pH-dependent conformation change that has been linked to readthrough efficiency. It was proposed that pH-dependent base-triple formation facilitate S1-L2 interaction crucial for read-through competence. This model was further proposed to extend to the pseudoknot stimulator of -1 PRF. We use the human telomerase pseudoknot (hTPK) DU177 as a model system to examine the pH-dependent -1 PRF efficiency. The UAU triples of DU177 can be replaced by isomorphic CGC triples while retaining the -1 PRF stimulation activity. The CGC triple can harbor an extra protonation-mediated tertiary hydrogen-bond to form a C+GC triple. The -1 PRF efficiencies of the CGC variants exhibit strong pH-dependency that correlates very well with their thermal stability. Two distinct thermal transitions were observed for the CGC variants with the first one being more pH-sensitive. Such a systems may serve as a platform for examining the role of S1-L2 interaction (involving conserved AACAA in L2 in several viral -1 PRF pseudoknot stimulators) in -1 PRF stimulation and its coupling to the base-triple formation. Particularly, it will be very informative to analyze the S1-L2 configurations in different pH values that can tune the -1 PRF efficiency to different levels to link a specific pseudoknot configuration to -1 PRF stimulation activity.

16 – POS Board 16

Atomic Force Microscopy Study of DNA knots in Confined Geometry

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DNA is a very important object of study for geneticists and biologists as well as for physicist. It is important to understand the role of topology of DNA in confined geometry, to better understand such processes as DNA migration in nanofluidics devices or DNA compaction in Viral capsids.
By combining Microfluidics device with Atomic Force Microscopy technique we were able to directly visualize and measure the effects of confining space on the statistical parameters of DNA with various topology (Linear, Circular relaxed & Knotted DNA)
Our method enabled us to separate DNA based on its size and topology, as well as based on the Knot complexity of DNA, with microfluidics device acting as a topological sieve.

17 – POS Board 17

Direct Coupling Analysis in Study of Knotted Proteins

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One of the most defining characteristics of proteins, one that enables them to perform their functions, is their structure. That holds especially true for the knotted proteins [1], since that structure requires a considerable "effort" on part of the protein. Both the evolutionary origin and the folding process of such molecule are still an unknown. One of the methods that holds the most promise for extracting such information is the Direct Coupling Analysis [2]. By indicating residue positions with high correlations it shows their conservation throughout the evolutionary history and suggests probable contact maps for both final structure and folding process of a protein.

Using direct information coefficient obtained from mean field DCA combined with structural information from Protein Data Bank we compare evolutionary conservation of the knotted and unknotted parts of the methyltransferases from the SPOUT clan with respect to the contacts present in the folded molecule, with most results suggesting better (or at least similar) correlations within the knot, which is nonetheless not reflected in the final protein structure.

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[2] Sulkowska J.I., Marcos F., Hwa T., Onuchic J.N., (2012) *Genomics Aided Structure Prediction (GASP)*, PNAS (USA), 109(26):10340-5.

18 – POS Board 18

Motif Identifier for Nucleic Acid Trajectory

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Many of the structural and dynamic properties of RNA and RNA-protein complexes can be investigated using molecular dynamics or Monte Carlo simulations. However, this requires post-processing and analysis of trajectories containing large number of molecular conformations. While there are many tools for the analysis of single RNA conformations, only a few of them enable analyzing multiple RNA conformations.

We present a software tool, Motif Identifier for Nucleic Acid Trajectory (MINT), for analyzing three-dimensional structures of RNA molecules, their full-atom molecular dynamics trajectories or other conformation sets (e.g. X-ray or NMR-derived structures). For each RNA conformation MINT determines the base pairing patterns, identifies secondary structure motifs (helices, triplexes, junctions, loops, bulges etc.) and pseudo-knots. Moreover, for stacked nucleotides the energy of interaction is calculated. If many conformations are analyzed, MINT provides average values of these parameters and description of their evolution.

MINT is written in Python programming language. The analysis of molecular dynamics trajectories can be run in parallel using any number of CPUs, limited only by the amount of available memory. The main output consists of several .csv type files that can be opened in any spreadsheet application. Visualizations of 3D structures can be made in popular molecular viewers thanks to the provided set of .pdb files with the occupancy column replaced with the values of the investigated parameters (e.g., the number of hydrogen bonds that the atom takes part in). For 2D visualizations MINT provides an .html file that can be opened in the VARNA (http://varna.lri.fr/) applet and the .xml input file for the RNAMovies (http://bibiserv.techfak.uni-bielefeld.de/rnamovies/) program.

MINT is a free software released under the terms of the GPLv2.0 license and available at http://www.mint.cent.edu.pl. The MINT package can be either downloaded or used as a webserver.

19 – POS Board 19

Persistence Length Dependence of Knot Probability in Compact Off-lattice Random Polymers

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The existence and spectrum of knots in proteins depends on the interplay of physics and evolution. In attempts to disentangle these two factors, an estimate of background propensity of knotting in a generic protein-like polymer of given length, devoid of evolutionary selection pressure, is highly desirable.

In this work, to obtain model polymers, a backbone trace was generated by filling a sphere uniformly at random with C^{α} atoms. Volume of the sphere was proportional to the protein chain length and realistic atomic density was assumed with minimal C^{α}--C^{α} distance of 0.38 nm. Virtual bonds were assigned by finding the minimal Hamiltonian path between positions of all C^{α} atoms (solved as a global optimization problem equivalent to the traveling salesman problem).

It turned out that generated structures, although matching well to global folds of real protein structures [Brylinski et al. *Phys. Chem. Chem. Phys.* 13, 17044 (2011)], have persistence length on average twice shorter than that of real protein chains. The spread of persistence lengths of generated structures allowed to observe that chains of shorter persistence lengths have their dependence of unknot probability on the (overall) chain length close to that of random self-avoiding polygons [Baiesi et al. *Phys. Rev. Lett.* 99, 058301 (2007)]. At the same time, chains of the same number of residues (and compactness) but larger persistence length have higher knotting probability.

This result suggests that prevalent self-avoiding walk-based approaches could underestimate the background propensity of knotting in proteins which, due to the usually non-negligible secondary structure content, have higher persistence lengths.

20 – POS Board 20

Internal Flexibility of Clindamycin in Water and in the 50S Subunit – An in Silico Study

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Clindamycin is an antibiotic from the lincosamide class used to treat diseases caused mostly by Gram-positive bacteria. Four crystal structures of this antibiotic are currently known [1,2,3,4]. Surprisingly, a comparison of all structures revealed two substantially different conformers of clindamycin in the bound form. Previously, we used quantum mechanical methods to investigate the probable conformations of clindamycin to explain the two binding modes in the 23S ribosomal RNA of the 50S subunit [5]. Here, we used molecular dynamics methods to understand why certain nucleotide substitutions hinder the binding of lincosamides and make bacteria resistant to these antibiotics.

In this work we describe the flexibility of lincosamide binding site in the 50S subunit and dynamic properties of clindamycin depending on the environment. To achieve this goal, we performed multiple 100ns long full-atom molecular dynamics simulations using the NAMD package. The following systems were simulated: clindamycin in a box of explicit water and a fragment of the 50S subunit containing clindamycin binding site with and without the A2058G mutation. The starting structure for the simulations was 3OFZ [3], containing a lower energy conformer of clindamycin.

The transformation of clindamycin geometry and hydrogen bond network were analysed for each trajectory. Root mean square deviation-based clustering and atom motion correlation analysis were also applied. In the simulations we observed a transition between the two conformers of clindamycin. The results also show that the stability of clindamycin-ribosome complexes is unaffected by G2058 mutations.

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21 – POS Board 21

Binding of Peptide Nucleic Acid Oligomers to Helix 69 of 23S Ribosomal RNA

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Targeting a fragment of bacterial rRNA called helix 69 (H69) with complementary peptide nucleic acid (PNA) oligomers seems to be a promising way to inhibit bacterial translation in a sequence-specific manner. H69 is a well conserved hairpin of 23S rRNA which influences initiation and accuracy of translation, peptidyl transferase reaction and ribosome recycling. From the structural point of view, H69 is a part of an intersubunit bridge B2a, which contacts the D stems of A- and P-site tRNAs. Deletion of H69 results in dominant lethal phenotype [1]. Several studies allowed to identify the residues A1912, U1917 and A1919 of H69 as essential for ribosomal functioning [2,3].

Based on the above mutational studies and accessibility of H69 in the ribosome structure we have designed a 13-nucleotide long PNA oligomer covering the sequence stretch G1907 – A1919 and investigated its interactions with isolated H69 hairpin. We performed melting temperature measurements, isothermal titration calorimetry, circular dichroism spectroscopy and non-denaturing gel electrophoresis to investigate the structural properties of the PNA-H69 complexes. Two PNA variants were tested: with and without a cell penetrating peptide $(KFF)_3K$. The PNA interactions with H69 of *E. coli* sequence were compared with the corresponding sequence in human ribosomes.

Results show that both PNAs invade the RNA loop and create stable complexes with H69. Binding of PNA to *E. coli* H69 was stronger than to human sequence. We have also confirmed the efficiency of translation inhibition of the PNA oligomers in cell-free *E. coli* extracts.

References:

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22 – POS Board 22

Theoretical Model of a Knotted Protein VirC2 Bound to a Double Stranded Plasmid DNA

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Agrobacterium tumefaciens's transfer system is the most common DNA delivery tool for genetic engineering of plants. Various researches suggest that VirC2 protein plays a crucial role in correct processing of the transfer DNA (T-DNA), however mechanism of its action remains unclear. The C-terminal domain of the VirC2 protein contains ribbon-helix-helix (RHH) motif, which is a common DNA-binding spot in transcription factors. Multiple experiments indicate that VirC2 binds to a double stranded plasmid DNA, but no direct evidence has been presented so far. Crystallographic structure revealed that VirC2 forms a trefoil knot in the DNA binding domain, within the RHH motif, although its role is yet to be determined.

We developed a tool for modeling of protein-DNA complexes. It is based on the CABS [1] model, which has been successfully used in protein structure prediction, modeling of protein dynamics [2], investigation of folding mechanisms [3] and protein docking [4]. The model incorporates a reduced representation of molecules, a very efficient sampling scheme and a statistical force field. Because of that, CABS allows for simulation of large molecular systems, such as protein-DNA complexes in long molecular events, which is inaccessible for Molecular Dynamics based methods.

In this work we present a theoretical model of interaction between VirC2 protein and a double stranded DNA molecule. The predicted structure of the complex may be helpful in understanding the role of the knot in the binding site and more generally the role of knots in proteins.

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23 – POS Board 23

The Iron-oxygen Switch in Anaerobic Bacterium Porphyromonas Gingivalis

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Bacterial regulation of oxidative stress response typically includes proteins like SoxRS, PerR and OxyR, while iron uptake mechanisms are regulated by iron-dependent regulators like Fur or DtxR-like proteins. Here we investigated the iron-dependent transcriptome of the anaerobic bacterium Porphyromonas gingivalis. We show that the bacterium regulates both iron uptake and oxidative stress mechanisms utilizing a homolog of the nitrate/peroxide stress regulator OxyR. We present the structure of the protein determined using x-ray crystallography. Transcriptional profiling reveals that the mode of regulation by OxyR is dependent on iron levels present in cell culture. We show that this OxyR protein binds iron, which we hypothesize coordinates the ligand in the redox cysteine 199 (Cys199) site analogous to that of Escherichia coli and Neisseria meningitides OxyR. A Cys199Ser mutation abolishes protein binding to iron as well as DNA and confers a bacterial phenotype like the OxyR deficient strain. Our data support a new mode of OxyR activation in the anaerobe P. gingivalis that depends on iron binding and leads to regulation of both iron uptake and oxidative stress response genes. Based on structural similarity of P. gingivalis and E. coli OxyR's our model may also shed light on previous modes of OxyR regulation in aerobic bacteria, which involve an oxidative stress induced cysteine switch or cysteine nitrosylation, as such modifications may be metal-catalyzed thus allowing for sensing low levels of peroxide or nitrite in vivo.

24 – POS Board 24

A Combinatorial Interpretation of the $\kappa_g^*(n)$ Coefficients

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Unicellular map and its shape have been applied to RNA pseudoknotted structure filtered by its topological genus. Studying the virtual Euler characteristic of the moduli space of curves, Harer and Zagier compute the generating function $C_g(z)$ of unicellular maps of genus g. They furthermore identify coefficients, $\kappa_g^*(n)$, which fully determine the series $C_g(z)$. The main result of this abstract is a combinatorial interpretation of $\kappa_g^*(n)$. We show that these enumerate a class of unicellular maps, which correspond 1-to-2^{2g} to a specific type of trees, referred to as O-trees, see Figure 1. We show how to generate from this specific class of O-trees to the class of shapes, see Figure 2. We prove the $\kappa_g^*(n)$ are positive integers that satisfy a two term recursion
$(n+1)\kappa_g^*(n) = (n-1)(2n-1)(2n-3)\kappa_{g-1}^*(n-2) + 2(2n-1)(2n-3)(2n-5)\kappa_{g-1}^*(n-3)$.
We furthermore prove that for any fixed g, the sequence $\{\kappa_{g,t}\}_{t=1}^{g}$ is log-concave, where $\kappa_g^*(n) = \kappa_{g,t}$, for $n = 2g+t-1$.

Keywords: unicellular map, fatgraph, O-tree, shape-polynomial, recursion

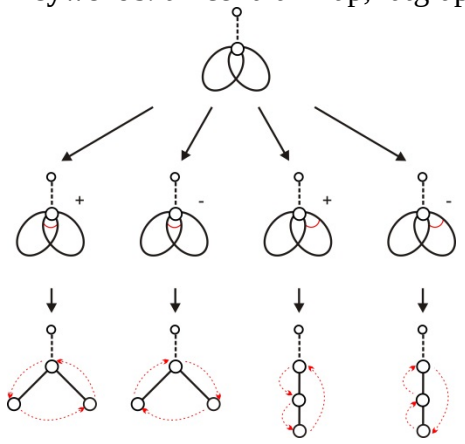

Figure 1.

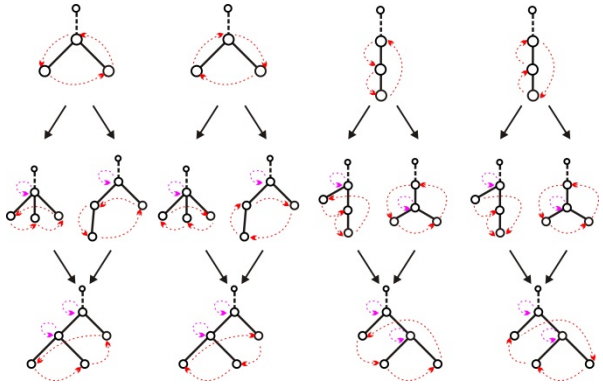

Figure 2.

25 – POS Board 25

Factors Governing Fibril Formation of Proteins: Insights from Lattice and All-atom Simulations

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Fibril formation of proteins and peptides is associated with a large group of major human diseases, including Alzheimer's disease, prion disorders, amyotrophic lateral sclerosis, type 2 diabetes etc. Therefore, understanding the key factors that govern this process is of paramount importance. The fibrillogenesis of polypeptide chains depends on their intrinsic properties as well as on external conditions. Using simple lattice and all-atom models we show that fibril formation times are strongly correlated with hydrophobicity, charges and population of the so called fibril-prone conformation in monomer state. The higher is the population the faster is the fibril elongation and this dependence may be described by a single exponential function. Our results open a new way to understand the fibrillogenesis of bio-molecules at the monomer level. We have shown that not all of proteins have the propensity to aggregation. We will also discuss the influence of environment with focus on the recently observed dual effect of crowders on aggregation rates of polypeptide chains.

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26 – POS Board 26

Mechanistic Insights into the Folding of Trefoil-knotted Proteins

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The past decade has seen the emergence of a new class of proteins that possess an intriguing knotted topological feature in their structures formed by the path of the polypeptide backbone. Elucidating when and how a polypeptide chain knots during the folding represents a significant challenge to the protein folding field as the knotted topology imposes additional complexity to the folding landscape. Most of the experimental investigations on knotted proteins have been focussed on two bacterial trefoil-knotted α/β methyltransferases, YibK and YbeA. Recently, with the use of a cell-free expression system and pulse-proteolysis kinetic experiments, Mallam and Jackson were able to investigate the folding rates of nascent chains of knotted proteins after they were first synthesised by the ribosome.

By using the same cell-free expression system and pulse-proteolysis kinetic experiments, this study investigates the mechanism of knotting by monitoring the effects of an additional protein domain on the folding rates of YibK and YbeA when it is fused to either the amino terminus, carboxy terminus or to both termini. Here, we demonstrate that the fusion of the additional protein domain to either the carboxy terminus or both termini of the knotted proteins retards the rate of folding, indicating that the threading motion is hindered. This suggests that the C-terminus is critical in the threading of the polypeptide chain to form a knot and thus provides the first experimental evidence of the knotting mechanism. In addition, we also investigate the effects of the GroEL-GroES chaperonin on the folding of these fusion proteins. Our results shed light on the role of molecular chaperones on the folding of knotted proteins, thus giving us more insights as to how knotted proteins have withstood evolutionary pressures despite their complex topologies and intrinsically slow rates of folding.

27 – POS Board 27

Untying Peptide Knots: Studies of Lasso Peptide Isopeptidase

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Lasso peptides are a class of ribosomally-synthesized and posttranslationally-modiifed peptide (RiPP) that is typified by a structure resembling a slipknot. These peptides consist of 15-25 amino acids, and the slipknot structure is established by the posttranslational installation of an isopeptide bond between the N-terminus of the peptide and a Glu or Asp sidechain 7-9 amino acids away. This remarkable structure endows the peptide with protease resistance, making lasso peptides of interest as scaffolds for peptide drugs. This talk will focus on efforts to discover new lasso peptides by genome mining. In addition, I will discuss a novel enzyme, lasso peptide isopeptidase, that specifically cleaves the lasso peptide into a linear form, effectively "untying" the peptide knot. Hypotheses about the biological role of these lasso peptides and their isopeptidase will also be discussed.

28A – POS Board 28

Effect of Knots on Binding of Intercalators to DNA

Shlomi Medalion and Yitzhak Rabin
Department of Physics and Institute of Nanotechnology and Advanced Materials, Bar-Ilan University, Ramat-Gan 52900, Israel

We study the effect of knots in circular dsDNA molecules on the binding of intercalating ligands. Using Monte Carlo simulations we show that depending on their handedness, the presence of knots can either suppress or enhance intercalation in supercoiled DNA. When the occupancy of intercalators on DNA is low, the effect of knots on intercalation can be captured by introducing a shift in the mean writhe of the chain that accounts for the writhe of the corresponding ideal knot. In the limit of high intercalator occupancy, the writhe distribution of different knots is strongly affected by excluded volume effects and therefore by salt concentration. Based on the finding that different knots yield well-separated probability distributions of bound intercalators, we propose a new experimental approach to determine DNA topology by monitoring the intensity of fluorescence emitted by dye molecules intercalated into knotted DNA molecules.

* S. Medalion and Y. Rabin, "Effect of Knots on Binding of Intercalators to DNA", J. Chem. Phys. (2014), 140, 205101; DOI:10.1063/1.4875804.

28B – POS Board 29

Discontinuities and Singularities in the Structure of the Most Tight Trefoil Knot

Sylwester Przybyl
Poznan University of Technology, Poland

The appropriately modified Finite Element Method has been used to find the most tight conformation of the trefoil knot tied on the perfect rope. The processed knot contains $N = 200640$ vertices. Each vertex is connected via longitudinally elastic beams with other vertices. The forces with which the beams act on the vertices shift them slowly in such a way that in the final conformation all forces acting on each of the vertices sum up to zero. The overlapping were prevented and curvature was not allowed to exceed $1/R$. Numerical calculations simulating the tightening of the knot lasted on a PC computer a few months. The final knot is equilateral. Its segment length $dl = 0.000\ 163\ 192\ 456\ 437 \pm 2 \cdot 10^{-15}$ and its total ropelenght $L = 32.742\ 934\ 477 \pm 1 \cdot 10^{-9}$.

The final knot contains 6 pieces, where curvature reaches its highest allowable value. The length of the pieces $l_{1,2} = 0.161$. The pieces contain almost 1000 verices.

Two, well supported by the numerical data, conjectures are most essential:

1. ***Curvature of the ideal trefoil knot is not continuous****. It reaches the maximum allowable value* $\kappa = 1$ *on six finite intervals. The plateaus of maximum curvature are separated at both sides by discontinuities.*

2. ***The torsion of the ideal trefoil is not a conventional function****: in points, where curvature displays primary discontinuities, torsion displays Dirac delta components.*

S. Przybyl and P. Pieranski, High resolution portrait of the ideal trefoil knot, J. Phys. A: Math. Theor. 47 (2014)

POSTER SESSION II
Friday, September 19
8:00 PM – 9:30 PM
Auditorium Lobby

All posters being presented in Poster Session II should be set up on the morning of September 19 and must be removed by 9:30 PM on September 19.

Mioduszewski, Lukasz	29-POS	Board 1
Morcos, Faruck	30-POS	Board 2
Németh, Eszter	31-POS	Board 3
Ngo, Son Tung	32-POS	Board 4
Niemyska, Wanda	33-POS	Board 5
Niewieczerzal, Szymon	34-POS	Board 6
Nowak, Wieslaw	35-POS	Board 7
Nowakowski, Adam	36-POS	Board 8
Perlińska, Agata	37-POS	Board 9
Pham, Dinh Quoc Huy	38-POS	Board 10
Plesa, Calin	39-POS	Board 11
Riehn, Robert	40-POS	Board 12
Sikora, Arthur	41-POS	Board 13
Strumillo, Marta	42-POS	Board 14
Strzelecki, Janusz	43-POS	Board 15
Sulkowski, Piotr	44-POS	Board 16
Taylor, Alexander	45-POS	Board 17
Trefz, Benjamin	46-POS	Board 18
Tubiana, Luca	47-POS	Board 19
Turoverov, Konstantin	48-POS	Board 20
Wabik, Jacek	49-POS	Board 21
Wojciechowski, Michal	50-POS	Board 22
Zhang, Wenhua	51-POS	Board 23
Zhang, Hongyu	52-POS	Board 24
Zhao, Yani	53-POS	Board 25
Ziegler, Fabian	54-POS	Board 26
Zuk, Pawel	55-POS	Board 27
Haglund, Ellinor	56-POS	Board 28
Kadlof, Michal	57-POS	Board 29
Szczasiuk, Agata	58-POS	Board 30

29 – POS Board 1

Unbinding and Unfolding of Adhesion Protein Complexes in Stretching Simulations

Bartosz Rózycki[1], **Lukasz Mioduszewski**[2], Marek Cieplak[1].
[2]University of Warsaw, Warsaw, Poland.[1]Polish Academy of Sciences, Warsaw, Poland,

We made coarse-grained simulations to analyze mechanically induced dissociation and unfolding of the protein complex CD48-2B4. This heterodimer is an indispensable component of the immunological system: 2B4 is a receptor on natural killer cells whereas CD48 is expressed on surfaces of various immune cells.

We find that the dissociation process strongly depends on the direction of pulling and may follow different pathways. When pulling with a constant speed, there are several force peaks before the subunits disconnect. In some pathways the final peak (from tensile forces involved in the act of separation) is lower than an earlier peak, associated with shear-involving unraveling of individual subunits (mainly CD48). These results suggest that measuring the highest force in AFM pulling experiments may not provide direct information about adhesion forces.
In living cells both subunits are anchored in cell membranes by their C-terminal domains. Interestingly, pulling by the C-termini results in the simplest scenario, with only one force peak and no different pathways. On the contrary, pulling by the N-terminus of the CD48 subunit always results in two pathways, no matter which other terminus is being pulled.
In constant force simulations, dissociation process changes if the pulling force exceeds the maximum force from constant speed simulations. Dependence of unfolding time on force is different in these two force regimes.

In both constant speed and force simulations, the CD48-2B4 interface can be divided into three distinct patches that act as separate units when resisting the pulling forces. They may break simultaneously or separately, depending on the pathway and pulling direction.
The simulation procedure was verified by simulating Synaptotagmin 1, a membrane-trafficking multidomain protein, which was also studied experimentally. Our simulation results agree with experimental findings.

30 – POS Board 2

A Relationship between Coevolutionary Information, Folding Landscapes and the Thermodynamics of Natural Sequence Selection

Faruck Morcos, Nicholas P. Schafer, Ryan R. Cheng, Jose N. Onuchic, Peter G. Wolynes.
Rice University, Houston, USA.

As a consequence of evolutionary sequence optimization, natural proteins are energetically more stable in their native state than in the molten globule states, resulting in an energy stabilization gap. Although the mechanisms behind evolutionary optimization are far from being resolved, it is possible to quantify the extent at which sequences have been optimized by such evolutionary mechanisms in a framework consistent with current theories of protein folding and experimental observations.

Methods: We compare the landscape statistics for a transferable energy function that is successful in structure prediction known as Associative memory, Water mediated, Structure and Energy Model (AWSEM) with direct residue-residue couplings obtained using Direct Coupling Analysis (DCA) and inferred from sequence alignments of protein families to estimate the effective temperature at which natural, foldable protein sequences have been selected in sequence space, T_{sel}. This temperature quantifies the importance of folding energetics to molecular evolution.

Results: We considered eight different families, with abundant number of sequences (> 4500), known experimental structures, lengths from 60 to 286aa and distinct folds. For each family, we estimated the selection temperature T_{sel} and the glass transition temperature T_g for the native sequences using experimentally determined folding temperatures T_f and the relationship between AWSEM and DCA Hamiltonians. We then computed T_f/T_g ratios, which are a metric of evolutionary folding optimization.

Conclusions: The resulting estimates of T_f/T_g are consistent with previous estimates based on setting up correspondences with lattice models and through comparisons with laboratory folding kinetics. Consistent estimates for T_{sel} and T_f/T_g are also obtained using an alternate scheme, which relies on the comparison of residue coevolution with experimental stability changes upon single site mutations. Our results provide new evidence for funneling and suggest new directions for protein modeling and design.

31 – POS Board 3

Cooperative DNA-binding of the Colicin E7 Nuclease Domain

Eszter Németh[1,2], Judit Kopniczky[3], Evelin Balázs[1], Béla Gyurcsik[1,2].
[1]University of Szeged, Szeged, Hungary, [2]MTA-SzTE Bioinorganic Chemistry Research Group of Hungarian Academy of Sciences, Szeged, Hungary, [3]University of Szeged, Szeged, Hungary.

Colicin E7 is a nonspecific bacterial nuclease. Although its catalytic site, i.e. the HNH motif, is located at the C-terminus of the protein, N-terminal point mutations remarkably decreased the nuclease activity. According to circular dichroism spectra and isothermal microcalorimetric titrations this phenomenon could not be attributed to a large conformational change or to a decreased metal-binding affinity. The mutant proteins bound a short double stranded DNA of 13 base pairs as strong as the wild-type enzyme in the gel mobility shift assays. However, increasing the length of the DNA substrate (21, 188, 306, 777 base pairs) revealed differences in the binding mode of the proteins. The gel eletrophoresis picture unexpectedly showed distinct bands of DNA/NColE7 complexes with long substrates. This indicates the possibility of cooperative binding process(es), resulting in favored supershifted DNA/NColE7 complexes of outstanding stability. The influence of N-terminal mutations on the electrophoresis pattern was also investigated. The more positive charged residues were removed from the N-terminus, the more pronounced smear appeared. Atomic force microscopy was applied to check the DNA conformation change upon protein binding. Our results provide better insight into the DNA-binding mechanism of the colicin E7 nuclease domain. Furthermore, we prove the importance of the selection of DNA molecules for protein/DNA-binding studies. It is a common way to apply short DNA sequences in these experiments. However the natural substrates for enzymes are mostly long DNA molecules. In this contribution we show that the K_d value may be affected by the length of the DNA substrates, and that the modified DNA conformation may be critical for enzymatic activity.

32 – POS Board 4

Effect of the Tottori Familial Disease Mutation (D7N) on the Monomers and Dimers of Aβ40 and Aβ42

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[1]Institute of Physics PAN, Warsaw, Poland, [2]Institute for Computational Science and Technology, Ho Chi Minh, Viet Nam, [3]Universite Paris 7, Paris, France, [4]Universite Denis Diderot, Paris, France, [5]Institut Universitaire de France, Paris, France.

Recent experiments have shown that the mutation Tottori (D7N) alters the toxicity, assembly and rate of fibril formation of the wild type (WT) amyloid beta (Aβ) Aβ40 and Aβ42 peptides. We used all-atom molecular dynamics simulations in explicit solvent of the monomer and dimer of both alloforms with their WT and D7N sequences. The monomer simulations starting from a random coil and totaling 3 μs show that the D7N mutation changes the fold and the network of salt bridges in both alloforms. The dimer simulations starting from the amyloid fibrillar states and totaling 4.4 μs also reveal noticeable changes in terms of secondary structure, salt bridge, and topology. Overall, this study provides physical insights into the enhanced rate of fibril formation upon D7N mutation and an atomic picture of the D7N-mediated conformational change on Aβ40 and Aβ42 peptides.

33 – POS Board 5

KnotProt: A Database of Proteins with Knots and Slipknots

Michal Jamroz[1], **Wanda Niemyska**[2], Eric J. Rawdon[3], Andrzej Stasiak[4,*], Kenneth C. Millett[5], Piotr Sułkowski[6,*], and Joanna I. Sulkowska[1,7,*]
[1] Faculty of Chemistry, University of Warsaw, Warsaw, Poland, [2] Institute of Mathematics, University of Silesia, Katowice, Poland, [3] Department of Mathematics, University of St. Thomas, Saint Paul, USA, [4] Center for Integrative Genomics, University of Lausanne, Switzerland, [5] Department of Mathematics, University of California, Santa Barbara, USA, [6] Faculty of Physics, University of Warsaw, Warsaw, Poland, [7] Centre of New Technologies, University of Warsaw, Warsaw, Poland

The protein topology database KnotProt, collects information about protein structures with open polypeptide chains forming knots or slipknots. The knotting complexity of the catalogued proteins is presented in the form of a matrix diagram that shows users the knot type of the entire polypeptide chain and of each of its subchains. The pattern visible in the matrix gives the knotting fingerprint of a given protein and permits users to determine, for example, the minimal length of the knotted regions (knots' core size) or the depth of a knot, i.e. how many aminoacids can be removed from either end of the catalogued protein structure before converting it from a knot to a different type of knot. In addition, the database presents extensive information about the biological function of proteins with non-trivial knotting and the families and fold types of these proteins. As an additional feature, the KnotProt database enables users to submit protein or polymer structures and generate their knotting fingerprints.

34 – POS Board 6

Folding Knotted Proteins in a Chaperonin Cage

Szymon Niewieczerzał [1] and Joanna I. Sulkowska [1,2]
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Thermodynamic properties of middle size knotted proteins are still not known. Recent results have shown that it is possible to determine the free energy landscape of the smallest knotted protein, MJ0366 from Methanocaldococcus jannaschii, with structure based models [1]. On the other hand, recent experimental results have shown that also bigger protein with much deeper knot, YibK, can tie itself, also observed with numerical simulations, however chaperonin-assisted folding can significantly speed this process up.

In this work we examine by molecular dynamics with structures based model of the chaperonin-assisted folding and thermodynamics of MJ0366, and its version with the extended C-terminal tail. We find out that assisted folding significantly increase probability of knotting. Moreover, the size of the confinement has a significant impact on the kinetics of folding-unfolding processes, which was also previously reported for proteins with an unknoted topology. Introduction of attraction forces between cavity walls and the protein shifts the equillibrium towards the denaturated state. Independently of an available space for the protein, hysteretic behavior is observed in folding-unfolding processes. Surprisingly the unknotting (not only unfolding) occurs at the presence of a larger fraction of native contacts (Q) than knotting (and folding). Those results suggest that chaperonin cage could have a significant influence on knotting probability for middle size proteins as was suggested experimentally for YibK [2].

1. Noel JK, Onuchic JN, Sulkowska JI (2013), “Knotting a protein in explicit solvent”, The Journal of Physical Chemistry Letters, 4(21), 3570-3573.
2. Mallam AL, Jackson SE (2011), “Knot formation in newly translated proteins is spontaneous and accelerated by chaperonins”, Nat Chem Biol 8:147–153

35 – POS Board 7

Balls and Sticks in Medically Important Proteins under Mechanical Stress

Wieslaw Nowak, Lukasz Peplowski, Rafal Jakubowski, Karolina Mikulska-Ruminska, Jakub Rydzewski.
Institute of Physics, Torun, Poland.

The human brain contains over 10**14 synapses between neurons. Hundreds of different protein pairs govern proper development and functioning of neuronal networks. Do they contain protein knots? Mechanical stability of cytoskeleton to large extent depends on spectrin repeats. Individual helices are tightly coupled to create microfibers. The lack of nanomechnical stability in both cellular environments may lead to serious disorders and health hazards. The data on important synaptic components, such as neurexins (NRXNs – related to autism), neuroligins (NLGNs -linking pre- and post-synaptic part of the synaptic cleft), contactins (CNTNs present in Ranvier nodes), reelins (RELNs - regulation of neuronal migration) and keratins (parts of epidermis) stemming from single molecule Virtual Atomic Force Microscopy computational experiments will be presented. In this presentation we will show how our efforts in understanding of nanomechanics of these systems led to developing steered molecular dynamics simulation protocols that may help to interpret real AFM spectra and to see performance of such modular proteins subject to extreme mechanical stress. Both all-atoms and coarse-grained simulations contribute to better mechanical models of these intriguing systems.
Supported by NCN (UMO-2012/05/N/ST3/03178), NCU grant 1142-F, NCN grants No. N202 262038 and No. N519 578138. KM acknowledges SCIEX fellowship.

36 – POS Board 8

Integrin Alpha-4 Subunit Gene Expression in Mesenchymal Stem Cells using Clinically Applicable mRNA-based Approach

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[1]MMRC PAS, Warsaw, Poland, [2]The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Mesenchymal stem cells (MSC) transplantation was found as a new approach to repair tissues and blood-based delivery is suggested as a minimal invasive approach. α4β1 integrin is involved in leukocyte extravasation. It is plausible to verify whether the increase of such molecules in the cell membrane could enable migration of exogenous engineered cells into the injured tissue. The aim of our work was to elicit overexpression of the ITGA4gene in MSC by mRNA mediated transfection.

ITGA4gene cDNA was cloned to pSP72vector (P2191-Promega) and used as a template for mRNA production in vitro. T7mMessage-mMachine Kit (AM1344,-Ambion) with poly(A) tailing kit (AM1350-Ambion) and mMessage mMachine®T7UltraKit (AM1345-Ambion) including ARCA cap were employed. SSBprotein (S3917-Sigma) was used for mRNA stabilization. HumanMSC (PT2501-Lonza) and HEK293cells were transfected with Lipofectamine®2000(Invitrogen). Transfection efficacy was assessed by RT-PCR and ITGA4 protein production was confirmed by ICC.

mRNA-ITGA4 was produced in vitro by T7mMessage-mMachineKit, but no ITGA4protein production was detected although mRNA-ITGA4 cellular delivery. mRNA-ITGA4 stabilization by SSBprotein resulted in ITGA4protein synthesis in HEK293cells only. The use of (ARCA)mRNA-ITGA4 containing anti-reverse-cap-analog(ARCA) resulted in ITGA4protein detection in MSC. The ITGA4protein MSC distribution was transient and gradually moving from inner cellular structures toward membrane where was present for up to 24h.

Cytoplasmic mRNA half-time and translation rate pose problems in mRNA-based transfections. The employment of ARCAcap seems to address some of these questions. Future studies will be performed to clarify whether this transient ITGA4protein presence in transfected cells is sufficient for the improvement of their migration from blood to injured tissue.

Supported by a National Centre for Research and Development grant No101 in ERA-NET NEURON project: "MEMS-IRBI".

37 – POS Board 9

Comparison of Orthologous Proteins: Knotted and Unknotted

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[1] Centre of New Technology, University of Warsaw, Poland. [2] Faculty of Chemistry, University of Warsaw, Poland

Knots are complex structures which exist both in macro and microscopic world. Since first knots in proteins were noticed 20 years ago many more proteins with even more complex knots have been discovered. To this day their origin is unknown but their presence in all three domains of life suggests that, since they weren't eliminated by natural selection, they must serve an important role in a cell. Knots appear to have more stable topology than other protein folds and it can be established by comparing orthologous proteins, knotted and unknotted. Using molecular dynamics on analogous pairs of proteins from methyltransferases we identified, that there is a difference in stability between knot fold and Rossmann fold, Rossmann fold is less stable. Additional difference is observed in dynamics between simulations with and without ligands. Also examined were the conformations of a ligand in the active site of those analogous proteins and it revealed clear trend where ligand prefers to take L-shaped conformations in knotted proteins and I-shaped in unknotted. This subject is worth investigating further because many of those methyltransferases are important enzymes and thanks to the different ligand conformations forced by the different folds it could be possible to create specific inhibitors for the knotted proteins.

38 – POS Board 10

Binding of Fullerene to Amyloid Beta Fibrils: Size Matters

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Carbon based nanomaterials, such as fullerene, nanotube and graphene have been found to recently interact with and influence assemblies of proteins and peptides. Their applicability and usage in biology and medicine is increasingly being considered. In this report the binding affinity of fullerenes C20, C36, C60, C70 and C84 to Aβ40 and Aβ42 fibrils is studied by docking and all-atom molecular dynamics simulations with the Amber 99SB force field and water model TIP3P. Using the molecular mechanic-Poisson Boltzmann surface area method one can show that the binding free energy linearly decreases with the number of carbon atoms of fullerene, i.e. the larger is the fullerene size, the higher is binding affinity. Overall, fullerenes bind to Aβ9-40 fibrils stronger than to Aβ17-42. The number of water molecules trapped in the interior of Aβ9-40 fibrils was found to be lower than inside pentamer 5Aβ17-42. C60 destroys 5Aβ17-42 structure to a greater extent compared to other fullerenes. Our study revealed that the van der Waals interaction dominates over the electrostatic interaction and non-polar residues of amyloid beta peptides play the significant role in interaction with fullerenes providing novel insight into the development of drug candidates against Alzheimer's disease.

39 – POS Board 11

Study of DNA Knots with Solid-State Nanopores

Calin Plesa[1], Daniel V. Verschueren[1], Menno J. Witteveen[1], Justus W. Ruitenberg[1], Magnus P. Jonsson[1], Alexander Y. Grosberg[2], Yitzhak Rabin[3], Cees Dekker[1].
[1]Delft University of Technology, Delft, Netherlands, [2]New York University, New York, NY, USA, [3]Bar Ilan University, Ramat Gan, Israel.

Knots play an important role in biology, particularly in the context of DNA molecules. Experimental techniques to observe DNA knots rely primarily on gel electrophoresis and are limited to circular molecules below 10 kbp in length. Here we show that solid-state nanopores can be used to directly observe DNA knots in both linear and circular single molecules with lengths above 10 kbp under high ionic strength conditions, something which has not been possible before. We use this technique to study the percentage of knots for different length molecules up to 165.5 kbp in length and compare these values to theoretical predictions for long polymers. We vary buffers and voltages to find how the measurement’s resolution limit influences the amount of knots observed. We demonstrate that the solid-state nanopore technique can provide information about the position, the size, and the number of DNA strands inside the knot.

40 – POS Board 12

Detecting DNA Knotting by Strong Confinement and Action of Loop-inducing Proteins

Maedeh Roushan, Zubair Azad, Parminder Kaur, Jianguo Lin, Hong Wang, **Robert Riehn**.
North Carolina State University, Raleigh, NC, USA.

DNA performs a carefully choreographed ballet during the cell cycle. The organization is driven by the specific binding of proteins to form tertiary DNA-protein-DNA complexes. The search process that precedes the formation must overcome the challenge of very low effective mobility of genomic-sized DNA pieces in the dense cellular environment, and potentially leads to complex topologies such as knots and loops.

We have developed nanofluidic devices that gently elongate single DNA molecules through a lateral force without tethering the molecule or blocking the molecule ends. Nanochannel cross-sections are 100x100 nm2, and channels are hundreds of microns long. Because DNA is elongated through confinement, loops and knots with a length down to 2 kb can be directly observed in real time. Channels are made of fused silica, enabling single-molecule observation of both DNA and proteins. Because the effective concentration of DNA inside channels exceeds 1 mg/ml with the channel at the point of DNA-DNA contact, protein-mediated capture cross-sections are very high.

We have investigated a range of proteins that have been proposed as part of the telomeric T-loop complex, as well as DNA ligases. We find that a subset of these induce knots that are free to travel along the elongated DNA molecule, but cannot leave the DNA strand at its end. We further find that we can distinguish these knots from simple loops, and that the diffusivity of knots and loops can be modified through transient protein bridges. Finally we are able to distinguish proteins with dense and sparse binding patterns.

41 – POS Board 13

Exploring the Mechanisms of Outer Membrane Transport through CW-EPR and High Resolution NMR

Arthur Sikora.
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TonB dependent transporters are a family of proteins that is essential for the success of many pathogenic bacteria, making these interesting targets for new antibiotics. TonB coupling to these proteins allows utilization of the proton-motive force of the inner membrane through a mechanism not yet fully understood. The Eschericha coli vitamin B12 transporter, BtuB, is a beta barrel outer membrane transporter and the focus of this work.

Although high-resolution crystal structures have been obtained for this protein, the mechanism of substrate transport is still unclear. These structures provide no information on the transient conformational states that play a big role in transport and crystallographical conditions result in an incorrect picture of the protein's native state. 1 Utilizing a combination of site-directed spin labeling and chemical denaturation we are able to characterize the energetics of the excited protein conformational states that facilitate transport. Previous studies have shown that one region of the N-terminus requires significantly less energy to unfold2 the goal of this work has been to define the structure of this unfolded intermediate. We focused in on the hatch domain, which occludes the barrel, with the goal of understanding the energetic landscape of this domain and nature of the B12 pore.

Additionally we use high resolution NMR to probe conformational changes directly under a variety of conditions and to understand substrate mediated motion and trapped states that might occur during transport. Taken together, these techniques provide the means to elucidate the transport mechanisms for this whole family of transporters.

42 – POS Board 14

Implementation and Evaluation of the New Protocol for Protein Homology Modeling

Marta Strumillo, Dominik Gront.
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Template-based modelling of a protein structure is one of ubiquitous tasks of structural bioinformatics. The methods can deliver model structures important for testing biological hypotheses, virtual docking and drug design. The performance of these methods is evaluated every two years during Critical Assessment of Protein Structure Prediction (CASP) experiment.

In this contribution we present a new automated protocol for template-based modelling, which combines computational tools recently developed in the Laboratory of Theory of Biopolymers: database of protein domain structured (BDDB) with one dimensional and three dimensional threading. At the first stage, template domain structures are assigned to the a sequence by the means of 1D Threading approach. Then the query-template pairs are subjected to a 3D threading procedure where a Monte Carlo search scheme is used to search for lowest scoring alignments. Finally, a number of model structures are build based on each of the alignments. Three different methods are used for this purpose: Modeller, Rosetta and CABS to ensure proper handling of various modelling scenarios that range from very easy to most difficult cases where only a remote homology may be detected. The models are clustered including possibility of knotted structures and the final solution is selected. The protocol is currently tested in ongoing CASP11 experiment.

Gniewek et al. BMC Bioinformatics 2014, 15:22 BioShell-Threading: versatile Monte Carlo package for protein 3D threading
Gront et al. NAR, 2012, Vol. 40 BioShell Threader: protein homology detection based on sequence profiles and secondary structure profiles

43 – POS Board 15

New Mechanical Transition Observed in Highly Stretched DNA with Constrained Linking Number

Janusz W. Strzelecki[1], Lukasz Peplowski[1], Robert Lenartowski[2], Wieslaw Nowak[1], Aleksander Balter[1].
[1]Nicolaus Copernicus University, Torun, Poland, [2]Nicolaus Copernicus University, Torun, Poland.

We report discovery of a new mechanical transition in stretched and topologically constrained DNA. We used a well established technique of stretching single molecules with an untreated Atomic Force Microscope tip to extend DNA. We explored the DNA mechanics under previously untested high stretching force regime, up to 1.8 nN of possible limit of sugar-phosphate backbone. We observed that if double helix is prohibited from rotation, then a transition is displayed as a small plateau in force vs. distance plot both during stretching and relaxation, at stretching forces approaching 1 nN. This transition was absent when molecules with rotational freedom were stretched, showing thus that underlying mechanism was directly connected with molecule linking number conservation. Such DNA behavior proves, that contrary to previous beliefs DNA duplex is not fully melted after undergoing two transitions reported before. Our all-atom steered molecular dynamics simulations of constrained and stretched DNA double helix showed the molecule forced to collapse and adapt a form similar to underwound P-DNA, with bases protruding outside of backbones at extensions correspnding to new transition. Thus, our preliminary hypothesis is that the observed increase in length in the new plateau is a result of reducing the diameter of a double helix through extreme stretching when unwinding is not possible. These results broaden our understanding of interplay of nanomechanics and topology of DNA. Additionally, recent research suggests that DNA could experience similar extreme conditions during abnormal mitosis. The behavior of highly deformed DNA is also important when considering this molecule as a potential bionanotechnology material.
Strzelecki, J., Peplowski, L., Lenartowski, R., Nowak, W., & Balter, A. (2014). Mechanical transition in a highly stretched and torsionally constrained DNA. Physical Review E, 89(2), 020701.

44 – POS Board 16

RNA, Topology and Random Matrices

Piotr Sulkowski[1,2].
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To a given biopolymer, or a complex composed of several interconnected biopolymers, one can associate an auxiliary two-dimensional surface. A genus of this surface provides a very interesting topological characteristic of a given biopolymer (or a complex). I will discuss how random matrix theory can be used to classify all possible configurations of interacting biopolymers and determine their genus. Furthermore, I will present a new classification of known RNA structures based on their genus, and discuss the role of various types of base pairs in this classification.

45 – POS Board 17

Knotted Defect Tangles in Three-dimensional Random Waves

Alexander Taylor, Mark R. Dennis.
University of Bristol, Bristol, Bristol, United Kingdom.

Many physical 3D space filling processes can be understood in terms of complex filamentary tangles. These include polymer strands in a dense melt, as well as disclinations in liquid crystals, and the topological defects in quantum condensed matter systems and optical fields. On large scales these systems appear statistically random, but certain properties appear universal despite the physically different origins of complexity.

We track the tangle of topological defects in numerical simulations of a random wave model [1]. These are the lines of zero intensity in the wavefield, and despite the linear input conditions form a dense filamentary tangle with nonlinear features that encompass the complexity of the field. As with other systems, the small scale conformations of the lines are described with a simple local model, but on global scales the tangling becomes random.

We observe that while many standard quantities reveal only a common statistical scaling on the large scale [2], the topology – particularly the occurrence of knots in vortex loops - discriminates between tangles with different origins. In fact, knotting is somewhat less common than in standard random walk models, though highly complex knots do occur.

[1] M V Berry and M R Dennis. *Proc R Soc A* 456, 2059-79 (2000)
[2] A J Taylor and M R Dennis. Geometry and scaling of tangled vortex lines in three-dimensional random wave fields, in preparation.

46 – POS Board 18

How Molecular Knots can pass through Each Other

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We suggest and explain a mechanism in which two molecular knots on a single DNA strand can pass through each other and swap positions along the strand: One of the two knots expands in size and the other diffuses along the contour of the former. This peculiar mechanism, which only requires a few k_BT, is not only interesting from an aesthetic point of view, but may also play a role in future technological applications such as nanopore sequencing once strand sizes exceed 100,000 base pairs and knots in DNA become likely.

47 – POS Board 19

Progressive Factorization of Composite Knots into Isolated Prime Components: A Systematic Computational Investigation

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Composite knots are known to dominate the knot spectrum of long polymer rings, but in spite of their ubiquity their behavior remains largely unexplored. One of the few standing points is that, in the limit of long polymers rings, their knotting probability tends to the product of the knotting probabilities of the single factor knots composing them. This factorization of the knotting probability has been justified with the assumption that in long polymers knots become localized and therefore behave like point-like decorations on the rings.

Here, using Monte Carlo simulations and advanced knot localization methods, we analyze the length and distribution of prime components in composite knots tied on Freely Jointed Polymer Rings. For increasing contour length, we observe the progressive factorization of composite knots into separated prime components. However, we observe that a complete factorization, equivalent to the "decorated ring" picture, is not obtained even for rings of contour lengths up to tens of times the most probable length of the prime knots tied on the rings.

Following our results, we suggest that the "decorated ring" hypothesis may not be necessary to explain the factorization of the knotting probabilities, at least when polymers excluded volume is not relevant. We rationalize the behavior of the system through a simple one dimensional model in which prime knots are replaced by sliplinks randomly placed on a circle, with the only constraint that the length of the loops has the same distribution of the length of the corresponding prime knots.

48 – POS Board 20

The Knott and Chromophore of Near-infrared Fluorescent Probe iRFP: Impact on Protein Folding

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The near-infrared fluorescent probes engineered from bacterial phytochrome photoreceptors (BphPs) make an advance in non-invasive imaging of biological processes in vivo. These proteins are able to develop the near-infrared fluorescence binding and utilizing as a chromophore the biliverdin IXa (BV) available in the mammalian cells. BphPs and their derivative probes contain a unique figure-of-eight knot between domains involved in chromophore binding. The BphPs knot is supposed to be important for chromophore binding and for transduction of conformational changes induced by light absorption by the chromophore to the effector domain of photoreceptors. Study of knotted proteins is of high importance for solving the fundamental problem of protein folding.

We investigated the influence of chromophore and knot on the folding of the near-infrared iRFP developed from BphPs, RpBphP2. To this aim we obtained and characterized iRFP in its holoform (in complex with the chromophore) and its apoform (chromophore-free). The spectral features of iRFP in the apoform suggest that it retains the secondary and tertiary structures inherent to the holoform of protein. Apoprotein incorporates BV at a molar ratio of 1:1 as indicated by equilibrium microdialysis. The conformation and microenvironment of BV in the apo-iRFP/BV complex was similar to that for native iRFP. Denaturation – renaturation experiments on iRFP in holoform and apoform revealed that the knot does not prevent efficient protein folding while the chromophore stimulates protein aggregation and thus inhibits protein refolding.

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49 – POS Board 21

Multiscale Flexible Docking of Troponin I to Troponin C

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Most of the current protein-protein docking procedures fail to reproduce large-scale motions of a modeled complex. Our approach based on the coarse-grained modeling of flexible proteins overcomes this limitation. The CABSDock procedure used in this work employs a coarse-grained CABS model, an efficient and versatile tool for modeling protein structure, dynamics and interactions [1-4].

In this work CABSDock was used to model the assembly process of troponin C (TnC) with the N-terminal helix of troponin I (TnI). TnC/TnI binding was investigated for both cardiac and skeletal troponin. The TnI fragment was modeled as fully flexible protein chain, without any restraints. The entire structure of the TnC was also treated as flexible, although each domain was restricted to near-native conformation. Our simulations show that binding of TnI fragment changes orientation between N-terminal and C-terminal domain of TnC. The obtained picture of the binding process provides a valuable insight into the mechanistic description of troponin function.

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50 – POS Board 22

Protein Unfolding by Biological Unfoldases: Insights from Modeling

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The molecular determinants of the high efficiency of biological machines like unfoldases (e.g. the proteasome) are not well understood.

We propose a coarse-grained model to study protein translocation into the chamber of biological unfoldases (e.g. AAA+-ATPases) represented as a funnel.

It is argued that translocation is a more efficient way of unfolding a protein than AFM-based force-clamp, as it allows for a conformational freedom while concentrating local tension on consecutive regions of a protein chain and preventing refolding. This results in a serial unfolding of the protein structures dominated by unzipping, which can be several orders of magnitude faster than AFM-induced unfolding in a low-force regime. Thus, pulling against the unfoldase pore is an efficient catalyst of the unfolding reaction. We also show that the presence of the funnel makes the tension along the backbone of the substrate protein non-uniform. Hence the stalling force measured by single-molecule force spectroscopy techniques may not reflect the traction force of the unfoldase motor. We also consider degradation of knotted proteins by the proteasome.

51 – POS Board 23

The Structural Basis of tRNA t^6A Modification in Bacteria

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The universal and essential N^6-threonylcarbamoyladenosine (t^6A) modification occurring on ANN-decoding tRNAs plays a pivotal role in translational fidelity. The enzymes responsible for t^6A modification of tRNA had remained a mystery for 40 years until recently, it was discovered that the Kae1/Qir7/YgjD and Sua5/YrdC protein families are related and responsible for biosynthesis of t^6A at cellular level. In archaea and eukarya, t^6A is biosynthesized by the KEOPS/EKC protein complex (Kae1, Bud32, Cgi121 and Pcc1 in archaea, with a fifth Gon7 specific in yeast) and in mitochondria by Qri7 (a KAE1 paralog) in conjunction with Sua5. In bacteria, the biosynthesis of t^6A is accomplished by YgjD, YeaZ, YjeE and YrdC using tRNA, ATP, *L*-threonine and bicarbonate as substrates. We used molecular cloning, biochemical assays, *X-ray* crystallography and SAXS to elucidate the molecular mechanism underscoring the tRNA t^6A modification in bacteria.

We show that the YgjD-YeaZ-YjeE ternary complex forms in presence of ATP and is dynamically tuned by both the heterodimer YgjD-YeaZ and the hydrolysis of ATP into ADP by ATPase YjeE that is characteristic of GTPase proteins. Our crystal structure of dimer YgjD-YeaZ shows that dimerization of YgjD and YeaZ induces a conformational change on YeaZ in proximity to the interface and thereby creates an atypical ADP-binding site. The activation of the ATPase activity of YjeE by heterodimer YgjD-YeaZ is reminiscent of GTPase Activating Proteins (GAPs). Guided by the similar orientation of ADPs bound in crystal structures of YjeE and in heterodimer YgjD-YeaZ, we built a model for the YgjD-YeaZ-YjeE ternary complex as validated by SAXS. The tRNA t^6A modification assay *in vitro* shows that the YgjD-YeaZ-YjeE ternary complex is essential for the biosynthesis of t6A in conjunction with YrdC while the ATPase activity of YjeE is dispensable.

52 – POS Board 24

Characterisation of the Folding Pathway of a Topologically Knotted Human Ubiquitin Hydrolase

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Proteins containing topological knots have been steadily increasing in numbers. Due to their complex topology, their folding mechanisms are less well characterised compared to small single-domain proteins. Earlier biophysical studies on proteins with simple trefoil knot have shown that they can fold efficiently via obligatory intermediates states. However, the folding of more complex knotted systems such as proteins with 41, 52 or 61 knots remains to be characterised. Human ubiquitin C-terminal hydrolase homolog L1 (UCH-L1), has one of the most complex knot topologies identified so far – a 52 so-called Gordian knot. Earlier equilibrium unfolding experiments have shown that an intermediate state is stably populated at moderate urea concentrations. However, characterisation of the folding pathway by kinetic studies has been impeded because the transition between the intermediate (I) and denatured (D) states is fast and the fluorescent signal change is very small. Therefore, we constructed a series of mutants, L3W, L70W, F108W, V113W, F160W, F181W, F214W, each of which contains a single tryptophan at different locations on the polypeptide chain which act as fluorescence reporter of local structure formation (the tryptophan 26 in wild-type was substituted by a phenylalanine in the mutants). The equilibrium unfolding studies have shown that the mutants have the same m-values for each structural transition as wild type, suggesting that the substitutions do not significantly change the nature of the intermediate state. However, the stability of the mutants varies. Mutants F160W, F181W and F214W show enhanced fluorescence for the I-D transition and thus can be used to characterise the folding mechanism in further detail. The results of single jump and double jump unfolding and refolding measurements suggest that UCH-L1 folds via a parallel pathway on each of which an intermediate is populated.

53 – POS Board 25

Thermal Properties of Polymer Knots in the Presence of Short-range Interactions

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Polymer rings forming complex topological configurations are studied in connection with several applications ranging from biochemistry to mechanical engineering. A brief account on the latest experimental results in the subject will be reviewed. The most difficult problem in dealing with the statistical mechanics of polymer knots is to distinguish their topological configurations. In this talk the results of extensive simulations of single polymer knots on a simple cubic lattice and with lengths up to thousand segments will be presented. To equilibrate the knots and for the Monte Carlo sampling, which is based on the Wang-Landau algorithm, a set of pivot moves is used. To preserve the topology of the knots after each move, two recently developed techniques have been employed. One is the so-called PAEA method. It is able to detect the changes of topology exactly and is very fast. The other method uses the Vassiliev knot invariant of degree 2 represented in the form of contour integrals. This invariant has the great advantage of allowing large pivot moves, which are able to accelerate the Monte Carlo sampling procedure. As an application of these methods, the calculation of the specific energy, the radius of gyration and the heat capacity of several types of polymer knots in a solution with poor or good solvents will be presented. Some consequences on the thermodynamics of polymer knots will be drawn. The problem of sampling certain rare knot conformations and of detecting pseudo phase transitions associated with these conformations will be discussed. A comparison with the previously known result will be made.

54 – POS Board 26

Unknotting the Gordian Knot: Single Molecule Force Spectroscopy on UCH-L1

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We perform Single Molecule Force Spectroscopy with Optical Tweezers to single molecules of the knotted protein UCH-L1, a protein knot with a 5_2 (so called "Gordian") topology.
Force offers ideal possibilities to control the conformation of the knotted chain. Here we apply force in the range of several piconewton to single molecules of UCH-L1.
With mutations of single amino acids in the sequence of UCH-L1 we can pull with different geometries. Pulling at the n- and c-terminus of the sequence leads to unfolding and tightening the knot, while other pulling positions can reduce the knot from a 5_2 to a 3_1 structure or even completely unknot the polypeptide chain. With these mutants, we study the folding kinetics in dependence of the knot formation of UCH-L1.

55 – POS Board 27

Regularized Rotne-Prager Tensor for Spherical Particles of Different Radii

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The Rotne-Prager-Yamakawa (RPY) approximation is commonly used to model the hydrodynamic interactions between small spherical particles suspended in a viscous fluid at a low Reynolds number. However, when the particles overlap, the RPY tensors lose their positive definiteness, which leads to numerical problems in the Brownian dynamics simulations as well as errors in calculations of the hydrodynamic properties of rigid macromolecules using bead modeling. Here we show, how to generalize the RPY approach to the case of overlapping spherical particles of different radii. Our method relies on the direct integration of force densities over the sphere surfaces and thus automatically provides the correct limiting behavior of the mobilities for the touching spheres and for a complete overlap, with one sphere immersed in the other one. This approach can then be used to calculate hydrodynamic properties of complex macromolecules using bead models with overlapping, different-sized beads, which we illustrate with an example.

56 – POS Board 28

Full Characterization of the Native State Dynamics of the Pierced Lasso Bundle Leptin

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Recently, we discovered a new structural complexity in the pleiotropic hormone leptin, where the C-terminal disulphide bridge creates a covalent-loop through which part of the polypeptide chain is threaded (as seen in knotted proteins, Figure bellow). We explored whether other proteins contain a similar intriguing knot-like structure as in leptin and discovered 11 structural homologous proteins in the PDB forming a new class of knot-like proteins. We call this new helical family class the Pierced Lasso Bundle (PLB). Structure based models showed that the dynamics of the native state are altered through oxidation, where the dynamics of the threaded state is more malleable then the unthreaded state, suggesting a mode of controlling receptor interaction and biological activity. To further investigate the malleable behavior of the PLBs we used relaxation/dispersion NMR experiments together with MD structure based models and explicit solvent simulations to fully understand the NSD of leptin. The results show increased dynamics in the covalent-loop controlling the threaded topology as well as in one of the receptor interphases engaging the second receptor to form the active quaternary receptor-ligand complex. Finally, complete comprehension of the native state of leptin and its complex topology can significantly influence *ab initio* predictions of newly identified protein targets as well as guide therapeutic research and drug design.

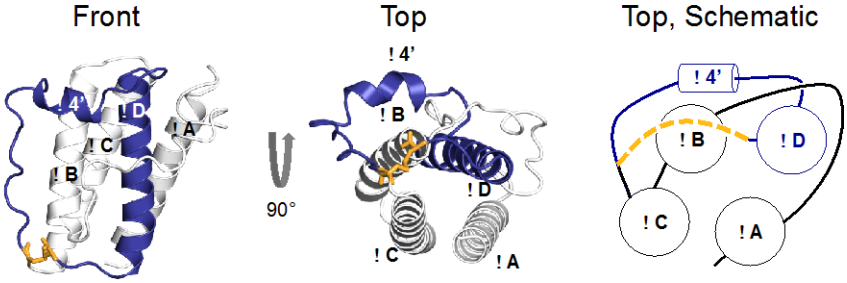

Figure. A cartoon representation of leptin. The front and top view of the protein in a cartoon representation, where the two cysteines are highlighted in yellow. Helix A through D are part of the four helix bundle while helix 4' is outside the bundle in loop 4. Under oxidizing conditions the cysteines forms a disulphide bridge creating a 50 residue long covalent-loop/lasso (blue). Examining the structure lead to the discovery of a threaded topology where helix C and half of helix B are threaded through the lasso creating a Pierced Lasso Bundle topology. The right panel shows a schematic figure of the top view colored in the same manner as the cartoon representation.

57 – POS Board 29

Discovering new knotted protein topologies by reconstruction incomplete X-RAY structures

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Knotted proteins are novel intriguing proteins with nontrivial topological structure. Studies show that topology of knot represents the next level of protein organization. Moreover current results show that topology is evolutionary conserved stronger than tertiary structure of proteins [1]. This suggests the existence of biologically significant function played by knots. Currently more than 900 of knotted structures have been identified in every branch of tree of life.

To determine topology of a protein we need to know its complete structure. However many structures in PBD are incomplete due to imperfection of crystallographic methods. There are thousands of incomplete structures, which miss information about spatial distribution of amino acids, and may “mask” information about real topology of a knot. In order to determine “true” topology of open chain of proteins we used methods of homology modeling based on MODELLER software. We designed methods to reconstruct missing parts of a protein chain, taking into account the amount of available information, quality of homologs, final energy; we also added a new variable: a conservation of knot topology in a template and final models. This model allows to correctly identify topology of proteins and leads to identification of new topologies in proteins.

In addition, we also identified a protein in which a slipknot appears and disappears during conformational changes, together with domain movements. This is the first example of such a protein and importance of knot topology in this process is not known yet.

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58 – POS Board 30

Bioshell Domain Database (BDDB) - Web interface

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BioShell Domain Database (BDDB) holds all the structural domains that may be extracted from the protein structures deposited to the PDB. After sequence-based and structural clustering procedures, 41% of the domains have been succesfully confirmed to belong to the SCOP classes, 49% have been automatically assigned to the SCOP classes and 10% remained not classified.

The web interface allows users to browse the domains in protein structures as well as to assign domains to a FASTA sequence provided by a user with PsiBlast. Finally, a protein structure (in the PDB format) may be used as a query to search in the database using TMAlign algorithm.

The main objective is to provide templates for template-based structural modeling of proteins and to learn what is the domain organization in a protein stucture. Another possible application of BDDB is to use it as high quality and updated source of the classification of proteins domains.

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