



# Multi-Pole Approach to Structural Science

## Multi-Pole Approach to Structural Science Conference

### PROGRAMME & ABSTRACTS

May 10 – 13, 2015  
Staszic Palace at Nowy Świat 72  
Warsaw, Poland

#### **Organizing Committee**

Janusz Lipkowski, Chair

Maria Gdaniec  
Mariusz Jaskólski  
Zbigniew Kaszukur  
Wojciech Kwiatek  
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**Under the Honorary Patronage of  
the Minister of Science and Higher Education**

**Prof. Lena Kolarska - Bobińska**

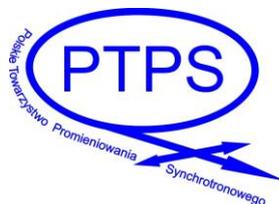


**Ministry of Science  
and Higher Education**

Republic of Poland

## CONFERENCE SPONSORS

We would like to thank the following organizations and companies for their financial support



## ACKNOWLEDGEMENTS

Throughout the program listing, the numbers next to the abstracts and in the author index refer to the corresponding presentation numbers in the Lecture (L) and Poster (P) Abstract sections of the book. Sponsors' abstracts are not numbered. Last-minute contributions of abstracts/posters are not included in this volume and will be made available on the conference website.

Abstracts from this volume should not be cited in bibliographies. Material contained herein should be used and cited ONLY with the consent of the author and ONLY as "personal communication".

The recording of lectures by any means, and the photography of slide or poster material is prohibited, unless explicit permission is obtained from the organizers or from the presenter / lecturer / poster author.

## INSTRUCTIONS FOR LECTURES

- Presentations files should ideally be compatible with the PowerPoint .ppt, .pptx, or Adobe Acrobat .pdf formats.
- The computer provided by the conference organizers will be equipped with Microsoft Windows (version 7) and Microsoft Office (version 2010) and its usage is highly recommended. We cannot guarantee the availability of any other platform for presentations and we cannot guarantee the presence of any special software for e.g. additional multimedia.
- Please contact the organizers at least one day before your presentation and provide all presentation files. A technician will assist you in placing your presentation on the main presentation computer and in testing the presentation file format compatibility.
- Should you wish to use any special format other than standard PowerPoint or Adobe Acrobat, please be prepared to have your own hardware (laptop) with appropriate software installed and tested.
- If you wish to use your own laptop for presentation, please bring all the necessary cable adapters and contact the organizers 24h in advance, to test the equipment and to learn the procedure for setting up your computer during the session with your lecture.

## INSTRUCTIONS FOR POSTERS

### **Poster Session venue:**

Exhibition Hall

Warsaw University Library

55/56 Dobra Street (In Polish: ulica Dobra 55/56)

Warszawa

The maximum poster size is 98 cm (width) and up to 150 cm (height).

Posters boards and pins will be provided at the site.

### **POSTER DISPLAY SCHEDULE:**

Posters will be assigned numbers prior to the conference.

Set up time: On the presentation day – since lunch time

Removal time: After the session or from morning until noon on Wednesday

Odd numbers: Poster presentations during the 1<sup>st</sup> hour of the session (18:00-19:00)

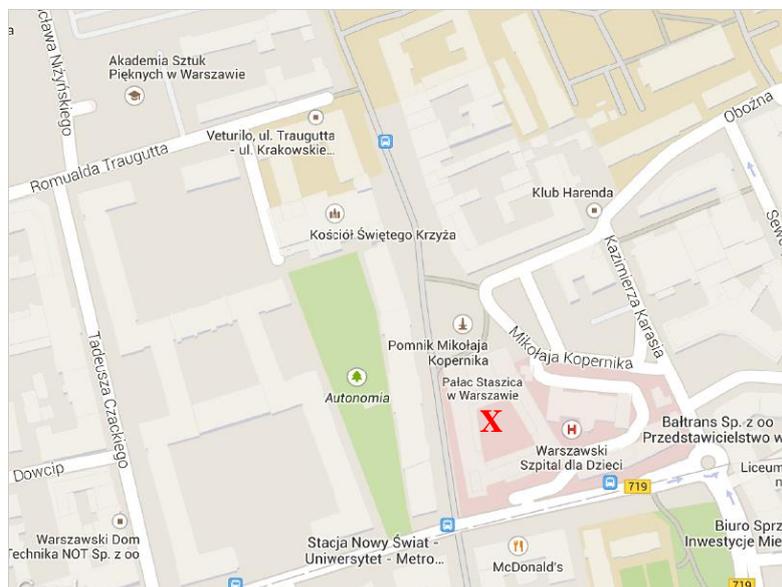
## CONFERENCE VENUE

### Address of the venue:

### THE STASZIC PALACE AT NOWY ŚWIAT 72, WARSAW, POLAND

The conference will take place in the Mirror Hall (in Polish: “Sala Lustrzana”), the main hall of the Staszic Palace.

GPS coordinates: latitude N 52°14'15" (52.2375), longitude E 21°1'5" (21.018056)



**X – Staszic Palace -Conference site**

### Emergency contacts:

From mobile phone 112 for all cases

From stationary phone:

- Ambulance 999
- Police 997
- Fire department 998

## INVITED SPEAKERS

### Special Plenary Speaker



**[Prof. Ada Yonath](#)**

**Weizmann Institute of Science, Rehovot, Israel**

**Nobel Prize in Chemistry, 2009**

### Special Guest



**[Prof. Iain Mattaj](#)**

**EMBL Heidelberg  
Director General**

### Speakers

(in alphabetical order):

- ❖ **Matthias Bachtler** International Institute of Molecular and Cell Biology, Warsaw  
Institute of Biochemistry and Biophysics, Warsaw, Poland
- ❖ **Dominika Borek**, University of Texas Southwestern Medical Center, Dallas, TX, USA
- ❖ **Krzysztof Brzezinski**, Institute of Chemistry, University of Białystok, Poland
- ❖ **Andrzej Marek Brzozowski**, University of York, United Kingdom
- ❖ **Grzegorz Bujacz**, Lodz University of Technology, Poland,  
Polish Academy of Sciences, Poznań, Poland
- ❖ **Janusz Marek Bujnicki**, International Institute of Molecular and Cell Biology, Warsaw,  
Poland, Adam Mickiewicz University, Poznań, Poland
- ❖ **Maksymilian Chruszcz**, University of South Carolina, Columbia, SC, USA
- ❖ **Marek Cieplak**, Institute of Physics, Polish Academy of Science, Warsaw, Poland
- ❖ **Tomasz Cierpicki**, University of Michigan, Ann Arbor, MI, USA
- ❖ **Mirosław Cygler**, University of Saskatchewan, Saskatoon, SK, Canada  
McGill University, Montreal Quebec, Canada

- ❖ **Zbigniew Dauter**, National Cancer Institute, Argonne, IL, USA
- ❖ **Zygmunt Derewenda**, University of Virginia, Charlottesville, VA, USA
- ❖ **Andrzej Dziembowski**, Institute of Biochemistry and Biophysics, Polish Academy of Sciences and Institute of Genetics and Biotechnology, University of Warsaw, Poland
- ❖ **Witold Filipowicz**, Friedrich Miescher Institute of Biomedical Research, Basel, Switzerland
- ❖ **Maria Gdaniec**, Adam Mickiewicz University, Poznań, Poland
- ❖ **Adam Godzik**, Sanford-Burnham Medical Research Institute, La Jolla, CA, USA
- ❖ **Jolanta Grembecka**, University of Michigan, Ann Arbor, MI, USA
- ❖ **Pawel Grochulski**, Canadian Light Source Inc., Saskatoon, SK, Canada  
College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada
- ❖ **Robert Janowski**, Helmholtz Zentrum, Munich, Germany
- ❖ **Mariusz Jaskólski**, Adam Mickiewicz University, Poznań, Poland  
Polish Academy of Sciences, Poznań, Poland
- ❖ **Andrzej Joachimiak**, Argonne National Laboratory, Argonne, IL, USA  
University of Chicago, Chicago, IL, USA
- ❖ **Krzysztof Jozwiak**, Medical University of Lublin, Poland
- ❖ **Zbigniew Kaszkur**, Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland
- ❖ **Andrzej Kolinski**, University of Warsaw, Poland
- ❖ **Janusz Lipkowski**, Cardinal Stefan Wyszyński University, Warsaw, Poland
- ❖ **Karolina Majorek**, Adam Mickiewicz University, Poznań, Poland  
University of Virginia, Charlottesville, USA
- ❖ **Karolina Michalska**, Argonne National Laboratory, Argonne, IL, USA  
University of Chicago, Chicago, IL, USA
- ❖ **Wladek Minor**, University of Virginia, Charlottesville, USA
- ❖ **Piotr Neumann**, University of Göttingen, Germany
- ❖ **Wieslaw Nowak**, Nicholas Copernicus University, Toruń, Poland
- ❖ **Marcin Nowotny**, International Institute of Molecular and Cell Biology, Warsaw, Poland
- ❖ **Zbyszek Otwinowski**, University of Texas Southwestern Medical Center, Dallas, TX, USA
- ❖ **Agnieszka Pietrzyk**, Freie Universität Berlin, Germany
- ❖ **Waldemar Priebe**, University of Texas MD Anderson Cancer Center, Houston, TX, USA
- ❖ **Urszula Rychlewska**, Adam Mickiewicz University, Poznań, Poland
- ❖ **Wojciech Rypniewski**, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland
- ❖ **Piotr Setny**, University of Warsaw, Poland
- ❖ **Tadeusz Skarzynski**, Agilent Technologies, Oxford, United Kingdom
- ❖ **Joanna Sulkowska**, University of Warsaw, Poland
- ❖ **Marcin Józef Suskiewicz**, Vienna Biocenter, VBC, Vienna, Austria
- ❖ **Marta Szachniuk**, Poznan University of Technology, Poland  
Institute of Bioorganic Chemistry, Poznań, Poland
- ❖ **Joanna Trylska**, University of Warsaw, Poland
- ❖ **Alexander Wlodawer**, National Cancer Institute, Frederick, MD, USA
- ❖ **Krzysztof Woźniak**, University of Warsaw, Poland

## CONFERENCE PROGRAMME

### Sunday, May 10

- 14:00 – 17:00 **Registration (at the Staszic Palace)**  
17:00 – 18:00 **Opening remarks from distinguished organizers, sponsors, and guests**  
Chair: Janusz Lipkowski
- 18:00 – 19:00** **Session I: History of macromolecular crystallography**  
Chair: Wlodek Minor
- 18:00 – 18:30 Alexander Wlodawer *A very abbreviated history of macromolecular crystallography*  
18:30 – 19:00 Zbigniew Dauter *Multipoles through the looking glass*  
19:00 – 21:00 Beer and wine

### Monday, May 11

- 8:30 – 9:15 **Opening Lecture**  
Chair: Joanna Trylska
- 8:30 – 9:15 Ada Yonath *From Zdunska Wola to Warsaw with the Ribosome*
- 9:15 – 9:30 Coffee Break
- 9:30 – 12:00 **Session II (Part 1): Proteins and nucleic acids**  
Chair: Marcin Nowotny
- 9:30 – 10:00 Matthias Bochtler *A structural biologist's view of DNA methylation and hydroxymethylation.*  
10:00 – 10:30 Witold Filipowicz *Structural insights to the role of the CCR4-NOT complex and GW182 and DDX6 proteins in miRNA-mediated repression.*  
10:30 – 11:00 Andrzej Dziembowski *Architecture of the CCR4-NOT complex from Schizosaccharomyces pombe, a multifunctional cellular machine*  
11:00 – 11:30 Robert Janowski *Structural basis for RNA recognition in roquin-mediated post-transcriptional gene regulation*  
11:30 – 12:00 Joanna Trylska *Interactions of aminoglycoside antibiotics with RNA*
- 12:00 – 13:00 Lunch Break
- 13:00 – 14:50 **Session III (Part 1): Modeling and theoretical studies**  
Chair: Witold Filipowicz

- 13:00 – 13:30 Andrzej Kolinski *Towards unrestrained protein-protein molecular docking*
- 13:30 – 14:00 Janusz M. Bujnicki *Coarse-grained modeling of 3D structure for RNA and macromolecular complexes*
- 14:00 – 14:25 Marta Szachniuk *RNApdbee and assessing the RNA secondary structure*
- 14:25 – 14:50 Marek Cieplak *Structure-based models of proteins in action: virus capsids, proteins with cavities, unfolding by proteasomes, formation of knots*
- 14:50 – 15:15 Coffee Break
- 15:15 – 16:00 Iain Mattaj – *EMBL - Europe's flagship laboratory for the life sciences and its role in structural biology*
- 16:00 – 17:30 **Panel Session – Accession of Poland to EMBL**  
Chair: Andrzej Legocki  
Participants: Iain Mattaj, Leszek Kaczmarek, Mariusz Jaskolski, Prof. Wlodzislaw Duch, Maciej Zylicz, Jerzy Duszynski
- 17:30 – 17:45 Coffee Break
- 17:45 – 19:30 **Session IV: Large and challenging structures**  
Chair: Matthias Bochtler
- 17:45 – 18:10 Zygmunt Derewenda *The structural biology of Ca<sup>2+</sup>-independent modulation of smooth muscle contraction*
- 18:10 – 18:35 Jolanta Grembecka *Structure-based development of menin-MLL inhibitors for cancer therapy.*
- 18:35 – 19:00 Pawel Grochulski *Crossing membranes: beta-cyclodextrin-gemini surfactant and the type II secretion system*
- 19:00 – 19:30 Mariusz Jaskólski *Tema con variazioni: modulated structures of Hyp-1 complexes*
- 19:30 – 21:00 Social program

## Tuesday, May 12

- 8:30 – 11:10 **Session III (Part 2): Modeling and theoretical studies**  
Chair: Mirosław Cygler
- 8:30 – 9:00 Adam Godzik *Understanding cancer mutations with protein and network modeling*
- 9:00 – 9:30 Janusz Lipkowski *Hydration patterns in supramolecular systems*
- 9:30 – 9:55 Krzysztof Woźniak *Crystallography beyond Independent Atom Model*
- 9:55 – 10:20 Piotr Setny *From hydration free energies to localised water molecules - a novel, semi-explicit hydration model*
- 10:20 – 10:45 Joanna Sulkowska *New complex topology in proteins and evolutionary analysis of their origin*
- 10:45 – 11:10 Wiesław Nowak *How to navigate inside protein tunnels - lessons from nature*

- 11:10 – 11:30 Coffee Break
- 11:30 – 12:20 **Session II (Part 2): Proteins and nucleic acids**  
Chair: Jolanta Grembecka
- 11:30 – 11:55 Marcin Nowotny *The mechanism of structure-selective nucleases in DNA repair*
- 11:55 – 12:20 Waldemar Priebe *Bench-to-Bedside Design, Synthesis, Evaluation, and Development of Novel DNA Binding Agents*
- 12:20 – 13:20 Lunch Break
- 13:20 – 15:35 **Session IV (Part 1): Methods and their improvement**  
Chair: Zbyszek Dauter
- 13:20 – 13:50 Wlodek Minor *Protein Crystallography with Speed and Finesse*
- 13:50 – 14:15 Dominika Borek *Solving structures from very low resolution data*
- 14:15 – 14:40 Maria Gdaniec *Exploring the wealth of specific intermolecular interactions for crystal engineering*
- 14:40 – 15:10 Andrzej Joachimiak *Structural Biology Using Light Sources Helps Combat Infectious Diseases and Antibiotic Resistance*
- 15:10 – 15:35 Tadeusz Skarzynski *Structural biology in drug discovery: current strategies and challenges*
- 15:35 – 15:50 Coffee Break
- 15:50 – 18:00 **Session IV (Part 2): Methods and their improvement (continued)**  
Chair: Zbyszek Dauter
- 15:50 – 16:20 Piotr Neumann *Multi-resolution refinement and fitting of atomic models into cryo-EM reconstructions*
- 16:20 – 17:45 Zbyszek Otwinowski *Advanced methods for molecular replacement.*
- 17:45 – 17:10 Agnieszka Pietrzyk *Identification of amino acid sequence by X-ray crystallography: crystal structures of silkworm proteins as case studies*
- 17:10 – 17:35 Urszula Rychlewska *Chiral, Shape Persistent Molecules as a Source of Inclusion and Microporosity in Crystals*
- 17:35 – 18:00 Zbigniew Kaszukur *True nanocrystallography*
- 18:00 – 20:00 **Poster Session and Oral Presentations by the Sponsors**
- 18:00 – 18:20 Piotr Wardega -NanoTemper Technologies GmbH  
*Quantitative analysis of biomolecular interactions with Microscale Thermophoresis (MST)*
- 18:20 – 18:40 Mariusz Milik - Head of Computational Chemistry, Selvita S.A.  
*Computer-aided Biotechnology: Example of Selvita S.A.*

## Wednesday, May 13

- 8:30 – 10:20 **Session V: Signaling, transport, and immunology**

Chair: Andrzej Joachimiak

- 8:30 – 9:00 Andrzej Marek Brzozowski *Insulin: molecular fossil or hormonal maverick?*
- 9:00 – 9:30 Maksymilian Chruszcz *Allergens - structure and function*
- 9:30 – 9:55 Krzysztof Jozwiak *Structural biology aspects of ligand directed signaling in G-protein coupled receptors*
- 9:55 – 10:20 Karolina Michalska *Solute-binding proteins recognizing products of lignin degradation*
- 10:20 – 10:50 Coffee Break
- 10:50 – 12:40 **Session VI (Part 1): Enzyme Structures**  
Chair: Alexander Wlodawer
- 10:50 – 11:15 Krzysztof Brzezinski *Structural characterization of microbial S-adenosyl-L-homocysteine hydrolases*
- 11:15 – 11:45 Grzegorz Bujacz *The structural studies of the two isoforms of laccase from the white-rot fungi *Pycnoporus sanguineus**
- 11:45 – 12:15 Mirosław Cygler *Effector proteins from pathogenic bacteria: focus on kinases*
- 12:15 – 12:40 Tomasz Cierpicki *Targeting E3 ligase activity of RING domain with small molecules*
- 12:40 – 12:50 **Award of the poster prizes**
- 12:50 – 13:40 Lunch Break
- 13:40 – 15:00 **Session VI (Part 2): Enzyme Structures (continued)**  
Chair: Alexander Wlodawer
- 13:40 – 14:05 Karolina Majorek *Structural and functional characterization of a GNAT acetyltransferase - reproducibility issues*
- 14:05 – 14:30 Marcin Józef Suskiewicz *The structural investigation of a novel protein kinase class phosphorylating arginine residues*
- 14:30 – 15:00 Wojciech Rypniewski *Crystal structures of chitinases from extremophiles*
- 15:00 – 15:30 Closing session**

**Panel discussion**  
**on**  
**the accession of Poland to European Molecular Biology**  
**Organization – EMBL**  
**May 11, 2015, 16.00 – 17.30**

As a part of the MultiPole Approach to Structural Biology, an international conference organized in Warsaw, a panel discussion on the accession of Poland to EMBL will be organized. The discussion will be moderated by Prof. Andrzej B. Legocki.

Following scientists confirmed their participation in the discussion:

Prof. Jerzy Duszynski – President of Polish Academy of Sciences

Prof. Iain Mattaj – Director General of EMBL

Prof. Włodzisław Duch – Undersecretary Ministry of Science and High Education,

Prof. Leszek Kaczmarek – Dean of PAS Biological Division

Prof. Mariusz Jaskólski – University of Adam Mickiewicz Poznań,

Prof. Maciej Zylicz – Foundation of Polish Science

Prof. Alex Włodawer – Cancer Research Center N.C.I Frederick, USA

**Discussion moderator Prof. Andrzej B. Legocki**



Professor of Biological Sciences, since 2011 Professor Emeritus. He graduated organic chemistry at Poznań University, Ph.D. in biochemistry. Prof. Legocki works on plant molecular biology, plant-bacterium interaction, structure and expression of plant genes, plant-based recombinant oral vaccines. Prof. Legocki was the director of the Institute of Bioorganic Chemistry PAS in Poznań (1988-2003), the President of the Polish Academy of Sciences (2003-2006). Prof. Legocki is member of the Polish Academy of Sciences, the European Molecular Biology Organization (EMBO), the ScanBalt Academy, the Academiae Europaeae. Prof. Legocki has been awarded honorary doctorates of the Agriculture Universities of Poznań and Warsaw and Universities of Lublin and Szczecin.

## **European Molecular Biology Laboratory (EMBL)**

(General information from the EMBL web page:

[http://www.embl.de/aboutus/general\\_information/index.html](http://www.embl.de/aboutus/general_information/index.html))

The European Molecular Biology Laboratory (EMBL) is one of the world's leading research institutions, and Europe's flagship laboratory for the life sciences.

EMBL operates from five sites across Europe:

- Heidelberg, Germany - main laboratory
- Hinxton, UK - European Bioinformatics Institute (EMBL-EBI)
- Grenoble, France - research and services for structural biology
- Hamburg, Germany - research and services for structural biology
- Monterotondo, Italy - mouse biology

EMBL is an intergovernmental organisation specialising in basic research in the life sciences, funded by public research monies from 21 member states, including much of Europe and Israel, and two associate members, Argentina and Australia. EMBL is led by the Director General, currently Professor Iain Mattaj, appointed by the governing body, EMBL Council. The Council is comprised of representatives of all member and associate member states.

The cornerstones of EMBL's mission are to:

- perform basic research in molecular biology;
- train scientists, students and visitors at all levels;
- offer vital services to scientists in the member states;
- develop new instruments and methods;
- actively engage in technology transfer.

Research at EMBL emphasises experimental analysis at multiple levels of biological organisation, from the molecule to the organism, as well as computational biology, bioinformatics and systems biology.

Research is conducted by approximately 85 independent groups covering the spectrum of molecular biology. EMBL is international, innovative and interdisciplinary. Its 1,760 employees from 60 nations represent scientific disciplines including biology, physics, chemistry and computer science.

Outstanding training is available at multiple levels: PhD students, postdocs and visiting scientists. EMBL hosts a comprehensive schedule of courses, conferences and workshops, many organised in collaboration with EMBL's sister organisation, the European Molecular Biology Organization (EMBO).

Services provided by EMBL include:

- core biomolecular databases and bioinformatics tools, particularly at EMBL-EBI;
- the provision of beamlines, instrumentation and high-throughput technology for structural biology at the Hamburg and Grenoble outstations;
- Core Facilities, which provide cost-effective and efficient access to methods and technologies that are expensive to set up or maintain, or that require considerable expense.

**LECTURES – ABSTRACTS AND SPEAKERS' MINI-CVS**



### **Matthias Bochtler**

Scientific career: 1990 – 1995 Student at the LMU Munich, Germany and Cambridge University, Cambridge, UK 1996 – 1999 Assistant at the Max Planck Institute of Biochemistry in Martinsried, Germany for Prof. Robert Huber 1999 – 2000

Postdoc at the Max Planck Institute of Biochemistry in Martinsried, Germany 2001 - 2007 Group Leader at the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden, Germany (on delegation to Warsaw, Poland) 2007-2011

Principle Investigator Structural Biology, Cardiff University 2011-present Professor and Group Leader at the International Institute of Molecular and Cell Biology (IIMCB) and at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences (IBB, PAS) in Warsaw, Poland

>3500 citations, H-factor 23 according to ISI

# **A structural biologist's view of DNA methylation and hydroxymethylation.**

Bochtler M<sup>1,2</sup>, K. Mierzejewska, A. A. Kazrani, W. Siwek, H. Czapinska

<sup>1</sup>International Institute of Molecular and Cell Biology, Warsaw, Poland

<sup>2</sup>Institute of Biochemistry and Biophysics

Abstract: DNA methylation and hydroxymethylation introduce relatively small changes in DNA bases, yet there are many proteins that distinguish between unmethylated, methylated and hydroxymethylated DNA. In the case of cytosine methylated DNA, solvation/desolvation effects are thought to play a major role in methylation specific binding. Using restriction endonuclease R. DpnI as an example, we have studied specific binding to adenine methylated DNA. From biochemical data, two crystal structures and hydrogen-deuterium experiments, we conclude that the high specificity of the enzyme relies on a “double readout” of methylation by two separate domains (catalytic and winged helix). Each domain relies partially on desolvation for methylation detection, but in addition, adenine methylation in the GATC context generates DNA methyl groups in close proximity, which enforces DNA deformation. We suspect that R. DpnI specifically recognizes the deformed conformation, and hence ultimately relies on a methyl-methyl clash in the substrate to specifically bind adenine methylated DNA. As a model for hydroxymethylcytosine specific DNA binding, we have used the PvuRtsII, a bacterial enzyme, which specifically cleaves the DNA with 5-hydroxymethylcytosine bases. A crystal structure of PvuRtsII shows a previously unrecognized SRA domain in the enzyme, which can be expected to flip the modified base. Although our own and another group’s biochemical data regarding nucleotide flipping are still confusing, currently the most plausible model is that specific 5hmC binding relies on scrutiny of the flipped base, which is highly reminiscent of the way how many DNA repair proteins detect damaged bases.



**Dominika Borek**

Education:

M.S., Adam Mickiewicz University, 1997;

Ph.D., Adam Mickiewicz University, 2001;

Postdoc, UT Southwestern Medical Center, 2001-2004.

Fields of interest:

methods in macromolecular crystallography, radiation-induced effects, higher-order chromatin organization, next-generation sequencing.

40 publications, ~1300 citations, H-index 17.

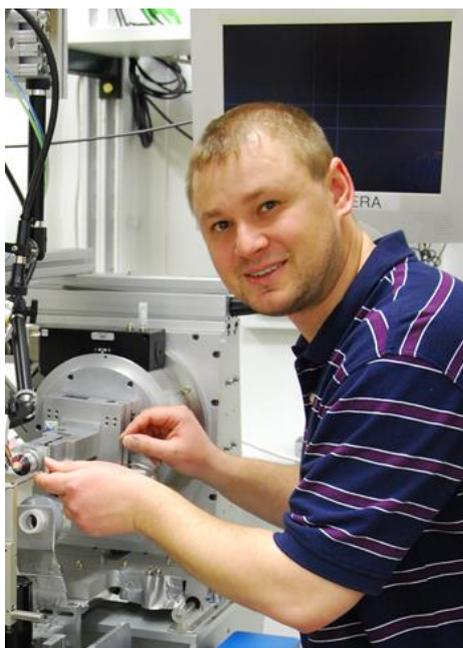
# **Solving structures from very low resolution data**

Dominika Borek, Zbyszek Otwinowski

University of Texas Southwestern Medical Center, Dallas, TX, USA

Only 1.6% of crystal structures in the PDB were solved at resolutions lower than 3.5 Å. Low resolution phasing and model-building are very difficult, despite the fact that alpha helices are identifiable at 9 Å resolution and beta strands at 4.7 Å. Our accumulated experience indicates that efficient use of low resolution data requires efficient propagation of phase information through every step of the structure solution process, so the phase can become an additional observable in the process of model-building. In addition, when information is sparse, as it is in the case of low resolution data, the detrimental impact of bias introduced at intermediate steps needs to be considered in a manner different from the way methods used for higher-resolution data handle it.

We will show how to decide whether low resolution model-building and phasing are feasible in a particular case and discuss our experience with projects ranging from 3.5 to 4.2 Å, for which atomic models have been built despite data scarcity.



### **Krzysztof Brzezinski**

Education: MSc in Pharmacy, Poznan University of Medical Sciences (2000), PhD in Chemistry, Adam Mickiewicz University, Poznan (2006).

Postdoctoral fellowship: Zbyszek Dauter laboratory, National Institutes of Health/Argonne National Laboratory, USA (2010-2012).

Employment: Center for Biocrystallographic Research at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznan (2008 – 2010), University of Bialystok (2012 – present).

Field of interest – structural studies of glycosyltransferases and enzymes involved in the regulation of methylation reactions, small molecule crystallography.

24 publications, ~170 total citations, H-index 7, 15 structures in PDB, reviewer for Acta Crystallographica section D and Molecules.

Recipient of the OPUS 5 grant (National Science Centre, Poland), Polish Biochemical Society J. K. Parnas' Prize (2005).

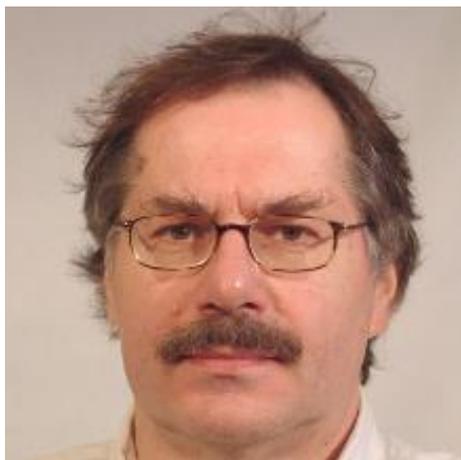
# **Structural characterization of microbial S-adenosyl-L-homocysteine hydrolases**

Krzysztof Brzezinski

Laboratory of Biochemistry and Structural Biology, Institute of Chemistry, University of Białystok, Poland

S-adenosyl-L-homocysteine hydrolase (SAHase) is an essential enzyme involved in the regulation of methylation reactions. This applies to both, healthy host cells and their invading pathogens form. Therefore, selective inhibition of SAHases in targeted cells is an excellent possibility for a drug intervention at the molecular level of cell metabolism. SAHases are highly-conserved enzymes with almost identical organization of the active site. This fact practically precludes design of highly selective inhibitors against the enzymes of pathogenic origin that would not affect the human cells. Therefore, the aim of this study is not to focus on the active site but to elucidate mechanisms of substrate and inhibitor delivery to the substrate-binding pocket of SAHases. For this purpose, targets were selected from various prokaryotic and eukaryotic human pathogens. Phylogenetic relationships and sequence differences including extra inserts within a substrate-binding domain have been taken into account during enzyme selection. The premises about various mechanisms which regulate the accessibility of the substrate binding pocket are based on crystallographic studies of SAHases from various organisms. However, the chemical nature of the different regulation mechanisms has not yet been explained. Additionally, apart from the active site, a role of a non-conservative entrance to the substrate-binding pocket in substrate delivery to the active site is proposed.

This project is supported by a grant from the Polish National Science Center (No. UMO-2013/09/B/NZ1/01880).



**Andrzej Marek Brzozowski**

Education - M.Sc., University of Lodz, 1977; Ph.D., University of Lodz, 1980.

Fields of interests - structural endocrinology, molecular cross-links of diabetes, cancer and life-span regulation, application of chemistry in modification/mimicking of protein hormones, art as an index of the real.

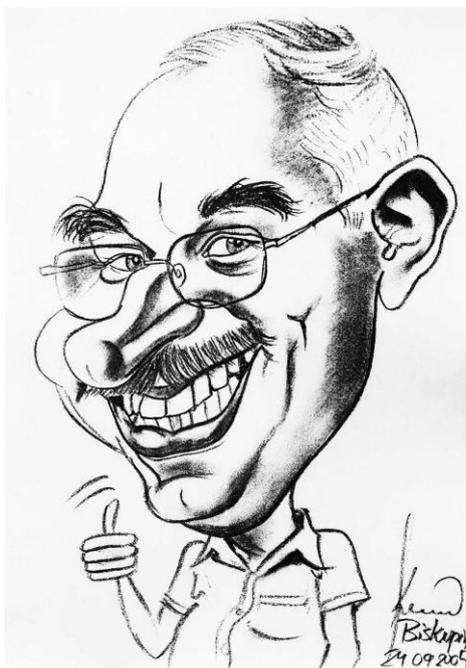
>100 publications, >8,500 citations, H-index 33, advised 12 Ph.D. and >35 M.Sc./M.Res. theses.

# **Insulin: molecular fossil or hormonal maverick?**

Andrzej Marek Brzozowski

:Structural Biology Laboratory, Department of Chemistry, University of York, United Kingdom

Abstract: Human insulin and its related Insulin-like Growth Factor-I and II (IGFs) are small protein hormones that are key regulators of human metabolism, growth and life-span. Remarkably, insulin/IGF axis signaling is very conserved in all animal kingdom. 40 years after D Hodgkin pioneering work on insulin structure, the first insight into 3-D organization of human insulin:Insulin Receptor (IR) complex has been achieved. It revealed an unexpected mode of the hormone:receptor engagement, and explained/unified the wealth of accumulated biochemical data concerning insulin functionality. Moreover, extension of this work into insulin-related insects' homologues confirmed structural and molecular bases of functional similarity between insect and human hormones. We have also been trying to address the issues related to (still unknown) storage form(s) of insulin in pancreas vesicles. Unexpectedly, this line of research linked insulin with its putative role in the Central Nervous System, and origins of this hormone in invertebrates. Highlights of all these aspects of insulin-based studies will be presented.



### **Grzegorz Bujacz**

Education - M.S., Technical University of Lodz 1986; Ph. D., Technical University of Lodz 1993; D.Sc., Technical University of Lodz 1999. Professor, 2005

Fields of interest: Protein crystallization, Protein structure-function relationship, enzymes from retroviruses and other pathogens; structural biology of plants and insect.

97 publications, 1,717 total citations, H-index 20, 67 structures in the PDB, Advised 7 PhD and 31 MSc theses.

Vice Dean, Faculty of Biotechnology and Food Sciences, Lodz University of Technology(2002-2008), Chairmen of Protein Crystallography Section at Crystallography Committee of the Polish Academy of Sciences,

# The structural studies of the two isoforms of laccase from the white-rot fungi *Pycnoporus sanguineus*

Grzegorz Bujacz

X-ray Analysis Laboratory, Institute of Technical Biochemistry, Lodz University of Technology, Poland  
Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences,  
Poznań, Poland

Laccases (EC 1.10.3.2, benzenediol oxygen oxidoreductases) are enzymes that have the ability to catalyze the oxidation of a wide spectrum of phenolic compounds with the four-electron reduction of molecular oxygen to water [1]. The active site is well conserved among all species. It contains four copper atoms: one paramagnetic type 1 copper (T1) that is responsible for their characteristic blue color and where the oxidation of the reducing substrate occurs, one type 2 copper (T2) and two type 3 coppers (T3) that conform a trinuclear cluster in which molecular oxygen is reduced to two molecules of water [2]. Laccases are widely distributed in plants, lichens, bacteria and insects. However, these enzymes are particularly abundant in fungi, having been found in almost all wood-rotting fungi analyzed to date. In most cases laccases are monomeric glycoproteins of around 500 amino acids with molecular weights in the range of 60-85 kDa. The various functions carried out by those enzymes include the antagonistic ones such as their involvement in lignin biosynthesis (in plants), lignin degradation, pigment production, fruiting body formation, pathogenesis (in fungi) and spore protection against UV light (in bacteria) [1, 3]. The diversified functions of laccases make them an interesting enzyme for study from the point of view of their structure, function and application. Laccases of white-rot fungi (WRF) are of special interest because one of their roles is to degrade lignin and most of them are extracellular enzymes helping purification procedures [1]. During the last two decades, there has been an increasing interest in the genus *Pycnoporus* for its ability to overproduce high redox potential laccases as the ligninolytic enzymes.

We present the crystal structures of two thermostable laccases produced by strain *Pycnoporus sanguineus* CS43 (LacI and LacII). The molecular weights of LacI and LacII, determined by SDS-electrophoresis, is 68 and 66 kDa, respectively [3]. Both isoforms show high amino acids sequence similarity (91%) and high thermal stability, at 50°C and 60°C. They remain active at high concentration of organic solvent (acetonitrile, ethanol or acetone). The unique properties make them promising candidates for industrial applications in wastewater treatment. LacI exerted a higher thermal and pH stability, tolerance against inhibitors and was a more efficient catalyst for ABTS and DMP (laccases substrate) than LacII [3]. Based on the structures we would like to understand the isoform differences that cause LacI's markedly better pH and thermal stability as well as better resistance to inhibitors.

[1] Baldrian F., *FEMS Microbiol. Rev.*, 30, 215–242 (2006)

[2] Mot A. & Silaghi-Dumitrescu R., *Biochemistry*, 77, 1395-497 (2012)

[3] Rivera-Hoyos E. et al., *Fungal Biol. Rev.*, 27, 67-82 (2013)

[4] Ramirez-Cavazos L. et al., *J. Mol. Catal. B Enzym.*, 108, 32-42 (2014)



## **Janusz Bujnicki**

Education: M.Sc., University of Warsaw, 1998; Ph.D., University of Warsaw, 2001; D.Habil., Polish Academy of Sciences, 2005; Professor, 2009.

Fields of interest: bioinformatics, structural and synthetic biology, development of methods for computational modeling of protein and RNA 3D structures, discovery and characterization of enzymes involved in RNA metabolism, and engineering of proteins with new functions.

~280 publications, >4800 total citations, H-index 35 (excluding self-citations). Supervised 26 PhD and 47

MSc theses and trained 13 postdoctoral fellows.

Recipient of EMBO/HHMI Young Investigator Programme award (2003-2005), ERC Starting Grant (2011-2015), Foundation for Polish Science MISTRZ award (2015-2017), Ministry of Science award (2012), Prime Minister award (2014), decorated with the Knight's Cross of the Order of Polonia Restituta (2014) for outstanding scientific achievements. Won the national plebiscite "Poles with Verve" in the Science category (2013). Selected as one of "25 leaders for the next 25 years" by "Teraz Polska" magazine of the Polish Promotional Emblem Foundation.

Has been involved in various scientific organizations and bodies, including Polish Bioinformatics Society (founding member, vice-president 2008-2010, president 2011-2013), Science Europe LEGS panel (2013-), Academy of Young Scientists of the Polish Academy of Sciences (2011-), Citizens of Science (2012-), and Scientific Policy Committee (2014-). Editor of the Springer series Nucleic Acids and Molecular Biology (2009-2013), executive editor of the journal Nucleic Acids Research (2013-).

# Coarse-grained modeling of 3D structure for RNA and macromolecular complexes

Janusz Bujnicki

Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology, Warsaw, Poland  
Bioinformatics Laboratory, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland

Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology, ul. Ks. Trojdena 4, 02-109 Warsaw, and Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Umultowska 89, PL-61-614 Poznan, Poland; email: iamb@genesilico.pl

In addition to mRNAs, whose primary function is transmission of genetic information from DNA to proteins, numerous other classes of RNA molecules exist, which are involved in a variety of functions, such as catalyzing biochemical reactions or performing regulatory roles. In analogy to proteins, the function of these RNAs often depends on 3D structure and dynamics, which are largely determined by the ribonucleotide sequence. Experimental determination of high-resolution RNA structures is both laborious and difficult, and therefore the majority of known RNAs remain structurally uncharacterized. To address this problem, computational structure prediction methods were developed. All computational methods suffer from various limitations that make them generally unreliable for structure prediction of long RNA sequences. However, in many cases the limitations of computational and experimental methods can be overcome by combining these two complementary approaches with each other. I will present computational methods for prediction of RNA 3D structures and RNA-protein complexes developed in my group, with emphasis on software that can utilize restraints derived from experimental analyzes (SimRNA and PyRy3D). I will also present a method for structure-based RNA sequence design.

## References:

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- Tuszynska and Bujnicki (2011) DARS-RNP and QUASI-RNP: New statistical potentials for protein-RNA docking. *BMC Bioinformatics*, 12, 348.
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- Chojnowski et al. (2012) RIBER/DIBER: a software suite for crystal content analysis in the studies of protein-nucleic acid complexes. *Bioinformatics*, 28, 880-881.
- Pietal et al. (2012) RNAmapping2D - calculation, visualization and analysis of contact and distance maps for RNA and protein-RNA complex structures. *BMC Bioinformatics*, 13, 333.
- Matelska et al. (2013) S6:S18 ribosomal protein complex interacts with a structural motif present in its own mRNA. *RNA* 19(10):1341-8.
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- Chojnowski et al. (2014) RNA Bricks - a database of RNA 3D motifs and their interactions *Nucleic Acids Res* 42(1):D123-31.
- Smietanski et al. (2014) Structural analysis of human 2'-O-ribose methyltransferases involved in mRNA cap structure formation *Nature Commun* 5:3004, doi:10.1038/ncomms4004
- Magnus et al. (2014) Computational modeling of RNA 3D structures, with the aid of

experimental restraints. *RNA Biol* 2014 Apr 23;11(5)

Walen et al. (2014) ClaRNA: a classifier of contacts in RNA 3D structures based on a comparative analysis of various classification schemes. *Nucleic Acids Res* 2014, Oct 29;42(19):e151.

Głów et al. (2015) Sequence-specific cleavage of dsRNA by Mini-III RNase. *Nucleic Acids Res.* 2015 Jan 29. pii: gkv009.



**Maksymilian Chruszcz**

Associate Professor

Department of Chemistry and Biochemistry, University of South Carolina

Education

M.Sc., Jagiellonian University, 1997

Ph.D., Jagiellonian University, 2002

Employment

2003-2012, University of Virginia

2012-present, University of South Carolina

Fields of interest

-structural biology, in particular macromolecular crystallography

-molecular basis of allergy, asthma and infectious diseases

~100 publications, ~2000 total citations, h-index 21, >200 structures in PDB

# **Allergens - structure and function**

Maksymilian Chruszcz

University of South Carolina, Columbia, SC, USA

The ability of X-ray crystallography to provide accurate information about macromolecular structures, including the molecular details of the interactions between proteins and small molecules, is unsurpassed. However, one has to remember that a crystal structure is merely a “snapshot” representing an "average macromolecule". In addition, there are other limitations of X-ray crystallography that can significantly affect our interpretation of biochemical or biological processes. Such limitations will be discussed in the examples provided by analysis of pathogenesis-related class 10 protein (PR-10) originating from peanuts. The PR-10 protein, which is also a minor peanut allergen (Ara h 8), has an unusually large binding cavity and functions as a small molecule carrier. Conformational changes and the large ligand-binding cavity allow the protein to bind molecules that significantly differ in their structure. For example, we were able to demonstrate that the protein binds flavonoids, steroids, fatty acids and some plant hormones like zeatin. It is also possible that the ligand binding affects the immunological properties of PR-10 related allergens.



### **Marek Cieplak**

Education - M.S., Department of Physics, University of Warsaw, 1973; Ph. D., Department of Physics, University of Pittsburgh, 1977; D.Sc., Department of Physics, University of Warsaw, 1984. Professorial title, 1994.

Fields of interest – condensed matter theory (spin waves, spin glasses, porous media, growth processes, atomic friction, river networks, nanofluidics, self-organized nanostructures) and biological physics (large conformational changes of biomolecules within coarse-grained models, especially as induced by stretching, proteins with knots and slipknots, protein folding, dynamics of virus capsids and other multi-proteinic structures such as a cellulosome, interaction of proteins with solids, proteins at air-water interface, modeling of proteasomes, inference of genetic networks from the microarray data). Textbook “Theory of Quanta” with I. Białyński-Birula and Jerzy Kamiński (Polish Scientific Company -1991, 2001; Oxford University Press -1992). Co-editor of EPL . On the advisory board of Journal of Physics: Condensed Matter. Fellow of the American Physical Society (1998) and its Outstanding Referee (2008). Summer lectures at Rutgers University 1991-2000 and at Johns Hopkins University, since 2001. Many Polish grants; in several European projects (such as NaPa, Funmol, CellulosomePlus, MissingLink). 235 papers, Hirsch factor 37.

# **Structure-based models of proteins in action: virus capsids, proteins with cavities, unfolding by proteasomes, formation of knots**

Marek Cieplak

Laboratory of Biological Physics , Institute of Physics, Polish Academy of Science, Warsaw, Poland

The talk will give examples of results obtained with coarse grained models constructed based on the native state geometry of proteins. Such simplified models allow for molecular dynamics studies of systems at much larger time- and length-scales than in the standard all-atom simulations. The first example is the study of mechanical response of 35 virus capsids of various symmetries and sizes: containing between 8 460 and 135 780 amino acids.

Nanoindentation by a broad AFM tip is modeled as compression between two planes. Plots of the compressive force versus plate separation show a variety of behaviors and molecular effects that are beyond the continuum shell model. A second application is an analysis of properties of proteins with cavities. The third – an analysis of the mechanics of protein degradation by proteasomes in the context of measurement of the stalling force. The fourth – a demonstration of multiple folding pathways in folding of proteins with shallow knots.



### **Tomasz Cierpicki**

Assistant Professor in the Department of Pathology.  
Education - M.S., Wroclaw University of Technology, 1998; Ph. D. University of Wroclaw, 2002. Postdoc; University of Virginia 2002-2006. Fields of interest – application of structural biology methods to study proteins involved in cancer; NMR spectroscopy, drug discovery

>60 publications, ~1500 total citations, H-index 22.

# Targeting E3 ligase activity of RING domain with small molecules

Tomasz Cierpicki<sup>1</sup>, Felicia Gray, Weijiang Ying, Qingjie Zhao, Shirish Shukla, Hyoje Cho, George Lund, Hongzhi Miao, Trupta Purohit, Shihan He, Joshua Abbott, Bohdan Boytsov and Jolanta Grembecka

<sup>1</sup>University of Michigan, Ann Arbor, MI, USA

Abstract: Bmi1 is a central component of the Polycomb Repressive Complex 1 (PRC1) and is required for E3 ligase activity to ubiquitinate histone H2A. Multiple studies identified Bmi1 as oncogene promoting tumor growth in a variety of in vitro and in vivo animal models. Small molecule inhibitors of the Ring1B-Bmi1 E3 ligase activity have not been reported to date, but are highly desired as potential therapeutic agents targeting cancer stem cells. Targeting the Ring E3 ligases with small molecules is a very challenging task due to the lack of well-defined substrate binding pockets and a complex biochemical assays required for enzymatic activity studies. We have identified compounds binding to Ring1B-Bmi1 based on NMR fragment screening. Further medicinal chemistry optimization resulted in potent inhibitors of E3 ligase activity. In cells, our compounds inhibit Ring1B-Bmi1 ubiquitin ligase activity at low micromolar concentrations. To our knowledge, these compounds represent the first small molecule inhibitors that directly bind to Ring1B-Bmi1 and inhibit its E3 ubiquitin ligase activity and have a strong potential for further development into anticancer agents.



### **Mirosław Cygler**

Education - M.Sc., University of Lodz, 1970; Ph.D., University of Lodz, 1976. Post-doctoral fellowship at the National Research Council of Canada in Ottawa, 1979-81; University of Alberta, Edmonton, 1981-87; Biotechnology Research Institute, NRC, Montreal, 1987-2011; Adjunct Professor, Department of Biochemistry, McGill University, Montreal, since 1992; University of Saskatchewan since October 2011, Canada Research Chair in Molecular Medicine Using Synchrotron Light since January 2012; Director of the Centre for Proteomics Research in Interactions and Structure of Macromolecules (PRISM)

Fields of interest - structural biology of protein complexes, enzyme mechanisms, host-pathogen

interactions.

~260 publications, ~10,500 total citations, H-index 49, over 200 structures in PDB, supervised 6 Ph.D. students, trained >40 postdoctoral fellows.

## Effector proteins from pathogenic bacteria: focus on kinases

Mirosław Cygler<sup>1,2</sup>, Andrey Grishin, Gary Shaw, Claude Parsot

<sup>1</sup>University of Saskatchewan, Saskatoon, SK, Canada

<sup>2</sup>McGill University, Montreal Quebec, Canada

Abstract: Pathogens modify host cell responses through a ensemble of proteins ejected into the host through a syringe-like bacterial secretion system. One of the ways the cellular responses are modified to assure pathogen survival and proliferation inside the host is to hijack and redirect host signaling pathways. Bacterial effector kinases are among the tools to do just that. Kinases NleH1 and NleH2 from pathogenic *E. coli*, OspG from *Shigella*, SteC and SboH from *Salmonella*, LegK1-4 from *Legionella* and YspK and YpkA from *Yersinia* represent currently known effector kinases. Sequence analysis of these kinases indicates that some of them were derived from eukaryotes via horizontal gene transfer (SteC, LegK1-4, YpkA). Other kinases (NleH, OspG, SboH and YspK) have been so far identified only in the pathogenic bacteria. Structural investigations showed that NleH and OspG contain only a core kinase fold and lack the regulatory activation loop. While NleH is fully active on its own, OspG activity is stimulated by ubiquitin and even more by the ubiquitin-conjugating enzyme E2-ubiquitin complex. The structure of OspG:UbcH7-Ub complex and mutational analysis of OspG suggest the mechanism of OspG activation. Both NleH and OspG inhibit the NF- $\kappa$ B pathway, however, their substrates are yet unknown.



### **Zbigniew Dauter**

Education - M.Sc., Technical University of Gdansk, 1971; Ph.D., TUG, 1975; Postdoc, University of York, 1981-1984; D.Sc., University of Poznan, 2004.

In York (England) 1981-84, in EMBL Hamburg (Germany) 1986-1995, in York again 1996-97, since 1997 in the US.

Fields of interest - methods development in structural biology especially utilizing synchrotron radiation: atomic resolution structures, use of anomalous signal in phasing.

>320 publications, >13,500 total citations, H-index 60, >240 structures in the PDB.

Trained >10 postdoctoral fellows.

Recipient of the Copernicus Medal of the Polish Academy of Sciences, 2009.

# **Multipoles through the looking glass**

Zbigniew Dauter

National Cancer Institute, Argonne, IL, USA

Abstract: A survey of the past and present achievements of several macromolecular crystallographers of Polish origin is presented as well as the some highlights of their scientific progress from the origins in Poland to their current positions. Some statistics of their publication and citation record is shown, including information on selected projects executed in collaboration between them.



## **Zygmunt Derewenda**

Education - M.Sc., University of Lodz, 1977; Ph.D., University of Lodz, 1982; D.Sc., University of Lodz, 2005. Past employment: University of Lodz, Poland (1976-1985); University of York, UK (1985-1990); University of Alberta in Edmonton, Canada (1990-1996). Current Position: Distinguished Harrison Teaching Professor, Professor of Molecular Physiology and Biological Physics, University of Virginia School

of Medicine, Charlottesville, Virginia, 22908, USA. Director of the Visiting Student Program for Polish students (Masters) in USA; Co-Editor of Acta Crystallographica D Fields of interest - macromolecular crystallization and crystallography, protein structure and function, enzyme mechanism, protein-protein interactions; signal transduction

>150 publications, >11,000 total citations; > 110 structures in PDB H-index 58; Advised 5 PhD students (Virginia);

>15 postdoctoral fellows; and >30 MSc. students; (visiting from Polish Universities)

# **The structural biology of Ca<sup>2+</sup>-independent modulation of smooth muscle contraction**

Zygmunt Derewenda, Avril V. Somlyo

University of Virginia, Charlottesville, VA, USA

Abstract: It is well established that smooth muscle contraction is initiated by the influx of Ca ions, leading to the activation of the myosin light chain kinase (MLCK) and the canonical cross-bridge cycle. However, during the last two decades, intense research revealed that this simple mechanism is tuned and modulated in each smooth muscle tissue, specifically through the so-called Ca-sensitization effect, whereby contraction is sustained as Ca-levels drop, due to the inhibition of the myosin light chain phosphatase. This process is controlled by agonists that activate G-protein coupled receptors, which in turn engage the small GTPase RhoA. A plethora of accessory proteins, that either up- or down-regulate RhoA may confer tissue-specificity on numerous regulatory phenomena. These proteins potentially offer new targets for the discovery of drugs that could more selectively and specifically control such disease states as hypertension and asthma. Our laboratories collaborated for over twenty years on the elucidation of physiology and structural biology of the regulation of RhoA. This led, among others, to the determination of the first crystal structure of RhoA, and a number of regulatory proteins including GAPs (GTPase activating proteins), GEFs (Guanine exchange factors) and RhoGDI (the guanine nucleotide-dissociation inhibitor). This presentation will review these studies and introduce new avenues, currently under investigation in our laboratories.



## **Andrzej Dziembowski**

Education - M.Sc., University of Warsaw, 1997; Ph.D. University of Warsaw 2002; Postdoc 2002-2006 Centre de Genetique Moleculaire, Gif-sur-Yvette, France; D.Sc., University of Warsaw, 2009 Fields of interest – eukaryotic RNA metabolism; proteomics, biochemistry and structural biology of protein complexes ~50 publications, ~1800 citations, H-index 21. Trained > 7 M.Sc students, 3 PhD students Currently supervises 7 postdocs and 6 Ph.D. students. Recipient of the EMBO Installation Grant and ERC starting grant

# **Architecture of the CCR4-NOT complex from *Schizosaccharomyces pombe*, a multifunctional cellular machine**

Andrzej Dziembowski<sup>1,2</sup>, Marta Ukleja, Jorge Cuellar, Aleksandra Siwaszek, Joanna M. Kasprzak, Mariusz Czarnocki-Cieciura, Janusz Bujnicki, Jose Maria Valpuesta

<sup>1</sup>Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

<sup>2</sup>Institute of Genetics and Biotechnology, University of Warsaw

**Abstract:** The CCR4-NOT complex is a large macromolecular assembly present both in the cytoplasm and the nucleus of eukaryotic cells and involved in a variety of distant processes related to expression of genetic information such as poly(A) tail shortening, transcription regulation, nuclear export and protein degradation. Herein we have performed a comprehensive structural characterization of the native CCR4-NOT complex from *S. pombe*. We have obtained a cryo-EM 3D reconstruction of the complex which combined with different techniques such as immunomicroscopy, and RNA-nanogold labeling and the docking of the available high-resolution structures of different subunits and domains, have allowed us to propose for the first time the full molecular architecture of CCR4-NOT complex and to provide a mechanistic insight into the multifunctional properties of this complex.



### **Witold Filipowicz**

Witold Filipowicz is working at the Friedrich Miescher Institute for Biomedical Research in Basel, Switzerland and is also a Professor Emeritus at the University of Basel. He graduated as M.D. from the Medical University of Lodz, and received his Ph.D. from the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, where he was running his lab until moving to Basel in 1984. He also worked at the New York University Medical School, Roche Institute of Molecular Biology in Nutley, NJ, and Institut Jacques Monod of CNRS in Paris, as either post-doctoral fellow or sabbatical visitor. His prior research was focused on tRNA, rRNA and mRNA processing, on enzymes regulating RNA metabolism, and a role of non-coding RNAs in gene expression. Currently, he investigates mechanisms of RNA interference and microRNA function and metabolism in mammalian cells and mouse retina. He published nearly 200 papers in this area. He received the Lifetime Achievement in Science Award of International RNA Society (2011), is a member of EMBO, Polish Academy of Sciences, and also acts as a Chair of the Biochemistry and Molecular Biology Section of Academia Europaea. He sits on Advisory Boards of several Institutes and Editorial Boards of many scientific journals, including Cell.

# **Structural insights to the role of the CCR4-NOT complex and GW182 and DDX6 proteins in miRNA-mediated repression**

Witold Filipowicz

Friedrich Miescher Institute of Biomedical Research, Basel, Switzerland

Structural insights to the role of the CCR4-NOT complex and GW182 and DDX6 proteins in microRNA-mediated repression Witold Filipowicz Friedrich Miescher Institute for Biomedical Research, 4002 Basel, Switzerland MicroRNAs (miRNAs) are ~20-nt-long regulatory RNAs expressed in eukaryotes. They regulate gene expression post-transcriptionally, by imperfectly base-pairing to 3'UTRs of mRNAs what results in translational repression and mRNA deadenylation and degradation. Most of mammalian genes are predicted to be subject to miRNA regulation. Clearly, discovery of miRNAs added a new dimension to the complexity and regulation of eukaryotic genomes. We will discuss current knowledge about the mechanism of miRNA-mediated repression, focusing on a role of GW182 proteins and the multi-subunit CCR4-NOT complex in translational repression and mRNA deadenylation. Our recent work performed in collaboration with Elena Conti's group at MPI in Martinsried and Marcin Nowotny and Andrzej Dziembowski in Warsaw, revealed the mechanism of the CCR4/NOT recruitment by GW182 proteins and a role of Trp-containing motifs (W-motifs) of GW182s in this process. Structural studies showed that the C-terminal repressive domain of GW182 associates with CCR4/NOT by directly interacting with the complex subunit CNOT9, which contains two Trp-binding pockets. We have also demonstrated that, further downstream, the CCR4-NOT complex recruits the DEAD-box RNA helicase/ATPase DDX6 to mRNA. Structural analyses of the apo form of DDX6 and its complex with the CCR4/NOT scaffold subunit CNOT1 revealed that the DDX6 interaction with CCR4/NOT is accompanied by a major conformational change in DDX6 resulting in activation of its ATPase activity. The DDX6 activation is required for its function in miRNA-mediated repression of translation.



**Maria Gdaniec**

Education: M.Sc., chemistry. A. Mickiewicz University, 1974; Ph.D., chemistry, 1978; D.Sc., physical chemistry, 1992; Professor, chemistry, 2004

Fields of interest: crystal engineering, structural and supramolecular chemistry, X-ray crystallography.

~350 publications, ~3,100 total citations, H-index 28.  
Supervised 3 Ph.D. and 37 M.Sc. theses.  
President of the Committee of Crystallography, Polish Academy of Sciences; Co-editor of Acta Crystallographica E

# Exploring the wealth of specific intermolecular interactions for crystal engineering

Maria Gdaniec

Department of Crystallography, Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland

Crystal engineering, a branch of supramolecular chemistry, exploits our present knowledge of intermolecular interactions and molecular recognition processes in the design and synthesis of crystalline phases. In recent years scientific interest in preparation of mono- and multi-component molecular crystals with controlled properties is rising rapidly and a number as well as a variety of applications of newly prepared solid phases is growing steadily.

Research during the past twenty years shows that cocrystallization of chemical compounds is a more common phenomenon than initially believed and the number of new chemical substances that can be prepared in this way is practically unlimited. Strategy for the synthesis of multicomponent crystals is analogous to the strategy for supramolecular synthesis. Selection of the reagents should lead to heteromeric recognition, resulting in the formation of cocrystals, instead of homomeric recognition, resulting in separate crystallization of the substrates. Therefore the reacting molecules should be equipped with complementary, but different, functional groups able to form heterosynthons. At the very beginning crystal engineering was mainly applying conventional hydrogen bonds to link complementary functional groups, however a variety of specific intermolecular interactions with strength comparable to hydrogen bonds is getting more and more abundant.

In this presentation, the design of new multicomponent crystals with supramolecular synthons based on hydrogen, halogen and chalcogen bonds, aromatic interactions, including aryl-perfluoroaryl synthon, will be presented. The obvious next step in crystal engineering is an introduction into the crystal design process of a combination of synthons based on diverse kinds of intermolecular interactions. As a result, better understanding of supramolecular chemistry of functional groups as well as determination of the hierarchy of supramolecular synthons can be achieved. This kind of research requires however creation of modular systems where cooperation and competition of intermolecular interactions can be regulated via a systematic alteration in the structure of reagents, allowing thus for tuning of the structure and the properties of multi-component crystals.



## **Adam Godzik**

Education - M.S. (1983) and Ph. D. (1990) Department of Physics, University of Warsaw; Postdoc – European Molecular Laboratory, Heidelberg 1990 (with Chris Sander), The Scripps Research Institute, La Jolla, 1991 (with Jeff Skolnick).

Fields of interest – evolutionary, structural and systems biology and ways to merge the three to study the emergence and evolution of biological processes such as apoptosis or innate immunity.

>320 publications, >21,000 total citations, H-index 70 (Google Scholar). Trained >20 postdoctoral fellows

# **Understanding cancer mutations with protein and network modeling**

Adam Godzik

Bioinformatics and Systems Biology Program, Sanford-Burnham Medical Research Institute, La Jolla, CA,  
USA

Cancer is characterized by extreme heterogeneity, with every individual tumor having a distinct pattern of genomic alterations. These patterns affect outcomes and treatment options, but except some well studied alterations we mostly don't understand their specific roles. We analyzed missense somatic mutations in a pan-cancer cohort of 5,989 tumors from 23 projects of The Cancer Genome Atlas (TCGA), mapping them on experimental or predicted models of human proteins and protein-protein complexes. We then searched for enrichment of cancer mutations on specific structural features, such as domains or PPI interfaces and their correlation with disease or treatment outcomes. We identified hundreds of novel domain or interface cancer drivers and found many examples how mutations in different regions in the same gene can have different effects, including patient outcomes. Results of this analysis are available on <http://cancer3D.org>.



**Jolanta Grembecka**

Assistant Professor, Department of Pathology, University of Michigan, Ann Arbor, MI, USA.

In the US since 2002.

Education - M.S., University of Opole, 1995; Ph. D. Wroclaw University of Technology, 2000.

Postdoc; University of Virginia 2002-2006.

Fields of interest – drug discovery, development of small molecules for targeted therapies in cancer, identification and development of inhibitors targeting protein-protein interactions.

55 publications, ~1100 total citations, H-index 19.

Recipient of the Leukemia and Lymphoma Society (LLS) Translational Research Program Award and LLS Therapy Acceleration Program, LLS Scholar and American Cancer Society (ACS) Research Scholar.

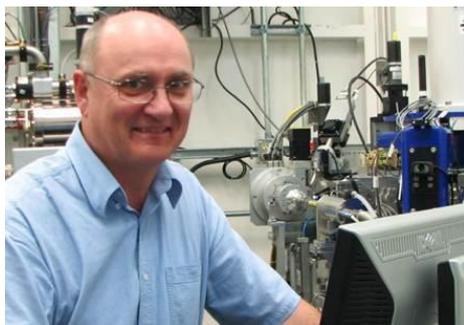
## **Structure-based development of menin-MLL inhibitors for cancer therapy.**

Jolanta Grembecka<sup>1</sup>, Dmitry Borkin, Hongzhi Miao, Jonathan Pollock,  
Katarzyna Kempinska, Trupta Purohit, Tomasz Cierpicki

<sup>1</sup>Department of Pathology, University of Michigan, Ann Arbor, MI, USA

Menin is a protein that directly interacts with the Mixed Lineage Leukemia 1 (MLL1) and MLL2 histone methyltransferases, and is required for their recruitment to the target genes. Menin also binds to MLL fusion proteins and this protein-protein interaction is required for development of acute leukemias with translocations of the MLL gene. Accumulating evidences suggest that menin complexes with MLL1 or MLL2 play a role in development of solid tumors. Therefore, small molecule inhibitors of the menin-MLL interaction might result in new therapeutics for leukemias and/or solid tumors.

By applying the high throughput screening followed by extensive medicinal chemistry we developed very potent small molecules that specifically bind to menin with low nanomolar affinities and inhibit the menin-MLL interaction in vitro and in human cells. Crystallography studies demonstrate that these compounds bind to the MLL binding site on menin and closely mimic the key MLL interactions with menin. Using structure-based design, we optimized both potency and drug-like properties of the thienopyrimidine class of the menin-MLL inhibitors, including pharmacokinetic profile, making them suitable for in vivo studies. These compounds demonstrate strong effect and specific mechanism of action in MLL leukemia cells, including inhibition of cell proliferation, induced differentiation and downregulation of MLL fusion protein target genes. More importantly, the menin-MLL inhibitors we developed block progression of acute leukemia in vivo in mice models of MLL leukemia, validating their therapeutic potential. Currently, broader applications of these compounds in various cancers are being explored. Our work provides another example of successful targeting of protein-protein interactions with small molecules for therapeutic applications.



## **Pawel Grochulski**

Employment: Technical University of Łódź, 1979-1990; National Research Council of Canada, 1991-1998; BioMep Inc., 1999-2001; Canadian Light Source, University of Saskatchewan, 2001- present.

Education - M.Sc., Technical University of Łódź, 1979; Ph.D., Technical University of Łódź, 1988;

postdoctoral fellowship, Medical Foundation of Buffalo (now Hauptman-Woodward Medical Research Institute), USA, 1991; Habilitation (D.Sc.), Technical University of Łódź, 1994.

Fields of interest: application of synchrotron radiation to structural biology including: structure-function relationships of biological molecules, mechanisms of enzymatic activities (including stereoselective enzymatic reactions), penetration of biological membranes, rational drug design and drug delivery systems, development and refinement of synchrotron-based phase determination techniques as well as synchrotron instrumentation

Techniques: chemical crystallography, macromolecular crystallography (MX), powder diffraction, small angle X-ray scattering (SAXS) and X-ray absorption spectroscopy (XAS).

~90 publications, ~3,450 total citations, H-index 18

Co-founder of the Canadian Macromolecular Crystallography Facility (CMCF), member of the IUCr Commission on Synchrotron Radiation, co-founder and President of the Polish-Canadian Scientific Society of Saskatoon, Member of the Program Committee for CIHR-THRUST, member of the Proteomics Research in Interactions and Structure of Macromolecules (PRISM ) at the University of Saskatchewan.

# Crossing membranes: beta-cyclodextrin-gemini surfactant and the type II secretion system

Pawel Grochulski<sup>1,2</sup>, Masoomah Poorgohorban, Ildiko Badea, Elizabeth Vanderlinde, Darek Martynowski, Peter Howard and Lee Wilson

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<sup>2</sup>College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada

Biological membranes can be crossed by ions, small molecules and proteins. There are two types of transport through biological membranes, passive and active transport. In the first type the transport is driven by diffusion, whereas in the latter case it is facilitated by other mechanisms. For example, the transport is by endocytosis in the case of the gemini-based drug delivery system and via a sophisticated protein machinery in the case of the type II secretion system (T2SS).

When designing a drug delivery system, one option would be to transport genetic material or an insoluble drug to a specific site inside the cell through a membrane using nanoparticles. In this process, the cell membrane engulfs the nanoparticle, forms a complete sphere around it, and then draws the membrane-bound vesicle, called an endosome, into the cell. Drugs loaded into nanoparticles can thereby be internalized efficiently by a large variety of cells, including cancer cells. Development and evaluation of novel  $\beta$ -cyclodextrin-gemini based nano-delivery systems for topical treatment of melanoma using synchrotron techniques will be presented. This includes the application of synchrotron WAXS (chemical crystallography, powder diffraction), SAXS and NMR in the characterisation of the system.

From a different perspective, transport of protein toxins and enzymes out of Gram-negative bacterial cells is performed via the type II secretion system, composed of 12-16 proteins depending on the species. *Vibrio vulnificus* utilizes the T2SS to translocate extracellular proteins from the periplasmic space across the outer membrane through a megadalton complex called the secretin. General secretion pathway (Gsp) proteins GspA and GspB are required for outer membrane localization and assembly of GspD, the protein that forms the secretin. The GspAB complex interacts with both peptidoglycan (PG) and the secretin to allow transport of proteins out of the cell. In *V. vulnificus* GspA and GspB are fused into one protein, EpsAB, whose crystal structure will be discussed. Another component of the T2SS in *Aeromonas hydrophila* is the inner membrane ExeC protein. The periplasmic domain of ExeC in *A. hydrophila* is composed of an N-terminal HR domain and a C-terminal PDZ domain. We have solved the crystal structure of the PDZ-domain expressed with a 6-His-tag at the C-terminus. Interactions of the 6-His-tag within the crystal structure of the PDZ domain may give some indication as to how the PDZ domain interacts with other components of the T2SS machinery. We also demonstrated that in *A. hydrophila* the PG-AB complex facilitates assembly of the secretin through direct interaction between ExeB and ExeD.



**Robert Janowski**

Staff Scientist,  
Institute of Structural Biology (STB),  
Helmholtz Centrum Munich

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Previous posts:

Adam Mickiewicz University (Staff Scientist),  
Institute of Bioorganic Chemistry, Polish Academy of  
Science (Staff Scientist),

EMBL (Postdoctoral Fellow),

Institute for Research in Biomedicine (Postdoctoral  
Fellow), Instituto de Biología Molecular de Barcelona (Postdoctoral Fellow)

Education: M.Sc. (chemistry) A. Mickiewicz Univ. (1997); M.Sc. (Structural Biology using  
the Internet) Birkbeck College, Univ. of London (2001); Ph.D. (chemistry) A. Mickiewicz  
Univ. (2002);

Fields of interest: structural biology of RNA localization and transport, viral proteins,  
structure based drug design

22 publications, 19 structures in the PDB.

# **Structural basis for RNA recognition in roquin-mediated post-transcriptional gene regulation**

Robert Janowski

Helmholtz Zentrum, Munich, Germany

Roquin function in T cells is essential for the prevention of autoimmune disease. Roquin interacts with the 3' untranslated regions (UTRs) of co-stimulatory receptors and controls T-cell activation and differentiation. The N-terminal ROQ domain from mouse roquin adopts an extended winged-helix (WH) fold, which is sufficient for binding to the constitutive decay element (CDE) in the Tnf 3' UTR. The crystal structure of the ROQ domain in complex with a prototypical CDE RNA stem-loop reveals tight recognition of the RNA stem and its triloop. Surprisingly, roquin uses mainly non-sequence-specific contacts to the RNA, thus suggesting a relaxed CDE consensus and implicating a broader spectrum of target mRNAs than previously anticipated. Consistently with this, NMR and binding experiments with CDE-like stem-loops together with cell-based assays confirm roquin-dependent regulation of relaxed CDE consensus motifs in natural 3' UTRs.



### **Mariusz Jaskólski**

Previous posts: Visiting Scientist (NCI); International Research Scholar of the HHMI; Faculty Scholar, Center for Cancer Research (NCI); Vice-President of the European Crystallographic Association

Education: M.Sc. (chemistry) A. Mickiewicz Univ. (1976); Ph.D. (chemistry) A. Mickiewicz Univ. (1979); D.Sc. (physical chemistry) A. Mickiewicz Univ. (1985); Full Professor (chemistry) 2005

Fields of interest: structural biology of hydrolases, especially applied to enzymes from retroviruses and other pathogens; structural biology of plants; conformational aberrations; crystallographic methodology and teaching

~330 publications, >5,700 total citations, H-index 35, >100 structures in the PDB.

Supervised 10 Ph.D. fellows. Trained 9 postdoctoral fellows.

Corresponding member of the Polish Academy of Sciences, Foreign member of Regia Societas Scientiarum Upsaliensis (Sweden), EMBO member. Jan Zawadzki Medal (Pol. Chem. Soc.), Leon Marchlewski Medal (Committee of Biochem. & Biophys., Pol. Acad. Sci.), Sniadecki Medal (Pol. Chem. Soc.). Prize of the Foundation for Polish Science. Polish-American Scientific Award of FNP/AAAS (with A. Wlodawer).

Photo M. Forecki

## Tema con variazioni: modulated structures of Hyp-1 complexes

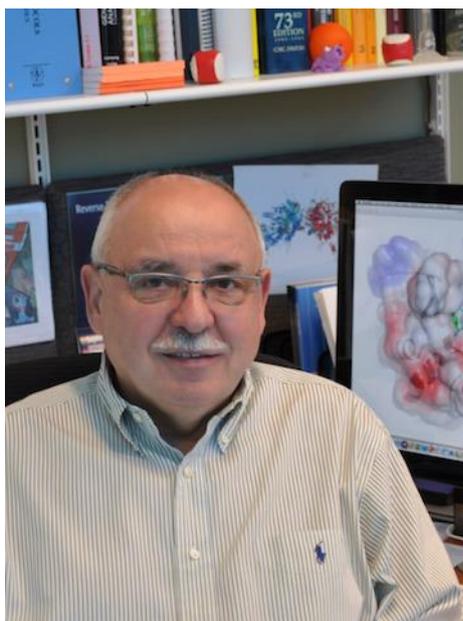
Mariusz Jaskolski<sup>1,2</sup>, Joanna Sliwiak<sup>2</sup>, Zbigniew Dauter<sup>3</sup>

<sup>1</sup>Department of Crystallography, Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland

<sup>2</sup>Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

<sup>3</sup>Synchrotron Radiation Research Section, MCL, National Cancer Institute, Argonne National Laboratory, Argonne, IL, USA

We reported previously that Hyp-1 (a PR-10 protein from St John's wort), when cocrystallized with ANS (8-anilino-1-naphthalene sulfonate), formed tetartohedrally twinned crystals with commensurately modulated structure in space group C2, in which seven tetrameric assemblies (each formed from two beta-sheet dimers related by  $\sim 180$  deg rotation and  $\sim 1/14$  translation along  $c$ ) were arranged according to translational non-crystallographic symmetry (tNCS) with nearly perfect repetition of  $\sim 1/7$  along  $c$ . This bizarre packing resulted in a very long  $c$  axis ( $\sim 298$  Å), which is also the direction of tetragonal pseudosymmetry. In reciprocal space, there is a salient intensity modulation, with peaks at  $l=7n+/-3$  and troughs in-between. In addition to the unique 28 copies of Hyp-1, the large cell also contains 89 copies of the ANS ligand, 60 of which are intimately bound in a variable pattern in three distinct docking sites of the protein molecules. The unique pattern of ANS docking in the NCS-related Hyp-1 molecules confirms that the modulation is real, and the excellent electron density of the ligands confirms that exact crystallographic periodicity is regained after seven pseudotranslational repetitions along  $c$ . There is also an intriguing correlation between the interstitial ANS ligands and a pattern of shorter-longer distances between the tNCS-related protein molecules. Recently, in a different crystallization experiment, which used a mixture of melatonin and ANS as competing ligands, Hyp-1 has been crystallized again producing crystals of the same symmetry and  $a$ ,  $b$  parameters, but with  $c$  (384 Å) elongated  $9/7$  times, and with  $l=9n+/-4$  intensity modulation in the diffraction pattern. The structure has been solved as well, revealing the same packing principle, but with the basic pattern repeated nine times along  $c$ . Refinement of this gigantic structure, with 36 unique protein molecules, is a daunting task but it is already obvious that the protein crystallized with selective binding of ANS, and that the same tetrameric NCS assembly is exploited to build a tNCS structure that is a rational (but difficult to explain!) variation of the previous case.



### **Andrzej Joachimiak**

Argonne Distinguished Fellow, Director of the Structural Biology Center and the Midwest Center for Structural Genomics at Biosciences Division, Argonne National Laboratory, Argonne, IL, USA, Senior Fellow at Computational Institute, cPI Center for Structural Genomics of Infectious Diseases and Professor, University of Chicago, Chicago, IL, USA.

Education - M.Sc., University of Adam Mickiewicz, Poznan, 1974; Ph.D., Institute of Bioorganic Chemistry, Poznan, Poland, 1980; Postdoctoral Fellow - University of Chicago 1980-1982; D.Sc., Institute of Biochemistry and Biophysics, Warsaw, 1991.

University of Chicago, Chicago, USA; 1980-86, Institute of Bioorganic Chemistry, Poznan (Poland) 1986-1990, at Yale University 1990-93, at Argonne

National Laboratory 1993-present, at the University of Chicago (Chicago, USA) 2004-present.

Fields of interest - structural biology using synchrotron radiation, enzymes specificity, protein-ligand interactions, protein-nucleic acid interactions, molecular chaperones.

>320 publications and book chapters, >16,600 total citations, H-index 63, >2,200 structures in the PDB. Trained >20 postdoctoral fellows. Associate Editor of Journal of Structural and Functional Genomics, Protein Science, Protein & Cell, Member, European Academy of Sciences, Polish Society of Arts and Sciences, Protein Data Bank Advisory Committee, NSLS-II Advisory Committee, ORNL Neutron Advisory Board, Recipient of the University of Chicago Award for Distinguished Performance and the Arthur H. Compton Award, Advanced Photon Source.

# Structural Biology Using Light Sources Helps Combat Infectious Diseases and Antibiotic Resistance

Andrzej Joachimiak

Argonne National Laboratory, Argonne, IL, USA  
University of Chicago, Chicago, IL, USA

Many aspects of protein function, including molecular recognition, assembly and catalysis, depend on the 3D atomic structure. X-ray crystallography remains the most powerful method capable of providing atomic information on interactions of proteins with other macromolecules and small ligands. Third generation light sources and dedicated macromolecular crystallography (MX) beamlines have expanded our competence in determining protein structures. New strategies developed allow data collection from highly demanding crystals using mini-beams and reduce radiation damage. Genome sequencing projects have accelerated significantly and now include studies of many human pathogens. Expanded protein sequence space allows comprehensive approaches to studies of the entire cellular systems. Structural Genomics efforts took advantage of these innovations and contributed a complementary array of the rapid, highly integrated and cost effective methods in molecular and structural biology and created structure determination pipelines. When combined with MX synchrotron facilities, advanced software and computing resources, these pipelines resulted in significant acceleration of protein structure determination and expanded the range of projects.

Antibiotic resistance has been discovered against key antibiotics used in the treatment of many pathogenic strains that are found in various environments and poses a major threat worldwide. The continued evolution of a complex array of antibiotic-resistance genes presents a formidable challenge and efforts to develop new antimicrobials have lagged behind. Structure determination pipelines can be applied to emerging diseases and drug resistance. These studies can aid mechanistic analyses and structure-based drug discovery. The New Delhi Metallo- $\beta$ -lactamase (NDM-1) gene makes multiple pathogenic bacteria resistant to all known  $\beta$ -lactam antibiotics. NDM-1 represents an example of extreme promiscuity - it is capable of efficiently hydrolyzing a wide range of  $\beta$ -lactams, including many "last resort" carbapenems; it can utilize different metal cofactors and seems to exploit alternative mechanisms. The structures of NDM-1 in complex with ligands revealed an enlarged and flexible active site capable of accommodating many  $\beta$ -lactam substrates. The zinc ions serve to activate a water molecule that hydrolyzes the  $\beta$ -lactam ring.

The development of new antibiotics that are effective against drug-resistant strains and the discovery of new drug targets are equally important. Recent progress on specific inosine-monophosphate dehydrogenase (IMPDH) inhibitors has prompted a new interest in bacterial IMPDHs as potential drug targets. IMPDH is considered a highly promising target because the protein controls the guanosine monophosphate pool and the gene is often found to be necessary for bacterial survival. Important differences between bacterial and eukaryotic enzymes can be utilized to design species-specific inhibitors. Structural studies of IMPDH in complex with different inhibitors combined with binding studies provide insight to how species-specific inhibitors can be developed.

This work was supported by NIH Grant GM094585, the NIAID/DHHS Contracts [HHSN272200700058C and HHSN272201200026C]. The use of SBC beamlines was supported by the U.S. DOE, BER [DE-AC02-06CH11357].



**Krzysztof Jozwiak**

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Previous posts:

Visiting Scientist (NIA/NIH); Postdoctoral fellow at Laboratory of Biomodeling / International Institute of Molecular and Cell Biology in Warsaw;

Postdoctoral fellow at Laboratory of Clinical Investigations/ National Institute on Aging, Baltimore, MD, USA

Education: M.Sc. (chemistry) Maria Curie-Skłodowska University. (1995); Ph.D. (Pharm. Sci.) Medical University of Lublin (2000); D.Sc. (Pharm. Sci) MU Lublin (2007); Full Professor (Pharm. Sci.) 2013.

Fields of interest: molecular mechanisms of drug – target interactions particularly for molecules targeting neuronal receptors, structural basis of drugs' subtype selectivity and/or pathway specificity (biased agonism), chemical biology and drug design.

~80 publications, ~850 total citations, H-index 15.

Supervised 6 Ph.D. students. Trained 6 postdoctoral fellows. Laureate of the UCB-Ehrlich Award for Excellence in Medicinal Chemistry (EFMC'2012).

# **Structural biology aspects of ligand directed signaling in G-protein coupled receptors**

Krzysztof Jozwiak<sup>1</sup>, Karolina Pajak, Anita Plazinska, Irving W. Wainer

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Biased agonism (a.k.a. ligand directed signaling) emerges as one of the most promising approach in design, discovery and development of new medicinal compounds targeting G-protein coupled receptors (GPCRs) in future therapies. Most GPCRs induce a myriad of intracellular signaling responses upon activation (e.g., G  $\alpha$ -protein coupling, beta-arrestin recruitment) and recent discoveries strongly suggest that different ligands may stimulate one or the other response in a selective manner. A lot of examples of ligands acting on various GPCRs for which biased effects has been reported; RCSB Protein Data Bank contains a growing number of crystal structures for various GPCRs. However, relatively little is known on structural basis of ligand directed signaling on molecular levels. Current presentation will review structural aspects of ligand directed signaling in GPCRs. Structural chemistry analyses allow in some cases to define molecular requirements for a ligand to evoke signaling biased between G protein and beta-arrestin pathways. Our study of newly developed beta2-adrenergic receptor agonists reveals that biased agonism manifested by the fact that some derivatives activate the receptor to couple exclusively the Gs protein is linked to specific interaction of a ligand with Y308 residue of the receptor. The interaction most likely interferes the conformational transition toward the active state and therefore may be responsible for selective recognition of Gs protein by the intracellular domain. The mechanism seems to be uniform for GPCR family as similar observation has been made for D2L-dopaminergic or M2-muscarinic receptor.



**Zbigniew Kaszkur**

Assoc.Professor in the Institute of Physical Chemistry of the Polish Academy of Sciences

Head of Laboratory of X-ray Powder Diffractometry and Spectrometry in the Institute of Physical Chemistry of the Polish Academy of Sciences

Head of workgroup “Dynamics of nanocrystal structure induced by surface chemistry” (IPC PAS),

Deputy Head of the Department of Catalysis on Metals in Institute of Physical Chemistry PAS,

Coordinator of Working Group 2 in COST Action MP0903,

Member of the Committee of Crystallography of Polish Academy of Sciences (term 2007-2010, 2011-2014)

Secretary of Polish Synchrotron Radiation Society for

the term 2012-2014

Secretary of Polish Crystallographic Association for the term 2010-2012, 2013-2016

# True nanocrystallography

Zbigniew Kaszku

Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland

A popular definition of nanocrystallography is - a branch of science applying methods of crystallography to nanocrystals (NC). This definition already does not apply to novel methods of electron nanocrystallography or femtosecond nanocrystallography that addresses studies of atomic and molecular arrangements in a scale of nanometers, using methods and tools not used previously. It becomes now apparent that structural studies of small nanocrystals of size below 10 nm rise specific questions and require specific methods. NC structure is dominated by surface and is very sensitive to a gaseous environment. With measurable effects of surface relaxation and reconstruction that can be chemically induced the classic tools like Bragg's law, Rietveld refinement, strain analysis etc. are not well applicable. Developed by us for in situ powder diffraction a method to monitor and interpret changes to NC surface structure allows detection of a chemically induced surface reconstruction as well as observations of surface induced symmetry violations and NC reshaping. As most of crystallographic rules for small NC is no longer strictly obeyed, the proposed method builds up new tools of 'true nanocrystallography' basing on atomistic simulations.

After our successful first observation of dynamics of Pt surface reconstruction [1] on hydrogen desorption we were able to measure a degree of surface relaxation (affecting the overall interplanar spacing) on adsorption and relate it to the adsorption energy and the coverage. The observation of changing on adsorption interplanar spacing much exceeding the change expected from adsorption energy and coverage, is indicative of a lateral surface reconstruction phenomenon [2]. Such a tool allowed us to propose an explanation of the observed quick coalescence of Pt in NO atmosphere at 80 deg.C, in terms of a turbulence caused by a self-canceling cyclic surface reconstruction, the reconstruction being detected by our method [3]. The cyclic phenomenon would be caused by a changing on reconstruction number of the atoms exposed to the adsorbate. The caused surface turbulence forms a likely driving force for a nanocluster transport and merger.

The developed tools allows also e.g. explanation and control of the reversible surface segregation phenomena in PdAg nanoalloy giving insight into elementary diffusion mechanisms [4].

This novel nanocrystallography can be well applied to NCs under pressure overcoming known in catalysis so called pressure gap and material gap.

1. Rzeszotarski P., Kaszku Z., *Phys.Chem.Chem.Phys.*, 11, 5416 – 5421 (2009).
2. Kaszku Z., Rzeszotarski P., Juszczyk W., *J.Appl.Crystallogr.*, (2014), 47, 2069-2077.
3. Kaszku Z., Mierzwa B., Juszczyk W., Rzeszotarski P., Łomot D., *RSC Adv.*, 4 (28), 14758 – 14765 (2014) .
4. Kaszku Z., Juszczyk W., Łomot D., *Phys.Chem.Chem.Phys.*, (2015), under review.



**Andrzej Kolinski**

Previous posts: Visiting Professor; Washington University, Saint Louis, US, 1985-89; Adjunct Member; The Scripps Research Institute, San Diego, US, 1989-99; Professor; Donald Danforth Plant Science Center, Saint Louis, US, 1999-02; Professor; State University of New York at Buffalo, Center of Excellence in Bioinformatics, SUNY, Buffalo, US, 2002-03.

Education - M.Sc., Ph.D., D.Sc., Faculty of Chemistry, University of Warsaw.

Fields of interest - theory of polymers and biopolymers, molecular modeling, development of new methods for large scale simulations of biomacromolecular systems.

~220 publications, ~7,000 total citations, H-index 48. Advised 19 Ph.D. and 38 M.Sc. theses.

Honors and awards: Gold Cross of Merit awarded by the President of Poland, Prize of the Foundation for Polish Science in life science, Jan Zawadzki Medal of the Polish Chemical Society, International Scholar's Award of Howard Hughes Medical Institute HHMI (1995-99).

# Towards unrestrained protein-protein molecular docking

Andrzej Kolinski

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Laboratory of Theory of Biopolymers develops novel molecular modeling methods which enable efficient simulations of flexible protein docking. The new methods are expected to qualitatively surpass the currently available docking protocols by including the full structural mobility of smaller molecules forming a complex (peptides and small proteins) and the substantial conformational flexibility of proteins (or protein complexes) that bind with them. No information about the approximate location of the binding site(s) will be required. The modeling procedures are not limited only to the structure prediction of protein complexes, but are also applicable to the investigation of the mechanisms of complex formation and characterization of their dynamic properties (1). Multiscale modeling schemes start from coarse-grained representation and continue on more detailed models, finally refining the results on all-atom level. Preliminary results show that the methods provide realistic pictures of protein-protein docking of small molecules (2) and enable fast and dependable structure prediction of protein-peptide complexes (3). Development of multiscale modeling tools for structure predictions of larger protein complexes is now in progress.

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

(2) M. Kurcinski, A. Kolinski & S. Kmiecik, "Mechanism of folding and binding of an intrinsically disordered protein as revealed by ab initio simulations", *J. Chem. Theo. Comp.* 10(6):2224-2231 (2014)

(3) M. Kurcinski, M. Jamroz, M. Blaszczyk, A. Kolinski & S. Kmiecik, "CABS-dock: server for flexible peptide-protein docking", *Nucleic Acids Research*, submitted



## **Janusz Lipkowski**

### Education:

1965 Warsaw University, Faculty of Chemistry (MSc),  
Institute of Physical Chemistry, Polish Academy of  
Sciences, Warsaw.

PhD. (1972), habilitation (1983), titular professor  
(1990).

Career/Employment: 1978-1992 and 2003-2013 Head of  
the Department of Physicochemistry of Supramolecular  
Complexes of the Institute of Physical Chemistry,  
PASci, Warsaw;

1992-2003 Director, Institute as above;

2003-2006 vice-President of Polish Academy of  
Sciences;

2007 – professor, Cardinal Stefan Wyszyński  
University in Warsaw, Faculty of Mathematical and

Natural Sciences

Specialization: main field CRYSTALLOGRAPHY, other fields SUPRAMOLECULAR  
CHEMISTRY

Current research interests INCLUSION COMPLEXES, NON-COVALENT BONDING,  
ENERGETICS OF INTERACTIONS

Selected Honours: Memberships Polish Academy of Sciences (corr. Member 1998, full  
member 2013), Polish Chemical Society (since 1970), Societas Scientiarum Varsoviensis  
President since December 2007, American Chemical Society (since 1999), American  
Crystallographic Association (since 1999), European Crystallographic Association (since  
1999) World Innovation Foundation (Fellow, since 2006), Polish Academy of Arts and  
Sciences (corr. Mem. 2012), Polish Crystallographic Society – chairman (since 2010), Dr hc,  
Institute of Inorganic Chemistry, Russian Academy of Sciences, Novosibirsk, Russia (1997),  
Honorary Professor, Siberian Branch of the Russian Academy of Sciences (2001) Honorary  
Member, Academy of Sciences of Moldova (2006)

Publications - Number of papers in refereed journals: 342 (updated 2014) - Number of  
communications to scientific meetings: 450 (updated 2009) - Books: chapters in: 11

# Hydration patterns in supramolecular systems

Janusz Lipkowski

Cardinal Stefan Wyszyński University, Warsaw, Poland

Hydration modes in supramolecular systems can be divided into two categories: hydrophilic and hydrophobic. Both types cooperate and, while the former is commonly discussed in structural papers, the latter is less well known. For this reason this part will be discussed here in more detail.

Hydrophobic hydration is the hydration of hydrophobic molecules and surfaces. Hydrophobic hydration (for reviews see [1]) produces a reduction in density and an increase in the heat capacity [2]. The expanded network causes the density decrease whereas the ordered bonds must be bent on increasing the temperature, so affecting the heat capacity. Hydrophobic hydration is accompanied by a negative enthalpy change, due in part to the multiple van der Waals interactions between water and the hydrophobic material, a negative entropy change due to the increased order in the surrounding water and positive heat capacity change (CP) due to the negative enthalpy change (i.e. the stronger hydrogen bonds at the surface). For example, adding CH<sub>2</sub> groups to aliphatic alcohols increases the heat produced on solution ( $\Delta H/CH_2 = -5.4 \text{ kJ mol}^{-1}$ ) but causes a greater decrease in the entropy ( $-T\Delta S/CH_2 = +7.1 \text{ kJ mol}^{-1}$ ) [this introductory text was taken from the ref. 3].

In its pure form the structure of hydrophobic hydration is observed in clathrate hydrates. In numerous examples a combination of hydrophobic and hydrophilic hydration has been found. In the present paper a series of x-ray structures will be used to demonstrate the characteristic of hydrophobic bonding of organic and metallorganic species. These include macrocyclic crown polyethers and diaza-crowns, cyclodextrins, cucurbiturils and selected metal complexes of the compounds listed above. The hydrophilic counterpart will be illustrated using x-ray structures of selected strongly ionic compounds.

A variety of interesting topologies were found and hydrogen bonding geometries characterized using statistics of bond lengths and angles.

Some dynamic aspects of selected structures will be illustrated with the use of temperature dependence of lattice parameters of highly hydrated structures (CD complexes). Open, zeolite-like structures, demonstrate fast solvent exchange in the systems, what can be observed microscopically as cracking of crystalline samples. This phenomenon will be displayed with the use of short microscope movies.

## References:

- [1] (a) V. V. Yaminsky and E. A. Vogler, *Hydrophobic hydration*, *Curr. Opin. Colloid Interface Sci.* 6 (2001) 342-349; (b) B. Widom, P. Bhimalapuram and K. Koga, *The hydrophobic effect*, *Phys. Chem. Chem. Phys.* 5 (2003) 3085-3093 (G. Graziano, Comment on "The hydrophobic effect" by B. Widom, P. Bhimalapuram and K. Koga, *Phys. Chem. Chem. Phys.*, 2003, 5, 3085, *Phys. Chem. Chem. Phys.* 6 (2004) 4527-4528.) (c) D. Chandler, *Interfaces and the driving force of hydrophobic assembly*, *Nature* 437 (2005) 640-647.
- [2] V. Gutmann, *Fundamental considerations about liquid water*, *Pure Appl. Chem.* 63 (1991) 1715-1724
- [3] (Martin Chaplin, "Water Structure and Science", [www.lsbu.ac.uk/water](http://www.lsbu.ac.uk/water)).



**Karolina Majorek**

Education: B.Sc. (bioinformatics) Adam Mickiewicz University, Poznań (2006); M.Sc. (bioinformatics) Adam Mickiewicz University, Poznań (2008); Currently: Ph.D. candidate (biochemistry) Adam Mickiewicz University, Poznań and Visiting Graduate Student at University of Virginia, Charlottesville, USA  
Fields of interest: structural biology, ligand screening, Gcn5-related N-acetyltransferases

16 publications and book chapters, ~128 total citations, H-index 6; 41 structures in the PDB

# **Structural and functional characterization of a GNAT acetyltransferase - reproducibility issues**

Karolina Majorek

Adam Mickiewicz University, Poznań, Poland  
University of Virginia, Charlottesville, USA

The Gcn5-related N-acetyltransferases (GNATs) catalyze the acetylation of a wide range of substrates, including both small molecules and proteins. GNATs are implicated in numerous aspects of eukaryotic and prokaryotic physiology. Bacterial species generally have many GNATs, but very few of them have been functionally characterized. These observations led us to initiate characterization of bacterial GNATs.

One of the enzymes characterized using several screening methods is PA4794 from a nosocomial pathogen *Pseudomonas aeruginosa*. Our results show that PA4794 is a protein acetyltransferase specific for C-terminal lysine residues. We also discovered that PA4794 is inhibited by cephalosporin antibiotics, which bind in the substrate binding site by mimicking the conformation of the substrates. We determined many high resolution structures of PA4794 in a variety of complexes, including with substrates, products, and inhibitors. Two notable structures include a complex with HEPES and a structure with a His-tag bound in the substrate-binding site. Our analysis allowed us to identify several sensitive elements of the experimental protocols that can affect the biophysical and kinetic analysis, potentially altering the interpretation of results. These observations prove how critical it is to track and adjust experimental conditions and investigate the influence of these factors on protein activity and structure.

The conditions of protein expression and purification can play a significant role in the outcome of protein functional studies. The presence or absence of affinity tags, the choice of buffer, and experimental methods, all can affect the biological activity of investigated proteins, but these effects are rarely discussed in the literature. Even small changes in the experimental protocols can generate unexpected artifacts and influence the results of the experiments and their interpretation. When subsequent studies are based on questionable data, a “ripple effect” is caused, as the problematic data is propagated.



**Karolina Michalska**

Education - M.Sc., Adam Mickiewicz University, Poznan, 2002; Ph.D., Adam Mickiewicz University, Poznan, 2007; Postdoctoral Fellow – Argonne National Laboratory, 2010-2013.

Employment - Adam Mickiewicz University, Poznan, 2009 – 2010; Argonne National Laboratory 2013 – present; Computation Institute, University of Chicago, Chicago, USA; 2014 – present.

Fields of interest - structure-function relationship in biomolecules, hydrolytic enzymes, protein-ligand interactions.

20 publications and book chapters, >265 total citations, H-index 9, 61 structures in the PDB. Reviewer and editor for structure notes for Journal of Structural and Functional Genomics, reviewer for Acta Crystallographica D, F.

START stipend, Foundation for Polish Science (2010), City of Poznan Young Scientist Scholarship (2008), Polish Biochemical Society J. K. Parnas' Prize (2005).

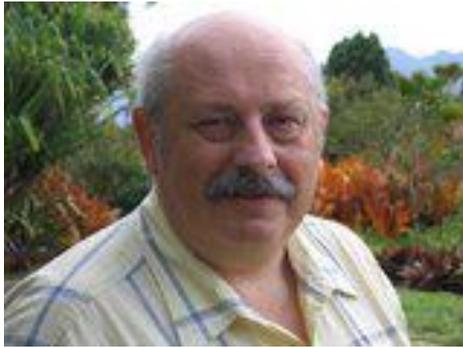
## **Presentation title: Solute-binding proteins recognizing products of lignin degradation**

Karolina Michalska

Protein Crystallographer at the Structural Biology Center and the Midwest Center for Structural Genomics at Biosciences Division, Argonne National Laboratory, Argonne, IL, USA  
Computation Institute and member of Center for Structural Genomics of Infectious Diseases, University of Chicago, Chicago, IL, USA

Lignin, a plant-produced aromatic polymer, represents a significant, yet difficult to utilize, reservoir of organic matter. Understanding lignin bioprocessing is important for comprehending carbon cycling. Biodegradation of this highly resistant material requires multiple classes of extracellular enzymes produced by certain species of fungi and bacteria. In free-radical-based random depolymerization of lignin a number of small aromatic compounds are produced. These molecules are further metabolized by microbes through relatively well-known aerobic and anaerobic pathways. However, mechanisms by which these molecules are imported into cells attracted little attention. We investigated a set of solute-binding proteins (SBPs) of ABC transporters from soil Alphaproteobacteria that mediate transport of lignin degradation products. Functional screens and structural studies were integrated into discovery framework. Detailed characterization enabled us to distinguish four functional groups that show binding preference based on the size of an aliphatic chain and a number of aromatic ring substituents. Multiple crystal structures for protein-ligand complexes for each of these groups provided details of molecular recognition that can be used to infer ligand specificity. The ligand-binding characterization allows for the assignment of specific functions of several SBPs, many of which have been misannotated as branched-chain amino acid-binding proteins. The results demonstrate that Alphaproteobacteria contain a broad spectrum of transport capabilities for lignin-derived compounds. A detailed analysis of this new class of transporters will advance genomic annotation projects and provide insight into the metabolic potential of soil bacteria.

This research has been funded in part by a grant from the NIH grant GM094585, and by the U.S. DOE, BER, under Contract DE-AC02-06CH11357.



**Wladek Minor**

Education: M.Sc., University of Warsaw, 1969; Ph.D., University of Warsaw, 1978.

Employment: University of Warsaw 1969-1985; Purdue University 1985-1995; University of Virginia 1995-present;

Fields of interest - development of methods for structural biology, in particular macromolecular structure determination by protein crystallography.

Data management in structural biology, data mining as applied to drug discovery, bioinformatics. Member of Midwest Center for Structural Genomics, Center of Structural Genomics of Infectious Diseases, New York Center for Structural Genomics and Enzyme Function Initiative.

>160 publications, >33,000 total citations, H-index 33, >300 structures in PDB. Trained >57 students, 10 post-docs (trainees) . There are five PI's among lab alumni.

Recipient of the Edlich-Henderson Inventor of the Year Award. Honorary Professor at University of Liverpool. Journal of Structural and Functional Genomics member of the editorial board.

# Protein Crystallography with Speed and Finesse

Wladek Minor

University of Virginia, Charlottesville, USA

X-ray crystallography is one of the most detailed “microscopes” available today for examining macromolecular structures. However, structures are only simplified models of target proteins and/or nucleic acids, and should be seen as a framework for generating hypotheses to be explored. Numerous biochemical and biophysical experiments, including new diffraction experiments, can and should be performed to verify or falsify these hypotheses. Processing of structural information, particularly when combined with functional, experimental, and sequential data in the context of pathways or interaction networks with other bio-macromolecules and/or bioactive chemical compounds, increasingly requires the use of Big Data paradigms for effective data management, as well as for checking data integrity and accuracy. This is easy to say but extremely difficult to implement, as development of these tools takes time, effort, and most of all, creativity of the leaders and developers of these projects. This is more expensive than just the purchase of supercomputers with petabyte storage. A combination of advancements in high-quality data harvesting, validation, mining, and data management tools would make it possible to convert high-throughput pipelines into high-output pipelines in target-based drug discovery and academic biomedical research.



**Piotr Neumann**

Ph.D., Department of Structural Biology, Institute of Microbiology & Genetics GZMB Georg-August-University Göttingen, Germany (pneuman2@uni-goettingen.de) Previous post: Martin-Luther-Universität Halle-Wittenberg Education: M.Sc. (chemistry) N. Copernicus University, Torun (1999); Ph.D. (chemistry) N. Copernicus University, Torun (2004) Fields of interest: structural biology of macromolecular complexes, crystallographic methodology of data processing and refinement ~ 60 publications, > 100 structures in the PDB

# Multi-resolution refinement and fitting of atomic models into cryo-EM reconstructions

Piotr Neumann

University of Göttingen, Germany

Recent progress in high-resolution structure determination of large macromolecular complexes by single particle electron cryo-microscopy (cryo-EM) made it possible to obtain cryo-EM reconstructions with the overall quality comparable to the crystal structure derived electron density maps of aforementioned complexes, like ribosomes. Although local resolution of the cryo-EM reconstructions can be non-uniform and structures available to date are usually reported to an overall resolution lower than 3 Å, the level of visible structural details legitimates building of atomic models, which can not be modeled by simple rigid body fit of known 3D structures or their fragments/domains. In order to be able to build a model observed in cryo-EM map conformational state of the macromolecular complex, the initial atomic model needs to be fitted and refined against the target cryo-EM density. This raises the need to develop new tools for cryo-EM based 3D modeling or customize the usage of existing powerful programs already available for X-ray crystallography. Here we present an atomic model of the 70S ribosome from *Escherichia coli* in complex with elongation factor Tu, aminoacyl-tRNA and the antibiotic kirromycin at 2.65 - 2.9 Å resolution obtained by cryo-EM reconstruction and a pseudo-crystallographic refinement approach. The superior quality of the cryo-EM reconstruction allowed for the first time to build all 35 RNA modifications in the bacterial ribosome explaining their roles in fine-tuning ribosome structure and function. The starting model of the crystal structure of *Escherichia coli* ribosome has been refined against reciprocal structure factors obtained from EM maps using a combination of efficient conformational sampling with “Deformable Elastic Network” restraints, manual modeling and standard crystallography-like refinement. Application of available crystallographic refinement programs (CNS, PHENIX) and newly established protocols to yield a reliable atomic model based on cryo-EM maps will be discussed.



### **Wieslaw Nowak**

Education - M.Sc., N. Copernicus University, Torun (NCU,chemistry), 1979; Ph.D., NCU (theoretical physics), 1980; D.Sc., NCU, 2000. Professor, 2009.

Fields of interest - development and application of methods involving computer simulations of medically important proteins, photoactive proteins and fluorescent probes, biophysics, quantum chemistry and bioinformatics, single molecule force spectroscopy, science, technology and society.

>60 publications, >600 total citations. Advised 5 Ph.D. and >25 M.Sc. theses. Recipient of the NIH, INSERM, JSPS postdoctoral awards, a member of Polish Physical Society, Biophysical Society, Open Systems and

Information Dynamics Society, founding member and vice-president (2008-) of the Polish Bioinformatics Society. Organizer of BioInformatics in Torun (BiT) workshops (15 meetings organized). Head of ZiFi - a group of interdisciplinary scientists (>30) working in the MIT-C (Modern Interdisciplinary Technologies Center of NCU).

# How to navigate inside protein tunnels - lessons from nature

Jakub Rydzewski, Łukasz Peplowski, Sławomir Orłowski, Wiesław Nowak

Theoretical Molecular Biophysics Group, Institute of Physics, Nicolaus Copernicus University, ul. Grudządzka 5, Toruń, Poland, (wiesiek@fizyka.umk.pl)

Life depends to large extent on biochemical processes happening in deeply buried active sites. Penetration of protein tunnels is an ubiquitous process, perhaps the most studied example is oxygen diffusion in heme globular proteins. Molecular dynamics (MD) simulations may help to elucidate ligand transport paths inside protein matrices and a sequence of events accompanying in this traffic. Unfortunately, plain MD is not practical to this end – hopping across energy barriers is often a rare event and extremely long simulation times are required to get decent statistics. In the talk we will present an overview of the recent progress in the simulations using accelerated enhanced MD methods, in particular those that help to calculate ligand diffusion paths. For example, Locally Enhanced Sampling brings better understanding of cytoglobin [1], neuroglobin [2] and nitrile hydratase [3] activities. Recently, we have suggested a few new memetic algorithms, based on the immune system activity and ant colonies behavior [4]. The new approach is based on Steered MD Simulations and Random Expulsion Method by R. Wade at al. [5], but preliminary calculations show that our approach outperforms the existing methods. Examples of ligands' navigation through cytochrome P450cam, nitrile hydrates, and GPCR muscarinic receptor M2 will be presented. We hope, that our new computational scheme will help others to grasp better dynamical phenomena tuned by proteins' architectures functionally refined through the ages of the Evolution.

[1] Orłowski, S.; Nowak, W.; *J. Mol. Model.* 2007, 13, 715-723.

[2] ref. in. Nowak, W. *Handbook of Computational Chemistry*; Springer, 2012; pp 1127-1153.

[3] Peplowski, L.; Kubiak, K.; Nowak, W. *Chem. Phys. Lett.* 2008, 467, 144-149.

[4] Rydzewski J; Nowak W., submitted (2015).

[5] Ludemann, S. K.; Lounnas, V.; Wade, R. C.; *J. Mol. Biol.* 2000,303, 797-811.



**Marcin Nowotny**

Education - M.S., University of Warsaw (1997); Ph. D., Nencki Institute of Experimental Biology (2002)  
Postdoctoral fellowship – Wei Yang laboratory, National Institutes of Health (2003-2008)

Field of interest – structural studies of nucleic acid processing

32 publications, >1300 total citations, H-index 15

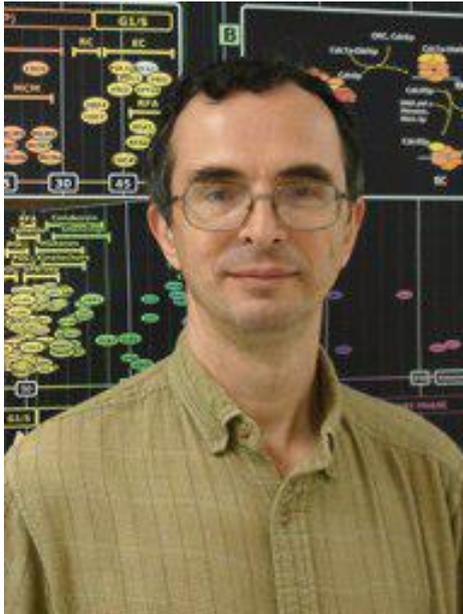
Recipient of the EMBO Installation Grant, Wellcome Trust Senior Research Fellowship, ERC Starting Grant, and Howard Hughes International Early Career Scientist Award

# The mechanism of structure-selective nucleases in DNA repair

Marcin Nowotny

Laboratory of Protein Structure, International Institute of Molecular and Cell Biology

DNA constantly undergoes chemical modifications, or DNA damage, that can distort genetic information. All organisms possess efficient pathways for the repair of the damage of the DNA. Critical elements of these pathways are nucleases that recognize and specifically cleave DNA structures: four-way DNA junctions (Holliday junctions), DNA flaps and structures corresponding to replication forks. In this presentation the structure and mechanism of selected structure-selective nucleases will be discussed. Nucleotide excision repair (NER) is a general DNA repair pathway that detects and corrects a wide spectrum of different DNA modifications. Its critical step is the excision of a DNA fragment that contains the lesion. This step involves two nucleases – one of them is XPG (or Rad2 in yeast). It specifically recognizes a junction between single-stranded and double-stranded DNA in so-called DNA bubbles – structures generated during earlier stages of NER. We have solved a series of crystal structures of Rad2 in complex with DNA substrates (1). They revealed that Rad2 does not specifically recognize the single-stranded part of the substrate but rather binds the last exposed base pair of the double-stranded portion of the DNA. Rad2/XPG is closely related to other structure-specific nucleases: FEN1, EXO1 and GEN1 but it is the only member of the group that is capable of the cleavage of DNA bubbles. The structures offer an explanation for this unique specificity. An element called helical arch adopts a different structure in Rad2 than in FEN1 and EXO1. It no longer blocks the exit from the active site which allows Rad2 to accommodate substrates without a free 5' end such as DNA bubbles. Homologous recombination is another fundamental DNA repair pathway. It uses homologous regions of the DNA to drive the repair of particularly dangerous DNA lesions: DNA breaks and interstrand cross-links. One of the intermediates of this pathway are four-arm DNA structures called Holliday junctions. They need to be removed and one way to achieve it is through the action of specialized nucleases called resolvases. In bacteria the canonical resolvase is RuvC. It functions as a dimer which symmetrically cleaves the DNA using two active sites. We solved the first crystal structure of RuvC in complex with its DNA substrate at 3.75 Å resolution and verified the structural model using thiol-based site-specific cross-linking (2). The structure showed that the HJ is in a novel tetrahedral conformation with two phosphates 1 nt from the HJ exchange point interacting with the active sites. Two helices forming the RuvC dimer also participate in the stabilization of the exchange point. The mode of DNA binding by RuvC is very different from phage resolvases for which complex crystal structures had been solved previously, indicating that multiple modes of HJ recognition evolved. In Eukaryotes one of the HJ removal mechanisms involves SLX1 and MUS81-EME1 nucleases whose concerted action is coordinated by scaffolding protein SLX4. We have solved the first crystal structure of yeast Slx1 and its complex with Slx4-interacting domain termed CCD (3). The structures demonstrated that the GIY-YIG nuclease and RING finger domains present in Slx1 form a compact structure reinforced by a long alpha-helix. Slx1 alone is inactive and it forms a homodimer in crystal and in solution in which the nuclease active site is blocked, offering a likely mechanism to control its promiscuous nuclease activity. Once the Slx1 binds the Slx4 CCD, the active becomes exposed and the nuclease is activated. Therefore, Slx1 is active only when bound to Slx4 platform which controls its activity. References: (1) Mietus M, et al.(2014) *Nucleic Acids Res.*, 42(16):10762-75. (2) Górecka KM, et al.(2013) *Nucleic Acids Res.*, 41(21):9945-55 (3) Gaur V, et al.(2015) *Cell Reports*, in press



**Zbyszek Otwinowski**

Education:

M.S., Warsaw University, 1978;

Ph.D., University of Chicago, 1989;

Postdoc, Yale University, 1989-1994.

Fields of interest:

Development of computational methods for macromolecular crystallography and next-generation sequencing.

85 publications, ~44,000 citations, H-index 36.

## **Advanced methods for molecular replacement.**

Zbyszek Otwinowski<sup>1</sup>, Wenlin Li, Andriy Kryshchak, Nick Grishin,  
Dominika Borek

<sup>1</sup>University of Texas Southwestern Medical Center, Dallas, TX, USA

Molecular replacement (MR) is a highly-developed and widely-used method of crystal structure determination with alternative approaches to structure solution requiring substantially higher experimental effort. The crucial factors for MR success are: (1) completeness of the search model and its structural similarity to the target structure, (2) completeness and sensitivity of the MR search, and (3) a strategy for testing alternative models and weighting schemes to navigate the combinatorial complexity of the associated assumptions. We identified the biggest obstacles and designed an integrated approach that addresses all identified problems. This advance is based on a novel approach to search models preparation and better conceptual approaches to combinatorial problems of testing multiple hypotheses in MR search.



**Agnieszka Pietrzyk**

Education: M.Sc. (biotechnology) Wrocław Univ. of Technology (2009); Ph.D. (biochemistry) Institute of Bioorganic Chemistry, Polish Academy of Sciences (2014)

Previous experience: Predoctoral Visiting Fellow, EMBL-Hamburg, Hamburg, Germany

Fields of interest: structural biology, DNA repair enzymes, structural proteomics of mulberry silkworm

6 publications, 20 total citations, H-index 3; 10 structures in the PDB

# **Identification of amino acid sequence by X-ray crystallography: crystal structures of silkworm proteins as case studies**

Agnieszka Pietrzyk

Laboratory of Structural Biochemistry, Department of Biology, Chemistry and Pharmacy, Universität Berlin,  
Germany

X ray crystallography provides the information about 3D structure of a protein and enables to determine its function, but it can also be extremely helpful in amino acid sequence identification of unknown proteins, for instance, as it was in the case of several major hemolymph proteins of mulberry silkworm *Bombyx mori*. Among the mentioned hemolymph proteins two major groups can be distinguished: high molecular weight storage proteins (SPs), which are hexamers (thus also referred to as “hexamerins”) and 30-kDa lipoproteins (LPs). As the presented studies aimed at the structural analysis of the most abundant proteins in the hemolymph, the identities of the proteins isolated from its natural source were initially unknown. The level of homology among 30-kDa LPs is high and the percent of similar residues ranges from 50 to 95 %, the final identification of isolated 30-kDa LPs was possible only due to precise analysis of electron density maps.

However, the studies on high molecular weight SPs provided even more interesting results. At the beginning the protein isolated from high molecular weight fraction of hemolymph was identified as a pure SP2 according to the results of LC/MS/MS analysis. Later on, electron density maps revealed that the investigated structure represented a complex of two different SPs, SP2 and SP3. The hexameric complex was formed by three SP2 and three SP3 molecules. This was an important discovery, because both proteins were previously always described as homohexamers and according to the crystal structure they were described as a heterohexamer for the first time.

This research was supported in part by the European Union within the European Regional Development Fund and by grant 2011/03/B/NZ1/01238 from the National Science Centre.



### **Waldemar Priebe**

Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA. Education - M.Sc., University of Warsaw, 1971; Ph.D., (Prof. A. Zamojski - advisor), Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland, 1978. Postdoctoral Training, Ohio State University 1979-1981. In the US since Dec. 7th, 1981. Fields of interest - drug discovery and development, cancer biology, translational research, carbohydrate chemistry and metabolism. His research merges biology with chemistry and focuses on the design and development of drugs that selectively target DNA and inhibitors of signaling and metabolic pathways important to tumor progression and survival. >200 publications, > 65 patents. More > 45 postdoctoral fellows and visiting scientists from Poland. Founder/founding scientist of 5 biotech companies in the USA; 5 new WP drugs in clinical studies and 2 in advanced preclinical. The University of

Texas Graduate School of Biomedical Sciences Dean's Excellence Award, 1995. American Chemical Society, Division of Carbohydrate Sciences - Chairman Elect, 2002 – 2004; Chairman, 2004-2006; Past Chairman, 2006-2008; Alternate Counselor 2009-present. Honorary Membership of The Sociedad Argentina de Investigaciones en Quimica Organica (SAIQQ), 1999. The Award of T. Sendzimir Gold Medal, Association of Polish Inventors, May, 2005. Melville L. Wolfrom Award, Washington DC, Awarded by American Chemical Society, Carbohydrate Division, 2009; Order of Polonia Restituta, Officer's Cross class, Official recognition by Polish Government and President of Poland, 2013; The Polish Academy of Sciences Medal, 2013. President of the Texas Chapter of the Kosciuszko Foundation – present. Trustee and board member of the Kosciuszko Foundation in New York- present.

# **Bench-to-Bedside Design, Synthesis, Evaluation, and Development of Novel DNA Binding Agents**

Waldemar Priebe

University of Texas MD Anderson Cancer Center, Houston, TX, USA

Abstract: Using our own unique modular approach we have designed and synthesized libraries of distinctively different DNA binding agents and assessed their biological activity. Our design and systematic screening of such libraries allowed identifying highly apoptotic compounds with drug-like properties and unique anticancer activity. Two of our compounds with promising clinical activity (Annamycin and Berubicin-WP744) are being evaluated in humans and several other agents are in preclinical development. Design of one of our library focused on the development of novel drugs for CNS malignancies. Existence of the blood-brain barrier (BBB) that blocks anticancer drugs from reaching brain tumors is one of the main reasons for the lack of clinically efficient treatment for CNS malignancies. So far, we have designed and identified two classes of the DNA-binding agents that are able to cross BBB. Other potential clinical indications of our DNA binding drugs that are currently in preclinical development include melanomas resistant to BRAF inhibitors and pancreatic cancer, which has the lowest 5-years survival of all cancers. Selected DNA-binding agents are also being tested in combination with our separate class of protein targeting agents that we named “Modulators of Transcription.” We will discuss our different approaches to the design of DNA interactive agents, their evaluation and potential applications.



**Urszula Rychlewska**

Previous posts: Research Associate, University of Florida, Gainesville, Florida, USA; Research Associate, University of North Carolina, Chapel Hill NC, USA; Visiting Professor, University of Wyoming, Laramie WY., USA.

Education: M.Sc. (chemistry) A. Mickiewicz Univ. (1970); Ph.D. (chemistry) A. Mickiewicz Univ. (1976); D.Sc. (physical chemistry) A. Mickiewicz Univ. (1987); Full Professor (chemistry) 2001

Fields of interest: Covalent organic rings and cages and supramolecular aggregates with chiral building blocks – conformational preferences, inclusion properties, types of interactions; Natural products as multidentate ligands; Metal complexes with amines, aminocarboxylates and peptides

185 publications, 1,719 total citations, H-index 21

Supervised 30 MSc fellows and 3 Ph.D. fellows.

Trained 1 postdoctoral fellow.

Awards: Doctor Honoris Causa of the University of Kragujevac, Serbia; Silver Medal of the Czechoslovak Academy of Sciences.

## **Presentation title: Chiral, Shape Persistent Molecules as a Source of Inclusion and Microporosity in Crystals**

Urszula Rychlewska<sup>1</sup>, Wioletta Bendzińska-Berus, Agnieszka Janiak, Beata Warżajtis

<sup>1</sup>Department of Crystallography, Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland

Abstract: Crystals of molecular rings, cages and rotors [1] display various kinds of packing peculiarities such as pseudosymmetry, intra- and intermolecular inclusion, porosity, etc. that result not only from the specific shape of these molecules but also from the deliberate presence in their skeleton of chiral and sterically demanding fragments. We will demonstrate that chiral, triangular-shape, methylene bridged amine (called trianglamine) is capable to include in its inner cavity linear or branched alcohol molecules belonging to the homologous series from ethanol to octanol. Solvent controlled grow of porous apohost crystals, their stability and inclusion ability will be discussed together with modifications of the host framework that accompany the uptake and loss of solvent molecules. Covalent cage compounds will be exemplified by the cuboctahedral-shape imine molecule containing 24 imine bonds. The molecule possesses unprecedentedly large inner cavity and displays both intra- and intermolecular inclusion in crystals. The family of rotor molecules will be represented by compounds that contain triphenylacetamide fragments attached to the chiral aliphatic backbone. The large triphenylmethyl rotors prevent the molecules from packing closely and are therefore a source of microporosity and intermolecular inclusion in crystals. Modifications of the host framework stimulated by included solvent molecules will be illustrated. A noteworthy observation from the crystal engineering point of view is a particular role of triphenylmethyl group as supramolecular protector responsible for the total absence of the classical amide...amide hydrogen bonds and rare involvement of the NH amide group in intermolecular interactions.

The work is partially sponsored by the Polish National Science Centre, grant MAESTRO 2012/06/A/ST5/00230.

[1] Samples for X-ray analysis have been obtained from a group led by prof. Jacek Gawroński, Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland



**Wojciech Rypniewski**

Education - M.Sc., York University (UK) (1983), Ph.D. Cambridge University (UK) (1987), D.Sc., Institute of Bioorganic Chemistry PAN (2001), Professorship (2007).

Fields of interest - crystallography of proteins and nucleic acids, structure-function relationship in biomolecules.

>70 publications, >3800 citations, H-index 24, 99 structures in the PDB

Promoted 6 Ph.Ds.

# **Crystal structures of chitinases from extremophiles**

Wojciech Rypniewski

Structure-Function Relationship of Biomolecules Group, Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Proteins from extremophiles hold the secret of protein stability and enzymatic efficiency. We have analysed chitinases from cold-adapted and thermophilic bacteria, compared them and looked for the features that give those enzymes their special characteristics.



**Piotr Setny**

Education: M.D. Medical University of Warsaw, 2001; M.Sc. (Physics) University of Warsaw, 2003; Ph. D. (Physics) University of Warsaw, 2008. Postdoctoral fellowship: University of California San Diego (2009), Technical University of Munich (2009 - 2013).

Fields of interest: biomolecular hydration, thermodynamics of receptor-ligand interactions, computer aided drug design.

18 publications, >350 citations, h-index 11.

Recipient of the Foundation for Polish Science, Polish National Science Centre, and EMBO grants.

# **From hydration free energies to localised water molecules - a novel, semi-explicit hydration model**

Piotr Setny

independent researcher, Centre of New Technologies, University of Warsaw, Poland

Biomolecules typically exist in aqueous environment. The influence of water needs to be taken into account in all theoretical studies concerning living matter at atomistic level. Computer simulations provide means for doing so, however, approaches involving water in atomic representation are often prohibitively expensive, while the existing simplified, so called implicit solvent models are only moderately successful.

We will present a novel method for modeling of biomolecular hydration. Based on discrete solvent representation and mean field approach, the method is capable of addressing deficiencies of the implicit solvent models, while maintaining their computational efficiency. We will demonstrate that the proposed model correctly reproduces experimental hydration free energies for an extensive set of roughly 700 diverse organic compounds, and accurately predicts the distribution of water molecules buried within protein structures. Those capabilities make it a useful tool for computational and structural biology, in particular as an aid for experimental methods aimed at the prediction of macromolecular structures such as X-ray crystallography or NMR, as well as in application oriented areas such as computer aided drug design.



### **Tadeusz Skarzynski**

Previous posts: Postdoc, Imperial College, London (1984-1992); Research Scientist, Glaxo, UK, (1992-2000); Head of Structural Biology, GlaxoSmithKline UK (2000-2009).

Education: M. Sc., University of Lodz (1977); Ph. D., University of Lodz (1981).

Fields of interest: methods development for structural biology, structure-guided drug discovery.

Over 40 publications; about 2,500 total citations; H-index of 17; 28 structures in the PDB; more than 100 unpublished structures.

Head of Scientific Advisory Board to CCP4 from 2005 to 2009.

# **Structural biology in drug discovery: current strategies and challenges**

Tadeusz Skarzynski

Agilent Technologies, Oxford, United Kingdom

Abstract: Discovery of new medicines is a very difficult, costly and time consuming process. Despite all the recent spectacular advances in the biological and medical research, finding a pharmacologically effective new chemical entity requires a significant multidisciplinary effort from both academic and pharmaceutical industry laboratories. Over the last three decades macromolecular crystallography has become an integral part of the process, used in target evaluation, discovery of new chemical leads and lead optimization. An overview of the role played by structural biology, especially protein crystallography, in the discovery of new drugs will be presented, along with current strategies utilized by leading pharmaceutical laboratories. Several examples of structure-assisted drug discovery projects will be shown to illustrate the power of crystallography to influence the direction of research, as well as challenges where it is powerless to help.



Joanna Sulkowska  
Head of Interdisciplinary Laboratory of Biological  
Systems Modelling,  
Chemistry Department and Centre of New  
Technologies  
University of Warsaw, Poland

Education: M.Sc. (Physics) University of Warsaw,  
2003; Ph. D. (Physics) Polish Academy of Science,  
2007. Postdoctoral fellowship: University of California  
San Diego (2008-2012).

Fields of interest: biophysic, free energy landscape of  
protein, molecular dynamics simulations and coarse-  
grained models of biomolecules, thermodynamics of  
receptor-ligand interactions, knot theory, topology.

35 publications, >649 citations, h-index 15.

Current lab members - 2 postdocs, 2 PhD students, 4 Master students.

Recipient of the Foundation for Polish Science, Polish National Science Centre, EMBO  
grants, Biophysical Society grants, Visegard.

# **Tadpoles: new entangled motifs in proteins**

Joanna Sulkowska

Chemistry Department and Center of New Technology, University of Warsaw, Pasteura 1, Warsaw, Poland

We identify new entangled motifs in proteins that we call tadpoles. Tadpoles arise in proteins with disulphide bridges (or in proteins with amide linkages), when termini of a protein backbone pierces through an auxiliary surface of minimal area, spanned on a covalent loop. We find that as much as 18% of all proteins with disulphide bridges in a non-redundant subset of PDB form tadpoles, and classify them into five distinct geometric classes. Based on biological classification of proteins we find that tadpoles are much more common in viruses, plants and fungi than in other kingdoms of life. During the talk I will also discuss possible functions of tadpoles. Tadpoles and associated surfaces of minimal area provide new, interesting geometric characteristics not only of proteins, but also of other biomolecules, with many potential applications.



**Marcin Józef Suskiewicz**

Education: MBiochem, Oxford University, UK (2010)

Previous experience: projects in groups of Mariusz Jaskólski (Poznań), Sir Philip Cohen (Dundee), Martin Noble (Oxford), Joel Sussman (Weizmann)

Fields of interest: protein phosphorylation; kinases, phosphatases, ATPases; protein folding and unfolding; chaperones; structural biology, especially crystallography; systems biology; philosophy of science

Just 1 publication (review)

# **The structural investigation of a novel protein kinase class phosphorylating arginine residues**

Marcin Józef Suskiewicz<sup>1</sup>, Débora Broch Trentini, Tim Clausen

Tim Clausen's group, Research Institute of Molecular Pathology, IMP, Vienna Biocenter, VBC, Vienna, Austria

Across evolution, cells adapt their proteome in response to internal and external signals. A simple model of such a process is provided by the heat-shock response in Gram-positive bacteria. In these species, sensing a temperature upshift results in the activation of the protein kinase McsB phosphorylating transcription factors that control expression of numerous genes.

McsB is of great interest for at least two reasons. First, it is the first kinase shown to phosphorylate proteins on arginine residues and, consistently, displays no homology to classical protein kinases, instead constituting a novel protein kinase class related to the eukaryotic metabolic enzyme creatine kinase. Second, as an efficient stress response is a prerequisite for a successful host invasion, McsB is important for virulence of notorious Gram-positive pathogens such as *Staphylococcus aureus* and may thus serve as a pharmacological target.

In order to investigate the unique function of McsB, we performed its structural and functional analysis. A series of X-ray structures of McsB reveals the molecular mechanism of protein arginine phosphorylation which is strikingly different from that of canonical protein kinases. Accompanying in vitro and in vivo functional studies of McsB shed further light on its mechanism and elucidate its biological role in coordinating the heat-shock response. Our work opens potential therapeutic avenues for combating antibiotic-resistant bacteria and poses an interesting question: whether arginine phosphorylation, a novel protein modification that has remained elusive until recently, is also present in eukaryotes.



**Marta Szachniuk**

Education: M.Sc. (computing science) Poznan University of Technology (1998); M.Sc. (mathematics) Poznan University of Technology (1999); Ph.D. (computing science) Poznan University of Technology (2005).

Recipient of the EDDA Award for the best doctoral dissertation in Europe within the area of Operations

Research (2006).

Vice-President of the Polish Bioinformatics Society. Member of the Managing Board of EURO WG CBBM.

Fields of interest: algorithms design and optimization, artificial intelligence, biological databases, structural biology of RNA, structure prediction and evaluation.

25 publications, >200 total citations, H-index 7, supervised 20 M.Sc. theses.

Representative bioinformatic tools: RNA FRABASE, RNAComposer, RNAnalyzer, RNAdbee, MCQ4Structures.

## **Presentation title: RNApdbee and assessing the RNA secondary structure**

Marta Szachniuk<sup>1,2</sup>, Tomasz Zok, Agnieszka Rybarczyk, Maciej Antczak, Natalia Szostak, Mariusz Popena, Piotr Lukasiak, Ryszard W. Adamiak, Jacek Blazewicz

<sup>1</sup>Institute of Computing Science, Poznan University of Technology, Poland

<sup>2</sup>Institute of Bioorganic Chemistry, Poznań, Poland

The function of RNA is strongly associated with its structure, therefore an appropriate recognition of the latter, on every level of organization, is of great importance. It particularly concerns the assessment of base-base interactions, described as the secondary structure, the knowledge of which contributes to an interpretation of RNA functions. It also allows for structural analysis on the three-dimensional level. The secondary structure of RNA molecules can be predicted from sequence using in silico methods often adjusted with experimental data, or derived upon 3D structure atom coordinates. In both cases, computational approaches consider mostly Watson-Crick and wobble base pairs to describe the structure. Handling of non-canonical interactions, important for a full description of RNA structure, is still found challenging.

Here, we introduce RNApdbee that supports processing of RNA structures, also within protein complexes. The method allows to work with unknotted and knotted RNAs, and is able to classify pseudoknot orders. It gives an information about extended secondary structure characterized by canonical and non-canonical base pairs. We also present the novel protocol for assessing an extended RNA secondary structure upon RNA sequence. Its idea is based on predicting the RNA 3D structure from user-provided sequence and next, assessing the extended secondary structure from foreseen atom coordinates. Our example implementation of the protocol involves RNAComposer for the 3D RNA structure prediction and RNApdbee for base pair annotation. This example performance reveals better accuracy in non-canonical base pair assessment than the compared methods that directly predict RNA secondary structure. Both webserver tools involved in the protocol are freely available to all users and can be accessed at: <http://rnadbbee.cs.put.poznan.pl/> (RNApdbee) and <http://rnacomposer.cs.put.poznan.pl/> (RNAComposer).

Acknowledgements: The authors acknowledge support from National Science Centre, Poland (2012/05/B/ST6/03026, 2012/06/A/ST6/00384).

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M. Popena, M. Szachniuk, M. Antczak, K.J. Purzycka, P. Lukasiak, N. Bartol, J. Blazewicz, R.W. Adamiak. Automated 3D structure composition for large RNAs, *Nucleic Acids Research* 40(14), 2012, e112.



### **Joanna Trylska**

Education - M. Sc. University of Warsaw, 1995; Ph. D. University of Warsaw, 2001; Postdoc, University of California San Diego, 2003-2005; D. Sc. (habilitation) University of Warsaw, 2009.

Fields of interest - antibiotics targeting the ribosome, antisense antibacterial oligonucleotides, molecular dynamics simulations and coarse-grained models of biomolecules, development of molecular modeling software.

~65 publications, ~950 citations, H-index 18. Trained 3 postdoctoral fellows, 3 PhD and 3 MSc students. Current lab members - 2 postdocs and 7 PhD students.

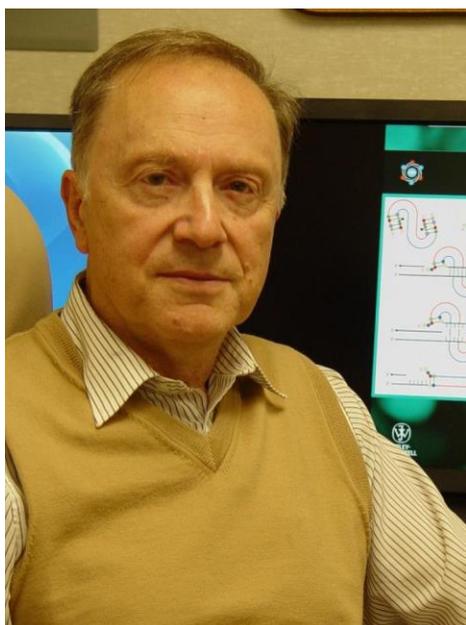
Recipient of the Foundation for Polish Science Focus and Team projects, founder member of the Polish Bioinformatics Society, associate editor of BMC Biophysics.

# **Interactions of aminoglycoside antibiotics with RNA**

Joanna Trylska

Biomolecular Machines Laboratory, Centre of New Technologies, University of Warsaw, Poland

Bacterial ribosomal RNA, with a complicated tertiary architecture, constitutes many functional sites in the ribosome. Therefore, it is a target for many antibiotics that inhibit the synthesis of bacterial proteins. A class of antibiotics targeting ribosomal RNA are aminoglycosides. They are most active against aerobic Gram-negative bacteria and due to their possible oto- and nephrotoxicity are mainly used to treat severe hospital-acquired infections. The primary binding site of 2-deoxystreptamine aminoglycosides is located in the aminoacyl-tRNA binding site in the small ribosomal subunit. However, they were also found to bind to a neighbouring region in the large subunit. I will describe our efforts to understand (thermo)dynamics of aminoglycoside recognition by ribosomal RNA and the molecular mechanism of aminoglycoside resistance due to RNA mutations and enzymatic modification.



**Alexander Wlodawer**

National Cancer Institute, Frederick, MD, USA

In the USA since 1969.

Education - M.Sc., University of Warsaw, 1968; Ph.D.

UCLA, Los Angeles, CA, 1974; Postdoctoral fellow,

Stanford University 1974-1976.

Fields of interest - crystallography applied to studies of proteins relevant to cancer and AIDS, methods development in structural biology.

>350 publications, >16,000 total citations, H-index 62,

270 structures in the PDB. Trained >20 postdoctoral

fellows.

Foreign member of the Polish Academy of Sciences,

recipient of the Heyrovsky Medal of the Czech

Academy of Sciences, Doctor Honoris Causa of the

Technical University of Lodz, Fellow of the American

Crystallographic Association. Polish-American Scientific Award of FNP/AAAS (with M. Jaskolski).

# **A very abbreviated history of macromolecular crystallography**

Alexander Wlodawer

Macromolecular Crystallography Laboratory, National Cancer Institute, Frederick, MD, USA

Abstract: X-ray crystallography is just over 100 years old as a scientific discipline. Successful determination of the first macromolecular structures (of oxygen carrier proteins, myoglobin and hemoglobin) was first reported about 55 years ago. To celebrate the achievements of crystallographers, the United Nations declared the year 2014 to be “The International Year of Crystallography”. Whereas the celebrations are now over, progress in the field is not slowing down, and close to 100,000 crystal structures of proteins and nucleic acids are now available in the Protein Data Bank. I will discuss the most important, often iconic, achievements of crystallographers that led to major advances in our understanding of the structure and function of biological macromolecules. At least 42 scientists received Nobel Prizes in Physics, Chemistry, or Medicine for their contributions that included the use of X-rays or neutrons and crystallography, including 24 who made seminal discoveries in macromolecular sciences. I will spotlight mostly, but not only, on the recipients of this most prestigious scientific honor. Technical advances that made this success possible, such as the use of synchrotron radiation and the recent introduction of free electron lasers as X-ray sources, will also be discussed.



### **Krzysztof Woźniak**

Previous posts: Postdoc – Chemistry Department Cambridge University(1994/95), Visiting professor - H. Poincare Univ, Nancy, France(2006); Sabbatical stay - Chemistry Department Cambridge University(2014)

Education: M.Sc. (chemistry) Warsaw Univ. (1986); Ph.D. (chemistry) Warsaw Univ. (1992); D.Sc. (physical chemistry) Warsaw Univ. (1998); Full Professor (chemistry) 2002

Fields of interest: experimental studies of charge density, weak interactions in the solid state, crystallographic methodology, supramolecular chemistry, minerals, proton sponges, structure and properties of catalysts, methods beyond IAM, XPS, solid state NMR and its applications

~270 publications, ~3400 total citations, H-index 31

Supervised 11 Ph.D. fellows (+ currently 6 under

supervision)

Member of: Polish Chemical Society, Polish Crystallographic Associations, American Crystallographic Association, Cambridge Philosophical

Awards: Foundation for Polish Science and NCN Master Awards, Invited participant of the 21st Solvay Conference in Chemistry (2007)

# Crystallography beyond Independent Atom Model

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Sławomir Domagała<sup>1</sup>, Paulina Dominiak<sup>1</sup>

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Although everything seems to be already well known in the field of routine structural single crystal X-ray analysis and ca. 1.1 mln structures have been solved and refined so far, even commonly used approaches and models should be critically re-evaluated. It is incredible that the Independent Atom Model (IAM) of electron density effectively introduced a century ago is still the most common model of electron density used in structural analysis. One would even say that its success has dominated the whole field. On the other hand, when this model was introduced Max von Laue, the Braggs and their colleagues were using home-made pieces of equipment which could have hardly supplied qualitative information on diffraction spots. In consequence the errors associated with the model of electron density used were overshadowed by far larger diffraction hardware errors. However, within the past century there is an overwhelming progress in design and production of X-ray hardware which is made for needs of both small laboratories and large scale facilities. This progress in sophisticated X-ray hardware should also accelerate progress in the quality and complexity of models of electron density used to interpret experimental results. However, it is very surprising that although the quality of diffraction information collected in X-ray experiments in the XXI century allows these days for far more thorough structural data quality, almost all crystallographers keep using 100 years old models of electron density effectively proving that even with the most modern scientific tools, one can step backward and do ca. 100 years old crystallography :).

In my presentation, I will discuss the main ideas of experimental charge density studies, Hirshfeld Atom Refinement, and Transferable Aspherical Atom Model. I will present a detailed comparison of structural, thermal and electronic parameters obtained for the same diffraction data sets when different models of electron density (IAM, TAAM, HAR, MM) are refined against collected intensities of reflections. Accuracy and precision of structural data obtained from routine and charge density studies going beyond IAM will be discussed. Some practical suggestions will be presented how to estimate and improve the quality of single crystal X-ray diffraction structural results.

## Acknowledgements

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**POSTERS - ABSTRACTS**

# Structural determination of 5' UTR RNA motifs

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5' untranslated regions of mRNA contain cis-regulatory elements frequently forming secondary structures including IRESes, binding sites for RNA binding proteins, uAUGs and uORFs. These sequences play an important role in translation regulation by promoting or inhibiting translation initiation, affecting mRNA's stability and also acting as riboswitches. As impairment of this regulation machinery perturbs cellular metabolism, leading to various physiological abnormalities studying it at a structural level seems to be an important research subject.

The aim of my research is to determine the structure of 5' UTR RNA motifs using mainly X-ray crystallography method, in combination with low-resolution structural probing methods and theoretical structure prediction. Although X-ray crystallography method for structure determination is widely used for protein structure determination it can be also used for RNA. X-ray crystallography technique employs single crystal X-ray diffraction (SXRD) to unambiguously determine the three dimensional structure of large biological molecules at atomic resolution. The strength of this method lies in the high level of accuracy it provides and lack of the size limitation for the molecules that are analyzed. The steps involved in this technique consist of sample preparation, crystallization, X-ray diffraction and structure determination. But as the surface of RNA molecules is dominated by a poor differentiated regular array of negatively charged phosphates, the crystallization of RNAs remains a formidable experimental challenge which makes low-resolution structural probing methods like SHAPE, CD etc. and theoretical structure prediction also important.

Structural insight obtain using different structural characterization methods will help in understanding the different mechanisms of translation regulation, impact of 5' UTR structure on gene expression and linkage between mutations in UTR coding sequence and expression abnormalities.

## **Crystal structure of the complex of GLPO with an anti-thyroid drug PTU**

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Department of Biophysics, All India Institute of medical Sciences, New Delhi-110029, India.

Lactoperoxidase is a member of mammalian heme peroxidase superfamily that consists of lactoperoxidase (LPO), myeloperoxidase (MPO), thyroid peroxidase (TPO), eosinophil peroxidase (EPO). So far crystal structures of only two mammalian peroxidases LPO and MPO have been determined. The structures of LPO have been determined from the samples obtained from bovine, buffalo, goat and sheep. Their complexes have also been determined with inhibitors and substrate analogues. However, the structures of EPO and TPO have not been obtained so far. Due to significant sequence identity between LPO and TPO, the structures of these proteins are expected to be similar. Similarly their substrates will also have similarity as well as the substrate and enzyme interactions will be similar. Therefore, a complex of LPO was prepared with propylthiouracil (PTU), a compound used as a drug in thyroid ailments. The complex was crystallized using co-crystallization. The crystals of native LPO were also soaked in the solution containing PTU. The crystals belonged to monoclinic space group with cell dimensions  $a = 80.2 \text{ \AA}$ ,  $b = 82.5 \text{ \AA}$ ,  $c = 95.0 \text{ \AA}$ ,  $\beta = 73.7^\circ$ . There were four molecules of LPO in the asymmetric unit. The structure determination of the complex revealed that PTU binds to the LPO at the distal heme site. It is held at this site through several hydrogen bonds and van der Waals contacts. The mode of binding and number of interactions suggest that a similar mode of binding may occur with TPO. Based on the information of interactions and the missing potential interactions, the modifications in the structure of PTU are suggested so that the improved design of the TPO inhibitor is obtained.

# Modeling of protein structure and flexibility using CABS-fold and CABS-flex web servers

Maciej Blaszczyk, Michal Jamroz, Sebastian Kmiecik and Andrzej Kolinski.

Faculty of Chemistry, University of Warsaw

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 utilized 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].

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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.
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# Multipole expansion convergence of electrostatic interaction energy from UBDB databank.

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Żwirki i Wigury 101, 02-089 Warszawa, Poland.

Hansen-Coppens multipole model (HCMM) [1] was invented as a response to insufficiencies of IAM (Independent Atom Model). Unlike the simple spherical densities in IAM, HCMM uses the finite spherical harmonics to represent the complexity of electron density in molecules. HCMM could not only be applied in high-resolution X-ray diffraction data refinement, but by its transferability can be a good starting point for creation of databanks storing multipole parameters. Koritsiansky, Volkov and Coppens created a databank [2] (UBDB - University at Buffalo DataBank) based on HCMM, and theoretically-calculated structure factors.

In our work we examined several possibilities of UBDB application, mainly in calculations of electrostatic intermolecular interactions in dimers. The transferability of the multipole parameters was extensively tested by examination of molecular dipole moments and electrostatic interaction energies (Ees)(using EPMM method) for dimers from S66/S66\_8 datasets. The EPMM method consist of two different approach: EP - Exact Potential - which evaluates the exact Coulomb integral and MM - Multipole Method - Buckingham-type multipole approximation.

We compared results obtained from direct HCMM refinements against molecular densities to those from densities reconstructed by the use of UBDB. The difference is slight, results from the UBDB have an similar RMSD to those from direct refinement, for Ees when related to referential quantum mechanical results. This suggests the averaging within particular atom type does not change the global electrostatic properties of the compounds.

Further investigations of EPMM, EP or pure MM methods with UBDB suggest that in most cases higher multipole moments (octupoles and hexadecapoles) are not essential from energetic point of view, but can be important in more complex interactions. In most cases calculations at Lmax=2 level are sufficient to achieve convergence. There are slight changes between global EPMM RMSD's for Lmax=2,3,4. Some examples, mostly with weak electrostatic interactions can be properly described using even Lmax=1.

Comparing the results from pure MM method makes visible that for such interactions as pi-pi benzene-benzene or benzene-ethyne T-shape multipole expansion should be truncate at least at Lmax=2, or even Lmax=3 for stacking dimers (pi - H) of uracil - and nonpolar pentane/neopentane/cyclopentane, whereas for H-bonded group (strong electrostatic) Lmax=1 is enough. That tendencies become stronger at lower distances, but in that area drawing conclusions should be more careful because of so called penetration phenomenon.

When EPMM method is applied, above trends differs and RMSDs are always lower. This is due to mentioned above penetration energy (Epen) which is included in EP. It is clearly visible that Epen is strongly sensitive to electron density subtleties. So that EPMM's results at lower truncation of multipolar expansion become uncorrelated with our reference values, whereas with Lmax=2,3,4 correlation coefficient is rather high. Possibility of proper penetration energy evaluation is one of the major advantage of our procedure. For most of the systems at equilibrium geometries the molecular electron densities are overlapping each other and multipole expansion must be inadequate.

Above results demonstrates that electron densities reconstructed from UBDB provide

the quantitative information, and pseudoatom types stored in UBDB has very well transferable multipole parameters.

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[1] Hansen N. K. ; Coppens P. *Acta Cryst.* 1978, A34, 909--921.

[2] Jarzembska K.N.; Dominiak P.,M. *Acta Cryst* 2011, A68, 139—147

# **SimRNA: a coarse-grained method for RNA folding simulations and 3D structure prediction.**

Michał Boniecki

Laboratory of Bioinformatics and Protein Engineering ,International Institute of Molecular and Cell Biology

The molecules of the ribonucleic acid (RNA) perform a variety of vital roles in all living cells. Their biological function depends on their structure and dynamics, both of which are difficult to experimentally determine, but can be theoretically inferred based on the RNA sequence. We have developed a computational method for molecular simulations of RNA, named SimRNA.

SimRNA is based on a coarse-grained representation of a nucleotide chain, a statistically derived energy function, and Monte Carlo methods for sampling of the conformational space. The backbone of RNA chain is represented by P and C4' atoms, whereas nucleotide bases are represented by three atoms: N1-C2-C4 for pyrimidines and N9-C2-C6 for purines. Despite the bases being represented by only three atoms, other atoms can be implicitly taken into account in terms of the excluded volume. All base-base interactions were modeled using discrete three-dimensional grids built on local systems of coordinates.

All terms of the energy function used were derived from a manually curated database of crystal RNA structures, as a statistical potential. Sampling of the conformational space was accomplished by the use of the asymmetric Metropolis algorithm coupled with a dedicated set of moves. The algorithm was embedded in either a simulated annealing or replica exchange Monte Carlo method. Recent tests demonstrated that SimRNA is able to predict basic topologies of RNA molecules with sizes up to about 50 nucleotides, based on their sequences only, and larger molecules if supplied with appropriate distance restraints. The user can specify various types of restraints, including restraints on secondary structure, distance and position.

SimRNA can be used for systems composed of several chains of RNA. It is also able to fold/refine structures with irregular (non-helical) geometry of the backbone (RNA pseudo-knots, coaxial stacking, bulges, etc.). As SimRNA is based on folding simulations, it also allows for examining folding pathways, getting an approximate view of the energy landscapes, and investigating of the thermodynamics of RNA systems.

# Thermal analysis of 2,6-diaminopurine crystals with the help of charge density.

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2. Biological and Chemical Research Centre, University of Warsaw, Żwirki i Wigury 101, 02-089 Warsaw, Poland

The aim of a project was to analyze temperature evolution of the structure of 2,6-diaminopurine (DAP). These compound crystalizes in triclinic system and asymmetric unit contains two DAP molecules and two water molecules. DAP is a nucleobase, a derivative of adenine present in nucleoacids, which can be used to compare Watson-Crick pairing in DNA. Investigating the properties of analogues of nucleobases may contribute to our knowledge about nucleic acid properties in general and give an opportunity to find novel ligands binding to DNA, what is essential for drug design.

Monocrystals of DAP were measured in different temperatures in the range from 100 to 320K. Subtle, untrival changes in diffraction pattern were observed due to temperature rise. The structures were refined with the standard IAM model and with multipole model transferred from charge density measurement at 100 K. Unit cell parameters and atomic displacement parameters variations within temperature changes were noticed. The accurate analysis of observed variations was performed.

# Diclofenac and naproxen – competitors in binding to equine serum albumin

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Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences,  
Lodz University of Technology, Stefanowskiego 4/10, 90-924 Lodz, Poland

Ligands bound to serum albumin are transported through the circulatory system to their place of function or disposal. Among compounds transported by albumin there are fatty acids, bilirubin, heme, hormones, metabolites and a number of drugs. This main transporter of numerous substances in plasma is under investigation at many laboratories.[1]

X-ray crystallography enabled observation of diclofenac (DIC) and naproxen (NPS) binding competition to equine serum albumin (ESA). Crystal structures of ESA complexes with diclofenac ESA/DIC, with naproxen ESA/NPS [2], and with both of these ligands in one complex ESA/DIC/NPS show interesting binding properties of this protein. ESA possesses two binding pockets responsible for the transport of these both anti-inflammatory drugs. One of them is located in Drug Site 2 - DS2 and is able to bind either of investigated drugs. The ESA/DIC/NPS complex revealed that the higher affinity to DS2 presents NPS. The other binding sites of these drugs are located in different compartments of the protein. DIC binding site is in the crevice between subdomain IIIA and IIIB while the NPS site, referred as fatty acid pocket (FA6), is placed at the interface between subdomains IIA and IIB.

The binding affinity of the investigated drugs to ESA were additionally studied by Isothermal Titration Calorimetry (ITC). The binding mode of these ligands to ESA in comparison to ITC results will be discussed.

This research was supported by grant 2013/11/B/ST5/02271 from the Polish National Science Centre.

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[2] Bujacz, A., Zielinski, K. Sekula, B. (2014) Structural studies of bovine, equine, and leporine serum albumin complexes with naproxen. *Proteins*, 82: 2199–2208.

# Studies of nucleobases interactions in crystalline state with experimental electron density analysis of cytosinium chloride

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1. Biological and Chemical Research Centre, Chemistry Department, University of Warsaw  
Żwirki i Wigury 101, 02-089 Warszawa, Poland.

My works consist of two parts – first is electron density analysis of cytosinium chloride with focus of used refinement methods. Second part comprises information based on CSD search and classification of interactions in cytosine homodimers.

Single crystals of cytosinium chloride were obtained by slow evaporation of water from solution containing cytosine and 4-thiouracil with small amount of hydrochloric acid. High resolution (0.5 Å) X-ray diffraction data were collected on monocrystal diffractometer at 90 K. The data were next subject to data reduction, structure solution, independent atom model refinement and finally multipolar refinement procedures. Several strategies were tested to obtain the best model of crystal electron density.

Cytosine chloride crystallizes in  $P2_1/n$  group in monoclinic system. Unit cell consists of one protonated cytosine molecule and one chloride ion located almost in the same plane. Hydrogen bonds, besides cation-anion interactions, play important role in building the crystal structure and determination of crystal lattice energy.

The purpose of search through CSD was to find all homodimers of cytosine interacting through Watson-Crick, Hoogsteen or sugar edge. Results were divided into groups to classify and describe particular patterns of interactions and observe nucleobase preferences to form distinct structures.

# Hypercoordinated triorganosilyl cations stabilized by pincer type ligands: structures and bonding

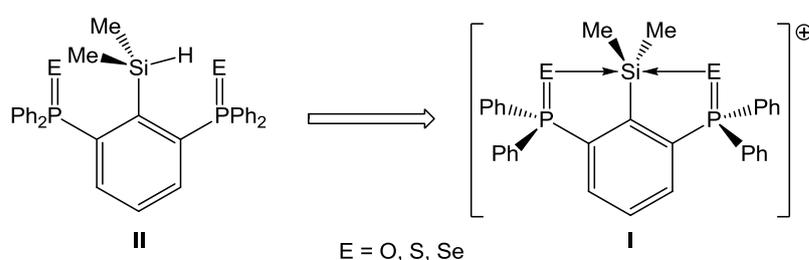
Lilianna Chęcińska<sup>1</sup>, Jens Beckmann<sup>2</sup>

<sup>1</sup>Department of Theoretical and Structural Chemistry, University of Lodz, Pomorska 163/165, 90 236 Lodz, Poland

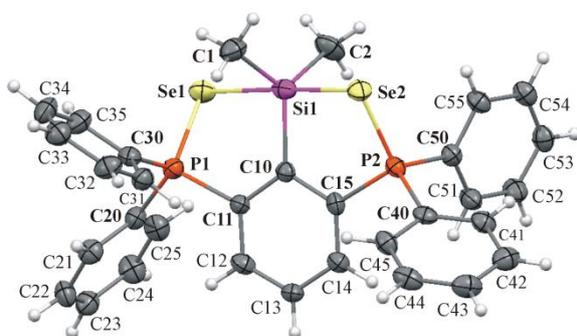
[lilach@uni.lodz.pl](mailto:lilach@uni.lodz.pl)

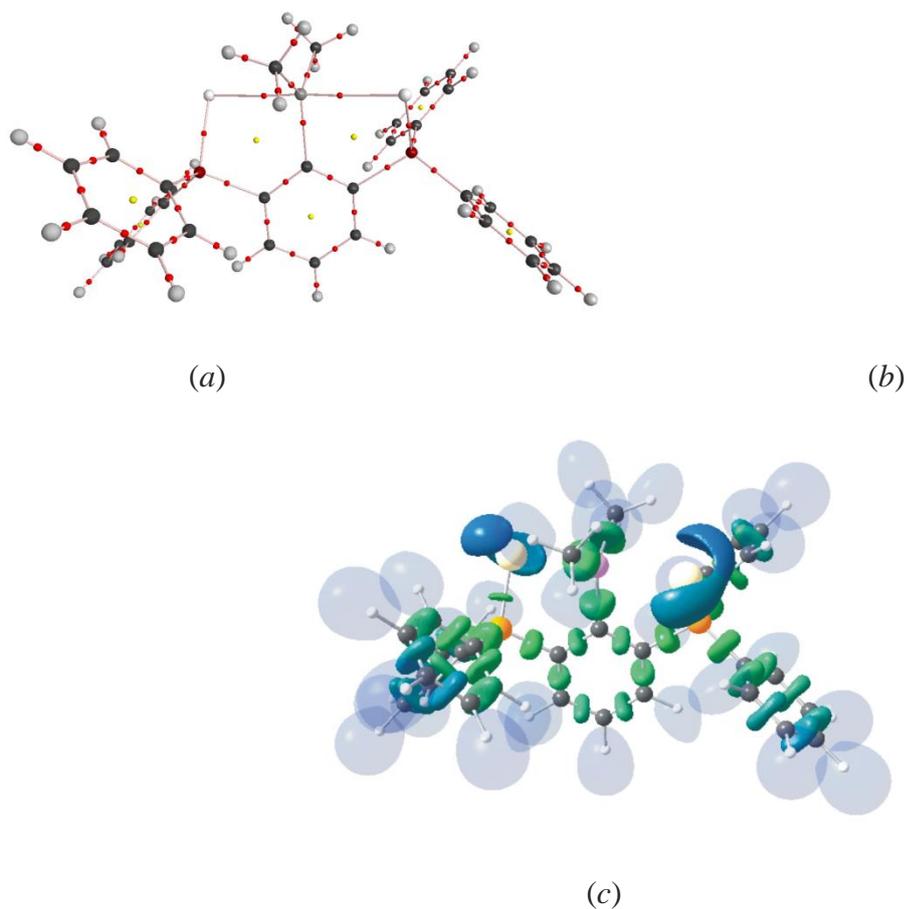
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Hypercoordinated triorganosilyl cations **I** stabilized by pincer-type ligands have been prepared by abstraction of hydride ions from the neutral precursors **II**. The stabilization of the cations is achieved by two intramolecularly coordinating P=E (E=O,S,Se) groups.



We report X-ray single-crystal structure determinations of precursors **II** and cations **I** studied. Based on their geometries from crystals the DFT calculations have been performed (full optimizations of geometry) at the B3PW91/6-311+G(2df,p) level of theory. The further investigation of the structurally relevant bonds based on various topological and integrated criteria (a set of real-space bonding indicators) deduced from theoretical calculations according to QTAIM theory [1] and ELI-D approach [2] has been performed. A combination of these two methods provides quantitative information about the nature of analyzed bonding C–Si, C–P, E–P and E–Si (where E = O, S, Se) (see Figures *a-c* showing graphical representations of methods used for an example of PSe-pincer derivative).





*Figures: X-ray single-crystal structure (a); molecular graph of gas-phase structure (b) and theoretical ELI-D localization domain representation (c) of PSe-pincer derivative.*

### **Acknowledgements**

We would like to thank the Deutscher Akademischer Austauschdienst (DAAD) for a two-month fellowship for L. Chęcińska.

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- [2] M. Kohout, Int. J. Quantum Chem., 97 (2004) 651-658.

# **Multipolar scattering factors for macromolecules - efficient implementation of direct summation**

Michał Chodkiewicz

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With increasing number of high resolution data for macromolecules a need for efficient implementation of accurate scattering factors (SF) calculation emerges. Therefore we have developed a C++ code for multipolar and spherical atom scattering factors computation via direct summation. Such an approach can result in relatively slow calculation of SF (compared to the most popular method combining spherical atom model with fast Fourier transform). We have proposed a few algorithms to overcome this problem. The code was optimized for use with databanks of aspherical atomic form factors (UBDB databank [1] was applied). The influence of the developments on execution time is discussed for model macromolecules.

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## **Brickworx builds recurrent RNA and DNA structural motifs into medium and low-resolution electron-density maps**

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BrickworX is a computer program that builds crystal structure models of nucleic acid molecules using recurrent RNA motifs extracted from RNA Bricks database (<http://iimcb.genesilico.pl/rnabricks>) or B-DNA double helices. In a first step, phosphate groups are detected in a user-provided electron-density map. Subsequently, comparing the three-dimensional patterns of the P atoms with a database of nucleic acid fragments, matching positions of the motifs in the unit cell are found. Finally, the matched motifs are merged and refined in real space to find the most likely conformations, including a fit of the sequence to the electron-density map.

The Brickworx program is available for download and as a web server at <http://iimcb.genesilico.pl/brickworx>.

# Heterodimerization of opioid receptor proteins investigated using coarse-grained MD simulations.

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G-protein coupled receptors (GPCRs) are members of the biggest known transmembrane receptor protein family in the human genome. Because of the role they play in the signalling pathways that are crucial for controlling both physiological and pathological conditions, the GPCRs have become a promising target for new drugs [1]. Furthermore, recent experiments suggest that the opioid receptors, among other GPCR family members, form functional dimers in vivo [2]. In consequence, the understanding of dimerization may potentially lead to pharmacological therapies precisely targeted at a chosen dimer type.

In this study we used coarse grained molecular dynamics (CG MD) simulations to investigate the phenomenon of dimer formation. The systems under investigation consisted of membrane model made of POPC lipid and cholesterol molecules, a number of embedded opioid receptors, water and ion molecules. All the systems were represented using the MARTINI [3] forcefield. The long simulation timescales exceeding 4  $\mu$ s for each simulation, allowed for the observation of spontaneous formation of GPCRs dimers. The resulting dimer structures were analyzed and most stable receptor-receptor interfaces were identified and compared to available experimental data.

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# **Minimal contact map as a way to facilitate the protein folding simulations**

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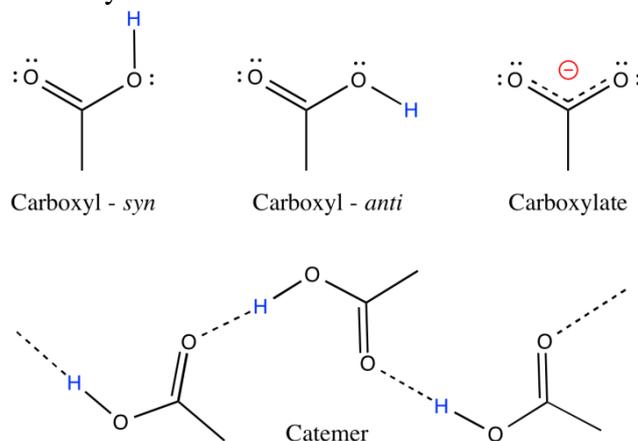
Protein molecular dynamics simulation often enables researchers to determine and explain key points of protein function and folding mechanism, which would otherwise remain unnoticed in pure experimental treatment. However, an explicit solvent simulations in most cases are computationally too demanding. One of the possible solution is to use coarse grained structure based models, in which the energy function is constructed based on native contacts (the contact map). The results obtained by such methods were shown to be consistent with the all-atom simulations, being far faster at the same time. But yet, for proteins with complex topology (e.g. proteins with knots), although the native contact approach can lead to the native state, it can be insufficient to explore the complete free energy landscape. In order to observe reversible folding/unfolding pathways it was suggested to enrich the contact map with some non-native contacts. In this work we propose entirely converse way to overcome the complex topology protein folding problem - construct the minimal contact map sufficient for the protein to fold. We found a map which enables efficient folding and unfolding of protein. The folded and unfolded states were determined by the shape of the free energy landscape characterized by two stable minima separated by a barrier. Another feature of minimal contact map is that the use of such a map additionaly can speed up the calculations, as it posses far less native contacts. Moreover, this analysis implies that the driving force to tie a knot is encoded in some subset of the native contacts.

# Carboxyl-carboxyl(ate) interaction modes in the CSD: structural aspects and biomolecular implications

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Carboxyl and carboxylate groups form important supramolecular motifs (synthons). Besides carboxyl cyclic dimers, carboxyl and carboxylate groups can associate through a single hydrogen bond. Carboxylic groups can further form polymeric-like catemer chains within crystals. Through an exhaustive exploration of the Cambridge Structural Database (CSD), the apparently infinite number of single hydrogen bond arrangements involving these groups could be reduced to 17 isolated carboxyl-carboxyl (13) and carboxyl-carboxylate (4) motifs. In addition, we show that only eight distinct catemer motifs involving repetitive combinations of syn and anti carboxyl groups can be formed. Statistical data related to the occurrence and conformational preferences of these motifs are presented along with data related to the strength of the hydrogen bonds they can form. Indeed, the carboxylic donor group form much stronger hydrogen bonds than the carboxyl(ate) acceptor groups. Such strong hydrogen bonds are found in proteins where Asp/Glu amino acids form recurrent carboxyl-carboxylate motifs that are part of complex interaction networks playing a role in structure and folding. We consequently present data emphasizing how the analysis of small molecules of high-resolution and containing hydrogen atoms can help understand structural aspects of larger and more complex biomolecular systems of lower resolution.



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D'Ascenzo L. & Auffinger P., *Acta Cryst. B* (2015) In press

# Validation of protein structures by analysis of side chain rotamers in coarse-grained Caresian representation.

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Taking into account the rapid increase in utility of three-dimensional protein models, it is important to develop tools to support correct modeling of high resolution protein structures. For this purpose, it is necessary to use a number of different filters that allow eliminate erroneous models. Currently interatomic distances and torsion angles are used for this purpose, but other geometric regularities such as space constraints may be implemented. One of the main sources of errors in protein structures is still related to incorrect side chains packing as well as wrong stereochemistry. We believe that a large part of these errors can be eliminated by the analysis in the local Cartesian coordinates of each amino acids rotamer found in the protein.

Rotamers of  $L\alpha$ -amino acids are low energy isomers resulting from rotation around single bonds in the side chain, on which the subsequent chi torsion angles are defined. The empirical observation suggest that the side chains of amino acids in protein structures avoid most of the available conformational space and appear frequently as clusters in chi-angle space. Our novel approach was to create a library of coarse-grained rotamers defined in the local Cartesian space (each CG virtual pseudo atom representing an amino acid side chain except for carbon  $C\beta$ ). When we investigate the outliers (cases outside of the range), we can easily detect errors in the experimental data or unique exceptions justified by the specific interactions.

Our tool revealed distinct errors in the structure of the seemingly very accurate experimental data (PISCES and Top500 databases; resolution not worse than 1.6Å), for instance incorrect spatial position  $C\alpha$ - $C\beta$  bond - placed in the plane of subsequent atoms: N- $C\alpha$ -C (1nxbA: LEU-52; 3a35A: LEU-93). For the first time in addition to the interpretation of the numerical results of the statistical analysis, we have the opportunity to observe in real-time the fragment of protein structure, which relate to specific points on the plot or histogram. Therefore we can have a visual analysis and evaluation of each residue in the experimental data: type of rotamer, stereochemistry, spatial restrictions and clashes. Our Biomolecular Features Explorer is available online as a part of Bioshell package (<http://www.bioshell.pl>).

# Structural insights into binding of N7-benzyl cap analogs by two isoforms of human initiation factor 4E: eIF4E1a and eIF4E1b

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All nuclear transcribed eukaryotic mRNAs possess at their 5' - end a common structure called cap, which consists of N7-methylguanosine bound by a 5'-5'-triphosphate bridge to the first transcribed nucleotide (m7GpppN) and its recognition by eIF4E is a key point of the translation initiation regulation. In eIF4E cap binding slot the N7-methyl guanine base of cap is sandwiched by the indol rings of conserved tryptophans (Trp56 and Trp102) via cation- $\pi$  stacking interaction. What is interesting, the biophysical and crystallographic studies [1] showed that eIF4E1a can efficiently bind the cap analogs with benzyl group instead of methyl in N7 position of guanine ring. The big benzyl group packs into hydrophobic pocket behind the Trp moieties involved in stacking interaction. What is more, the N7 benzyl cap analogs are tested as potential anticancer therapeutics in oncogenic cells with increased eIF4E1a expression where are employed to inhibit translation by blocking the interaction between canonical translation factor, eIF4E1a and 5' mRNA cap [2].

In our research we extend the investigation of specificity for the translation initiation factor eIF4E1a and its close homolog eIF4E1b, which acts in cells as a translational repressor, toward different variants of N7- benzyl mono and dinucleotide cap analogs. We observed similar affinity of eIF4E1a for N7-benzyl cap analogs and their methylated counterparts and, unexpectedly, an exceptionally higher specificity of eIF4E1b toward N7-benzyl cap analogs. What is more, for both proteins phosphate groups and presence of the second nucleotide in cap derivatives modulate the stability of eIF4E-cap analogs complexes. The homology modeling did not reveal any significant differences in cap binding slot between both isoforms, however, the analysis of tertiary structure using near-CD spectroscopy showed different conformational changes of eIF4E's tryptophan residues as a results of binding N7-methyl and benzyl cap analogs.

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# Structural modeling of human Splicing Factor 3b using PyRy3D software

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One of the major challenges in structural biology is to determine the structures of macromolecular complexes and to understand their function and mechanism of action. However, structural characterization of macromolecular assemblies is very difficult. For this reason a hybrid computational approach is used to incorporate spatial information from a variety of experimental methods into modeling procedure.

We developed PyRy3D (see <http://www.genesilico.pl/pyry3d>) - a computational tool that applies hybrid approach in order to build low-resolution models of large macromolecular complexes. The model building procedure applies a Monte Carlo approach to sample the space of solutions. Spatial restraints are used to define components interacting with each other, and a simple scoring function is applied to pack them tightly into contours of the entire complex (e.g. cryoEM density maps).

Splicing Factor 3b (SF3b) is a protein complex responsible for the recognition of the intron's branch site in U2- and U12-dependent introns. Human SF3b complex consists of seven proteins: SF3b155, SF3b145, SF3b130, SF3b49, SF3b14a, SF3b14b and SF3b10. However, high-resolution structures have been determined experimentally only for a few SF3b components or their fragments. A structure of the whole complex has been determined by cryoelectron microscopy at 9.7 Å resolution. However, despite intensive research on SF3b, its complete structure and mechanism of action remain unknown.

Thus far, we modeled the spatial structures of all spliceosomal proteins, including SF3b components. Now, we applied the hybrid modeling approach implemented in PyRy3D software in order to build ensembles of structural models of the human SF3b complex that agree with currently available experimental and theoretical data. Our model identified likely positions of all proteins that form the SF3b complex, with SF3b155 serving as a scaffold, and interacting with most of the SF3b proteins. SF3b14a is located in the center of the complex, surrounded tightly by SF3b155. We predict a mechanism that includes a movement of SF3b155 and SF3b145, which allows for revealing of SF3b14a and its interaction with pre-mRNA at the initial steps of splicing.

# The same optimal model of electron density distribution for fast interaction energy calculations and X-ray diffraction data interpretation - is it possible?

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Fast but accurate computation of electrostatic interactions in macromolecular systems is a continuous challenge. Various efforts have been put in developing new generation force fields which treat electrostatics in more sophisticated way than atomic point charge approximation. Application of higher-order electric multipole moments seems to be natural way of improvement [1]. But the point multipole expansion has also its limits. It is not accurate enough to properly describe interactions at “short distances” due to penetration effects.

Pseudoatom databanks were primary developed to improve scattering model used in analysis of X-ray diffraction data [2-4]. They allow to go beyond neutral and spherical representation of atoms while modelling crystal electron density. The methodology rely on the concept that aspherical charge densities of atoms having similar chemical environment are transferable enough to build a databank. The Hansen-Coppens pseudoatom model [5] based on a finite spherical harmonic expansion of the electron density around each atomic center is used in this approach.

The University at Buffalo Databank (UBDB) of pseudoatoms is developed not only to provide better interpretation of diffraction data but also as a more general tool to reconstruct electron density of any (macro-) molecule and to compute electrostatic properties from it, electrostatic interaction energies (Ees) in particular. Having access to charge density, instead of point multipole moments, direct integration over density distributions can be used for short distances within the Exact Potential / Multipole Moments (EPMM) scheme of Ees computation [6]. Thus penetration of charge density can be properly accounted for. But such method of Ees computation, although still faster than quantum mechanics, is considerably slower than point charges.

We will propose a hybrid method of Ees computations, the method which seems to be almost as good as UBDB+EPMM, but much faster. We will show very first results of its verification in Ees prediction and discuss, on exemplary X-ray diffraction dataset if this hybrid approach can also be used to improve density models in charge density analysis to maintain dual applicability of one pseudoatom databank.

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# Non-catalytic domains of the RNA-guided DNA methyltransferase DRM2

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RNA guided DNA methylation (RdDM) is found in plants and adds a layer of epigenetic control that is missing in animals. The process is biologically well characterized, and involves two RNA polymerases, Pol IV and Pol V (which are unique to plants), argonaute protein (AGO4), and the DNA methyltransferase DRM2 (DOMAINS REARRANGED METHYLTRANSFERASE2) [1]. DRM2 is the catalytic engine for RNA mediated DNA methylation in plants, and Arabidopsis has been extensively used as a model organism for RdDM studies. The enzyme methylates cytosines in the CHH context (where H is A, T or C), but not (or to a much lesser extent) in the CG and CHG contexts. The protein consists of three N-terminal UBA domains, and a C-terminal methyltransferase catalytic domain. A study published in 2014 by Zhong and co-workers has already revealed the atomic structure of the methyltransferase domain, and has also shown that its three UBA domains are essential for the activity of the methyltransferase in vivo [2]. A loss of these domains results in plants with global losses of non-CG DNA methylation, especially in the CHH context (comparable with a catalytic mutant). However, the reasons for this surprising observation have remained unclear.

The role of UBA domains of DRM2 on the last step of the non-CG DNA methylation mechanism will be investigated. Genetic and biochemical/biophysical experiments will elucidate the network of protein partners of DRM2 and bring to light the individual contributions of individual DRM2 UBA domains to non-CG DNA methylation. For structural characterization, all combinations of single, double and the triple UBA domains of DRM2 (six in total) have been cloned. Initial expression (recombinantly in *E. coli*) and solubility tests indicate that all protein fragments are soluble. Two were purified and used for crystallization screens.

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- 1 - Matzke MA & Mosher RA (2014) RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. *Nature reviews Genetics* 15, 394-408.
- 2 - Zhong X, Du J, Hale CJ, Gallego-Bartolome J, Feng S, Vashisht AA, Chory J, Wohlschlegel JA, Patel DJ & Jacobsen SE (2014) Molecular Mechanism of Action of Plant DRM De Novo DNA Methyltransferases. *Cell* 157, 1050-160.

# **Development of StrAlign – an algorithm for structural alignment of proteins**

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StrAlign – a part of BioShell utility library for bioinformatics - is a program for obtaining structural alignment between pairs of the proteins, using TM-score rotation matrix. Our target was to utilize StrAlign to compute alignments of similar accuracy to TM-align but at lower CPU cost and further use it to cluster PDB deposits into structurally similar groups (domains).

StrAlign was tested on protein domains obtained by cutting PDB deposits into domains. Its results were compared with TM-align. CPU time of the two methods was also recorded. Program was modified to improve these parameters.

In this work we also used BioShell package to explore the space of structural alignments as calculated by TM-score and StrAlign methods.

## **Structural basis of 5'mRNA cap interaction with Decapping Scavenger enzyme (DcpS)**

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Decapping Scavenger enzyme (DcpS) takes part in degradation of short mRNA fragments containing the 5'mRNA cap structure. It is the last stage of 3'-5' mRNA decay pathway. Products of DcpS-mediated hydrolysis are 7-methylguanosine monophosphate (m7GMP) and nucleoside diphosphate (NDP). DcpS is a member of the histidine triad family (HIT). In this work we examined mutant DcpS enzymes from three different organisms: human, *Caenorhabditis elegans* and *Ascaris suum*. Within all three proteins mutation of the middle histidine to asparagine resulted in loss of hydrolytic activity. That enabled investigating molecular mechanism of DcpS interaction with four chemically synthesized cap analogs: m7GpppG, m7GpppA, m7GpppC and m7GpppU. Affinity constants were determined by fluorescence time-synchronized titration. The aim of this work was to gain insight into the role of first transcribed nucleotide (second nucleotide in the cap structure) in short mRNAs degradation by Decapping Scavenger enzyme.

# Non-catalytic domains of the RNA-guided DNA methyltransferase DRM2

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RNA guided DNA methylation (RdDM) is found in plants and adds a layer of epigenetic control that is missing in animals. The process is biologically well characterized, and involves two RNA polymerases, Pol IV and Pol V (which are unique to plants), argonaute protein (AGO4), and the DNA methyltransferase DRM2 (DOMAINS REARRANGED METHYLTRANSFERASE2) [1]. DRM2 is the catalytic engine for RNA mediated DNA methylation in plants, and Arabidopsis has been extensively used as a model organism for RdDM studies. The enzyme methylates cytosines in the CHH context (where H is A, T or C), but not (or to a much lesser extent) in the CG and CHG contexts. The protein consists of three N-terminal UBA domains, and a C-terminal methyltransferase catalytic domain. A study published in 2014 by Zhong and co-workers has already revealed the atomic structure of the methyltransferase domain, and has also shown that its three UBA domains are essential for the activity of the methyltransferase in vivo [2]. A loss of these domains results in plants with global losses of non-CG DNA methylation, especially in the CHH context (comparable with a catalytic mutant). However, the reasons for this surprising observation have remained unclear.

The role of UBA domains of DRM2 on the last step of the non-CG DNA methylation mechanism will be investigated. Genetic and biochemical/biophysical experiments will elucidate the network of protein partners of DRM2 and bring to light the individual contributions of individual DRM2 UBA domains to non-CG DNA methylation. For structural characterization, all combinations of single, double and the triple UBA domains of DRM2 (six in total) have been cloned. Initial expression (recombinantly in *E. coli*) and solubility tests indicate that all protein fragments are soluble. Two were purified and used for crystallization screens.

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# Crystal structure of a DNA/RNA chimera in complex with Ba<sup>2+</sup> ions: a case of unusual multi-domain twinning

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Ribonucleotides are misincorporated into replicating DNA due to the inherent imperfect accuracy of DNA polymerases, the similarity of ribonucleotides and deoxyribonucleotides, and the high concentration of ribonucleotides in the nucleus. Such misincorporated ribonucleotides are targeted by the cell for removal, but recently new evidence has emerged for a functional role of misinserted ribonucleotides in DNA.

We have synthesized chimeric self-complementary (dCrG)<sub>3</sub> hexanucleotides and crystallized their duplexes in complex with barium and strontium cations. X-ray diffraction data for both complexes were collected using synchrotron radiation. All crystals were isomorphous and showed identical multi-domain twinning with the same unit cell parameters. The best data set with the highest resolution (1.09 Å) collected for the Ba<sup>2+</sup> complex was tentatively indexed and scaled, with a low R<sub>sym</sub> value of 7%, in the orthorhombic space group C2221 with unit cell parameters a = 35.72 Å, b = 61.88 Å, c = 44.10 Å.

However, several attempts to solve and refine the structure in the orthorhombic space group have failed. Subsequently, the data were processed in the monoclinic (P21) and triclinic (P1) space groups with unit cell parameters a = 35.72 Å, b = 44.10 Å, c = 35.72 Å, alpha=gamma=90, beta = 120 deg, allowing the structure to be successfully solved by molecular replacement (MR), and refined. The molecular model used corresponded to duplex (3p4j) and the MR found 4 copies of it in the monoclinic cell and 8 copies in the triclinic cell. Analysis of the intensity statistics clearly showed the presence of multi-domain twinning, with six twin domains for space group P21, or even twelve twin domains for space group P1. A monoclinic lattice with a and c approximately equal and the beta ~120 deg. may emulate a hexagonal metric with the possibility of a 3-fold twin operator along the b axis and three pseudomerohedral twin domains. In combination with an additional two-fold pseudomerohedral twinning generating the apparent orthorhombic diffraction pattern, this leads to a crystal composed of six twin domains, with the two-fold and three-fold twin operators perpendicular to each other.

A refinement of the crystal structure of the DNA/RNA chimera in complex with Ba<sup>2+</sup> ions carried out in the P21 space group with SHELXL using the "HKL F 5" command, converged with the final R factor of 13.8%. Subsequent refinement in REFMAC5 with automatic twin law detection reduced the R factor to 11.5%. The six twin fractions obtained from SHELXL and REFMAC are equivalent, and range from 0.09 to 0.28.

The DNA/RNA chimera has a crystal structure characteristic of Z-DNA d(CG)<sub>3</sub> duplexes, with four duplexes packed in the asymmetric unit of the monoclinic cell, and with typical duplex-duplex stacking. However, the presence of 2'-OH groups in the purine nucleotides leads to additional interactions, which have a stabilizing effect on the crystal packing. Some of the nucleotides have an unusual C1'-exo or C4'-exo sugar pucker, not found in Z-DNA duplexes.

# **Viral RNA binding by the human IFIT1-IFIT3 protein complex in the innate immune response.**

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The interferon-induced proteins with tetratricopeptide repeats (IFITs) have recently emerged as a potent innate immune effectors that bind non-self RNA, which results in the inhibition of translation of viral transcripts. The structure of IFIT5 reveals the mode of recognition of the 5' triphosphate (PPP) group on RNA, whereas IFIT1 can recognize both 5'-PPP or cap 0 groups. IFIT1 interacts with IFIT3, which has no known RNA binding capability on its own, and for which the role in the larger multi-IFIT complex is elusive. Here, we begin the dissection of the role of the higher-order IFIT complexes and demonstrate that the IFIT1-IFIT3 complex binds RNA with a higher affinity than IFIT1 alone. The IFIT1-IFIT3 interaction, which is mediated by the last tetratricopeptide repeat motifs in both proteins, is necessary for the full antiviral effect of IFIT1 against VSV. In cells, IFIT1 and IFIT3 associated together, and re-distributed and co-localized together with PPP-RNA. We propose a new role for IFIT3 as an enhancer of IFIT1 activity. Regulation of the IFIT1-IFIT3 complex may provide additional possibility for signal integration in the antiviral response.

# High resolution structure of M23 peptidase with substrate analogue

Maja Grabowska

International Institute for Molecular and Cell Biology

LytM is an autolysin from *Staphylococcus aureus* that belongs to M23 family of zinc dependent metallopeptidases (MEROPS) comprising primarily bacterial peptidoglycan hydrolases. LytM is a two-domain protein: N-terminal domain is preceded by a signal peptide while a C-terminal domain can be divided into an occluding region and a region of high similarity to the lysostaphin catalytic domain. LytM occurs naturally in a latent form, but can be activated by cleavage of an inhibitory N-terminal proregion. We present a 1.4 Å crystal structure of LytM catalytic domain with transition state analogue, tetraglycine phosphinate, bound in the active site. The LytM active site is clearly occupied by analogue of tetraglycine with very strong electron density for the phosphinate phosphorus, oxygen atoms and the “diglycine” fragment on the primed side, while the electron density is weak or even absent on the non-primed site. Based on the structure analysis we proposed grounds of substrate recognition and binding, as well as possible mechanisms of catalysis with the involvement of His291 and/or His260 as the general base and a possible catalytic role for Tyr204.

# **BioShell software in structure biology applications.**

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Faculty of Chemistry, University of Warsaw

BioShell project has been started in 2005 as a set of stand-alone programs aimed on simplification of typical bioinformatics tasks. Since its beginnings it has been focused on biomolecular modelling and structural bioinformatics. The software provides a wide range of methods to handle, analyse and model structures of biomolecules, most notably proteins. The most recent version of BioShell includes a few web applications (i.e. programs operated from a web-browser) devised to visualise various biomolecular features.

# Crystal structure of inorganic pyrophosphatase from *Arabidopsis thaliana* AtPPA1

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*Arabidopsis thaliana* inorganic pyrophosphatase (AtPPA1) coding DNA (ppa1 gene) was cloned into bacterial expression vector and overproduced in *E. coli* cells as a His-tagged protein. The recombinant protein was purified from the bacterial lysate by two consecutive chromatographic steps: chelating chromatography on Ni<sup>2+</sup>-charged resin followed by FPLC size exclusion chromatography. The homogenous protein was submitted for crystallization. X-Ray diffraction data extending to 1.9Å resolution were collected using synchrotron radiation. The structure was solved by molecular replacement using *Pyrococcus furiosus* structure coordinates (PDB code: 1twl) having the highest sequence identity to AtPP1 (49%) and refined to R-factor below 15.6%. The structure coordinates of AtPPA1 have been



Crystal structure of AtPPA1  
(PDB code: 4lug)

deposited in PDB with code: 4lug. The structure of AtPP1 represents an OB-fold which overlaps with other structural models for known bacterial and yeast inorganic pyrophosphatases. Ppases are oligomeric enzymes that are active as homohexamers, or homotetramers composed of about 20 kDa subunits in prokaryotes. Eukaryotic PPases act as homodimers with 30-35 kDa subunits. Plant PPases are an exception because they function as 75 kDa trimers. Moreover, the analysis of AtPPA1 sequence using PsiPred (signal peptide predictor) revealed that it possesses N-terminal putative transit peptide of mitochondrial targeting, and a possible cleavage site at Val31. *In vitro*, cleavage of short (few kDa) fragment is observed during protein storage. Mutant with substitution D98N shows delayed autoproteolysis compared to wild type (WT) protein. Crystal

structure refinement and protein sequencing revealed that the N-terminal fragment corresponding to the predicted mitochondrial targeting peptide is cleaved.

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# Characteristics of the energy landscape of proteins with non-trivial topology based on tRNA (guanosine-2'-O-) methyltransferase

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Proteins with knots form a new, interesting class of proteins. Although knotted proteins were studied both theoretically and experimentally, the role knots still remains unknown. To elucidate the mystery of the biological function of the knot, first one has to understand how those proteins can efficiently fold and unfold. One of the best known knotted protein group is the SPOUT superfamily, which consists of methyltransferases with deep trefoil knot. The least complicated member of this superfamily is the RLMH\_STAAU protein (PDB id: 1vh0) which is the large subunit of ribosomal RNA methyltransferase H in *Staphylococcus aureus*. On the other hand, to elucidate the role of the knot, this protein can be compared with unknotted protein (PDB id: 1ej0), which is structurally and functionally similar to 1vh0. In this work, in order to understand folding/unfolding pathways we perform structure based model (SBM) simulations for both mentioned proteins. We show that the unfolding of the knotted protein is a three step process. The intermediate state possesses neither secondary nor tertiary structure except for the knot. Moreover, we show that in this state the size of knot forming loop remains constant. By analyzing the unfolding times of both proteins we were able to define the unfolding probability curves. By comparing these curves we conclude that the kinetics of both processes must be different.

## Structural studies about new fibrillogenic drugs

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The aim of this project are the studies of amyloidogenic peptides, which possess regenerative properties. For this purpose peptides with N-terminus sequence derived from collagen or elastin sequence and C-terminus with an aggregation sequence was designed. The active forms of these peptides are essential for the reconstruction of the elastic layer of skin [1, 2, 3]. Fibrils formed by short peptides derived from collagen or elastin sequence can be used as potential reservoir of drugs which can display accelerated skin wound healing. Peptide fibrils demonstrate remarkable stability and they could be employed for controlled and slow release of active peptides, which opens the perspectives of application as long-acting drugs [4].

In this part of work we decided to check whether the amyloidogenic protein fragments, which is a fragment of human cystatin C (hCC), have amyloidogenic properties and can be used as potential pharmaceuticals. Research on a fragment of hCC 52-65 shows that sequences present within the L1 loop of hCC has strong amyloidogenic properties (not published data). In this part of the project we performed biochemical and structural studies for nine hexapeptides from the residue 52 to 65 shifted by one aminoacid residue in sequency of hCC. Results showed that some of those peptides really have high tendency to aggregation and form fibrils in solution and in the crystals.

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# Crystal structure of the antimicrobial peptidase lysostaphin

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*Staphylococcus simulans* lysostaphin efficiently cleaves *Staphylococcus aureus* cell walls, causing its death. *Staphylococcus aureus* is a common human and animal pathogen of major clinical significance. Lysostaphin antistaphylococcal properties have been proven to be effective in several mouse and rat models of staphylococcal infection. Moreover, the gene has been both stably engineered into and virally delivered to mice or livestock to obtain resistance against staphylococci. Here, we present the first crystal structure of mature lysostaphin (3.5 Å) and its isolated catalytic domain (1.26 Å). The crystal structure shows the expected overall organization of lysostaphin into an N-terminal catalytic domain and a C-terminal CWT domain, which are mobile with respect to each other because of the presence of a highly flexible peptide linker. The catalytic domain shares key features of the M23 family peptidases and its high resolution structure provides information about Zn<sup>2+</sup> coordination in the enzyme active site. Biochemical and structural studies indicate that CWT domain of lysostaphin interacts directly with peptidoglycan interpeptide bridges. Overall structural analysis of lysostaphin may help in further enzyme engineering to improve its biotechnological applications.

# Structural studies on CB1 receptor-ligand interactions

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Cannabinoid receptors are typical examples of GPCRs (G-Protein Coupled Receptors) with hydrophobic ligands. Although a crystallographic structure for the S1PR1 (sphingosine-1-phosphate type 1) receptor has been published, for CB1 receptors the homology models have to be constructed. We have studied the early activation stages of CB1 and particularly how ligand molecule affects the configuration of residues in the transmission switch, associated with the highly conserved CWxP motif in the transmembrane helix TM6. Each GPCR contains several molecular switches and also water molecules and ions participate in the internal hydrogen bond network.

Here, we docked several ligands to different models of CB1 receptor (based on S1PR1 structure) and performed several molecular dynamics (MD) simulations with agonists and antagonists. We observed movement of residues involved in transmission switch as the first activation event. We have also identified two probable sodium allosteric binding sites in CB1 receptor – in vicinity of residues D2.50 and D6.58, accordingly. Since CB1 is expressed at high levels in the central nervous system to affect cognition, memory, motor, and metabolic functions a knowledge of its activation is indispensable.

# **Aggrescan3D web server for protein aggregation prediction taking into account protein structure and its dynamic fluctuations**

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Protein aggregation underlies an increasing number of disorders and constitutes a major bottleneck in the development of therapeutic proteins. Our present understanding on the molecular determinants of protein aggregation has crystallized in a series of predictive algorithms to identify aggregation-prone sites. A majority of these methods rely only on sequence. Therefore, they find difficulties to predict the aggregation properties of folded globular proteins, where aggregation-prone sites are often not contiguous in sequence or buried inside the native structure. The AGGRESKAN3D (A3D) server overcomes these limitations by taking into account the protein structure and the experimental aggregation propensity scale from the well-established AGGRESKAN method. Using the A3D server, the identified aggregation-prone residues can be virtually mutated to design variants with increased solubility, or to test the impact of pathogenic mutations. Additionally, A3D server enables to take into account the dynamic fluctuations of protein structure in solution, which may influence aggregation propensity. This is possible in A3D Dynamic Mode that exploits the CABS-flex approach for the fast simulations of flexibility of globular proteins.

# Comparison of evolutionary data of analogous proteins with different topologies

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One of the top current mysteries of the structural biology is the formation of non-trivial topologies - namely knots and slipknots.

Although the central dogma of molecular biology suggests that the structure (and its quirks) must be encoded within the protein sequence, we still do not know why the complex structures are preserved throughout the tree of life. To try to find the answer to these questions we compared two pairs of analogous (with respect to enzyme classification) families - methyltransferases (with one family characterized by a 3<sub>1</sub> topological knot) and phosphoglycerate mutases (one family containing a 3<sub>1</sub> slipknot). Through application of Direct Coupling Analysis we have found differing patterns in the evolutionary conservation of sequence families, which reinforces the theory that knots appeared very early in the evolution and were preserved independently.

# Direct observation of the excited state structure of a Ag(I)-Cu(I) complex

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Heterodentate coordination complexes have been extensively studied because of their rich electronic and luminescent properties, which are of importance in the design of molecular devices. The short metal-metal contacts found in such complexes determine the nature of the lowest lying emissive states, and must be explored in order to understand their physical properties. Recent advances in time-resolved (TR) synchrotron techniques supported by specific data collection strategies and data processing procedures[1] allow for elucidation of molecular excited state geometries in the solid state. The approach has been so far successfully applied to several high-quality Laue-data sets collected at the 14-ID BioCars beamline at the Advanced Photon Source.[2]

In this contribution we present synchrotron TR experiment results obtained for a new solvent-free crystal form of a model complex containing Ag(I) and Cu(I) (Ag<sub>2</sub>Cu<sub>2</sub>L<sub>4</sub>, L = 2-diphenylphosphino-3-methylindole ligand).[3] This system exhibits red solid-state luminescence with a lifetime of about 1 μs. This is one of the shortest-lived excited states we have studied so far with the Laue technique. The relatively short lifetime goes along with significant structural changes observed upon irradiation, such as, the Ag...Ag distance shortening of about 0.26 Å for the excited state. The results clearly show strengthening of the Ag...Ag interactions suggesting a bond formation upon excitation. The photocrystallographic findings are supported by spectroscopic measurements and quantum computations. The results confirm the triplet nature of the emissive state originating mainly from a ligand-to-metal charge transfer.

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# Thermodynamics of oligonucleotide complexes studied by replica exchange and classical molecular dynamics

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Nucleic acid analogs that can bind, in a sequence-specific manner, to natural nucleic acids receive increased attention due to their applications as probes in molecular biology and biochemistry. Molecular Dynamics (MD) and Replica Exchange Molecular Dynamics (REMD) simulations have been successfully used to study the dynamics of the complexes formed by natural and synthetic oligonucleotides. However, predicting thermodynamic stability of such complexes still poses difficulties due to the flexibility of the oligomers and lack of force field parameters for synthetic nucleotides.

We investigate the thermodynamics of binding and structural properties of a peptide nucleic acid (PNA) oligomer hybridizing with a functional fragment of the ribosomal RNA. We studied PNA/RNA, RNA/RNA and PNA/PNA ten-base-pairs-long duplexes of similar sequence. The RNA strand sequence, in the PNA/RNA complex corresponds to the bacterial ribosomal A-site, which is essential in the decoding process. Oligonucleotides targeting this region of the ribosome can inhibit bacterial proteins synthesis, and they can be potentially used as antibiotics.

We performed all-atom MD simulations of these complexes with the Amber99 force field in the temperature range from 300 K to 500 K. The 300 – 400 K range was additionally studied with the REMD method. Generated trajectories were analyzed using the recently presented MINT package [1]. The analyses of the number of hydrogen bonds, the number of base pairs, the stacking interactions and the average secondary structure in each temperature allowed us to estimate the thermodynamic parameters of these complexes. Furthermore, we compared the computational results with the melting temperatures that we derived from the UV-monitored thermal denaturation experiments. This allowed us to assess advantages and disadvantages of the applied computational methods in predicting the thermodynamic stabilities of various oligonucleotide duplexes.

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# Dynamic peptidic capsules and switching of their inherent chirality driven by self-assembly

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Construction well-defined discrete peptidic molecular structures, e. g. virus-like cages, by means of self-assembly usually requires using large protein motifs and programmed self-assembly by genetic fusion of proteins endowed with oligomerization properties. Formation of discrete non-fibrilous shapes by means of dynamic combinatorial chemistry driven by self-assembly of short peptides remained an unexplored area.

Recently, we reported the results of our efforts to create discrete, closed-cup  $\beta$ -barrel-type structures by biomimetic self-assembly and self-sorting of short peptides. Eight chains of short peptides (up to tetrapeptides) spontaneously self-sort and covalently attach the macrocyclic scaffold (tetraformylresorcin[4]arene) and, finally, self-assemble to form molecular capsules in non-polar solvents. We showed that unnatural heterochiral self-assembly motifs can be uncovered by this approach. Self-assembly is a powerful driving force capable of controlling the stereochemical routes of chemical reactions and tautomeric equilibria.

Here we show also crystallographic structures of the capsules and one monomeric compound. Comparison of ECD calculated and experimental spectra and crystallographic structures of the capsules allowed to find out that during self-assembly of the capsules switching of inherent chirality takes place.

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# Crystallographic analysis of a novel bacterial cytochrome P450 monooxygenase to understand selective steroid hydroxylation

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The cytochrome P450 superfamily (P450s or CYPs) of heme-containing mono-oxygenases, found in all domains of life, plays key roles in the biotransformation or biosynthesis of drugs, xenobiotics, steroids, fatty acids, eicosanoids, fat-soluble vitamins and bile acids. They catalyze oxidation reactions of unactivated C-H bonds, often with high regio/stereoselectivity. Manifold crystal structures solved to date prove that all P450s present a common, triangular, helix-rich fold, despite rather low sequence identities among isoforms belonging to different families (< 40% identity). Flexible regions surrounding the entrance to the heme in the active site pocket are associated with a wide range of selective oxidations. [1] Broad enzymatic properties explain the increasing interest for P450s by the pharmaceutical and chemical manufacturing industries. Unfortunately, application of P450s as green biocatalysts is often hindered by low enzymatic activity, insoluble gene expression and the dependence on supporting electron transport proteins for full activity [2].

In the EU FP7 funded project called “P4FIFTY”, industrial and academic researchers are looking to develop methods to overcome limitations mentioned above [3]. One of the targets of the project is to understand the structural basis of substrate specificity and regio/stereoselectivity of selected cytochromes P450, and to apply these insights in protein design strategies towards improved enzymes. We applied X-ray crystallography to study a promising novel P450 enzyme from *Bacillus megaterium*, and successfully determined its crystal structure in the absence and presence of steroid ligands, at resolutions ranging from 2.55 - 2.2 Å. The substrate-free structure revealed a common P450 overall fold with a wide-open active site, while the two ligand-bound structures show large conformational changes, providing an insight into protein flexibility and selective ligand binding. Results obtained drive the ongoing structure-inspired protein engineering efforts.

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## **The HTX Lab**

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The high throughput crystallization facility (The HTX Lab) at the EMBL Grenoble outstation is one of the largest in Europe. The HTX Lab is the key platform of the Partnership for Structural Biology (PSB) and is located next to the European Synchrotron Radiation Facility (ESRF) and the Institute Laue Langevin (ILL). It offers automated nanovolume crystallization screening to both academic and industrial users. Dedicated operators perform the crystallization experiments which are followed up through automated imaging systems. Results along with all the experimental details are made available through the web interface of the Crystallization Information and Management System (CRIMS). In collaboration with the Cipriani Team, the platform developed Crystal Direct™, an approach that enables full automation of the crystal harvesting process. Integration of crystallization screening service and synchrotron data collection is under development.

# **Gapped protein models reconstruction server with topology correctness assessment**

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Protein models that come from X-RAY crystallography are the main source of data for structural biology studies. However some parts of proteins for example loose loops, intrinsically disorder regions and region involved in non trivial topology not always have clear interpretation. In consequence coordinates of those amino acids are not determined and thus we sometimes observe "gaps" in structures deposited in Protein Data Bank. There are thousands of structures with such missing regions. Many research techniques to understand free energy landscape of proteins by molecular dynamics approach or drugs virtual screening require complete - ungapped models. Here we present a web server designed to reconstruct such missing fragments of protein models.

Protein modelling is difficult task and quality of the model highly depends on many factors like availability of homologue structures or size of missing region. This process is even more challenging when missing region is knotted. There are a few tools that support modelling process and quality assessment, but usually they have heavy learning curve and/or require building complexed pipelines. Our server provide easy-to-use homology modelling tool dedicated for modelling gapped regions.

To our current best knowledge none of the existing tools take into account the topology. Currently it is know that about 2% of proteins deposited in the PDB posses a non trivial topology - (slip)knotted. Protein modelling which take into account topology is a novel challenge in this field. Our server provided a first method to face this task.

# **PyRy3D - a software tool for modeling of macromolecular complexes**

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One of the major challenges in structural biology is to determine the structures of macromolecular complexes and to understand their function and mechanism of action. However, structural characterization of macromolecular assemblies is very difficult.

Thus far, we developed PyRy3D, a method for building low-resolution models of large macromolecular complexes. The components (proteins, nucleic acids and any other type of physical objects including e.g. solid surfaces) can be represented as rigid bodies (e.g. based on atomic coordinates of structures determined experimentally or modeled computationally) or as flexible shapes (e.g. for parts, whose structures are dynamic or unknown). The model building procedure applies a Monte Carlo approach to sample the space of solutions. Spatial restraints are used to define components interacting with each other, and a simple scoring function is applied to pack them tightly into contours of the entire complex (e.g. cryoEM density maps). This approach enables the construction of low-resolution models even for very large macromolecular complexes with components of unknown 3D structure, such as human mitochondrial RNA polymerase gamma.

# Structural insights into CAG and CUG repeats with antisense PNA oligomers

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RNA-mediated diseases are a specific class of genetic disorders, in which a mutation does not lead to gene silencing or abnormal protein but results in toxic mRNA. The main cause of mutations is an abnormal expansion of specific microsatellite repeated sequences located in certain genes. Toxic microsatellites can be trinucleotide repeats (TNR) or tetra-, penta- and hexanucleotide repeats. A typical number of repeated units in a gene is less than 30 but when it exceeds 40-50, toxicity begins. In extreme cases, the number of repeats can reach several thousand.

The first identified RNA-mediated disorder is myotonic dystrophy type 1. It is linked to CTG repeats located in the 3'UTR of DMPK gene. The CAG repeats are mainly located in translated regions of the genes and are associated with polyglutamine (polyQ) disorders in which the protein products are toxic. However, there are indications that RNA is also involved in the pathogenesis of polyQ diseases. Both CAG and CUG expanded repeats are transcribed and present in mRNA. They form hairpin structures whose main part is the double-stranded stem. It shows abnormal affinity for several cellular proteins, which upsets the balance in the cell.

Myotonic dystrophy and polyglutamine diseases are neurological disorders which usually affect adults. They are progressive with no effective treatment but in recent years therapeutic approaches are under intensive development. One of them is using antisense oligonucleotides (ASO) which can directly bind to toxic RNA. ASO block the binding sites of proteins or inhibit translation of toxic protein. In this approach the oligomer has to be allele-specific and bind only to the mutated mRNA, not affecting the normal function of the non-mutated mRNA. The antisense oligonucleotides can contain different modifications such as LNA, derivatives of morpholino or PNA. The latter shows the highest allele-specificity for CAG repeats in mRNA of the HTT gene.

The main goal of our research was to solve the crystal structures of RNA containing CNG repeats in complex with their antisense PNA oligomers. We wanted to see how the PNA, as a potential therapeutic, recognises the CNG repeats and how it affects the structure of the repeats. We will present crystal structures of two duplexes of complementary RNA-PNA. We will discuss the properties of the PNA oligomers important in antisense therapy.

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# **New methods to improve modeling and prediction of protein structure, dynamics and function**

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We have developed and combined several novel methods to improve protein structure prediction from the amino acid sequence, and modeling of protein dynamics. One of the most promising developments in protein structure prediction are many-body potentials that take into account dense packing, and cooperativity of interactions in protein cores. We developed a method that uses whole protein information filtered through machine learners to score protein models based on their likeness to native structures. These results were published by us [1], and tested successfully in CASP 9, where our prediction group 4\_BODY\_POTENTIALS was among top three predictors in the category of template-free modeling for the most difficult targets. Recently we have significantly improved our potentials by considering electrostatic interactions and residue depth and used them for the prediction of protein structure and blind tested them in CASP 10. Our prediction group Kloczkowski\_Lab was ranked as the third one in prediction of structure (based on the single model) for all targets, and ranked also as the second one for template free-modeling (see: [http://www.predictioncenter.org/casp10/groups\\_analysis.cgi](http://www.predictioncenter.org/casp10/groups_analysis.cgi) ) [2]. By combining statistical contact potentials with entropies from the elastic network models of proteins we can compute free energy and improve coarse-grained modeling of protein structure and dynamics [3]. The consideration of protein flexibility and its fluctuational dynamics improves protein structure prediction, leads to a better refinement of computational models of proteins, and significantly improves protein docking [4,5]. We studied also the self-assembly of FVFLM peptide and its influence on the kinetics of A $\beta$ 16-20 oligomerization.

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## **Presentation title: Efficient docking of fully flexible peptides to flexible proteins without prior knowledge of the binding site: CABS-dock web server**

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Protein-peptide interactions play a key role in cell functions. Their structural characterization, although very challenging, is important for discovery of new drugs. Based on our highly efficient simulation methodology [1, 2], we developed the CABS-dock protocol and web server for flexible protein-peptide docking [3, 4]. While other docking algorithms require pre-defined localization of the binding site, CABS-dock doesn't require such knowledge. Given a protein receptor structure and a peptide sequence (and starting from random conformations and positions of the peptide), CABS-dock performs simulation search for the binding site allowing for full flexibility of the peptide and small fluctuations of the receptor backbone [3, 4]. This protocol was extensively tested over the largest dataset of non-redundant protein-peptide interactions available to date (including bound and unbound docking cases) [4]. For over 80% of the dataset cases, we obtained models with high or medium accuracy (sufficient for practical applications). Our unique method for coupled binding site search and protein-peptide docking can be easily complemented by other computational tools (e.g. high-resolution docking refinement protocols) or experimental data to improve the results of the docking experiment. CABS-dock web server is freely available at <http://biocomp.chem.uw.edu.pl/CABSdock>

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# **Preformed template fluctuations promote fibril formation: Insights from lattice and all-atom models**

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Fibril formation resulting from protein misfolding and aggregation is a hallmark of several neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Despite the fact that the fibril formation process is very slow and thus poses a significant challenge for theoretical and experimental studies, a number of alternative pictures of molecular mechanisms of amyloid fibril formation have been recently proposed. What seems to be common for the majority of the proposed models is that fibril elongation involves the formation of pre-nucleus seeds prior to the creation of a critical nucleus. Once the size of the pre-nucleus seed reaches the critical nucleus, its thermal fluctuations are expected to be small and the resulting nucleus provides a template for sequential (one-by-one) accommodation of added monomers. The effect of template fluctuations on fibril formation rates has not been explored either experimentally or theoretically so far. In this paper we make the first attempt at solving this problem by two sets of simulations. To mimic small template fluctuations, in one set, monomers of the preformed template are kept fixed, while in the other set they are allowed to fluctuate. The kinetics of addition of a new peptide onto the template is explored using all-atom simulations with explicit water and the GROMOS96 43a1 force field and simple lattice models. Our result demonstrates that preformed template fluctuations can modulate protein aggregation rates and pathways. The association of a nascent monomer with the template obeys the kinetics partitioning mechanism where the intermediate state occurs in a fraction of routes to the protofibril. It was shown that template immobility greatly increases the time of incorporating a new peptide into the preformed template compared to the fluctuating template case. This observation has also been confirmed by simulation using lattice models and may be invoked to understand the role of template fluctuations in slowing down fibril elongation *in vivo*.

# CheckMyBlob: toward an expert automatic ligand recognition system

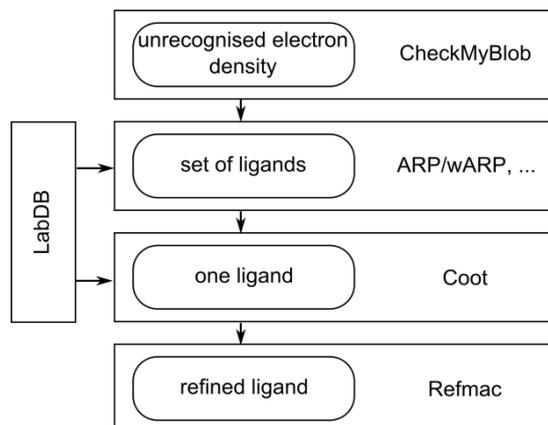
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The interpretation of macromolecular electron density maps generated by X-ray crystallography experiment is a complicated process. With automated, complex model building procedures such as ARP/wARP, Resolve, Phenix, Buccaneer or systems like HKL-3000, the regions of macromolecular structures containing polypeptides and nucleic acid chains may often be built with high accuracy and speed. However since a macromolecular structure may contain many different small molecule ligands in different conformations, automatic ligand recognition and placement may be difficult. Localization and identification of small ligands (containing up to 10 non-hydrogen atoms) is difficult because often they cannot be distinguished from one another on the basis of the electron density map alone. This recognition process is particularly challenging when the resolution of the diffraction data is relatively low (2.5Å or worse). Therefore the development of new methods is needed. Most current methods for automatically fitting small molecules into electron density require that the molecules be specified *a priori* (either a single ligand or a set of ligands; Fig. 1). The aim of the CheckMyBlob project is to predict the ligand or set of ligands that can fit into a region of unmodeled electron density when a protein environment is well defined. The set of potential ligands generated by CheckMyBlob can be used later as input for a pipeline of automatic ligand placement procedures (Fig. 1) such as the one implemented in the HKL-3000 system.

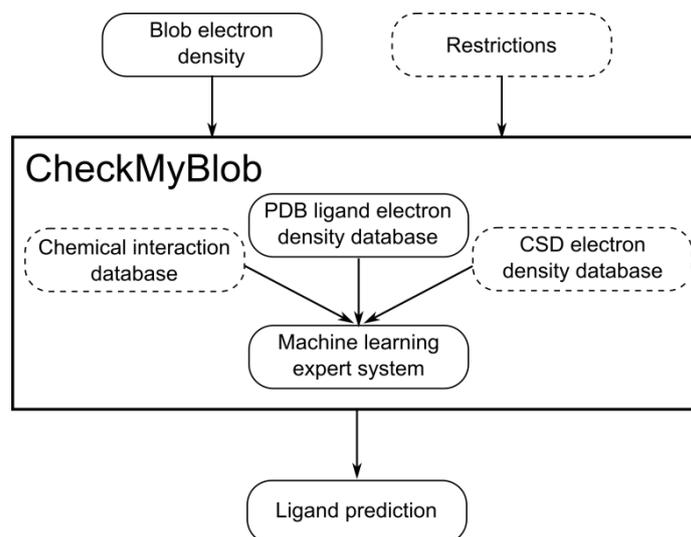


**Figure 1.** General ligand detection framework.

Within CheckMyBlob, we combine many different approaches for automatic ligand recognition to improve prediction of ligand identities in medium and low resolution structures (Fig. 2). First, we build a “blob descriptor” database for the most common ligands in the PDB. For identification of less common ligands, we plan to build an additional descriptor database based on small molecule databases such the Cambridge Structure Database (CSD). Moreover, the prediction algorithm will also use a database of known ligand chemical environments when the recognition of the ligand based on blob shape alone is problematic. These multiple different approaches will be unified within a state-of-the-art machine learning

system.

We present the current state of the project and our approach to automated blob recognition. We identify a few challenges and common pitfalls in automatic ligand detection, and demonstrate how we overcome them. In particular we show problems regarding: selection of the appropriate electron density map, ligand (blob) density masking, choosing optimal electron density thresholds, selection of rotational density invariants and descriptors, grouping of similar molecules, the description of the chemical environment, and the choice of machine learning algorithms.



**Figure 2.** The CheckMyBlob ligand identification framework. Part of the system during active development marked with a solid line.

# **Preliminary test of physics-based UNRES force field performance in domain packing and protein docking**

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UNRES (UNited RESidue) is a coarse-grained physics-based force field, in which each amino-acid residue is represented by two interaction sites, a peptide group (located in between two consecutive Calpha atoms) and a united side-chain (SC) attached to the corresponding Calpha atom. UNRES performs reasonably well in biannual CASP experiments, especially when the bioinformatics approaches fail to predict domain packing correctly; on the other hand, the UNRES-predicted structures have, generally, a lower resolution compared to those predicted by bioinformatics methods.

Consequently, we decided to develop an approach in which comparative-modeling information is applied to full extent for those sections of a target protein for which bioinformatics-based prediction is reliable and rely on UNRES prediction for other parts.

To combine the UNRES force field with the information from comparative modeling, we implemented MODELLER-like local-restraint components of the pseudo-energy function. With these terms, the secondary and tertiary-structure elements are held close to those of the respective knowledge-based model(s), while the UNRES force field governs mainly the packing of these elements. We also implemented a similar approach to protein-protein docking, by imposing restraints on whole or parts of the protein structures that assemble in a quaternary complex. Preliminary results of the prediction of the structure of single-chain proteins and protein complexes will be presented.

# Crystal structure of peptide-peptoid inhibitor in complex with trypsin: evidence for direct involvement of the Ser214 carbonyl group in proteolysis.

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Peptoids (poly N-substituted glycine residues) and peptide-peptoid hybrid polymers are interesting compounds mimicking the structure and function of biologically active peptides. STFI-1 is a serine protease inhibitor isolated from sunflower seeds. It contains only 14 amino acid residues and it is the smallest known naturally occurring serine protease inhibitor from the Bowman-Birk inhibitor family.

In this report we present the crystal structure of [NLys]5-SFTI-1 analogue (in which the side chain of Lys5 has been shifted from C $\alpha$  to N) in complex with bovine  $\beta$ -trypsin at 1.29 Å resolution. It was shown previously that peptoids are resistant to enzymatic degradation but up-to-date there has been no report in the literature for direct explanation of the proteolytic resistance of peptoids or peptide-peptoid hybrids.

Surprisingly, the reported structure is almost identical with the structure of native SFTI-1•trypsin complex. The compact structure of the SFTI-1 analogue, stabilized by a cis peptide bond between residues Pro7-Pro8 and by a disulfide bridge between Cys3 and Cys11, is not influenced by the more flexible N-substituted glycine residue. Moreover, the flexibility of the NLys side chain compensates the effect of the C $\alpha$ -to-N shift, allowing the  $\epsilon$ -amino group to occupy the same place as in the native structure. However, there is one interesting difference between the two structures, namely the absence of a hydrogen bond between the secondary amino group of NLys and the carbonyl O atom of the Ser214 residue from trypsin. It is well known that Ser214 is essential for substrate/inhibitor binding and that it forms (together with Trp216 and Gly216) a short antiparallel  $\beta$ -sheet interaction with the ligand molecules. Based on the present results, one can conclude that Ser214 plays an important role in binding of the P1 residue and in its preparation for enzymatic processing. This unexpected role of the main-chain carbonyl at residue Ser214 adds a new aspect to our view of the catalytic mechanism of serine proteases.

# Investigating electrostatic interactions in aminoglycoside-RNA crystallographic complexes

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Improving the binding affinities and selectivity of aminoglycoside antibiotics to bacterial ribosomes is necessary due to growing bacterial resistance. Therefore, it is crucial to capture and understand the interactions that govern aminoglycoside binding to ribosomal RNA. Electrostatic interactions are claimed to be the main driving force in the recognition of ligands by nucleic acids [1].

To obtain insight into electrostatic interactions of aminoglycosides with their RNA targets, we used the following approach [2]. From the crystal structures of twelve aminoglycoside-RNA complexes we reconstructed the electron densities using a pseudoatom database (University at Buffalo Databank - UBDB) [3]. This database reproduces electron densities for various atom types following the assumption that electron density is similar for atoms that are in chemically equivalent vicinities. These aspherical electron densities served to calculate per-atom contributions to the electrostatic energy of interactions between RNA and aminoglycosides using the Exact Potential Multipole Method (EPMM) [4]. Calculated interaction energies between various aminoglycosides and their binding sites revealed a significant correlation with experimentally obtained binding free energies. Analysis of water molecules mediating interactions between antibiotic and RNA allowed us to propose modifications which would enhance the binding specificity.

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# Recognition and structural adaptation within RNA – protein complexes: from crystal structure to dynamic functional modes of interactions.

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Retroviruses encode a regulatory protein Tat (the viral transcriptional transactivator protein), which activate the host cell transcriptional machinery. The Tat AD (Tat activation domain) binds to the cellular CycT1 (Cyclin T1) subunit of pTEFb complex (the positive transcriptional elongation factor) and induces specific binding of pTEFb to TAR RNA element (the transactivation response RNA) located at the 5'-end of all viral transcripts. The formation of TAR RNA - Tat - CycT1 complex stimulates viral gene expression at the level of transcription elongation.

Apparently the structures of the components of this tripartite complex change adaptively to optimize intermolecular interactions. TAR RNA have relatively stable double strand stem structure, while the loops appear highly dynamic in the absence of a ligands. The Tat protein is flexible in the absence of CycT1 or TAR.

The information at the atomic level of the molecular basis how a retroviral Tat protein interacts with both cellular CycT1 and a viral TAR RNA element to activate transcription was gained from the crystal structure of a tripartite ribonucleoprotein complex from EIAV (equine infectious anemia virus) and its corresponding equine host cell factor (2W2H in Protein Data Bank). Based on this structure we have studied by molecular modeling and extensive molecular dynamics (MD) simulations several models of the TAR RNA - Tat - CycT1 complex: the wild-type complex from EIAV and the mutant constructs with substitutions located within the apical loop of TAR, in correlation to the HIV1 TAR RNA sequence. The hexameric HIV-1 TAR apical loop is composed of CUGGG34A sequence instead of UCUGC15G, as in EIAV. G34 present in human HIV-1 TAR is crucial for CycT1–Tat binding.

In the result of extensive MD simulations we have identified a number of dynamic modes of structural adaptation within all components of the complexes not present in the crystal structure, especially in the Tat/TAR recognition motif (TRM) of CycT1 and N terminal of Tat fragment, which may have implications for the mechanism of TAR binding and the formation of the full functional complex.

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# Intermolecular interaction between nucleic acid bases in crystalline state from experimental charge density perspective.

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X-ray diffraction experiments not only give atomic position to determine geometry of chemical structure but the exact charge density distribution also be deduced. Accurate electron density studies provide information on chemical bonding that can be used to develop models for the stability and chemical reactivity of molecules and molecular aggregates. Nucleic acids are condensation polymers of nucleotides. To understand the interaction between these nucleotides a subatomic level study is required. For this purpose, X-ray diffraction data have been collected at 100 K for adenine, guanine and thymine and was interpreted in terms of the multipole formalism [1, 2]. The structures were refined to full convergence, first using the fixed model density composed of University of Buffalo pseudoatoms [3, 4], followed by fit of all parameters including multipole with constrained local symmetries. The multipolar refinement and subsequent quantum theory of atom in molecules (QTAIM) gave a comprehensive description of charge density in studies crystals. Further topological studies were performed to understand the interaction between the molecules.

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# Efficient docking of fully flexible peptides to flexible proteins without prior knowledge of the binding site: CABS-dock web server

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Protein-peptide interactions play a key role in cell functions. Their structural characterization, although very challenging, is important for discovery of new drugs. Based on our highly efficient simulation methodology[1, 2], we developed the CABS-dock protocol and web server for flexible protein-peptide docking[3, 4]. While other docking algorithms require pre-defined localization of the binding site, CABS-dock doesn't require such knowledge. Given a protein receptor structure and a peptide sequence (and starting from random conformations and positions of the peptide), CABS-dock performs simulation search for the binding site allowing for full flexibility of the peptide and small fluctuations of the receptor backbone[3, 4]. This protocol was extensively tested over the largest dataset of non-redundant protein-peptide interactions available to date (including bound and unbound docking cases)[4]. For over 80% of the dataset cases, we obtained models with high or medium accuracy (sufficient for practical applications). Our unique method for coupled binding site search and protein-peptide docking can be easily complemented by other computational tools (e.g. high-resolution docking refinement protocols) or experimental data to improve the results of the docking experiment. CABS-dock web server is freely available at <http://biocomp.chem.uw.edu.pl/CABSdock>

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# **Computational tool for modeling multi-chain biopolymer systems.**

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Mucines are large extracellular, heavy glycosylated proteins that play a role in human immune system by forming a selective molecular barrier among their other functions and are a target of cancer treatment research. Theoretical study of mucus properties are therefore necessary to enhance the ongoing effort for mucine-based medical applications. Mucines consist of different but repetitive domains and are capable of polymerizing further by forming disulfide bridges. To better understand the dynamics of mucus net forming and its structural properties it is essential to study a multi-chain system with interactions between different domains evaluated accordingly

Due to size of examined system we introduce a coarse grained off-lattice model employing Monte Carlo sampling with Metropolis algorithm. Part of this work is aimed to compare the performance and results of the Rouse model and a fixed bond-length model utilizing Verdier-Stockmayer like moves in Monte Carlo simulations.

# Structural and energetic landscape of a series of hydrated cocrystals of phenylenediboronic acids with aromatic N-oxides

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Phenyleneboronic acids are used in numerous branches of science. The best known chemical application is the Suzuki coupling reaction.[1] Phenyleneboronic acids have also found applications in biology and medicine, as supramolecular receptors, enzyme inhibitors, membrane transport.[2] Similarly, N-oxides of various heterocyclic compounds are important due to their vast applications as protective groups, oxidants or ligands in coordination complexes.[3] It is therefore interesting to testify whether it is possible to combine both classes of compounds into solid-state systems of desired properties (e.g. increased biological activity). Such a combination can be achieved by means of crystal engineering via formation of cocrystalline solids. In this respect the phenylenediboronic acids are of a particular interest due to their unique abilities to form complex hydrogen-bonded networks.

Consequently, the purpose of this study was to co-crystallize para- and ortho-phenylenediboronic acids with a series of aromatic N-oxides. Over ten new synthesized cocrystals were first analysed using single crystal X-ray diffraction. All of the complexes form centrosymmetric crystal structures belonging to either triclinic or monoclinic space groups of symmetry. The structures are characterized by an extended net of hydrogen bonds further stabilized by pi-stacking contacts. The obtained structural information allowed for periodic and dimer single-point computational studies which enabled a comprehensive analysis of crystal packing and energetic features of the reported systems. The nature of intermolecular interactions was additionally studied via Hirshfeld surface approach. The obtained theoretical results were confronted with that of the TGA-DSC experiments. Furthermore, water contribution to the lattice stability was deeply investigated. In all the systems water molecules play a role of a 'molecular glue' mediating the interactions between the acid and N-oxide moieties. However, in two of the studied systems water molecules additionally form one-dimensional substructures. The calculation results indicate the strength of different intermolecular interactions and reveal the great contribution of the solvent molecules to the crystal lattice formation. In addition, following the structural diversity of ortho-phenylenediboronic acid structures,[4] the very first cocrystals of this compound present surprising results in terms of crystal components. A remarkable result of the fused ortho-phenylenediboronic acid and its semi-anhydride incorporated into the cocrystal structure is reported.

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# RedMDStream: a tool for Effective Design of Coarse-Grained Models

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Computational models complement structural biology methods in understanding biomolecular systems. Molecular dynamics (MD) simulations add the information about internal motions to a static X-ray-derived picture. Capabilities of such *in silico* methods extend each year with an increase of computing power. However, with longer simulations the reliability of the models becomes an important issue.

This reliability problem is especially important for coarse-grained (CG) MD models. In such models better performance is achieved by adopting a simplified representation of molecules, e.g., each nucleotide or nucleic acid is represented by a single bead, rather than having to simulate motions of all the atoms separately, as in full-atomistic MD models. CG representation allows simulations of large systems, like the ribosome, and achieving timescales long enough to be relevant for folding of small proteins and RNAs. However reliability of the models is still questionable, at least when compared with full-atomistic models. Therefore, there is a need for a more robust parameterization schemes for such CG models and systematic understanding of limitations of the CG MD approaches.

We offer a set of programs (RedMDStream) [1] designed to aid in the most time consuming steps of making CG MD models. This is achieved by streamlining the design process:

1. The user defines how to create CG pseudo-atoms and connectivity patterns between the beads with assigned potential energy function. The definition can be based on data inferred from 2D structure prediction methods (RNA/proteins), experimental information about residue contacts, and full-atomistic structural data or MD trajectories.
2. RedMDStream provides a CG MD simulation engine, allowing running MD simulations in various ensembles and thermostats (NVE, NVT). Langevin and Brownian dynamics integration schemes are also possible. We provide tools to analyze the trajectories and distance distribution functions. An objective measure of the quality of a CG MD model can be easily set up by the user to select the best force field for a particular task.
3. RedMDStream provides also force field optimization tools: evolutionary algorithm, particle swarm and local optimization. [1,2] These are helpful in the trial and error tests to find optimal numerical parameter values, as well as help in deciding on minor modifications of the CG representation.

Topology definition, simulation engine, trajectory assessment and optimization tools are provided as a single application. Options of serial and parallel execution and in-memory calculations, saving on disk performance, are also available.

The application is designed not only to search for an optimal force field but also to better understand the model itself. By analyzing RedMDStream outcome, one can learn about the importance and correlations of potential energy function terms, as well as provide an estimate of the relationship between individual parameters and overall quality of the CG model.

The RedMDStream application can be downloaded for free from the following webpage: <http://bionano.cent.edu.pl/Software/RedMD> .

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# Impact of Mutations on Structure and Dynamics of Amyloid Beta Peptides: Insights from all-atom simulations

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The self-assembly of the amyloid beta (A $\beta$ ) peptides into senile plaques is the hallmark of Alzheimer's disease. Recent experiments have shown that the Tottori (D7N), English familial disease (H6R) and Taiwan (D7H) mutations alter the toxicity, assembly and fibril formation rate of the wild type (WT) A $\beta$ 40 and A $\beta$ 42 peptides. To understand these results at the atomic level we have performed all-atom replica exchange molecular dynamics simulations for monomer and dimer of WT and mutation sequences [1-4]. Our study revealed different mechanisms behind modulation of aggregation rates by mutations. In the H6R case, for instance, the rate of fibril formation of A $\beta$ 42 increases due to increased  $\beta$ -structure at the C-terminal and enhanced stability of salt bridge Asp23-Lys28 in monomer, while the enhancement of turn at residues 25–29 would play the key role for A $\beta$ 40. Overall, the reason behind the self-assembly acceleration is common that upon mutation the net charge is reduced leading to the weaker repulsive interaction between chains that facilitates the peptide association. The estimation of the solvation free energy shows that the mutation enhances the hydrophobicity of both peptides speeding up their aggregation. The obtained results further support our hypothesis that the propensity of proteins to self-assembly is governed by the population of the so called fibril-prone conformation in monomeric state [5].

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# New $\beta$ -lactoglobulin variants possessing single mutations in the binding site

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Bovine  $\beta$ -lactoglobulin (BlgB) belongs to lipocalin family and as other proteins from this group can be re-engineered to gain specificity to selected molecular targets. Analysis of substitutions occurrence in structurally related lipocalins was utilized to design new BlgB variants. Single mutations were introduced to the BlgB binding site to investigate impact of amino acid substitutions on BlgB structural stability and its binding properties.

New BlgB variants were expressed in *Origami B(DE3)* and purified by ion-exchange and size-exclusion chromatography. BlgB mutants and their complexes with ligands were crystallized using vapour diffusion method in hanging-drop setup. X-ray diffraction data were collected on *SuperNova* diffractometer (1.54 Å). Structures were solved by molecular replacement (*Phaser*) and refined using *Refmac5*.

Crystal structures of new BlgB variants revealed that selected amino acid replacements in  $\beta$ -barrel interior, especially in positions 56, 58, 92, 105 and 107, stabilize protein structure and affect binding of aliphatic (105) and aromatic (56) compounds, mostly by altering the shape of the binding pocket. Modifications introduced at the binding site entrance (positions 39, 62 and 69) did not affect binding of aromatic and aliphatic ligands but destabilized protein structure and altered crystallization process.

Results of structural and in-solution studies of single-site lactoglobulin mutants revealed that lactoglobulin affinity to selected compounds can be increased by modifying the shape of binding site rather than by introducing polar residues to  $\beta$ -barrel. Polar residues substituted to the region of binding site usually destabilize lactoglobulin structure or cause improper folding.

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# **The molecular characteristics of the nuclease from extreme psychrophilic bacterium *Desulfotalea psychrophila* LSv54**

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Nucleases are important molecular tools belonging to the group of hydrolase-degrading nucleic acids. Currently, only two nucleases degrading every form of nucleic acids are commercially known. They are Cryonase® (Takara, Japan) and Benzonase® (Merck, USA). Cryonase®, which originates from the psychrotrophic strain *Schewanella* sp. AC10, has an optimum activity temperature of 20°C, while Benzonase® which was isolated from the mesophilic microorganism *Serratia marcescens*, is most active at a temperature of 37°C. Both nucleases have found applications as tools used in molecular biology and genetic engineering techniques, and as industrial enzymes used in the elimination of nucleic acids from protein solutions, the elimination of genomic DNA, the degradation of DNA templates, the synthesis of DNA libraries, the footprinting method, the reduction of protein extract viscosity and the pretreatment of samples for two-dimensional electrophoresis.

We report the identification and characterization of the nuclease from psychrophilic bacterium *D. psychrophila* that grows exponentially at 4°C and may well grow at even lower temperatures. Psychrophilic microorganisms, living in extremely cold environments, produce enzymes which are adapted to performing reactions at a low temperature. This is extremely important in the case of nucleases, as it will allow their utilization in the reaction for the elimination of nucleic acids from thermosensitive preparations.

Nuclease from *D. psychrophila* is protein consisting 246 amino acid residues with a calculated molecular mass of 26 kDa. In comparison with the commercially available protein Cryonase®, the endonuclease from the strain *D. psychrophila* shows a high degree of homology with Cryonase® (35% identity and 51% similarity).

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## **Acrolein DNA adduct - 1,N6- $\alpha$ -hydroxypropanoadenine subjects to Dimroth rearrangement**

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Exocyclic DNA adducts 1,N6- $\alpha$ -hydroxypropanoadenine ( $\alpha$ -HPA) and 3,N4- $\alpha$ -hydroxypropanocytosine ( $\alpha$ -HPC) can originate exogenously as adducts of dietary and environmental toxin acrolein and endogenously during cellular oxidative stress from reaction of lipid peroxidation products:  $\alpha$ ,  $\beta$ -unsaturated- and epoxy- aldehydes with DNA bases. Both adducts contain an asymmetric carbon atom in the hydroxypropano ring, so they can exist as two stereoisomers. Unlike  $\alpha$ -HPC,  $\alpha$ -HPA appeared to be unstable in basic conditions. As we have recently shown by combination of 1D and 2D <sup>1</sup>H NMR (TOCSY, ROESY),  $\alpha$ -HPA spontaneously converts, at moderately basic conditions, to final product (adenine), and two additional transient forms:  $\gamma$ -HPA and acyclic adduct to the exocyclic N6. According to Mass Spectrometry data, the most probable acyclic adduct is N6-(1,3-dihydroxypropano)-adenine. 2-oxoglutarate- and iron-dependent AlkB dioxygenase is DNA/RNA repair enzyme which remove alkyl lesions from bases via an oxidative mechanism restoring native nucleic acid structure. Our previous data have shown that both stereoisomers of  $\alpha$ -HPA are repaired by AlkB, however the putative R stereoisomer more efficiently than the alternative one S does. Although, according to Molecular Modeling S isomer of  $\gamma$ -HPA may resemble R of  $\alpha$ -HPA at AlkB active site, preliminary experiments did not show any AlkB activity against  $\gamma$ -HPA.

# Characterization of efficiency of binding the ligand based on proteins with the same function and different topology

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tRNA guanine methyltransferases belong to enzymes which participate in a process of great biological significance. The methylation of tRNA is needed in the translation because the nucleic acid without attached methyl group blocks this process. In this group of enzymes can be found two types of topology: knotted and unknotted. Presence of a knot in a binding site forces a bent conformation of a ligand. In contrary the enzymes with trivial topology bind cofactor in an open conformation. This property was used to design a selective inhibitor of the knotted enzymes.[1] Knotted structures occur mainly in bacteria when unknotted mostly in mammals thus designed chemical compound could be used as a new antimicrobial drug for a proteins with non trivial topology. The efficacy of the ligand can be study theoretically with numerical simulation. We performed inhibitor and natural substrate docking to proteins from different organisms with distinct topologies, and on this basis predicted their selectivity and effectiveness.

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# Search for non-trivial topology in CASP competition – a key element to improve protein structure prediction

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The Critical Assessment of protein Structure Prediction (CASP) is a well-known competition for groups involved in developing efficient methods for predicting tertiary structure of proteins. It was created to evaluate existing methods and select the most promising approaches in protein structure prediction. Currently, it is well known that proteins with non-trivial topology (knots or slipknots) exist and they are found in 2% of structures [1] deposited in PDB. This unusual topology makes knotted proteins especially interesting in analysis of protein structure prediction. Based on polymer study, it is expected that knotted chains encounter more kinetics difficulties during folding and thus their folding time should be long. Therefore, knotted proteins, with a very long expected folding time, are less favored by hosting organism and thus could be eliminated during evolution. Here we search the CASP database for theoretical methods that could predict proteins with complex topologies. Additionally we show that topology is an important element, which has to be taken into consideration during protein structure prediction.

The analysis of over 600 000 structures, submitted to CASP for presence of knots and slipknots have shown that more than 2,5% of structures is knotted. We found that only fifth from those 2.5% were predicted correctly. The rest of knotted chains were predicted for unknotted targets thus these structures were incorrect. Based on our analysis and the fact that knotted protein chain is strongly conserved [2] the best chance to predict proper tertiary structure is to take into consideration topology: knot can only arise from knotted template and unknotted chain can only be used to predict trivial topology.

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# Electron density analysis of chromone and oxaphosphinane derivatives

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An experimental electron-density study of three chromone derivatives and one oxaphosphinane derivative were made on the basis of a topological analysis of its electron density distributions. The electron density was determined from a high-resolution X-ray diffraction data sets measured with synchrotron radiation at 100K collected at beamline F1 at Hasylab/DESY. Firstly, the spherical refinement was performed with SHELXL[1] and then the obtained model (IAM) was used as the input for the aspherical atom multipole refinement according to Hansen and Coppens multipole formalism [2].

A common characteristic feature of the crystal structure of chromone and oxaphosphinane derivatives is the intramolecular hydrogen bond which is a part of planar six-membered rings and which could be classified as resonance assisted hydrogen bond (RAHB).[3] An analysis of the experimental electron density by the Source Function [4] allowed to characterized the intra-molecular hydrogen bonds.

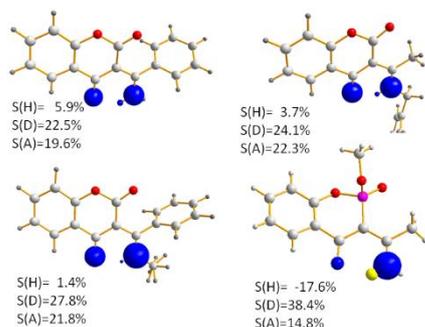


Fig. 1 The Source Function (SF) values  $S(\mathbf{r}, \Omega)$  are often reported in terms of their percentage contribution to the electron density at point  $\mathbf{r}$  (in this case in bond critical point-BCP): Percentage atomic source contributions in BCP are displayed as spheres whose sizes are proportional to the percentage contribution from each atom involved in the hydrogen bond. Blue color represents a positive contribution and yellow represents negative contribution of the source function

positive contribution and yellow represents negative contribution of the source function

The charge density distribution and analysis of topological properties revealed that the P-O bond is of the transit closed shell type with partially ionic character. Additionally, the spatial arrangement of valence shell charge concentration (VSCC) together with the ellipticity value indicates that bond between P atom and O atom seems to be a banana or bent bond.

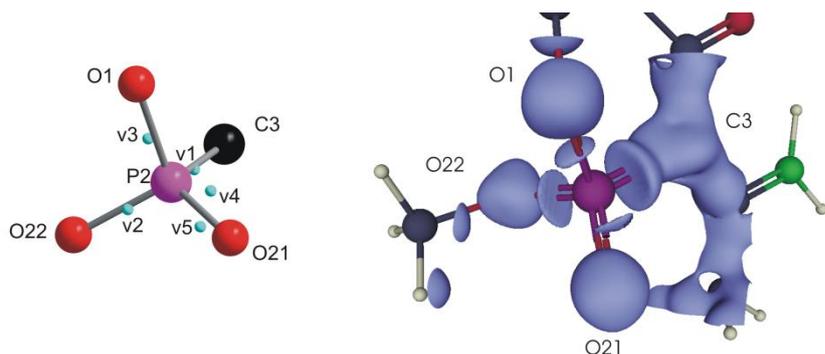


Fig. 2 Graphical representation of the VSCCs around the P atom with numbering scheme. The values of  $\nabla^2$  are:  $v1 = -14.04$ ,  $v2 = -8.30$ ,  $v3 = -3.32$ ,  $v4 = -1.12$ ,  $v5 = -0.41 \text{ e}/\text{\AA}^5$ ; (b) isosurface of negative

Laplacian in VSCC of the P atom at  $-0.5 \text{ e}/\text{\AA}^5$  isolevel

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## Facilities for Macromolecular Crystallography at the HZB

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The Macromolecular Crystallography (MX) group at the Helmholtz-Zentrum Berlin (HZB) has been in operation since 2003. Since then, three state-of-the-art synchrotron beam lines (BL14.1-3) for MX have been built up on a 7T-wavelength shifter source [1,2]. Currently, the three beam lines represent the most productive MX-stations in Germany, with close to 1400 PDB depositions (Status 03/2015). BLs14.1 and 14.2 are energy tuneable in the range 5.5-15.5 keV, while beam line 14.3 is a fixed-energy side station operated at 13.8 keV. All beam lines are equipped with state-of-the-art detectors: BL14.1 with a PILATUS 6M detector and BL14.3 with a large CCD-detector. The HZB-MX beamlines are in regular user operation providing close to 200 beam days per year and about 600 user shifts to approximately 70 research groups across Europe. During the first half of 2015 the endstation of BL14.2 is completely rebuilt. After the re-opening it will feature a DESY-type nanodiffractometer, a G-ROB sample changer and a PILATUS 2M detector. BL14.3 has been equipped with a HC1 crystal dehydration device since 2011. In addition to serving the user community mainly as a screening beam line, it is currently the only MX beamline in Europe with a HC1 device permanently installed [3]. Additional user facilities include office space adjacent to the beam lines, a sample preparation laboratory, a biology laboratory (safety level 1) and high-end computing resources. On the poster, a summary on the experimental possibilities of the beam lines and the ancillary equipment provided to the user community will be given.

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# **Transferable aspherical atom model refinement of protein and DNA structures against ultra-high-resolution X-ray data**

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In contrast to the independent atom model (IAM), in which all atoms are assumed to be spherical and neutral, transferable aspherical atom model (TAAM) takes into account charge transfer and deformation of the valence charge density resulting from the chemical bond formation, the presence of lone electron pairs, or intra- and intermolecular interactions. Both models can be used for refinement of small and large molecules e.g. proteins and nucleic acids, against ultra-high-resolution X-ray diffraction data. The University at Buffalo theoretical databank of aspherical pseudoatoms has been tested in the refinement of the tripeptide Phe-Val-Phe, Z-DNA hexamer duplex, Z-DNA dodecamer and aldose reductase. Application of the TAAM to these data improves quality of density maps and visibility of hydrogen atoms. It also slightly lowers the conventional R factor, improves the atomic displacement parameters and the results of the Hirshfeld rigid-bond test. Additional advantage is that the transferred charge density permits to estimate Coulombic interaction energy and electrostatic potential.

# **Crystal Structure of C/EBP $\epsilon$ bound to the Taz2 domain of p300 and the model of p300–C/EBP $\beta$ –HIPK2 ternary complex**

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The acetyltransferase activity of the transcriptional coregulator, p300/CBP, is modulated by phosphorylation that is triggered by binding of certain transcription factors such as C/EBP proteins. C/EBP activators bind to the Taz2 domain of p300/CBP through short, conserved sequence motifs, termed homology boxes A and B that comprise their minimal transactivation domains (TADs). Here, we describe the interactions between the minimal TAD of C/EBP $\epsilon$  and the Taz2 domain of p300, as determined from the crystal structure of a chimeric protein composed of residues 1723-1818 of p300 Taz2 and residues 37-61 of C/EBP $\epsilon$ . The position of the C/EBP $\epsilon$  peptide on the Taz2 protein interaction surface suggests that the N termini of C/EBP proteins are unbound in the C/EBP–p300–Taz2 complex. This observation is in agreement with the known location of the docking site of protein kinase HIPK2 on the C/EBP $\beta$  N terminus. We propose a structural framework for C/EBP $\beta$ -dependent phosphorylation of p300 by the protein kinase HIPK2 and suggest that the phosphorylation status of the p300/CBP C terminus may be regulated in part by proline-directed kinases and phosphatases which dock to sites located N-terminally to the homology box A of C/EBP proteins. This study provides structural insights that are important for understanding the molecular mechanisms underlying the

# MOLECULAR DYNAMIC STUDIES OF SMOOTHENED RECEPTOR IN LIQUID CUBIC PHASE

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Smoothened receptor (SMO) belongs to huge family of the G-Protein Coupled Receptors (GPCRs), also called the seven-transmembrane receptors (7TM). GPCRs play an important role in a signal transduction from extracellular side of a cell to its interior and possibly a formation of oligomers can modulate these transduction processes. SMO receptors takes part in a very important signalling pathway called hedgehog signalling, which is very important in the process of tissue creation. The significance of hedgehog signalling, however, persists over longer time periods, as it is crucial to the maintenance and regeneration of organs. Dysfunction of this receptor may lead to abnormalities, such as formation of a tumour. It was first crystallized in May 2013 in a specific environment: liquid cubic phases (LCP) [1]. It appears that the LCP [2, 3] is a new and very promising method for crystallization of proteins, particularly transmembrane proteins, such as GPCRs. During the process of typical crystalization the protein is being exposed to solution conditions which differ greatly from the native, membrane environment. Once the membrane protein is solubilized, it becomes vulnerable to slightest perturbations, which might result in denaturation, aggregation or even degradation. Any of those may spoil the protein sample. It is argued, that the instability of protein is caused by the removal of lateral pressure from the membrane so usage of LCP is highly required.

The molecular dynamics (MD) simulations [4] for systems in all-atom representation allow to study such systems in nano scale (both in space and time). MD is used to simulate behaviour of proteins, using the information gathered by experimental methods like X-ray crystallography. We studied a formation of GPCR monomers and oligomers in monoolein cubic phase (Pn3m) to get knowledge of dynamics of GPCRs in LCP environment. Lipid cubic phases are commonly used for crystallization of many membrane proteins, and especially GPCRs so a knowledge of their behaviour, including oligomerization and ligand binding is very important.

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## **Presentation title: Investigation of proteins with sulfur to $\alpha$ -carbon bonds with the example of Thurincin H using UNRES force field**

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Thurincin H is a protein from bacteria *Bacillus thuringiensis* SF361 with strong insecticide properties. Toxins produced by *B. thuringiensis* are widely used in agriculture as e.g. natural preservatives in dairy products. Thurincin H is a strong bacteriocin against *Listeria* spp. like *Listeria monocytogenes*, which are human pathogens, and also against other *Bacillus* species. Previous study on proteins Trn- $\alpha$  and Trn- $\beta$  demonstrated the presence of covalent bonds between the sulfur atoms of cysteine residues and  $\alpha$ -carbon atoms (Rea et al. 2010). Such bonds are also present in Thurincin H (31 amino-acid residue), between: S(Cys4)-C $\alpha$ (Ser28), S(Cys7)-C $\alpha$ (Thr25), S(Cys10)-C $\alpha$ (Thr22), and S(Cys13)-C $\alpha$ (Asn19).

We implemented the potentials for the sulfur to  $\alpha$ -carbon thioether bridges in physics-based UNRES coarse-grained force field. Because use of the coarse-grained UNRES force field results in the speed up of the calculations around 1000 times compared to all-atom force field (Liwo et al. 2007), the folding pathway of protein Thurincin H could be investigated extensively. The order of the formation of the sulfur to  $\alpha$ -carbon thioether bonds and the influence of the formation of these bonds on the stability of the structure of Thurincin H were determined.

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# **Estimation of the Binding Free Energy of AC1NX476 to HIV-1 Protease Wild Type and Mutations Using Free Energy Perturbation Method**

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The binding mechanism of AC1NX476 to HIV-1 protease wild type and mutations was studied by the docking and molecular dynamics simulations. The binding free energy was calculated using the double-annihilation binding free energy method. It is shown that the binding affinity of AC1NX476 to wild type is higher than not only ritonavir but also darunavir, making AC1NX476 become attractive candidate for HIV treatment. Our theoretical results are in excellent agreement with the experimental data as the correlation coefficient between calculated and experimentally measured binding free energies  $R = 0.993$ . Residues Asp25-A, Asp29-A, Asp30-A, Ile47-A, Gly48-A, and Val50-A from chain A, and Asp25-B from chain B play a crucial role in the ligand binding. The mutations were found to reduce the receptor–ligand interaction by widening the binding cavity, and the binding propensity is mainly driven by the van der Waals interaction. Our finding may be useful for designing potential drugs to combat with HIV.

# **Heterologous expression of steroid C25 dehydrogenase from *Sterolibacterium denitrificans* - a MGD, FeS and hem containing heterotrimer**

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Steroid C25 dehydrogenase (S25DH) from *Sterolibacterium denitrificans* catalyzes the oxygen independent hydroxylation of the tertiary C25 atom of the side chain of cholesterol and other steroid compounds to the respective tertiary alcohol. The enzyme is a heterotrimer ( $\alpha\beta\gamma$  composition), containing a molybdopterin guanine dinucleotide cofactor (MGD), 5 FeS clusters and a heme. The regioselective oxidation of cholesterol and its derivatives with S25DH provides opportunities for application of the hydroxylation products in medical treatment, as these compounds have an important role in the biosynthesis of signal compounds and hormones such as 25-hydroxycholesterol and vitamin D3 derivatives. The current purification protocol of S25DH from natural sources requires several chromatographic steps in an anaerobic glove box, a procedure which is too complex to be used in the biotechnological industry. Additionally, due to the slow growth of *S. denitrificans*, the final amount of the purified enzyme is not sufficient for the large scale application or further biophysical studies. Therefore, we aimed at the development of recombinant expression system for S25DH. Here, we describe the approach that was used to maximize the yield of protein expression using an *E. coli* production system. We have begun the optimization of S25DH overexpression from defining a protocol for molybdenum cofactor biosynthesis in *E. coli*. Using the molybdenum content in MDB loading chaperone of S25DH as an indicator of the level of synthesized and incorporated cofactor, we checked the medium composition, *E. coli* strain and the importance of electron acceptor when cultured under anaerobic conditions. Having defined the growth conditions suitable for MGD synthesis, the crude extract of *E. coli* overexpressing S25DH was assayed for enzymatic activity with cholesterol derivative used as a substrate. So far only residual activity of recombinant S25DH was detected with unexpected and undetermined by HPLC (and MS) analysis product of cholesterol conversion, so further effort will be directed toward obtaining higher yield of S25DH production suitable for affinity purification.

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# **KnotProt: a database of proteins with knots and slipknots**

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The protein topology database KnotProt, <http://knotprot.cent.uw.edu.pl/>, collects information about protein structures with open polypeptide chains forming knots or slipknots. The knotting complexity of the cataloged 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 (knot's core size) or the depth of a knot, i.e. how many amino acids can be removed from either end of the cataloged protein structure before converting it from a knot to a different type of knot. In addition, the database presents extensive information about the biological functions, families and fold types of proteins with non-trivial knotting. As an additional feature, the KnotProt database enables users to submit protein or polymer chains and generate their knotting fingerprints.

## Towards understanding diversity of tau amyloid strains.

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Tau (microtubule-associated protein tau) amyloidogenesis is the process which leads to the formation of amyloid fibrils – ordered protein aggregates which are able to adopt a number of distinct morphologies. Such structural polymorphism underlies the existence of amyloid strains – various stably propagating variants of amyloid with distinct biological activities (e.g. toxicity). Tau forms such structures. Conformational conversion of monomeric tau molecules into amyloid fibrils templated by seeds is an interesting research objective. Seeds induce transition of native molecules into filamentous assemblies. Such structures can also be obtained by fragmentation of amyloid fibrils by means of sonication.

Studying tau ordered aggregation in vitro requires inducers. Among them heparin (glycosaminoglycan) is the most common one. On the other hand fibrillization of tau induced by polyglutamic acid (PGA) is poorly characterized. In order to direct tau (a recombinant human 2N4R isoform) onto different assembly pathways we have used enantiomers of PGA: poly-L-glutamic acid (PLGA) and poly-D-glutamic acid (PDGA). We have used an interdisciplinary approach: kinetic measurement, transmission electron microscopy (TEM) and sedimentation. We have characterized unseeded and seeded processes of PLGA-/PDGA-induced tau aggregation to find out significantly different kinetics. The seeded processes remain more effective in the presence of PLGA but not when the process is induced by PDGA. The differences between kinetics of aggregation correspond to distinct architectures of obtained fibrils observed in TEM images. Moreover, PLGA-/PDGA-induced seeds propagate the structural features through seeded processes. To further characterize properties of obtained fibrils we have performed sedimentation experiments. These fibrils display distinct sedimentation properties. Taken together, these results show that a single tau isoform can aggregate and form polymorphic structures, reminiscent of amyloid strains.

Using chiral forms of PGA we have investigated molecular basis of tau amyloid polymorphism. Our results have implications for understanding diversity of tau amyloid strains.

# Zinc finger Com–RNA complex as an example of specific protein–RNA interaction

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RNA-binding proteins play a role at every stage of RNA life cycle: transcription, splicing, editing, export, degradation and regulation of translation. Many of them bind RNA molecules using RNA binding domains (RBDs). One of the most abundant RNA-binding domains is a zinc-finger (ZnF). ZnFs comprise about 30 amino acid residues and are characterized by a wide variety of mechanisms of substrate recognition and binding. In many cases the ZnF–RNA binding involves protein loops and  $\alpha$ -helices, utilizes hydrogen bonds, and stacking interactions. An important question is whether such a small domain is able to specifically recognize the nucleotide sequence of its substrate and to what extent the ZnF–RNA binding mechanism is shared by different ZnFs?

The bacteriophage Mu Com-RNA complex is our model system for studying ZnF–RNA interactions. The Com protein consists of 62 amino acid residues, including an N-terminal zinc finger module and a C-terminal intrinsically disordered segment. Com regulates the expression of the Mom system, which is responsible for chemical modification of the phage DNA, which results in phage genome protection against a wide variety of restriction endonucleases. So far, it is known that Com targets the hairpin structure just above the mom mRNA translation start site, contributes to the changes in the mRNA secondary structure and, consequently, to the exposition of the translation start signals. We aim to investigate the molecular basis of sequence and structure specificity in Com–RNA interactions using computational predictions, biochemical assays and X-ray crystallography.

Based on structure predictions for Com, we speculated that the N-terminal zinc finger is sufficient to bind RNA and, consequently, we used the truncated Com variant. Its specificity was established by in vitro selection (SELEX). Based on the in vitro selection results and RNA secondary structure predictions, two RNA molecules were designed and synthesized: RNA I forming a monomeric hairpin structure and RNA II forming a homodimeric duplex with several bulged nucleotides. Binding of Com to both designed RNA molecules, as well as to its natural RNA substrate, was confirmed using e.g., EMSA assay. It has been observed that Com indeed preferentially binds RNA hairpins (i.e. the monomeric version of the substrate).

Crystallization trials were set for the complexes of Com with its natural substrate as well as with RNA I and RNA II. So far crystal growth was observed only for Com with RNA duplex. The crystal diffracted to a resolution of 2.3 Å and belonged to the orthorhombic space group P 21 21 21, with unit-cell parameters  $a = 31.804$ ,  $b = 38.759$ ,  $c = 101.9$  Å. The size of the asymmetric unit suggests that only RNA crystallized, and one RNA duplex molecule is present per asymmetric unit.

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## Structure and mechanism of reverse transcriptases.

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Reverse transcription is a complex process in which single stranded RNA is converted into integration competent double-stranded DNA. This process is exclusively performed by enzyme called reverse transcriptase (RT). The RT-s are a multidomain proteins which consist of N-terminal polymerase domain and a C-terminal RNaseH domain. The multifunctional RTs possess two active sites, first is placed in polymerase domain, where all DNA elongation steps occur and the second is within RNaseH domain which is responsible for RNA hydrolysis within DNA/RNA hybrid. All of the structural information available for RTs concern the retroviral enzymes (1,2). In contrast there is paucity in equivalent studies on counterpart enzymes of LTR-containing retrotransposons, from which they are evolutionarily derived. We recently solved the first crystal structure of Ty3 RT in complex with its RNA/DNA substrate (3). In contrast to its retroviral counterparts, Ty3 RT adopts an asymmetric homodimeric architecture, whose assembly is substrate-dependent. More strikingly, our structure and biochemical data suggest that the RNase H and DNA polymerase activities are contributed by individual subunits of the homodimer.

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# Solid supports for serial protein crystallography at free electron lasers

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Serial femtosecond crystallography based on x-ray free-electron laser sources (XFELs) provides new opportunities for structural sciences, in particular in the view of time-resolved investigations of dynamic processes [1-3]. Even though after every ultrafast shot of very intense, coherent laser pulse the sample is destroyed due to Coulomb explosion, x-ray diffraction data can be collected in the so-called diffract-before-destroy regime.

High efficiency measurements at XFELs require high density and very well ordered deposition of the tested biological material – usually 2D or 3D crystals of proteins – on a substrate, which is scanned through the beam. This approach gives much higher hit rates and much lower sample consumption than commonly used liquid jets.

Microfabricated solid supports (fixed targets) filled with crystals protected from dehydration allow performing measurements at room temperature, enabling structure determination of macromolecules in a close-to-natural environment at high resolution [4]. Well-established microfabrication technology based on materials like silicon and silicon nitride gives opportunity to create ultrathin, low background packaging, which is especially useful for supporting 3D nanocrystals, as well as 2D crystals, which have too low diffraction yields for reasonable measurements using synchrotron radiation. However, further development towards highly x-rays transparent, less fragile, easy to mass-fabricate devices is required to allow future cost-efficient high throughput measurements at FELs.

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## **Presentation title: Purification and initial crystallization studies of molecular chaperone Hsp104 essential for protein disaggregation**

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The process in which a newly synthesized polypeptide chain transforms itself into a perfectly folded protein depends both on the properties of the amino-acid sequence and on multiple contributing influences from the crowded cellular milieu. Uncovering the mechanism of protein folding and unfolding is one of the grand challenges of modern science. The three-dimensional arrangement of the polypeptide chain and its conformation decide about the specific biological function of the protein in the cell. Only correctly folded proteins are fully functional, randomly arranged polypeptide chain doesn't have biological activity. The state of protein folding is controlled and regulated by the protein quality control system. The system is formed by chaperones involved in protein folding and the proteasomal degradation system. The proper functioning of the system is required because its dysfunction may lead to neurodegenerative diseases (Creutzfeldt-Jakob disease, Alzheimer's disease, Huntington's and Parkinson's disease).

Many chaperones are heat shock proteins. Their expression is increased when cells are exposed to elevated temperatures or other stress conditions [1]. Heat-shock proteins are named according to their molecular weight (for example: Hsp40, Hsp70, Hsp100). The project focus on the protein Hsp104, which belongs to the Hsp100 family and AAA+ super family. The importance of Hsp104 in the cell is due its ability to solubilize and refold, in cooperation with the Hsp70 chaperon system, proteins trapped in aggregates formed during heat stress. Active form is ring-shaped hexamer, which is thought to drive protein disaggregation by directly translocating substrates through its central channel. However, there is still no general consensus about the domain organization within hexameric molecular machine. A point of particular contention is the location and orientation of the unique M-domain, within hexameric assembly. Substantial efforts have been made to understand location of domain M and its role in the process but results are contradictory [2,3]. The domain has been subjected to a number of genetic and biochemical analyses but to answer all outstanding questions we need detailed structural information.

We aim to provide details information about the molecular mechanism of protein disaggregation, in which protein Hsp104 is involved. We are going to show the location and the orientation of the unique M domain by solving crystal structure of the protein using X-ray crystallography. At this stage of the project we are trying to obtain good quality crystals of Hsp104 and its variant stabilized by disulfide bridge.

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## **Major allergen from house dust mite Der p 1 complexed with Fab fragments of monoclonal antibodies 5H8 and 10B9**

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House dust mites (HDM) are a common source of indoor allergens in households around the world and a major cause of chronic asthma worldwide. Specific digestive enzymes of the HDM are potent proteases that are abundant in their feces, and are highly allergenic. Der p 1 is a Group 1 HDM allergen that belongs to the papain-like cysteine protease family, and its enzymatic activity affects its allergenicity. Der p 1 catalyzes the cleavage of amide linkages in a variety of substrates, including  $\alpha$ 1 antitrypsin, the CD23 receptor on human B cells, the IL 2 receptor (CD25) on human T cells, and the Der p 1 pro-polypeptide. The evidence strongly suggests that allergenicity of Der p 1 is exacerbated by Der p 1-related cleavage of these receptors. Exposure to Der p 1 occurs by inhalation of HDM fecal matter, and if it is persistent, it may cause the production of IgE antibodies in susceptible individuals. We identified specific conformational changes in Der p 1 that accompany antibody binding by determining structures of antigen-antibody complexes combined with additional analyses. By comparing the surfaces of the epitopes and the paratopes with all known protein-mAb and peptide-mAb complexes, we gained new insight into the nature of Der p 1 antigen-antibody interactions. The identification of IgE binding epitopes makes it possible to design modified allergen molecules, which may be used in immunotherapy for dust mite allergies.

# The charge density analysis of ferrocene taxol analogue

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The ferrocene analogue of taxol (named TAX1) was investigated. The most important fragment in the investigated molecule is a beta- lactam ring, which constitutes the core of the structure of many antibiotics families (for example penicillins, cephalosporins, carbapenems, and monobactams). The compound is also considered as a candidate for cancer treatment.

The structural and high resolution X-ray diffraction experiments were performed (on single crystal diffractometer in 100 K and 90 K, respectively) and analysis of the experimental electron density distribution was performed. Five different approaches were tested in order to characterize the distribution of the electron density. Modeled were electron density distributions for molecule with local symmetry imposed on atoms or without symmetry (while the iron atom was assigned an initial charge of either zero or plus two). For the last model in which the UBDB bank was applied, were calculated electron density distributions for a molecule with a local symmetry imposed on atoms when all multipole populations were allowed and iron atom has a neutral valance.

Preliminary results suggest that the model without imposed local symmetry, in which the iron atom has a zero valance population was the best. In all models the iron atom has positive charge, but the exact value of the charge is different in each model. It is worth to notice, that some of the populations of multipole parameters prohibited due to the local symmetry were statistically significant, when the model without imposed symmetry was tested. The atomic charges in the current best model are negative on both oxygen atoms and, on nitrogen atom, while the carbon atoms in the beta- lactam ring have positive charges. The distribution of the electrostatic potential around the TAX1 molecule and the network of intermolecular hydrogen bonds will be presented.

## Acknowledgements

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# The role of the hydrogen atom model in crystallographic studies

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Hydrogen bond (HB) is the most important stabilization interaction for proteins. Thermodynamic measurements showed that energies associated with HB formation in the liquid and solid states are similar [1], so single crystal diffraction methods are an efficient tool supplying not only geometry of a given interaction but also information on its relative strength. However, truly quantitative studies of both geometry and electron density distribution in organic crystals are also possible when experimental charge density approach is applied [2].

The proper description of the H atom in the charge density model must take into account two factors: thermal motion of atoms (which can be approximated using SHADE server [3]) and—in case of refinement with the Hansen-Coppens model - the expansion-contraction coefficients of the valence density ( $\kappa$ ). The role of the second factor is usual underestimated and  $\kappa$  parameters for H atoms are set to 1 during a whole refinement procedure. However, there are three theoretical approaches to  $\kappa$  modelling: Volkov model [4], UBDB databank [5] and theoretical  $\kappa$  parameters obtained from Crystal 09 calculations [6]. A comparison of the interaction energy of hydrogen bonds using all three models indicates that Volkov model and UBDB databank results are the most reliable.

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# Characteristics of ligand conformation in a knotted homodimeric methyltransferase

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Methyltransferases are enzymes that are crucial for several biological processes including modifications of nucleic acids. This group of proteins can form several different folds – both knotted and unknotted. There are over 60 distinct proteins from various organisms that possess a non-trivial topology [1] – trefoil knot, which is responsible for binding the cofactor essential for methylation of RNA. All of them create homodimeric structures capable of binding two ligands. On the contrary, methyltransferases without knotted topology are mostly monomers. It is still not fully understood what is the function of knot and thus whether the complexity of the structure is affecting rate of methylation. Despite the symmetry of those knotted proteins, as it has been shown [1], there is a difference in binding constants between ligands, indicating that one site is more favorable than the other. Using molecular dynamics simulation we were able to mimic this behavior and suggest possible mechanism behind it. In order to maintain the dimeric structure it appears that some amino acids within the active sites has to choose between two roles: binding the ligand or binding within dimer interface. It results in differences both in conformation and stability of the ligands.

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# Binding of fullerenes to amyloid beta fibrils: Size matters

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Binding affinity of fullerenes C<sub>20</sub>, C<sub>36</sub>, C<sub>60</sub>, C<sub>70</sub> and C<sub>84</sub> for amyloid beta 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 demonstrate 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 the binding affinity. Overall, fullerenes bind to Ab<sub>9–40</sub> fibrils stronger than to Ab<sub>17–42</sub>. The number of water molecules trapped in the interior of 12Ab<sub>9–40</sub> fibrils was found to be lower than inside pentamer 5Ab<sub>17–42</sub>. C<sub>60</sub> destroys Ab<sub>17–42</sub> fibril 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.

# **Structural studies on DNA cleavage-and-ligation nucleases of mobile genetic elements involved in spread of antibiotic resistance**

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Plasmids and integrative and conjugative elements (ICEs) are major mobile genetic elements (MGEs) that provide routes for rapid acquisition of new genetic information in bacteria and therefore contribute to the spread of antibiotics resistance. Essential for their action are plasmid/ICE-encoded site- and strand-specific one-metal-ion endonucleases called relaxases. Conjugative relaxases cleave a single strand of the DNA substrate by formation of an intermediate covalent adduct with the scissile phosphate of the DNA nic site. After the ssDNA-relaxase molecule is transferred to the recipient cell, relaxases ensure re-ligation of their DNA cargo. Additionally, plasmids and some ICEs encode for DNA replication relaxases, crucial for their maintenance. Understanding plasmid/ICEs conjugal transfer and replication may aid in combating the spread of antibiotics resistance as well as contribute to the development of new tools for DNA delivery into human cells. Structures of replicative and conjugative relaxases RepB, MobM and TrwC that were solved in our lab are compared herein.

# **Fitmunk: Automatic side chain conformation modeling and sequence recognition in protein crystallography using knowledge-based algorithms.**

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Improvements in crystallographic hardware and software have allowed automated structure solution pipelines to approach a "one-click" experience for determination and initial model building of macromolecular crystal structures. However, in many cases the resulting structures require a laborious, iterative process of refinement and validation. We have developed new methods for automatic modeling of side chain conformations and recognition of protein sequence from the electron density maps. These methods have been implemented in program Fitmunk, which utilizes a hybrid energy function derived from experimental data and prior information about rotamer frequencies. In contrast to existing methods, which incorporate the electron density term into protein modeling frameworks, we propose a new type of algorithm specifically designed to take advantage of the highly discriminatory nature of electron density. These algorithms allow Fitmunk to use extensive conformational sampling, which improves the accuracy of the modelling and makes it a versatile tool for crystallographic model building, refinement and validation. Together with new, adaptive side chain conformation libraries and dead-end elimination (DEE) theorem optimized for use in a crystallographic context, it allows for rapid placement of conformations that simultaneously fit well into electron density and conform to our chemical knowledge, like conformation distribution. Here we present several applications of Fitmunk that has been successfully used during model building and refinement of over 150 different models of 65 different proteins from CSGID, MCSG and NYSGRC structural genomics centers. Our approach allowed us to streamline the process of model building and refinement and resulted in improved quality of modeled structures. Fitmunk is available as a web server at [fitmunk.bitbucket.org](http://fitmunk.bitbucket.org)

# **Topology, geometry and free energy of intermolecular halogen bond formed between halogenated ligand and protein kinase.**

## **Analysis of PDB structures and solution study.**

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Halogenated ligands are potent, and frequently selective, inhibitors of protein kinases. Halogen bonding, the recently identified type of non-covalent interactions between halogen atom and an electron-rich partner, is currently widely exploited, both in drug design or supramolecular chemistry. However, there is still a controversy concerning contribution of halogen bond to ligand binding, and estimates free energy for creation of a separate halogen bond vary from 2 up to 30 kJ/mol. Interestingly, almost no attention has been directed to other effects that may result from replacement of a hydrogen by a strongly electronegative halogen. We have performed statistical analysis of all complexes of protein kinases deposited in PDB to estimate the contribution of halogen atom located on a ligand, both the direct and indirect, to protein - ligand interactions. We have clearly shown that presence of halogen atom(s) does modify the strength of hydrogen bonds created between ligands' nitrogen or oxygen neighboring the halogen atom and a protein. In general, halogenation increases the acidity of the proximal amino/imino/hydroxyl groups making them better, i.e. stronger, H-bond donors.

The analysis of halogen bond topology led to identification of two reasonably populated types of halogen bonds, both of which have been rarely reported yet for protein-ligand systems. We have also shown that a pattern of interactions rather than the location of particular halogen atoms is preserved along protein kinases complexes with halogenated ligands. And cumulative distributions of halogen-acceptor distances show that structures displaying short contacts involving a halogen atom are slightly overpopulated, contributing together to clearly defined shoulders at 2.82, 2.91 and 2.94 Å for chlorine, bromine and iodine, respectively.

In parallel, binding of nine halogenated benzotriazoles (TBBt and its analogues representing all possible patterns of halogenation on the benzene ring of benzotriazole) to the catalytic domain of human protein kinase CK2 (hCK2 $\alpha$ ) was monitored in solution by calorimetric (DSC and ITC) and optical (DSF and MST) methods. In general, the thermal stability of protein-ligand complexes follows the inhibitory activities determined by biochemical assays. DSC- and ITC-derived apparent heats for ligand binding ( $\Delta\Delta H_{\text{bind}}$ ) are driven by intermolecular electrostatic interactions, as indicated by a good correlation between  $\Delta\Delta H_{\text{bind}}$  and ligand pKa. Overall results, additionally supported by molecular modeling, confirm that a balance of hydrophobic and electrostatic interactions (~40 kJ/mol) predominates possible intermolecular halogen/hydrogen bonding (less than 10 kJ/mol) in binding of halogenated benzotriazoles to the ATP-binding site of hCK2 $\alpha$ .

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# STRUCTURAL AND SPECTROSCOPIC INVESTIGATIONS OF CYCLOBUTYLAMINE AND CYCLOBUTYLAMINE HYDRATES

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Clathrate hydrates are fascinating group of compounds where the guest molecules are trapped in the water cage. The most known example are clathrate hydrates of methane [1]. This research is focused on the synthesis of compounds of this kind with cyclobutylamine as the guest molecule. The structure of the amine hemihydrate is already known. It crystallizes in monoclinic P21/n space group [2]. The crystal is stabilized by a hydrogen bond and weak van der Waals interactions.

To screen the possibility of hydrate/clathrate formation and the amine/water ratios some preliminary XRD experiments have been made. The samples suitable for single crystal X-ray diffraction were grown with use of the *in situ* crystallization method assisted by IR laser [3]. In this way we were able to obtain crystals of the cyclobutylamine and its four new hydrates containing: 1.00, 6.43, 7.67 and 9.50 water molecules per one amine molecule. Their crystal and molecular structures were solved with use of the X-ray diffraction experiment on single crystal. The structures containing larger amount of water (6.43, 7.67 and 9.50 hydrates) resembled clathrate hydrates of methane. Contrary to true clathrates the guest molecules interacted with the water framework via N...O hydrogen bonds. These structures are in addition severely disordered. All systems were also characterized using Raman spectroscopy.

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# Comprehensive analysis of the active site architecture of metallo- $\beta$ -lactamases

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Antibiotic resistance is a serious and steadily growing threat to human health. It can be mediated by various mechanisms, one of them being the employment of especially designed enzymes to disarm the offending compound.  $\beta$ -lactamases are an example of such enzymes that hydrolyze  $\beta$ -lactam antibiotics. Current clinical strategy is to use a combined drug therapy consisting of an antibiotic plus a  $\beta$ -lactamase inhibitor (eg. clavulonic acid). However,  $\beta$ -lactamases are a broad group of enzymes and one particular class, metallo- $\beta$ -lactamases, is immune to the clinically used inhibitors so novel compounds need to be found. Metallo- $\beta$ -lactamases have been extensively studied, also structurally, and they exhibit a significant diversity of their active sites and substrate specificity. Since a thorough understanding of the active site features is necessary for structure-guided inhibitor design we performed a detailed analysis of their active site architectures.

The most clinically relevant class of metallo- $\beta$ -lactamases is B1 because it is often encoded on promiscuous plasmids. It is the best-researched class and out of the total 165 structures present in the pdb, 126 are of the B1 class enzymes. They bind two zinc ions that are essential for their catalytic activity. The zinc binding sites are well conserved but coordination modes can differ between structures depending on such factors as cysteine oxidation or ligand binding.

We present a comparison of the active sites of various metallo- $\beta$ -lactamase structures which is a useful information in our project of structure-guided inhibitor design.

# **IRON, PIRACY AND CONFLICT ON THE BACTERIAL HIGHSEAS: FERREDOXIN CONTAINING ANTIMICROBIAL PROTEINS PROVIDE INSIGHT INTO A NOVEL VIRULENCE RELATED IRON ACQUISITION SYSTEM IN PECTOBACTERIUM**

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The pectocins are a class of antimicrobial protein recently discovered in our lab, consisting of a fusion between an enzymatic, cell wall disrupting toxin domain and an iron-containing plant-like ferredoxin. These toxins are produced by the Gram-negative phytopathogen *Pectobacterium* for intra-species competition and cross the outer-membrane of their target cells using an unprecedented ‘Trojan-horse’ strategy [1]. We show that the plant-like ferredoxin domain of these proteins acts as bait, delivering the toxin domain to the periplasm by parasitising a previously undescribed ferredoxin import system. Further to this discovery, we have utilized these unusual toxins to identify the integral outer membrane protein responsible for ferredoxin import, which we have designated the ‘Ferredoxin uptake protein A’ (FupA). Additionally, using a genetic approach we have identified the periplasmic protein responsible for the liberation of the ferredoxin iron-sulphur cluster and the inner membrane transporter responsible for its import to the cytoplasm. *Pectobacterium*, a notorious plant pathogen, utilizes this Fup system to obtain iron from its plant host during infection [2]. Recently (unpublished results), we have solved the crystal structure of FupA, showing it to be a 22-stranded  $\beta$ -barrel of the TonB-dependent nutrient receptor family. To complement these data we have utilized a combination of NMR and X-ray crystallography to determine the ferredoxin/FupA binding surface in atomic detail. Further, utilizing a combination of X-ray crystallography, SAXS and molecular dynamics simulations we have solved the structure of pectocin M2, a representative member of these toxins, showing that it is flexible in solution and adopts dimensions compatible with traversing the lumen of FupA [3]. These data provide insight into the molecular mechanisms by which the ferredoxin domain individually (or in combination with the toxin domain of the pectocins) is imported through FupA into the periplasm of the cell. Ferredoxin import through FupA is to our knowledge, the first example of protein import by the proteobacteria and represents a hitherto undescribed means by which Gram-negative bacterial pathogens obtain iron from their hosts during infection.

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# Active site of psychrophilic aminotransferase in complexes with substrate and inhibitor

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Nowadays, more and more efforts are focused on searching for the enzymes' homologs that exhibit additionally beneficial features, without a loss of efficiency. Extremophilic enzymes are those, who maintain enzymatic activity in harsh environments. Therefore they are natural source for enzymes with special features concerning working environment. When the temperature of a process is to be lowered, the use of psychrophilic enzymes may allow it. The most desirable are the ones that possess significantly lower optimum reaction temperature, but their enzymatic activity remains at a comparable level to their mesophilic analogs [1].

Aminotransferase's (AT) catalytic activity depends on the presence of tightly bound pyridoxal-5'-phosphate (PLP) as coenzyme. The transamination reaction, which is ping-pong, bi-bi reaction, can be divided into several distinctive steps such as: formation of internal aldimine, external aldimine and unbound PMP. [2,3]

We have recently determined the crystal structure of aminotransferase from *Psychrobacter* sp. B6 with malic acid (PsyArAT/Ma) at resolution of 1.62 Å. Which, together with previously presented crystal structures of the native enzyme (PsyArAT - 2.19 Å) and its complex with aspartic acid (PsyArAT/D - 2.76 Å) [4], was further used for comparative studies. The resulted crystal structures differ by unit cells and crystal packing. The PsyArAT possesses a dimer in the asymmetric unit, while the PsyArAT/D complex consists of four dimers. The PsyArAT/Ma contains just a monomer in the asymmetric unit and the dimer is reconstituted with symmetry related monomer.

The malic acid, used as a ligand, is a substrate's analogue that lacks amino group, but still binds within the active site of the enzyme. Therefore the enzyme's activity becomes inhibited, since no transfer of the amino group occurs. The analysis of obtained PsyArAT/Ma structure enabled structural characterization of the competitive inhibition of aminotransferase with malic acid.

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## **Presentation title: Molecular modeling of interactions between selected fenoterol derivatives and human cytosolic sulfotransferases**

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First-pass metabolism refers to the process of rapid elimination of orally administered drugs and involves the activity of gastrointestinal and hepatic enzymes. This effect is one of the problems associated with the pharmacotherapy of  $\beta_2$ -selective adrenoceptor ( $\beta_2$ -AR) agonists, e.g. terbutaline, salbutamol or fenoterol. For example, the oral bioavailability of (R,R;S,S)-fenoterol in humans is only ~2%. The poor bioavailability of (R,R;S,S)-Fen is due to extensive presystemic sulfation by the sulfotransferase (SULT) enzymes.

Several fenoterol derivatives were designed to optimize the pharmacological profile of this group of compounds. (R,R)-Methoxyfenoterol, an analog of (R,R)-fenoterol is currently in clinical trials for use in the treatment of congestive heart failure. Since (R,R)-methoxyfenoterol and other studied analogs are of clinical importance we attempted to predict their metabolic profile. Crystal structures of SULT responsible for sulfation of this group of compounds, namely SULT1A1 and SULT1A3 were selected to model the substrate-enzyme interactions. Based on the docking simulations the metabolic preference resulting from substituent modification will be discussed.

# Structure and properties of azepane hydrates

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Azepane, also known as hexamethyleneimine, is a seven-membered aliphatic amine (C<sub>6</sub>H<sub>13</sub>N). It crystallizes in the P2<sub>1</sub>/n space group with eight molecules in the unit cell [1]. The structure is dominated by closed cycles of N–H...N hydrogen bonds. Such case can favor the formation of a whole range of various hydrates. Using the in situ crystallization technique [2] assisted by IR laser focused radiation we obtained three hydrates of azepane (with one, three and eleven molecules per one amine). The crystals of monohydrate undergo phase transition. Both phases belong to the space group and have unit cell volumes equal to 1494 Å<sup>3</sup> and 761 Å<sup>3</sup> in low and high temperature polymorph, respectively. In the monohydrate polymorphs hydrogen bonds group the amine and water molecules in columns. The trihydrate crystallizes in P2<sub>1</sub>/c space group (V=947 Å<sup>3</sup>) and H<sub>2</sub>O molecules form layers. The amine molecules are attached to these layers via N...O hydrogen bonds. The undecahydrate belonging to Pnma space group (V=1864 Å<sup>3</sup>) contains 3D network of interacting water molecules. In the structure the amine molecules are incorporated to this network thus the undecahydrate is example of semiclathrate [3]. In all presented structures both azepane and water molecules are disordered. Disorder of H<sub>2</sub>O molecules is manifested in alternative positions of hydrogen atoms.

## Acknowledgements

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# Specific plant hormone binding proteins with PR-10 fold - structural and thermodynamic studies

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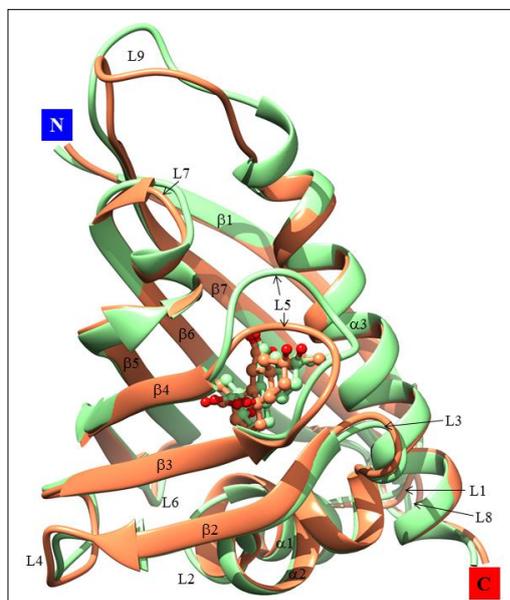
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Pathogenesis-related proteins of class 10 (PR-10) are a family of plant proteins with the same fold characterized by a large hydrophobic cavity that allows them to bind various ligands, such as phytohormones (Fernandes et al., 2013; Ruszkowski et al., 2013). A subfamily with only 20% sequence identity but with a conserved canonical PR-10 fold have been previously recognized as Cytokinin-Specific Binding Proteins (CSBPs) (Pasternak et al., 2006), although structurally the binding mode of *trans*-zeatin (a cytokinin phytohormone) was found to be quite diversified.

Here, it is shown that two CSBP orthologues from *Medicago truncatula* and *Vigna radiata* bind gibberellic acid (GA3), which is an entirely different phytohormone, in a conserved and highly specific manner. In both cases a single GA3 molecule is found in the internal cavity of the protein. The structural data derived from high resolution crystal structures are

corroborated by isothermal titration calorimetry (ITC), which reveals a much stronger interaction with GA3 than with *trans*-zeatin and pH dependence of the binding profile. As a conclusion, it is postulated that the CSBP subfamily of plant PR-10 proteins should be more properly linked with general phytohormone binding properties and termed phytohormone-binding proteins (PhBP) (Ruszkowski et al., 2014).



Note that the GA3 molecules (ball-and-stick representation) are in the same position and orientation in the internal binding cavity in both complexes (Ruszkowski et al., 2014).

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# The structure of hybrid materials containing SBA-15 and diatomite

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Hybrid materials are compounds containing two constituents mixed on a molecular scale. Typically, one component is an organic and second is an inorganic. Hybrid materials may be a mixture of heterogeneous or homogeneous. A more detailed definition takes into account the interaction between the components. So, hybrid materials can be divided into two classes:

- Class I- have a weak influence; between phases (van der Waals forces, hydrogen bonds, weak electrostatic interactions)

- Class II- strong chemical interactions.

Hybrids have been originated so long ago. But it was the development of physico-chemical studies contributed to the increased interest in these materials. This enabled a deeper understanding of their properties. A special role is played the synthesis of sol-gel. Developed in 1930 using silicon alkoxides as a precursor silica products. This process is very similar to the organic polymerization.

Organic-inorganic compounds are materials with unusual properties. The twentieth and twenty-first century through the development of sciences relating to materials paved the way for molecular engineering and contributed to the development of nanotechnology. Special interest refers to materials that combine glass and plastics. Very developing research area of functional hybrid materials. Mechanical properties play a secondary role. The most important are the chemical, electrochemical, electronic or biochemical activity. Innovative functional hybrids are used in optics, electronics, for storage and conversion of energy, as protective coatings, sensors, catalysts and in biomedicine.

# Non-canonical imperfect RNA base pair predictor

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One of the most important aspects in RNA 3D model building is the intramolecular interactions identification and validation. In this work I propose a method which can improve this stage of model building, and may result in creation of better final three-dimensional RNA models.

In my work I constructed a predictor that can identify both canonical and non-canonical base pair interactions within a given structure. The main advantages of this predictor are:

- 1) the ability to work with incomplete input structures,
- 2) the ability to correctly predict base pair type even for imperfect (fuzzy) input atoms coordinates.

The predictor is based on the set of SVM multi-class classifiers. For each input base pair the classifier chooses one of 18 recognized pair types. The predictor was trained on the experimental high quality data and tested on different, imperfect and incomplete (coarse-grained) structures. The average quality of predictor for tested fuzzy nucleotide pairs is at the level about 96% of correct recognitions.

# CHEMISTRY OF PIPERIDINE HYDRATES

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Piperidine is the heterocyclic aliphatic amine with six-membered ring. Its crystal structure has been known [1]. The molecules constituting crystals form columns, while the most important interaction stabilizing the system are hydrogen bonds. The aim of this report was to expand our knowledge about the molecular interactions in the solid state by formation of hydrates. Since both piperidine and water are liquids under ambient conditions we used the in situ crystallisation technique with IR laser [2] at lowered temperatures. Due to course we were able to obtain five different hydrates! They contained 0.50, 2.00, 8.10, 9.75 and 11.00 water molecules per one amine molecule. Their molecular and crystal structures were determined with use of single crystal X-ray diffraction measurements.

Interestingly, hydrates with high concentration of water were very similar to gas clathrates [3]. The difference between pure and reported clathrates is in hydrogen bond, which is formed between the amine molecule and the water framework. We have found that piperidine hydrates consisting of 9.75 and 11.00 water molecules were isostructural with analogous structures of tert-butylamine hydrates [4,5], whereas piperidine hydrate 8.10 was isostructural with izo-propylamine hydrate 8.00 [6, 7]. All received crystals was also characterised with Raman spectroscopy and X-ray powder diffraction.

## Acknowledgements

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# Structure determination and functional studies of D-isomer specific 2-hydroxyacid dehydrogenase from *Sinorhizobium meliloti*

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The 2-hydroxyacid dehydrogenase family (2HADH) contains a broad enzyme group of NAD(P)(H)-dependent oxidoreductases with key roles in many organisms metabolism. Those enzymes perform reversible reduction of 2-ketocarboxylic acids to hydroxyacids. The stereospecific reduction of carbonyl group is of interest for the production of chiral compounds which are broadly used as pharmaceuticals. Many members of the family are well characterized both structurally and biochemically. However, the function of multiple members cannot be predicted solely by sequence alignments due to low sequence identity to characterized members and remains to be identified. This study is focused on 2HADH family member from *Sinorhizobium meliloti*: a symbiotic bacterium living in common soil and infecting nodules on the roots of legumes. It has an ability to convert atmospheric nitrogen into ammonia which is used by the plants as a source of nitrogen.

The goal of the study is to determine the crystal structures of a member of 2HADH from *S. meliloti* (NYSGRC target 011884), identify its best cofactor and substrates based on kinetic parameters, classify according to the identified catalytic function, and characterize its function in the symbiotic organism.

The protein was successfully expressed, purified, crystallized and subsequently 3-D structures were determined by X-ray crystallography. Two structures have been solved, refined and deposited in the PDB: complex with NADP and sulfate (4WEQ, 2.0 Å) and complex with NADPH and oxalate (4Z0P, 1.7 Å). The complex with with NADPH and oxalate is of particular importance, because it was crystallized with oxalate bound in active site of the enzyme. This compound contains chemical group on which enzyme is supposed to act, and therefore the triple complex protein-NADP-oxalate represents the dead-end analogue of Michaelis complex. Comparison of those structures reveals important conformation changes during substrate binding. Further analysis using substrate screening, kinetics measurements, isothermal titration calorimetry, and fluorescence quenching are being performed. The complex analysis, broadens the structure-functional knowledge about the 2HADH family.

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# **DCAF (decaf) – Discrimination, Comparison, Alignment algorithm for small molecules**

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Predicting biological activity of small molecules is a key element of computer-aided drug design. Existing methods often fail to identify ligands with similar physicochemical properties but different structures. Many of the current approaches rely on generating 3D conformations, which leads to sampling problems and unacceptably high computational costs for large sets of molecules.

Herein we present DCAF (decaf) - a novel method for describing ligand properties and a fast and effective tool for comparing multiple molecules. To describe a molecule, DCAF substitutes its functional groups with pharmacophoric points (hence the "F" in the algorithms name), which correspond to nodes in an undirected graph. Weights of the edges in the graph are determined by the number of bonds between pharmacophoric points. Such pharmacophoric description puts an emphasis on ligands' physicochemical properties, rather than on structural details. It also does not require generating 3D conformations. This allows DCAF to provide a fast and robust similarity measure.

We tested DCAF in 35 case studies taken from the DUDE database, to evaluate its power to discriminate between active and inactive datasets. We confronted DCAF's ability to measure ligand similarity with the standard Tanimoto coefficient. We proved that our method is more accurate as it finds molecules with similar physicochemical properties, despite differences in details of their structures. We also used DCAF as a classifier and compared it to the SEA (Similarity Ensemble Approach) algorithm [1]. We demonstrate that DCAF is more sensitive and performs better for 23 (out of 35) receptors. Additionally, we show that DCAF can be used to filter compound databases based on similarity to single- or multiple-ligand model. Last but not least, it can also serve as a fast general alignment tool for multiple ligands. DCAF is written as a Python module and can be easily combined with OpenBabel or RDKit to facilitate ligand-based drug design.

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# **A comparison of the biotin-ferrocene conjugate and 2-(4'-hydroxyazobenzene)benzoic acid binding specificity to glycosylated avidin**

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Avidin is a biotin binding protein and belongs to the structural superfamily of calycins. This protein has been the object of various biochemical investigations due to its unique biotin binding properties. It is one of the strongest interactions in nature ( $K_d \approx 10^{-15}$  M) [1]. The various ligands are synthesized to modulate the strength of the interaction or to obtain desired conjugates which are of increasing importance regarding their biological applications [2, 3].

Here we present two crystal structures of avidin, a protein from hen egg white, in the form of a complex with two different ligands (biotin-ferrocene conjugate and 2-(4'-hydroxyazobenzene)benzoic acid). The overall fold of avidin consists of an eight-stranded antiparallel  $\beta$ -barrel, characterized by the conventional right-handed twist. The barrel provides a well-defined cavity for ligand binding, which is roughly oriented along the barrel axis.

The presented crystal structures show that the interactions of avidin with investigated ligands are different. A number of significant differences are visible what is connected with the structural constitution of ligand and the L3-4 loop conformation change.

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The interconnection between transcription and splicing is a subject of intense study. We report that Arabidopsis homologue of spliceosome disassembly factor NTR1 is required for correct expression and splicing of DOG1, a regulator of seed dormancy. Global splicing analysis in *atntr1* mutants revealed a bias for downstream 5' and 3' splice site selection and an enhanced rate of exon skipping. A local reduction in PolII occupancy at misspliced exons and introns in *atntr1* mutants suggests that directionality in splice site selection is a manifestation of fast PolII elongation kinetics. In agreement with this model, we found AtNTR1 to bind target genes and co-localise with PolII. A minigene analysis further confirmed that strong alternative splice sites constitute an AtNTR1-dependent transcriptional roadblock. Plants deficient in PolII endonucleolytic cleavage showed opposite effects for splice site choice and PolII occupancy compared to *atntr1* mutants, and inhibition of PolII elongation or endonucleolytic cleavage in *atntr1* mutant resulted in partial reversal of splicing defects. We propose that AtNTR1 is part of a transcription elongation checkpoint at alternative exons in Arabidopsis.

# **Aggrescan3D web server for protein aggregation prediction taking into account protein structure and its dynamic fluctuations**

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Protein aggregation underlies an increasing number of disorders and constitutes a major bottleneck in the development of therapeutic proteins. Our present understanding on the molecular determinants of protein aggregation has crystallized in a series of predictive algorithms to identify aggregation-prone sites. A majority of these methods rely only on sequence. Therefore, they find difficulties to predict the aggregation properties of folded globular proteins, where aggregation-prone sites are often not contiguous in sequence or buried inside the native structure. The AGGRESKAN3D (A3D) server [1] overcomes these limitations by taking into account the protein structure and the experimental aggregation propensity scale from the well-established AGGRESKAN method [2]. Using the A3D server, the identified aggregation-prone residues can be virtually mutated to design variants with increased solubility, or to test the impact of pathogenic mutations. Additionally, A3D server enables to take into account the dynamic fluctuations of protein structure in solution, which may influence aggregation propensity. This is possible in A3D Dynamic Mode that exploits the CABS-flex approach for the fast simulations of flexibility of globular proteins [3]. The A3D server can be accessed at <http://biocomp.chem.uw.edu.pl/A3D/>

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# Artificial peptidic capsules by chiral self-assembly and self-sorting

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Diversity of peptides, together with their biocompatibility, motivates intensive research on application of peptides as building blocks in the field of nanomaterials. Numerous examples of application of peptide-based materials have been reported including targeted drug delivery systems, materials for regenerative medicine, retroviral gene transfer and even capturing carbon dioxide from fuel gas. The limiting factor for many practical application is the synthesis. Synthesis of long peptides by classical methods (solid phase synthesis, NCA polymerization and genetic engineering) is labor intensive and often not applicable to the large scale. In this regard, utilizing relatively easily available short peptides in combination with dynamical covalent chemistry (DCC) is an appealing alternative. This approach can take advantage of peptides' natural tendency for self-assembly and of reversible character of chemical reactions to amplify the formation of complex functional structures. We report the results of our efforts to create discrete, closed-cup beta-barrel-type structures by biomimetic self-assembly and self-sorting of short peptides. Chirality of the peptides is used as an additional player, that allows for exploitation of new unnatural motifs. We also show that high thermodynamic and kinetic stability of the capsules results in highly hampered complexation abilities. Although the capsules retain their dynamic character allowing processes like chiral self-sorting and chiral self-assembly to work with high fidelity, guest complexation is not observed in non-polar environment. However, utilization of a reversible character of covalent bonds or application of mechanochemical methods results in quantitative complexation of very large guests, like fullerenes C60 and C70.

# Crystal forms of the Ovine and Caprine Serum Albumins

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Serum albumin is a three-domain, heart-like shape protein with mostly an alpha-helical structure. It represents up to 60% of all other proteins in the mammalian blood plasma. Such a high concentration of albumins is possible due to their very good solubility in water. The most interesting attribute of serum albumin is its capacity for binding a wide variety of ligands. Albumin can bind metal cations ( $\text{Ca}^{2+}$ ,  $\text{Na}^{+}$  and  $\text{K}^{+}$ ), fatty acids, hormones, metabolites, drugs and toxins. This protein has the greatest affinity for hydrophobic organic anions, with an average molecular weight (100-600 Da). Broad binding properties are a result of high flexibility and presence of three domains, which have numerous binding sites.

Until now, among even-toed ungulates' serum albumins, only crystal structure of bovine serum albumin (BSA) has been determined. [1] Sequential differences between even-toed ungulates' albumins are small. The identity between BSA and ovine serum albumin (OSA) equals 92%, this value is the same for BSA and caprine serum albumin (CSA). Sequences of OSA and CSA are identical in 98.5%. It gives only 8 different residues in all protein sequence which consists of 583 amino acids. Even these small differences influence flexibility of structure, capacity of binding pockets and affinity to ligands.

Such small differences in sequences between OSA and CSA affect also their crystallization properties. Both proteins crystallize in one common crystal form - P1, in the same crystallization conditions. Each of them produces also the crystals with higher symmetry: CSA in orthorhombic P212121 and OSA in trigonal P3221 space groups.

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# Structure and dynamics of newfound plant specific calcium sensor protein - SCS studied by mass spectrometry monitored hydrogen/deuterium exchange

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Plants as any other organisms are frequently exposed to harmful environmental conditions. To survive, plants developed complex defense mechanisms which are induced through specialized signaling pathways. The SnRK2 kinases subfamily is considered as a major player in plant response to osmotic stress. Recently negative regulator of SnRK2s activity was discovered in our Institute, a novel type of plant specific calcium sensor (SCS, SnRK2-interacting Calcium Sensor). SCS interacts directly with members of the SnRK2 subfamily causing their inactivation which was confirmed by in vitro studies. Currently there is no structural model of SCS, moreover SCS is quite unique because it possesses low sequence homology to proteins of known structure. According to Prosite prediction SCS contains one classical EF-hand motif and three putative non-canonical EF-hand.

Hydrogen deuterium exchange mass spectrometry (HDX-MS) was applied to investigate structure and dynamics of SCS protein in solution in spatially resolved manner. The pattern of hydrogen deuterium exchange along the protein sequence was obtained. Information about structural dynamics enable classification of specific regions in SCS in terms of flexibility. The most rigid regions with stable hydrogen bonds crucial for protein structural integrity, as well as unstructured parts of the protein was identified. In the absence of Ca<sup>2+</sup> ions the N-terminal part of SCS which contains classical EF-hand motif undergoes the fastest amide hydrogen deuterium exchange, which can be explained by the lack of stable hydrogen bonds in that region; in other words lack of secondary structure elements. Addition of Ca<sup>2+</sup> leads to dramatic conformational changes in the N-terminal part of the protein, which was inferred from decreased deuterium uptake. Changes in hydrogen deuterium exchange was not only observed in the region of canonical EF-hand but also in the regions classified as putative EF-hand motifs. HDX-MS results are in good agreement with molecular model of SCS obtained by homology modeling.

# Impact of sickle-cell mutation on structure and conductivity of DNA

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Single point mutations are not so uncommon in DNA structure. Most frequently, mutations arise from copying errors during DNA replication. Those mistakes could lead to a number of genetic disorders (e.g., color blindness, mucoviscidosis, sickle-cell anemia, etc.). The single A-T to T-A replacement in the 6th codon of the  $\beta$ -hemoglobin encoding sequence results in a dramatic misfolding of hemoglobin. This results in the overall red-blood cell shape-deformation (sickle shape) that leads to sickle cell anemia (SCA) [1]. Death rates caused by sickle cell anemia have increased significantly since 1990 [2].

Since DNA is known to be capable of conducting current, it is of great interest to examine the effect of SCA mutation on its conductivity. In particular, SCA detection at an early stage of child development in a noninvasive way (e.g., biosensors with nontoxic hybridization indicator, for instance riboflavin) is highly desirable.

We selected a 15 nucleotide single stranded DNA chain from the hemoglobin sequence (HBB) to create a sickle cell anemia biosensor. We observed that the conductivity of DNA is sensitive to the SCA mutation. Our experimental studies are accompanied by molecular dynamics simulations and ab initio calculations that shed further light on the nature of the observed conductivity of SCA mutated DNA.

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# Monte Carlo simulations of peptide docking associated with large conformation transitions of the binding protein

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Most of the current docking procedures are focused on fine conformational adjustments of assembled complexes and fail to reproduce large-scale protein motion. In this paper we test a new modeling approach developed to address this problem. CABS-dock is a versatile and efficient tool for modeling the structure, dynamics and interactions of protein complexes. The docking protocol employs a coarse-grained representation of proteins, a simplified model of interactions and advanced protocols for conformational sampling. CABS-dock is one of the very few tools that allow unrestrained docking with large conformational freedom of the receptor.

In an example application we modeled the process of complex assembly between two proteins: Troponin C (TnC) and the N-terminal helix of Troponin I (TnIN-helix) which occurs *in vivo* during muscle contraction. Docking simulations illustrated how the TnC molecule undergoes significant conformational transition on complex formation, a phenomenon which can be modeled only when protein flexibility is properly accounted for. This way our procedure opens up a new possibility for studying mechanisms of protein complex assembly, which may be a supporting tool for rational drug design.

# SUPRAMOLECULAR ARCHITECTURES BASED ON HALOGEN BONDING INTERACTIONS

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The basic task of crystal engineering is the design and synthesis of new materials with the desired architecture. Significant attention is given to the porous materials like zeolites, metal-organic frameworks, organic frameworks, due to their useful properties, e.g. sponge-like behavior, molecular storage or separation. Low framework density, low toxicity and controllable synthesis make the organic frameworks very attractive materials.

The approach that enables control of the supramolecular architecture of the porous materials relies on the building blocks with specific complementary functional groups that, in most cases, utilize directional nature of hydrogen bonds in self-assembly process. However, in recent years, some other directional interactions with strength comparable to hydrogen bonds, like aromatic interactions, halogen and chalcogen bonds, became a new popular tool in crystal engineering.

In the course of our research on organic porous materials, new frameworks based on two different halogen-bond donors, 1,3,5-trifluoro-2,4,6-triiodobenzene and 1,3,5-tris(iodoethynyl)-benzene, and tritopic halogen-bond acceptor, 2,4,6-tri(4-pyridyl)-1,3,5-triazine, were designed. Cocrystallization of these D<sub>3h</sub>-symmetric components resulted in the formation, via N $\cdots$ I halogen bonds, of porous layers with slightly distorted hexagonal symmetry. More importantly, no interpenetration of the neighboring layers was observed and spacious channels, filled with solvent molecules, constituting more than 50% of the crystal volume were formed.

# A study of the low resolution structure of human SGT1 protein in solution

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The human SGT1 (HsSGT1) protein (suppressor of G2 allele of SKP1) is involved in numerous cellular processes, including participation in the kinetochore assembly during cell division and innate immunity by the interaction with NB-LRR receptors [3, 6]. This protein works as co-chaperone of HSP90 protein as well as interacts with the SCF E3 ubiquitin ligase complex [6]. SGT1 also forms complex with the S100A6 (calcyclin) – protein involved in the calcium signalling [2]. Full length SGT1 protein possesses three domains: TPR (tetratricopeptide repeat), CS (CHORD- and SGT1-containing) and SGS (SGT1-specific), connected by variable regions denoted as VR1 and VR2, which are less conserved in comparison to the previously mentioned domains [1]. CS domain was shown to be responsible for the interaction with HSP90 protein. For SGS domain, the involvement in interactions with LRR domains of NB-LRR receptors and S100 protein was found [7]. In addition, the SGT1 protein was shown to be overexpressed in cancer cells and to reduce their sensitivity to chemotherapy [5], which makes it a possible drug target. There is little information about conformation of the full-length HsSGT1 protein in solution.

In this study we investigated the low-resolution structure and conformation of human SGT1 protein in solution using the small angle X-ray scattering (SAXS) technique. The data from SAXS experiments confirmed that HsSGT1 protein exists as a monomer in the experimental conditions, which is opposite to its plant and yeast orthologs that form dimers in solution [4]. Using *ab initio* and rigid-body modelling approaches, we were able to obtain a low-resolution model of the HsSGT1 protein. In addition, we also studied its truncated form without the SGS domain (HsSGT1 $\Delta$ SGS). Both proteins exhibit elongated shape in solution. Using the rigid body modelling and circular dichroism experiments, we showed that the variable regions and the SGS domain are mostly unstructured. Ensemble modelling using the EOM software confirmed that the full-length HsSGT1 and HsSGT1 $\Delta$ SGS proteins are highly dynamic in solution, which is due to flexibility of the VR1 and VR2 regions as well as the SGS domain. The flexible structure could account for the observed function, especially the diversity of interaction and co-chaperone activity, which requires binding of structurally different partners. Despite some differences in sequence, for example significantly shorter VR1 [1], the HsSGT1 shares the shape and flexible structure with its plant ortholog [4].

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# Synthesis, structure and complexation properties of peptidic molecular capsules

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Self-assembled capsules composed of amino acid or peptide derivatives connected with resorcin[4]arene scaffolds using acylhydrazone linkers were synthesized using a dynamic covalent chemistry (DCC) approach. The reversible formation of the linker combined with non-covalent self-assembly of the peptidic fragments result in effective self-sorting and quantitative formation of homochiral dimeric capsules from mixtures of substrates (hydrazides) of different chirality. These acylhydrazone capsules exhibit higher thermodynamic and kinetic stability and have larger cavities as compared with similar peptidic capsules based on imine linkers. The high thermodynamic and kinetic stability of the capsules results in highly hampered complexation abilities. In consequence, guest complexation is not observed in solution. However, utilization of a reversible character of covalent bonds or application of mechanochemical methods results in quantitative complexation of even very large guests, like fullerenes C<sub>60</sub> and C<sub>70</sub>. The structures of capsules as well as complexation of fullerenes was studied using single crystal X-ray crystallography and various NMR techniques. Due to high inherent symmetry, chirality and lack of heavy atoms the structures present a significant challenge for a crystallographer. Determination of two of them included X-ray diffraction measurements using a synchrotrone radiation source (MaxLab II synchrotrone in Lund, Sweden). In the NMR spectra the influence of chiral environment on the symmetry of fullerenes is clearly visible, resulting in differentiation of diastereotopic carbon atoms in C<sub>70</sub> (in agreement with reduced D<sub>5</sub> symmetry).

# **Enzyme stability enhancement: A case study on l-threonine aldolase**

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Threonine aldolase (TA) is a very promising enzyme that could be used to prepare biologically active compounds or building blocks for pharmaceutical industry. TA can be used to catalyze aldol reaction between glycine and a variety of aromatic and aliphatic aldehydes, thus creating various alpha-amino-alcohols. Rational design was applied to thermophilic TA from *Thermotoga maritima* to improve thermal stability by the incorporation of salt and disulfide bridges between subunits in the functional tetramer and design fully functional dimer. An activity assay together with CD analysis and Western-blot detection was used to evaluate mutants. Incorporation of disulfide bond was confirmed by MALDI-MS. Two of the proposed single point mutants, P56C and A21C displayed significantly enhanced stability compared to the wild type (WT). Its initial activity was not affected and persisted longer than WT, proportionally to increased stability.

# Human Prolidase Mutations <sup>2</sup>C Structural basis of Prolidase Deficiency

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Prolidase is a multifunctional enzyme whose biological relevance, its structure and mechanism of catalysis are still only partially understood. In humans prolidase is the only metalloenzyme that cleaves the iminodipeptides containing a proline or hydroxyproline residue at the C-terminal end. Prolidase deficiency (PD) is a rare recessive disorder characterized by diminished prolidase activity and manifested by variety of clinical symptoms.

Several mutations responsible for loss of prolidase activity were identified, but the structural basis of the enzyme inactivation mechanism remains unknown. The aim of this study is to determine the influence of single amino acids substitutions or deletions on prolidase structure. Those structures will help in understanding the mechanism of enzyme inactivation. In this study selected mutants will be studied by X-ray diffraction.

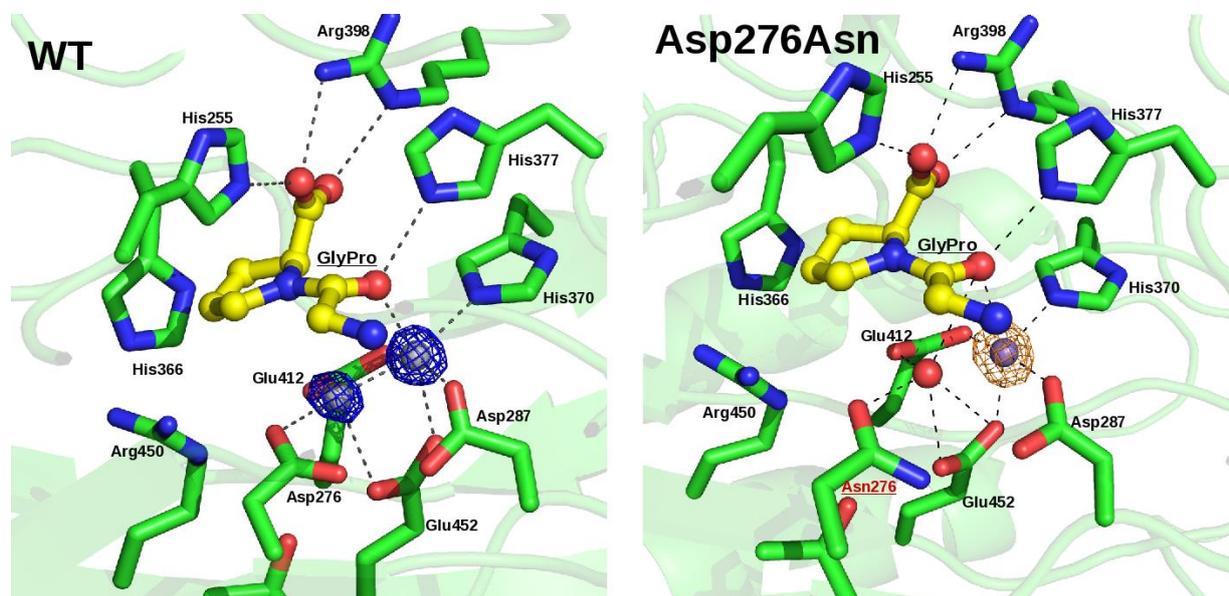


Figure 2: In order to identify ions in the active site crystals were soaked with  $MnCl_2$  and GlyPro solution and diffraction images were collected above and below Mn absorption edge. For wild-type (WT) 2Fo-Fc maps contoured at  $2.5\sigma$  shows position of two sodium ions. For mutants anomalous difference maps (AnoDe) contoured at  $10\sigma$  shows positions of manganese ions. Active site mutation is highlighted red. Anomalous difference maps were calculated with the use of *ANODE*.

# Crystallographic approach to identification of the 20S proteasome non-covalent binding sites

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The proteasome is a molecular machine that is a central for degradation of proteins involved in the regulation of the cell cycle as well as misfolded or otherwise damaged proteins whose accumulation is cytotoxic for the cell. Defects in functioning of the proteasome proteolytic system play a causal role in a number of diseases, including various cancers, autoimmune, cardiovascular and neurodegenerative diseases, what makes the proteasome a very promising therapeutic target.

So far, the only proteasome-targeting agents used in clinics are competitive inhibitors, directly blocking the enzyme's active sites. However, they are not selective enough and can block all active sites causing cell apoptosis. The 20S proteasome has active sites hidden in its interior. This feature of its structure encourages to test compounds which can target allosteric interactions between subunits and influence the gating mechanism, involved in the control of the substrates intake. Such modulators may provide a more precise and substrate-specific regulation and enable not only inhibition but also activation of the proteasome.

A variety of biomolecules, able to interact with the outer ring of the 20S proteasome and influence the enzyme activity, is already known. Among such natural regulators are Blm10 protein and PR-39 peptide. We have utilized them as templates for a design of low- molecular mass modulators of proteasome activity. Some of the synthesized compounds occurred to be very efficient in the 20S proteasome inhibition and the others were quite potent proteasome activators. The interesting results encourage us to crystallize complexes of 20S proteasome with the most effective allosteric modulators.

The enzyme was isolated from *Saccharomyces cerevisiae* and then purified in three steps: anion exchange chromatography, hydroxyapatite chromatography and gel filtration. Hanging drop vapor diffusion technique was used for the crystallization of proteasome. The best diffracting crystals were obtained under following conditions: 25 mM Magnesium acetate, 0.1 M MES 6.5, 14% (v/v), MPD. The obtained crystals were soaked with the selected compounds. Protein concentration used for the experiment was 4.5 mg/ml and data were collected using synchrotron beamline (BESSY II, Helmholtz-Zentrum Berlin and the Advanced Photon Source Argonne National Laboratory, Chicago). The structure was solved using molecular replacement technique. We determined the place of binding of our two small-molecule allosteric activators, which bind at the same place and in a similar manner as the protein activator Blm10.

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# QTAIM properties in amino acids and tripeptides derived from X-ray crystallography – comparison of various refinement methods

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The most commonly applied method of electron density refinement based on high resolution X-ray data, the multipole model (MM) proposed by Hansen and Coppens [1], constitutes a huge progress in comparison with the Independent Atom Model (IAM). The major improvement results from the description of valence electron density thanks to using aspherical atomic scattering factors; however the multipole model has still certain limitations such as problems with modeling polar bonds, heavier atoms or estimation of interaction energies. The remedy for these problems could be applying a model of electron density enabling more flexibility in the atomic core region. A good example of such a model is the one based on Hirshfeld partition of electron density utilized in the procedure of Hirshfeld Atom Refinement (HAR) [2] subsequently followed by experimental wave function fitting (XCW) [3]. The first stage, HAR, is used to obtain better positions of atoms exclusively from X-ray data but skicks to purely theoretical electron density; in the second phase, XCW, further refinement of electron density, introducing experimental contribution to the wave function is performed.

In this work a comparison between MM, HAR and XCW is made with reference to the quality of electron density reconstructed from crystallographic measurements for a set of 4 amino acids and 6 tripeptides, for which high resolution X-ray data is available. QTAIM [4] is used as a tool to analyze the obtained electron density basing on the atomic charges, dipoles and volumes, as well as properties calculated in the bond critical points. The influence of isotropic and anisotropic refinement of hydrogen thermal motions is investigated, as well as the effect of removing the most outlying reflections. The results are checked against benchmark properties from periodic DFT calculations in CRYSTAL09. The outcome of the research is not unambiguous and straightforward to interpret. In the overall view HAR appears as the method most matching the benchmark theoretical calculations, the other methods diverge significantly more and for MM the discrepancy seems slightly bigger than for XCW. There is a general tendency for XCW to shift the obtained values away from HAR and theoretical values and make them approach the MM results. This can be interpreted as the capability of XCW to reveal the differences between purely theoretical and experimental electron density. The surprising similarity of the XCW to the MM results encourages not to underestimate the potential of MM. It also begs the question whether theoretical electron density is the right benchmark to decide about the quality of electron density reconstructed from crystallographic experiments.

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# Interactions of peptide nucleic acids with ribosomal RNA helix 18

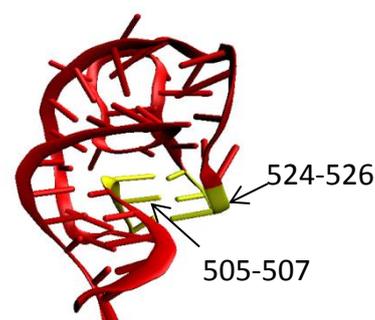
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Due to constantly developing bacterial resistance to known antibiotics, new antibacterial agents have to be developed. Our goal is to use peptide nucleic acid (PNA) oligomers to target functional sites of bacterial ribosomal RNA (rRNA) and inhibit bacterial translation. PNA is a neutral DNA mimic containing a pseudo-sugar backbone so targeting is based on complementary pairing of PNA with ribosomal RNA and steric blocking of the functional RNA fragment. However, the binding strength depends on the secondary and tertiary structures of the targeted rRNA region<sup>1</sup>.

We chose to target an rRNA site that is important for ribosome function and accessible to hybridization with PNA. Helix 18 is a conserved fragment of 16S rRNA formed by nucleotides 500 to 545 (according to *Escherichia coli* numbering)<sup>2</sup>. Its secondary structure contains a bulge and internal loop and nucleotides 505-507 and 524-526 form a three-dimensional pseudo-knot (see Figure). Distortion of this pseudo-knot affects ribosome function<sup>3</sup>. Therefore, targeting nucleotides in helix 18, especially those taking part in the pseudo-knot, should be an effective strategy to inhibit translation.



First, we theoretically verified the structural stability of isolated helix 18, i.e. without the ribosome context, using Motif Identifier for Nucleic acids Trajectory (<http://mint.cent.uw.edu.pl>). Second, we analyzed hybridization of PNA (of a sequence GCG GCT GCT GGC A-Lys) with helix 18 in solution using non-denaturing polyacrylamide gel electrophoresis, thermal denaturation, and fluorescence and circular dichroism spectroscopy. Third, we used PNA covalently linked with a cell-penetrating peptide (KFF)<sub>3</sub>K. PNA is often attached to a positively charged cell-penetrating peptide that ensures its transport to bacterial cells. We added this peptide to see if it influences the interactions between the PNA and helix 18. We have also tested the antibacterial effects of the PNA-peptide conjugate. The *E. coli* K-12 strain was used for determine the concentrations at which this conjugate inhibits bacterial growth<sup>4</sup>.

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# One conformer to rule them all? A study on ligands multiple conformations in molecular docking experiments.

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Molecular docking proved its value many times, although still provides too many false positives. Therefore, a lot of work must be done in terms of high throughput virtual screening (HTVS), to improve both precision and accuracy. In this work we evaluate a popular belief that taking into account many conformations during virtual screening/docking experiments can be beneficial for the analysis outcome.

Herein we present a comprehensive analysis of DUDE database, a popular benchmark for docking performance. Ligand conformations were obtained for entire DUDE database by downloading native DUDE conformations, and by using two docking programs; UCSF Dock 6.5 and Autodock Vina. Ligand-receptor complexes were evaluated with 3 native scoring functions and three methods of ranking ligands were evaluated: (1) best single conformer; (2) mean score and (3) RankScore1 [1], which discretizes ligands-receptor score to conformer position in a ranking list. Analysis results were compared by the area under receiver operating characteristic curve (ROC AUC).

All evidence derived from our analysis point out that taking into account many diverse conformations does not increase performance in any predictable and statistically significant manner. Although there are examples of impressive performance boosts, no correlations with common ligand nor protein features seem to be present. This lack of correlation basically suggests the observed effect is rather a scoring/docking method error than any other reason. Therefore, we conclude that while using any state of the art scoring function in HTVS campaigns, a single best conformer should rule all the other conformers out of the docking pipeline.

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# **Unfolded N-terminal Domain as Important Factor for Analysis Molecular Dynamic Processes in Folded C-terminal Part in Human Prion Protein.**

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Prion diseases observed in humans and other animals named as Transmissible spongiform encephalopathies (TSE) are a group of neuropathies caused by conformational changes in the prion protein (PrP). The initiation and propagation mechanisms of conversion of normal cellular (PrPC) to the protease resistant PrP<sup>Sc</sup> form and role of unfolded N-terminal domain in that transformation are still very illusive. Molecular dynamic processes constitute one of the important factor which facilitates and controlled all steps of the conversion. In presented study, <sup>15</sup>N Relaxation Dispersion data sets were acquired on two magnetic fields (16.4 T and 18.8 T) and used to extract information about backbone low frequency motions in the human wild-type PrPC (23-231, M129) protein. Evaluated data for whole length PrPC protein were analyzed together with previously obtained <sup>15</sup>N relaxation data for shorter PrPC (90-231, M129) construct with Reduce Spectral Density Mapping and ModelFree formalisms. Performed analysis reveals effects of existence N-terminal domain on molecular dynamic processes in whole PrPC protein. The low frequency motions could triggered the initial steps of transforming the cellular PrPC into the pathogenic PrP<sup>Sc</sup> form.

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# **Mammalian serum albumin complexes with naproxen, a representative of nonsteroidal anti-inflammatory drugs**

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Serum albumin, extracellular protein constituent predominant in the blood plasma of vertebrates, is one of the most extensively investigated macromolecules. A heart shape of serum albumin, divided into three  $\alpha$ -helical domains, is a characteristic structural feature of all mammalian albumins. Albumins are known as transport proteins, responsible for binding both endogenous and exogenous ligands and play a crucial role in the delivery of many molecules such as fatty acids, drugs, metal ions, steroids, metabolites or toxins [1]. These multidomain macromolecules attract great interest from the pharmaceutical industry since they show extraordinary ligand binding properties and have a major influence on the pharmacokinetics and pharmacodynamics of various compounds.

Naproxen, (S)-2-(6-methoxynaphthalen-2-yl) propanoic acid (NPS), a member of the class of nonsteroidal anti-inflammatory drugs (NSAIDs), is an effective and well-tolerated drug with low hepatotoxicity, commonly used for the reduction of pain and fever. Its mechanism of action is based on the inhibition of cyclooxygenase isoenzymes resulting in blocking the activity of prostaglandins, which act as messenger molecules in the process of inflammation.

We present three crystal structures of bovine (BSA), equine (ESA) and leporine (LSA) serum albumin complexes with naproxen (NPS) [2]. Our structural investigations show that non-human serum albumins bind more than one NPS molecule in the absence of fatty acids indicating high binding affinity of naproxen to these three serum albumins. Small differences in sequences of mammalian serum albumins influence the affinity and the binding mode of naproxen to this transport protein. The comparative structural analysis of BSA, ESA, LSA and HSA complexes with naproxen has been discussed.

This research was supported by grant 2013/11/B/ST5/02271 from the National Science Centre.

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## Better quality structures from routine X-ray data collection

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Routine structural single crystal X-ray analysis is now rather technical than scientific activity. However, it still can be improved. In this communication, a critical discussion of the dependence of structural parameters on resolution and model of electron density is presented.

A multiple data sets are analysed originating from 12 charge-density-quality data sets of  $\alpha$ -oxalic acid dihydrate ( $C_2H_2O_4 \cdot 2H_2O$ ). These high resolution data sets are trimmed to lower resolution. Independent Atom Model (IAM) and Transferable Aspherical Atom Model (TAAM), Hirshfeld Atom Refinement (HAR) are tested. It will be shown how structural and thermal parameters (and their errors) obtained for different models of electron density (IAM, TAAM, HAR, MM) refined against the same diffraction data sets are dependent on the  $2\theta_{max}$  diffraction angle. Accuracy and precision of structural data will be discussed. Some practical suggestions will be presented how to estimate and improve the quality of single crystal X-ray diffraction structural results.

We will show that in order to get more precise and accurate results of refinement, single crystal X-ray data should be collected to the higher, than commonly used,  $2\theta_{max}$  diffraction angles (for  $MoK\alpha$   $2\theta_{max} > 65^\circ$ ).

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Keywords: IAM, TAAM, HAR, accuracy, precision,



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