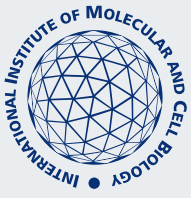
# Annual Report 2011

INTERNATIONAL INSTITUTE
OF MOLECULAR AND CELL BIOLOGY




Director
**Jacek Kuźnicki**

Deputy Scientific Director
**Michał Witt**

Deputy Director
**Jacek Jaworski**

Financial Manager
**Hanna Iwaniukowicz**

Chaiperson of the International Advisory Board
**Anna Tramontano**

Deputy Chairperson of the International Advisory Board
**Ineke Braakman**

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# Directors' Note

Last year's motto could relate to the Institute's significant additions to its resources of equipment. The structural funds obtained for the Centre for Preclinical Research and Technology (CePT) project made it possible for us to equip our laboratories with state-of-the-art equipment worth approximately EUR 3.5m. Thanks to these new additions, we will strengthen our capacity to carry out multi-faceted research into proteins and nucleic acids and their macromolecular interactions. First of all, we should mention the protein crystallography platform with crystallization robot and microfocus high-flux X-ray diffractometer equipped with a CCD detector, which replaced the long-serving Rigaku machine. Still, it is worth mentioning that the latter was, at the time of its purchase, the most advanced item of equipment of this type in Poland. But, if you have two dynamic protein crystallography groups on board you have to be up to their standard! Thanks to the expansion of our set of equipment, including mass spectrometers, laser gel scanners, spectrophotometers, ultracentrifuges (including an analytical one) and other advanced units, our Centre for Protein Structure and Function Analysis can position itself among the best-equipped research centres which specialize in proteins/nucleic acids, on a scope beyond the national scale. This will also open up opportunities for new relationships of scientific cooperation, including cooperation at the implementation stage. A continuation of this trend is being launched at the moment, in cooperation with the Max Planck Institute for Heart and Lung Research in Bad Nauheim, in the form of a zebrafish lab, which will be the first Polish laboratory to work on this universal animal model. In the Institute, there is a huge demand for work on this model and, therefore, we have great expectations regarding the new lab.

Among other important events this year we should also specifically mention two new, very prestigious grants from the European Research Council and yet another publication in *Nature*. Thanks to our cooperation agreement with the Intercollegiate Faculty of Biotechnology at the University of Gdańsk – Medical University of Gdańsk, we will run a joint PhD School project, which is a move that has always been strongly recommended to us by our International Advisory Board. Moreover, the unit which deals with technology transfer (Biotech-IP) has to handle an increased workload related to the preparation of patent applications and negotiations with potential users, which is a clear indication that some aspects of our research may have an innovative side to them. These and other ventures, as well as the successes recorded by our staff in terms of research results and grant money, all contribute to our sense of satisfaction and our belief that the Institute continues to develop on the right path.

This sense of satisfaction is somewhat disrupted by the fact that we have no opportunities to expand our premises, since the issue of the new seat of the Institute remains unresolved. We are aware that, despite the obvious scientific successes of the Institute, this restricting factor may become a decisive hindrance to further development in a time perspective view. In respect to this, we count on support from the Ministry of Science and Higher Education and the authorities of the Polish Academy of Sciences, and on ongoing cooperation with the municipal authorities of the Capital City of Warsaw.

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# Directors and Administration

**Jacek Kuźnicki**
Director

**Michał Witt**
Deputy Scientific Director

**Jacek Jaworski**
Deputy Director

**Hanna Iwaniukowicz**
Financial Manager

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Katarzyna Dąbrowska
Domestic Grants Administrator

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Agnieszka Karbowska
Director's Representative for Administrative Matters
Robert Banasiak
Maintenance Specialist
Dorota Makulska
Secretary

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Marcin Ogonowski
International Cooperation Specialist
Magdalena Powierża
International Cooperation Specialist, Technology Transfer Unit – Bio & Technology Innovations Platform, Unit Manager

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Accounting Specialist
Hanna Iwaniukowicz
Financial Manager
Monika Nowicka
Payroll Specialist
Agnieszka Kuna
Accounting Specialist
Renata Knyziak
Accounting Specialist
(not on the picture)

**Human Resources Unit**
Beata Tkacz
Human Resources Specialist
Monika Domańska-Paśko
Human Resources Specialist

# International Advisory Board of the International Institute of Molecular and Cell Biology

## 2010-2014 term

**Participants of the meeting of the International Advisory Board, May 2011**
From left (first row): I. Braakman, A. Tramontano, H. Saibil; (second row): A. Wlodawer, J.G. Sutcliffe, J. Kuźnicki (non-member), K. Hahlbrock, I. Dikič; (third row): O.A. Krishtal, F. van Leuven, W. Filipowicz, J. Mallet, D. Picard, N. Blin, W. Huttner, M. Witt (non-member).

***Chairperson:*** **Anna Tramontano**
***Deputy Chairperson:*** **Ineke Braakman**

***Members:***

**Francisco E. Baralle.** Director-General of International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

**Nikolaus Blin.** Institute of Human Genetics, University of Tuebingen, Tuebingen, Germany; Foreign member of Polish Academy of Sciences

**Ineke Braakman.** Department of Cellular Protein Chemistry, Utrecht University, Utrecht, Netherlands

**Ivan Dikič.** Institute of Biochemistry II, Goethe University Medical School, Frankfurt am Main, Germany

**Robert P. Erickson.** Department of Pediatrics, Section of Medical and Molecular Genetics, The University of Arizona, Health Sciences Center, Tucson, USA

**Witold Filipowicz.** Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

**Klaus Hahlbrock.** Professor Emeritus, Max-Planck Institute for Plant Breeding Research, Köln, Germany

**Wieland Huttner.** Executive Director, Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

**Oleg Aleksandrovich Krishtal.** Deputy Director of the Bogomoletz Institute of Physiology, Head of the Department of Cellular Membranology, Bogomoletz Institute of Physiology, Kiev, Ukraine

**Fred van Leuven.** Experimental Genetics Group, Department of Human Genetics, Katholieke Universiteit Leuven, Leuven, Belgium

**Jacques Mallet.** Directeur de recherché, Laboratoire de Genetique Moleculaire de la Neurotransmission et des Processus Neurodegeneratifs, CNRS, Hopital de la Pitie-Salpetriere, Paris, France

**Maciej J. Nałęcz.** Director, Division of Basic and Engineering Sciences, UNESCO, Paris, France

**Didier Picard.** Department of Cell Biology, University of Geneva, Sciences III, Geneve, Switzerland

**Helen Saibil.** Department of Crystallography, Birkbeck College London, Institute for Structural and Molecular Biology, London, UK

**J. Gregor Sutcliffe.** Department of Molecular Biology, The Scripps Research Institute, La Jolla, California, USA

**Adam Szewczyk.** Director, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

**Anna Tramontano.** I Medical Faculty, University of Rome "La Sapienza", Rome, Italy

**Alexander Wlodawer.** Chief, Macromolecular Crystallography Laboratory, National Cancer Institute, Frederick, USA

# Description of the Institute's Activities

**Relation of IIMCB to PAN**

The International Institute of Molecular and Cell Biology is directly subordinate to the President of the Polish Academy of Sciences (PAN), who supervises the organization and activities of the Institute. The President of PAN nominates members of International Advisory Board (IAB) and the Institute's Directors.

**The Organization of Research at IIMCB**

Nine research groups comprised the structure of IIMCB in 2011: Department of Molecular Biology (Żylicz), Laboratory of Bioinformatics and Protein Engineering (Bujnicki), Laboratory of Structural Biology (Bochtler), Laboratory of Neurodegeneration (Kuźnicki), Laboratory of Cell Biology (Miączyńska), Laboratory of Molecular and Cellular Neurobiology (Jaworski), Laboratory of Cell Cortex Mechanics MPG/PAN in Dresden (Paluch), Laboratory of Protein Structure (Nowotny) and Laboratory of Mitochondrial Biogenesis (Chacińska). The research carried out at IIMCB is mainly focused on fundamental biomedical problems. The major research topics include the following:

1. Role of molecular chaperones in cell transformation, including analysis of interactions between human wildtype p53 and mutant p53 with molecular chaperones and oncogenic activity of MDM2 (Żylicz group).
2. Development and application of computer software for structural bioinformatics of proteins and nucleic acids and theoretical and experimental studies of enzymes that act on nucleic acids (protein and RNA structure prediction and modeling, protein engineering, evolutionary analyses, and structure and function determination) (Bujnicki group).
3. Crystallographic structure determination of biological macromolecules (Bochtler group).
4. Studies of calcium and β-catenin signaling in the brain and molecular mechanisms of neurodegeneration (Kuźnicki group).
5. Interdependence between intracellular endocytic transport and nuclear signal transduction (Miączyńska group).
6. Molecular processes, including gene transcription, kinase-dependent cell signaling, cytoskeleton dynamics, intracellular trafficking that underlies neuronal development and plasticity, and central nervous system pathologies (e.g., tuberous sclerosis, epilepsy, and neurodegenerative disorders) (Jaworski group).
7. Mechanics of the actomyosin cortex, study of cortical contractility and the role of cortical mechanics during cytokinesis and migration (Paluch group).
8. Structural and biochemical studies of nucleic acid enzymes (Nowotny group).
9. Biogenesis of mitochondrial proteins, protein transport mechanisms, redox processes in mitochondria (Chacińska group).

**Awards and Honors**

- **Maciej Żylicz** received the title of **Doctor Honoris Causa of the University of Gdańsk** for his outstanding contribution to the development of biochemistry and molecular biology, especially for his research on chaperone proteins, also for creating an environment for promoting the development of science in Poland and his contribution to the development of the University of Gdańsk
- **Michał Witt** was elected the **Chairman of the Advisory Board for Molecular Genetic Tests and Biobanking**, established at the Ministry of Science and Higher Education. The Board is comprised of geneticists, lawyers, representatives of parental support groups, bioethicists, oncologists and laboratory diagnosticians
- **Jacek Jaworski** received the **Award of the Prime Minister for the habilitation thesis**
- **Maciej Żylicz** was elected a **member of the German National Academy of Sciences Leopoldina, and a member of the Senate of the German Max Planck Society**
- **Janusz Bujnicki** was elected a **member of the Young Scientists Academy** (Akademia Młodych Uczonych), an appendix to the Polish Academy of Sciences (PAN)
- **Marcin Nowotny** received prestigious **International Early Career (IECS) award granted by Howard Hughes Medical Institute**
- **Elżbieta Purta** from the Laboratory of Bioinformatics and Protein Engineering and **Monika Sokołowska** from the Laboratory of Structural Biology have received **scholarships for Outstanding Young Scientists funded by the Ministry of Science and Higher Education**
- **Łukasz Świech** from the Laboratory of Molecular and Cellular Neurobiology and **Jakub Sędziński** from the Laboratory of Cell Cortex Mechanics MPG/PAN in Dresden have received **EMBO Long Term Fellowships**
- **Grzegorz Chojnowski, Stanisław Dunin-Horkawicz, Katarzyna Kamińska, Irina Tuszyńska, Grzegorz Łach, Tomasz Waleń** from the Laboratory of Bioinformatics and Protein Engineering, **Małgorzata Figiel** and **Marcin Jaciuk** from the Laboratory of Protein Structure, **Monika Sokołowska** and **Marek Wojciechowski** from the Laboratory of Structural Biology have received funding from the Ministry of Science and Higher Education within the **Iuventus Plus Initiative**
- **Marta B. Wiśniewska**, **Katarzyna Misztal**, **Wojciech Michowski**, Marcin Szczot, **Elżbieta Purta**, Wiesława Leśniak, **Monika E. Klejman**, Michal Dąbrowski, Robert K. Filipkowski, **Andrzej Nagalski**, Jerzy W. Mozrzymas and **Jacek Kuźnicki** received the **2011 Jerzy Konorski Award** of the Polish Neuroscience Society and Neurobiology Committee PAN for the best research paper in the field of neurobiology for their paper: *LEF1/ß - Catenin Complex Regulates Transcription of the Cav3.1 Calcium Channel Gene (Cacna1g) in Thalamic Neurons of the Adult Brain*, published in the Journal of Neuroscience 2010 (30) 14: 4957-69
- **Elżbieta Purta** from the Laboratory of Bioinformatics and Protein Engineering has received the **Drabikowski Award** of the Polish Biochemical Society for the best PhD thesis in 2010 *Identification and characterization of new RNA modifying enzymes* (advisor: Janusz M. Bujnicki)

- **Iwona Cymerman** from the Laboratory of Molecular and Cellular Neurobiology, **Adam Sobczak** former postdoctoral fellow in the Laboratory of Neurodegeneration and **Magdalena Powierża** from the International Cooperation Unit received a nomination for the first forty participants of the **Top 500 Innovators - Science Management Commercialization Programme**. Nominations were handed by the Prime Minister Donald Tusk and the Minister of Science and Higher Education, Barbara Kudrycka
- **Nikola Brożko**, a graduate student in the Laboratory of Neurodegeneration, was awarded with the **"Girls of the Future" prize** by the Ministry of Science and Higher Education and ELLE magazine
- **Katarzyna Kamińska** received the **L'Oreal Award for Women in Science**
- **Elżbieta Purta** received the **award** from Polish Biochemical Society and MERCK **for best doctoral thesis in biochemistry** defended in 2010
- **International Institute of Molecular and Cell Biology** is a laureate of the **Funds for Science 2011 awards** in the commercialization of scientific research category for the project: "Tools and methods useful in characterising the immunotoxic activity of xenobiotic substances" implemented under the Operational Programme Innovative Economy, sub-measure 1.3.2 Support for the protection of industrial property generated in scientific entities as a result of R&D works
- **Katarzyna Kamińska** and **Grzegorz Chojnowski** were awarded with **START Fellowship** for young scientists by Foundation for Polish Science (FNP).

## Bio-Technology Innovations Platform

Technology Transfer Unit "BioTech-IP" (Bio Innovations & Technology Platform) was established in 2010 to support commercialization of research results of scientists working in Warsaw in six institutes affiliated to the Ochota Biocentre research consortium.

Biotech-IP is the first contact point for companies interested in carrying out research in Ochota Biocentre institutes and for scientists who want to sell their technologies and patents in areas such as biotechnology, biomedicine, bioinformatics, bioengineering, material technologies and bionanotechnology.

**Tasks of the unit:**

- to encourage creative and entrepreneurial attitude in the academic environment by supporting creative activities and to promote the commercial exploitation of research results
- to raise awareness of intellectual property protection among academics through series of scouting and promotional activities
- to search for and to verify research projects with a strong commercial potential as well as commercialization through spin-off companies formation or licensing of technologies to industrial partners
- to support spin-off companies created by researchers commercializing research results and technologies generated by the Ochota Biocentre institutes
- to initiate science-business networking activities and to get in contact with business angels, venture funds and business institutions
- to promote research services offered by Ochota Biocentre.

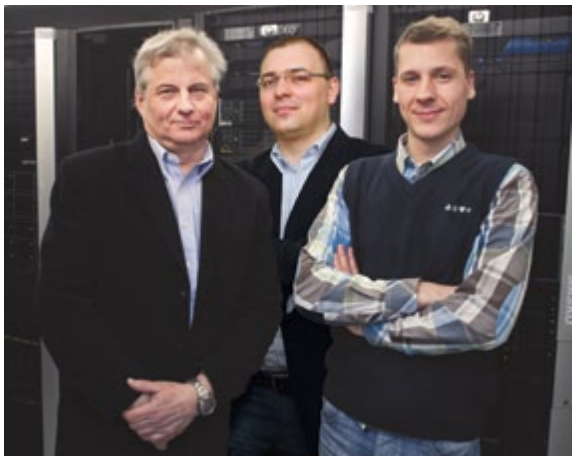
Two of BioTech-IP managers completed a prestigious 2 month internship at Stanford University (October-December 2011), organized by the Ministry of Science and Higher Education, where they learned technology transfer techniques and IPR protection.

IIMCB owns one patent that resulted from earlier scientific projects by Grzegorz Kudła ("The method of enhancement of expression of recombinant proteins in mammalian cells"; P370282). Another important invention was authored by Jarosław Dastych ("Cells and methods useful in characterising the immunotoxic activity of xenobiotic substances"; PCT/PL 03/00098). The invention is subject to a pending patent procedure in seven European countries and the USA and was commercialised by the formation of Proteon Pharmaceuticals Ltd. (a spin-off company; http://proteonpharma.com/). The patent procedure is supported by the Operational Programme Innovative Economy 1.3.2 in a project that was awarded by the ProRegio Foundation as the best commercialization project funded by the structural funds.

With the help of BioTech-IP, the Institute applied for five additional patents.

**Magdalena Powierża** Head of the Unit
**Leszek Lipiński** Industrial Cooperation Manager
**Adam Sobczak** Project Manager

## IT Unit



**Roman Szczepanowski** Director's Representative for Information Technology & Research Equipment
**Michał Romiszewski** IT Specialist
**Jakub Skaruz** IT Specialist

After upgrading of Institute's file servers and computer the new server room was arranged with support of the Polish Ministry of Science and Higher Education. It was built according to the highest technical standards, with raised floor, two independent power lines, water detection system, data center grade cooling, power control with UPS, automatic fire suppression system to control and extinguish fires without human intervention and secure, camera controlled access. Computing power of our cluster increased to 14 TFLOP - 1444 cores, 3,36 TB of RAM memory and an additional 30 TB of hard drive. The newly installed single-mode fiber optic cables, running from Internet provider – Interdisciplinary Centre for Mathematical and Computational Modelling – to our new server room are used to obtain a faster, more reliable Internet connection, are becoming an important part of the new Biocentrum Ochota Cluster Computing Grid.

## Foreign scientists at IIMCB

- **Frank King**, MSc (USA) – PhD student in the Department of Molecular Biology, 1999-2001; graduated in Oct., 2001
- **Sanne Mikkelsen**, MSc (Denmark) – involved in Polish Centenarians Program PolStu99, then in the Laboratory of Neurodegeneration, 1999-2001
- **Sophie Chiron** (France) – senior technician at Cell and DNA Bank, Polish Centenarians Program PolStu99, 1999-2000
- **Matthias Bochtler**, Prof. (Germany) – Head of the Laboratory of Structural Biology MPG/PAN Junior Research Group, 2000-present
- **Sergey Odintsov**, MSc (Belarus) – SMM's PhD student in the Laboratory of Structural Biology MPG/PAN, 2001-2004
- **Ahmad Noor Jalili**, MD (Iran) – PhD student in the Laboratory of Molecular Neurology, 2002-2003
- **Tiziana Cacciamani**, PhD (Italy) – Post-doctoral fellow in the Laboratory of Department of Molecular Biology, 2003 (2 months)
- **Gang Zhao**, PhD (China) – Post-doctoral fellow in the Laboratory of Neurodegeneration, 2003-2004
- **Michael Kreutz**, PhD (Germany) – Senior researcher in the Laboratory of Neurodegeneration, 2004 (2 months)
- **Rashid Sajid**, PhD (Pakistan) – Post-doctoral fellow in the Laboratory of Cell Biology, 2006-2009
- **Kristian Rother**, PhD (Germany/Finland) – Post-doctoral fellow in the Laboratory of Bioinformatics and Protein Engineering, 2006-2009
- **Neli Kachamakova**, PhD (Bulgaria) – Post-doctoral fellow in the Laboratory of Neurodegeneration, 2006-2007
- **Laura Lopez Munoz**, BSc (Spain) – MSc student in the Laboratory of Bioinformatics and Protein Engineering 2006-2007 (one semester)
- **Tran Cat Dong**, PhD (Vietnam) – Post-doctoral fellow in the Laboratory of Neurodegeneration, 2007 (2 months)
- **Nguyen Trong Hung**, MD (Vietnam) – PhD student in the Laboratory of Neurodegeneration, 2007 (1 month)
- **Dario Piano**, PhD (Italy) – expert involved in EU grant MEMPROT, the Laboratory of Structure Biology, 2007-2009
- **Elisa Tomat**, PhD (Italy) – visiting researcher (Dept. of Chemistry, MIT) in the Laboratory of Molecular and Cellular Neurobiology, July, 2008
- **Sabah El Alaoui**, PhD (Spain) – expert involved in EU grant – MEMPROT, the Laboratory of Structure Biology, 2008–2009
- **Umesh Ghoshdastider**, MSc (India) – PhD student involved in EU grant SBMPs within 7th FP "Marie Curie Networks for Initial Training", the Laboratory of Biomodelling, since Aug. 2009
- **Dragos Trinca**, PhD (Romania) – experienced researcher involved in EU grant SBMPs within 7th FP "Marie Curie Networks for Initial Training", the Laboratory of Biomodelling, 2009 (3 months)
- **Jean-Philippe Borges**, PhD (France) – researcher involved in EU grant MEMPROT, the Laboratory of Structure Biology, since Jan. 2010
- **Inmaculada Mora Espi**, MSc student (Spain) – volunteer in the Laboratory of Mitochondrial Biogenesis, March – August 2010
- **Shuguang Juan**, MSc (China) – PhD student involved in EU grant SBMPs within 7th FP "Marie Curie Networks for Initial Training", Chemistry Dept, Faculty of Chemistry, University of Warsaw, since Feb. 2011
- **Sonja Obranić**, PhD (Croatia) - volunteer in the Laboratory of Bioinformatics and Protein Engineering, 2011 (3 months)
- **Aksana Varabyova**, MSc (Belarus), PhD student in the Laboratory of Mitochondrial Biogenesis, since Feb. 2010
- **Xavier Lucas**, BSc in Chemistry (Spain)- volunteer in the Laboratory of Bioinformatics and Protein Engineering, since Sept. 2010
- **Sam Dinesh Stephen**, MSc (India) – PhD student involved in EU grant ITN Transpol within 7th FP "Marie Curie Networks for Initial Training" the Laboratory of Cell Biology, since July 2011
- **Rongliang Wu**, PhD (China) – researcher involved in EU grant SBMPs within 7th FP" Marie Curie Networks for Initial Training", Chemistry Dept, Faculty of Chemistry, University of Warsaw, since June 2011
- **Ulrike Topf**, PhD (Germany) – volunteer, the Laboratory of Mitochondrial Biogenesis, since Feb. 2012
- **Mahmoud Tawilla**, (Egypt) – MSc student, the Laboratory of Neurodegeneration, 2011 (2 months)
- **Karthik Shanmuganandam**, MSc (India) – PhD student, the Laboratory Structural Biology, since Dec. 2011
- **Asgar Abbas Kazrani**, MSc (India) – PhD student, the Laboratory Structural Biology, since Dec. 2011

# Lab Leader Competitions

The principles of organization of the Institute are distinct and differ from other research institutes in Poland: an important body of the Institute is the International Advisory Board, acting as the Scientific Council; the leader of each research team of the Institute is selected in an international competition; group leader's initial employment is limited to five years; the progress of research is assessed by the International Advisory Board after the first 3 years and then every 2 years, and the professor's contract may be either terminated or extended. There are no permanent scientific positions at the Institute, however a successful lab leader after seven years can be promoted to a senior position and fall into a rolling-tenure mechanism of employment.

According to the IIMCB bylaws, the lab leader position constitutes an equivalent of a professorial position of other Polish research institutes. Each competition is advertised in internationally visible media.

The applicants are initially screened formally at the Institute, and later, get evaluated by the Selection Committee, made up of several members of the International Advisory Board (IAB). Short-listed candidates with the highest score receive invitations to give a presentation in a symposium run publicly with the participation of IAB members. The final recommendation is made by IAB and passed to the Director, who comes with the binding decision.

We believe that the sharp selection criteria and objective and completely factual recruitment process of lab leaders is key to the success of such an institution as IIMCB. It is the starting point for dynamic growth, opening new lines of research and introduction of modern technology at the Institute. Only such a way of recruitment enables hiring of the most talented researchers - providing them with appropriate conditions of development in IIMCB often becomes the first step to independent, international scientific careers.

| Competition | Year | Number of candidates | Winners employed at IIMCB |
|---|---|---|---|
| I | 1998 | 6 | **Jarosław Dastych** |
| II | 1999 | 3 | **Maciej Żylicz** |
| III | 2000 | 6 | **Michał Hetman** |
| IV[1)] | 2000 | 7 | **Matthias Bochtler, Leszek Rychlewski** |
| V | 2002 | 9 | **Janusz M. Bujnicki, Sławomir Filipek** |
| VI[2)] | 2002 | 9 | – |
| VII | 2003 | 18 | **Marta Miączyńska** |
| VIII[3)] | 2004 | 26 | – |
| IX | 2005 | 26 | **Jacek Jaworski** |
| X[1)] | 2005 | 17 | **Ewa Paluch** |
| XI | 2006 | 25 | **Marcin Nowotny** |
| XII[3)] | 2007 | 16 | – |
| XIII | 2008 | 14 | **Agnieszka Chacińska** |
| XIV | 2010 | 20 | - |
| XV[4)] | 2012 | 18 | **in negotiations** |

[1)]these competitions fulfilled the MPG/PAN agreement
[2)]no result
[3)]the winner did not accept the offer
[4)]this competition fulfilled the MPG/IIMCB agreement

# Scientific Meetings and Lectures

- Health-Prot Research Symposium, 13.05.2011, Warsaw, IIMCB
- IIMCB Annual Report Session, 11.06.2011, Jachranka, Poland
- International Conference "The Modern Techniques for Drug Design Purposes", 4-5.10.2011, Warsaw, IIMCB, organized by S. Filipek
- International Conference "Multi-Pole Approach of Structural Biology", 16-18.11.2011, Warsaw, IIMCB, organized by JM. Bujnicki
- "Health-Prot Symposium on Inherited Disorders of Ciliary Function", 25-26.11.2011, Warsaw, IIMCB, organized by M. Witt
- Health-Prot Workshop "Summary of scientific results of the project and domestic actions beyond", 26.03.2012, Sopot, Poland

## Seminars of invited speakers

**• Special Lecture Series: Frontiers of Polish Biosciences***

**Leszek Kaczmarek** (The Nencki Institute of Experimental Biology, Warsaw, Laureate of 2010 Prime Minister Award for Outstanding Research Achievements) "Learning and memory: From c-Fos to MMP-9 to synaptic plasticity", 01.12.2011.

**• Regular IIMCB seminars**

**Leonora Bużańska** (NeuroRepair Department Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw) "How to create biomimetic neural stem cell niche?", 27.01.2011

**Przemko Tylzanowski** (Department of Musculoskeletal Sciences University of Leuven, Belgium) "Wnts of change - Gastrulating fish and a synovial joint", 03.02.2011

**Kathryn Ball** (Institute of Genetics and Molecular Medicine, University of Edinburgh, UK) "Pathways leading to post-translational activation of the IRF-1 tumour suppressor pathway", 14.02.2011

**Ola Söderberg** (Department of Immunology, Genetics and Pathology Uppsala University) "Visualization of cellular activity status", 03.03.2011

**Ewa Snaar-Jagalska** (Institute of Biology, Leiden University, the Netherlands) "Tumor vascularization and micrometastasis are cooperatively controlled by myeloid cells and VEGF signaling in a zebrafish xenograft model", 04.03.2011

**Amy Lee** (Department of Molecular Physiology and Biophysics University of Iowa USA) "New roles for neuronal $Ca^{2+}$ binding proteins", 15.03.2011

**Marcin Majka** (Department of Transplantation, Jagiellonian University Collegium Medicum, Kraków) "Role of MET receptor in tumorigenesis", 24.03.2011

**Giovanni Blandino** (Translational Oncogenomic Unit, Regina Elena Cancer Institute, Rome, Italy) "Molecular targets in breast cancer", 08.04.2011

**Michał Żółkiewski** (Department of Biochemistry, Kansas State University, USA) "AAA+ ATPase ClpB: a protein disaggregation machine", 14.04.2011

**Brigitte M. Jockusch** (Cell Biology, Zoological Institute Technical University of Braunschweig, Germany) "The Actin Modulator Profilin: Differential Activities of Isoforms in the Regulation of Synaptic Plasticity", 28.04.2011

**Harald Jockusch** (Developmental Biology and Molecular Pathology Bielefeld University, Germany) "Wobbler, a Mouse Model for Neurodegeneration: Pathology, Molecular Genetics, Mechanisms", 29.04.2011

**Stanislav Kalinin** (Institute of Molecular Physical Chemistry Heinrich Heine University Duesseldorf, Germany) "Structure and dynamics of the four-way RNA junction studied by smFRET", 05.05.2011

**Tobias Ost** (UK Senior Field Applications Specialist, Pacific Biosciences) "Eavesdropping on the polymerase: a single molecule approach to sequencing", 09.05.2011

**Daisuke Kihara** (Department of Computer Sciences Purdue University, USA) "Surface representation for molecular global and local shape comparison and docking", 25.05.2011

**Ceslovas Venclovas** (Institute of Biotechnology, Department of Bioinformatics Vilnius University, Lithuania) "Is there a link between the genome size and the nature of DNA replicases? Computational study of double-stranded DNA viruses", 01.06.2011

**Jens Meiler** (Departments of Chemistry and Pharmacology, Center of Structural Biology, Venderbilt University, Nashville, USA) "New Methods in Computational Structural and Chemical Biology", 01.06.2011

**Emidio Capriotti** (Department of Bioengineering, Stanford University, Palo Alto, USA; Department of Mathematics and Computer Sciences, University of Baleanic Islands, Palma de Mallorca, Spain) "Computational methods for RNA 3D structure comparison and prediction", 29.06.2011

**Johannes Herrmann** (University Kaiserslautern, Germany) "Oxidation-driven protein import into mitochondria", 11.07.2011

**Adam Kowalczyk** (National ICT Australia, University of Melbourne, Australia) "Genome Wide Search for Disease Associated Biomarker", 13.07.2011

**Robin Haw** (Department of Informatics and Bio-computing at the Ontario Institute for Cancer Research) "Reactome: a knowledgebase of biological pathways", 15.07.2011

**Małgorzata Wiweger** (ZF-SCEENS Leiden and Leiden University Medical Center, The Netherlands) "The zebrafish: a powerful model for human skeletal diseases", 25.07.2011

**Ulrike Topf** (Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland) "Teneurin, a novel player in maintenance of the basement membrane integrity", 22.08.2011

*A seminar series entitled „Frontiers of Polish Bioscience" was coordinated by Dr. Marta Miączyńska and Dr. Jacek Jaworski. These seminars provided an opportunity to listen to and meet the top Polish scientists who received prestigious awards or grants in a broad field of bioscience.

**Nicholas Ingolia** (Carnegie Institution for Science, Department of Embryology, Baltimore USA; Johns Hopkins University, Dept. of Biology, Baltimore, USA) "Genome-wide Profiling of Translation Initiation and Protein Synthesis", 06.09.2011

**Jordi Villa i Freixa** (Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain) "Towards a multiscale schema in the modelling of biomolecules and their function", 08.09.2011

**Roosa Laitinen** (Max Planck Laboratory, Jagiellonian University Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland) "Unraveling the molecular mechanisms underlying adaptation using A. thaliana hybrids", 27.09.2011

**Kaisa Haglund** (Centre for Cancer Biomedicine Institute for Cancer Research Oslo University Hospital, Norway) "Understanding in vivo roles of the CIN85/CD2AP family of adaptor proteins in endocytosis and cell division", 13.10.2011

**Mark Helm** (Johannes Gutenberg-Universität Mainz, Germany) "Biological functions of tRNA methylation", 20.10.2011

**Henri Grosjean** (Professor Emeritus at the Centre de Génétique Moléculaire, CNRS and Université de Paris-Sud, Gif-sur-Yvette and Orsay, France) "Deciphering the genetic code in organisms of the three domains of life: evolutionary aspect", 27.10.2011

**Michał Wasilewski** (Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padua, Italy) "Steroidogenesis, a moonlight job of mitochondria-shaping protein Opa1", 15.11.2011

**Jorg Hohfeld** (Institute for Cell Biology, University Bonn, Germany) "From creator to terminator - chaperone-assisted degradation in health and disease", 24.11.2011

**Tomasz Prószyński** (Deptartment of Molecular and Cellular Biology, Center for Brain Science, Harvard University, UK) "How to make a pretzel: the molecular machinery that orchestrates synaptic maturation", 19.12.2011

# IIMCB researchers' seminars

**Agata Góźdź** (Laboratory of Molecular and Cellular Neurobiology) "GSK3 as a regulator of neuronal plasticity – protein stability or cytoskeleton?", 10.02.2011

**Paweł Wiśniewski** (Department of Molecular Biology) "ATP-dependent MDM2 activity in the regulation of cellular signaling in NSCLC cells", 24.02.2011

**Tomasz Węgierski** (Laboratory of Neurodegeneration) "Search for STIM-interacting proteins in neurons", 10.03.2011

**Marek Wojciechowski** (Laboratory of Structural Biology) "Specific and non-specific DNA recognition", 17.03.2011

**Roman Szczepanowski** (Laboratory of Structural Biology) "Analytical ultracentrifugation. New face of the old method", 31.03.2011

**Krzysztof Skowronek** (Laboratory of Bioinformatics and Protein Engineering) "Mass spectrometer in IIMCB", 19.05.2011

**Tomasz Węgierski** (Laboratory of Neurodegeneration) & **Łukasz Sadowski** (Laboratory of Cell Biology) "Imaging at IIMCB: Licor Odyssey and fluorescence microscopes", 26.05.2011

**Agnieszka Chacińska** (Laboratory of Mitochondrial Biogenesis) "Redox-driven transport of mitochondrial proteins - nothing by chance", 02.06.2011

**Michal Miętus** (Laboratory of Protein Structure) "Structural studies on bacteriophage lambda DNA replication initiation protein O", 16.06.2011

**Jacek Kuźnicki** (Laboratory of Neurodegeneration) "Small ion and large problem – calcium and Alzheimer's disease", 06.10.2011

**Kamaszewska Agata** & **Pianka Dariusz** (Laboratory of Bioinformatics and Protein Engineering) "The road to patenting new enzymes", 10.10.2011

**Ewa Liszewska** (Laboratory of Molecular and Cellular Neurobiology) "N-cadherin induces oncogenic properties in mouse trophoblast stem cells", 08.12.2011

**Marta Małuszek** (Department of Molecular Biology) "DNA damage response is modulated by MDM2", 15.12.2011

# IIMCB Annual Report Session, 11.06.2011, Jachranka, Poland

**Urszula Hibner** (Institute of Molecular Genetics, Montpellier, CNRS UMR 5535, France) "Epithelial to mesenchymal transition in liver tumorigenesis"

**Lidia Wróbel** (Laboratory of Mitochondrial Biogenesis) "Biogenesis of mitochondrial membrane proteins – are the redox processes involved?"

**Agnieszka Skałecka** (Laboratory of Molecular and Cellular Neurobiology) "mTor kinase role in dendrite arbor formation of adult born neurons"

**Wojciech Siwek** (Laboratory of Bioinformatics and Protein Engineering) "What is the mechanism of action of R.DpnI, a Type II restriction enzyme specific for methylated DNA?"

**Maciej Lipko** (Laboratory of Cell Biology) "RNAi screen of endocytic genes based on transcriptional regulation of p53"

**Mirosław Śmietański** (Laboratory of Protein Structure) "Structural studies of mRNA cap methylation"

**Katarzyna Dębowska** (Laboratory of Neurodegeneration) "Calmyrin2 regulates Rab5-mediated endocytosis"

**Milena Wiech** (Department of Molecular Biology) "Control of the stability of mutant p53R175H by coaggregation with HSP70"

**Małgorzata Sztolsztener** (Laboratory of Mitochondrial Biogenesis) "MIA pathway, an evolutionary conserved system for protein import into mitochondria"

**Iwona Cymerman** (Laboratory of Molecular and Cellular Neurobiology) "GSK3 kinase shapes mature neurons"

**Beata Pyrzyńska** (Laboratory of Cell Biology) "Do cancer cells need appl to survive?"

**Wojciech Potrzebowski** (Laboratory of Bioinformatics and Protein Engineering) "Fitting macromolecules into electron-density maps: New tools and their applications to elucidate structures of Type I DNA restriction enzymes"

**Matthias Bochtler** (Laboratory of Structural Biology MPG/PA) "Is Anbu the missing link in proteasome evolution".

# Grants

## 7th Framework Programme

- NERCOMP "Structural studies of Nucleotide Excision Repair complexes" ERC, (281500); 1,498,000 EUR; 2012-2016; M. Nowotny
- RNA+P=123D "Breaking the code of RNA sequence-structure-function relationships: New strategies and tools for modelling and engineering of RNA and RNA-proteincomplexes" ERC, (261351); 1,500,000 EUR; 2011-2015; J.M. Bujnicki
- COMBIOM "Strengthening Cooperation in Molecular Biomedicine between EU and Ukraine" ERA.WIDE, (294932); 80,036 EUR; 2011-2014; J. Kuźnicki
- EXGENOMES "Exgenome Molecular Enzymes" Research for SME (286556); 156,000 EUR; 2011-2013; J.M. Bujnicki
- NeuConnect "Novel strategies for the treatment of schizophrenia based on genetic variation of the neural cell adhesion molecule NCAM and enzymes involved in its posttranslational modifications" (ERA-NET-NEURON/01/2011); 973,080 PLN; 2011-2014; J. Kuźnicki/M. Wiśniewska
- AMPREPACELL "Development of new experimental models for mental retardation and autism by iPS technology: generation of human affected and animal model neurons by reprogramming skin fibroblasts and testing gene correction using in vitro and in vivo models" (ERA-NET-NEURON/03/2011); 1,419,075 PLN; 2011-2014; J. Jaworski
- ImageNinND "Imaging Neurogenesis in Neurodegenerative Disease: In vivo imaging of dopaminergic adult-born neurons in the olfactory bulb of animal models of Parkinson's disease" (ERA-NET-NEURON/03/2010); 1,085,875 PLN; 2010-2013; J. Jaworski
- TRANSPOL "Transport and signalling mechanism in polarized cells" (ITN, 264399); 225,523 EUR; matching funds 475,200 PLN; 2010-2014; M. Miączyńska
- HEALTH-PROT "Proteins in Health and Disease" (Research Potential, 229676); 954,100 EUR; matching funds 4,099,289 PLN; 2009-2012; J. Kuźnicki
- NEURO.GSK3 "GSK-3 in neuronal plasticity and neurodegeneration: basic mechanisms and pre-clinical assessment" (Collaborative Project, 223276); 280,840 EUR; matching funds 363,315 PLN; 2008-2011; J. Jaworski
- SBMPs "Structural Biology of Membrane Proteins" (ITN, 211800); 263,284 EUR; matching funds 870,120 PLN; 2008-2012; S. Filipek

## 6th Framework Programme

- EURASNET "European alternative splicing network of excellence" (LSHG-CT-2005-518238); 143,000 EUR, matching funds 612,792 PLN; 2006-2010; IIMCB participation 2008-2011; J.M. Bujnicki

## Other International Funds

- Howard Hughes Medical Institute, International Early Career Award "Structural and Mechanistic Studies of Nucleic Acid Processing"; 715,000 USD; 2012-2017; M. Nowotny
- Polish Swiss Research Fund "The role of tumor susceptibility gene 101 (Tsg101) in transcriptional regulation in health and disease" (PSPB-094/2010); 5,365,200 PLN; 2012-2016; M. Miączyńska
- DFG Program Sensory and Regulatory RNAs in Prokaryotes "Single-molecule fluorescence analysis of the temperature dependent structure and dynamics of an RNA thermometer: consequences for its molecular function" (SE 1195/12-2); 90,450 EUR; 2010-2013; J.M. Bujnicki
- EMBO Installation Grant "Protein biogenesis and redox homeostasis in mitochondria" (1966); 150,000 EUR; 2010-2012, A. Chacińska
- Polish Norwegian Research Fund "Screening for novel functions of endocytic and autophagic proteins in the regulation of gene expression, cell growth and carcinogenesis" (PNRF-27-AI-1/07); 672,572 EUR; 2010-2011; I. Pilecka
- EMBO Installation Grant "Structural and biochemical studies of UvrA DNA repair protein" (1476); 250,000 EUR; 2007-2012; M. Nowotny
- Wellcome Trust International Senior Research Fellowship "Structural and functional studies of two members of integrase superfamily – type 2 RNase H and Ruvc resolvase – from substrate recognition to catalysis" (081760); 4,106,806 PLN; 2007-2012; M. Nowotny
- Howard Hughes Medical Institute, International Research Scholars "Signaling from endosomes to the nucleus: The role of APPL proteins and their interacting partners"; 500,000 USD; 2006-2011; M. Miączyńska
- Wellcome Trust International Senior Research Fellowship "Communication between intracellular organelles in trafficking and signalling: The role of APPL proteins" (076469); 4,315,706 PLN; 2005-2012; M. Miączyńska

# Structural Funds

- IE OP 1.2. Programme POMOST "Characterization of selected endocytic proteins as novel regulators of AP-1 mediated transcription" (POMOST/2011-3/11); 430,000 PLN; 2012-2014; E. Szymańska
- IE OP 1.1.2 TEAM Programme "Structural biology of methylation and hydroxymethylation"; (TEAM/2010-6/1); 2,023,940 PLN; 2011-2015; M. Bochtler
- IE OP 1.2 Programme VENTURES "The acquisition of chemotherapy resistance in non-small cell lung cancer – role of the p53 family proteins" (VENTURES/2010-6/8) 231,000 PLN; 2011-2014; Z. Tracz
- IE OP 1.2. Programme POMOST "The Elmo1 function of regulated by mTOR kinase activity in neuronal plasticity" (POMOST_C/60); 6,480 PLN; 2011; M. Błażejczyk
- IE OP 1.1.2 TEAM "Modeling of RNA and protein-RNA complexes: from sequence to structure to function"; (TEAM/2009-4/2); 2,200,000 PLN; 2010-2014; J.M. Bujnicki
- IE OP 1.1.2 Programme MPD "PhD Programme in Molecular Biology: Studies of nucleic acids and proteins – from basic to applied research"; (MPD/2009-3/2); 2,265,421 PLN; 2010-2015; M. Witt (7 PhD fellowships)
- HC OP 8.2.1 "Support for bio tech med. scientists in technology transfer" (UDA-POKL.08.02.01-14-041/09-00); 2,586,221 PLN; 2010-2013; M. Powierża
- IE OP 1.2. Programme POMOST „Functional characterization of the interactions between endosomal adaptor proteins APPL and Dvl proteins in the Wnt signaling pathway" (POMOST/2010-1/1); 420,000 PLN; 2010-2013; M. Banach-Orłowska
- IE OP 1.2. Programme POMOST „The role of mitochondria in biogenesis and pathogenesis of superoxide dismutase Sod1" (POMOST_C/35); 4,860 PLN; 2010-2011; M. Kaus-Drobek
- IE OP 1.1.2 Programme WELCOME "Biogenesis and turnover of mitochondria intermembrane space proteins" (WELCOME/2009/1); 5,940,670 PLN; 2009-2014; A. Chacińska
- IE OP 2.2.3 "Biocentrum Ochota – IT infrastructure for development of strategic directions of the biology and medicine", (POIG.02.03.00-00-003/09); 4,834,300 PLN; 2009-2013; J.M. Bujnicki and S. Filipek
- IE OP 2.2.2 "Centre of Pre-clinical Research and Technology (CePT)" (POIG.02.02.00-14-024/08-00); 14,625,545 PLN; 2008–2013; J. Kuźnicki
- IE OP 1.3.2 "Support for patent procedure of the invention: Tools and methods useful in characterising the immunotoxic activity of xenobiotic substances" (UDA-POIG.01.03.02-00-063/10-00); 230,315 PLN; 2011-2015; M. Powierża

# NCN Research Grants

- MAESTRO "Transgenic mice with elevated basal level of calcium ions in neurons as a model of aged-induced neurodegeneration of sporadic Alzheimer's disease"; (2011/02/A/NZ3/00144); 2,989,800 PLN; 2012- 2017; J. Kuźnicki
- MAESTRO "New functions of endocytic proteins in transcriptional regulation"; 2,875,000 PLN; 2012-2017; M. Miączyńska
- "The relationship between GSK3α and GSK3β activities and neuronal plasticity in chronic stress" (2011/01/M/NZ3/05413); 499,964 PLN; 2011-2014; I. Cymerman
- "Defining the mechanism of GSK3 dependent regulation of mTOR kinase activity in neurons in physiology and pathology" (2011/01/N/NZ3/05409); 150,000 PLN; 2011-2014; M. Urbańska
- "Characterization of mTOR-dependent phosphorylation of ZBP1 and CLIP-170 and description of its contribution to dendritic arbor development" (2011/01/N/NZ3/05405); 180,000 PLN; 2011-2014; A. Urbańska
- "Investigation of arc protein role in synaptic plasticity of hippocampal and cortical neurons and mechanisms regulating arc expression with the special focus on GSK3-dependent degradation" (2011/01/B/NZ3/05397); 450,000 PLN; 2011-2014; A. Góźdź
- "Role of transcription factor TCF7L2 in establishment of thalamocortical connectivity and identity of thalamic neurons" (2011/01/N/NZ3/05345); 96,000 PLN; 2011-2013; A. Nagalski
- "The contribution of STIM proteins and the role of store-operated calcium entry (SOCE) in calcium homeostasis of neurons" (2011/01/D/NZ3/02051); 684,000 PLN; 2011-2016; J. Gruszczyńska-Biegała
- "Structural and functional characterization of novel non-coding RNAs from Helicobacter pylori" (2011/01/D/NZ1/00212); 550,000 PLN; 2011-2014; G. Chojnowski
- "Generation of knockouts of HMTR1 and HMTR2 genes in human somatic cells and functional analysis of cap1 and cap2 methyltransferases encoded by these genes" (2011/01/N/NZ1/00211); 100,000 PLN; 2011-2013; M. Werner
- "Sequence specificity and its determinants in dsRNA endoribonucleases" (2011/01/B/NZ1/00209); 350,000 PLN; 2011-2014; K. Skowronek
- "The role of HSP70 in the stabilization of p53 mutants in cancer cells" (2011/01/N/NZ1/00202); 192,000 PLN; 2011-2013; M. Wiech

# Ministerial Research Grants

- "Structural analysis of RNase H3 in complex with a substrate - the mechanism of action and substrate specificity in the context of an enzyme family" (IP2011060971); 150,000 PLN; 2012-2013; M. Figiel
- "Development and application of new methods for protein-RNA and protein-DNA complexes modeling" (IP2011057071); 175,000 PLN; 2012-2014; I. Tuszyńska

- "Structural studies of mechanism of action of UvrC protein from bacterial DNA repair system called nucleotide excision repair system" (IP2011018671); 150,000 PLN; 2012-2013, M. Jaciuk
- "Structural analysis of the RNA-RNA and RNA-protein interactions" (IP2011006671); 145,000 PLN; 2012-2013; G. Chojnowski
- "Practical algorithms for graph isomorphism testing in the computational biology" (IP2011058671); 160,000 PLN; 2012-2013; T. Waleń
- "Casimir-Polder effect in scattering of atoms on liquid surfaces" (IP2011030771); 150,000 PLN; 2012-2013; G. Łach
- "Coordinating proteasome subunit expression: structural biology of the master regulator Rpn4" (IP2011050971); 400,000 PLN; 2012-2013; M. Sokołowska
- "Structural biology of anti-cancer DNA methyltransferase inhibitors" (IP2011060971); 200,000 PLN; 2012-2013; M. Wojciechowski
- "Bioinformatics analysis of sequence-structure-function relationships in the GIY-YIG nuclease superfamily" (IP2011021871); 100,000 PLN; 2012-2012; K. Kamińska
- "Analysis of the relationship between sequence and structure in coiled-coil protein domains" (IP2011011071); 178,000 PLN; 2012-2014; S. Dunin-Horkawicz
- "Changes in cell cycle and apoptosis as a basis for diagnosis and potential therapeutic targets in Alzheimer's disease" (NN401596840); 408,000 PLN; 2011-2014; U. Wojda
- "Is there a "universal" RNA-guided DNA endonuclease?" (NN302654640); 400,000 PLN; 2011-2014; M. Bochtler
- „The Elmo1 function of regulated by mTOR kinase activity in neuronal plasticity" (NN301779040); 250,000 PLN; 2011-2014; M. Błażejczyk
- "Function of STIM proteins in capacitative calcium entry to the ER of healthy neurons and of cells with calcium dyshomeostasis in Alzheimer's disease" (NN301190039); 480,000 PLN; 2010-2013; J. Kuźnicki
- „The role of multifunctional adaptor proteins APPL1 and APPL2 in the regulation of cell growth and tumorigenic potential" (NN301189839); 336,000 PLN; 2010-2013; B. Pyrzyńska
- "Experimental characterization of hMTcap1 and hMTcap2 – last missing enzymes taking part in biosynthesis of the cap structure of human mRNA" (NN301425338); 500,000 PLN; 2010-2013; J.M. Bujnicki
- "Biochemical and structural studies of lentiviral reverse transcriptases" (NN301439738); 599,800 PLN; 2010-2013; M. Nowotny
- "Mechanism of oncogenic activities of mutated *TP53*" (NN302621838); 600,000 PLN; 2010-2013; A. Żylicz
- "A new type of antibacterial drugs: a search for inhibitors of Arm methyltransferases that confer resistance to aminoglycosides" (0160/H03/2010/70); 100,000 PLN; 2010-2011; K.H. Kamińska
- "Searching for compounds abolishing bacterial resistance for MLSb antibiotics" (0337/P01/2010/70); 150,000 PLN; 2010-2011; E. Purta
- "Identifiction of the genetic program activated by Lef1/ β-catenin complex in mature neurons" (NN301424538); 372,000 PLN; 2010-2013; M. Wiśniewska
- "Structural studies of ββα-Me restriction endonucleases" (NN3014250378); 400,000 PLN; 2010-2012; H. Czapińska
- "Towards a new drug against influenza: Identification and characterization of compounds which abolish the activity of the influenza virus mRNA polymerase by the inhibition of virus endonuclease" (NN401585738); 150,000 PLN; 2010-2011; K. H. Kamińska
- "Innovation Creator (Kreator Innowacyjności) – to encourage entrepreneurship among scientists"(31/PMKI/U/30-06.09/2010); 422,990 PLN; 2010-2013; M. Powierża
- "The role of mitochondria in biogenesis and pathogenesis of superoxide dismutase Sod1" (NN301298337); 476,000 PLN; 2009-2012; A. Chacińska
- "Identification and characteristics of endocytic proteins involved in regulation of gene transcription" (NN301296437); 340,740 PLN; 2009-2012; I. Pilecka
- International Project Grant (MPG Program) "The role of cell cortex mechanics in cell motility" (454/N-MPG/2009/0); 4,692,929 PLN; 2009-2012; E. Paluch
- Polish-German Special Grant „Development and implementation of methods for improving protein's crystals quality by engineering of protein-protein contacts"; 940,000 PLN; 2008-2011; J.M. Bujnicki
- "Modulation of activity of transcription factors involved in tumorigenesis, by MDM2 and other E3 ubiquitin ligases" (NN301032534); 750,000 PLN; 2008-2011; M. Żylicz
- "Structural and biochemical studies of restriction enzymes specific for pseudopalindromic sequences" (NN301029534); 344,400 PLN; 2008-2011; M. Bochtler
- "Functional characterization of Exonuclease G - the role in the apoptosis and diabetes" (NN401061535); 290,400 PLN; 2008-2011; I. Cymerman

# Ministerial Doctoral Grants

- "Searching for β-catenin regulators in thalamic neurons" (NN301191739); 48,000 PLN; 2010-2012; J. Kuźnicki/K. Misztal
- "Function of calmyrin 2 in Ca2+-dependent exocytosis" (NN301335239); 60,000 PLN; 2010-2012; U. Wojda/K. Dębowska
- "Functional analysis of proteins responsible for processing of mRNA 3'termini. Identification of domains and intrinsically disordered regions" (NN301190139); 37,600 PLN; 2010-2012; J.M. Bujnicki/L. Kozłowski
- "Automated creation and implementation of data flow schemes between bioinformatics tools" (NN301297337); 49,680 PLN; 2009-2011; J.M. Bujnicki/J. Orłowski

# Ministerial Research-and-Development Grant

- AriaDNA 2010 Project (OR00002712); 9,904,670 PLN; 2010-2012; M. Witt/J. Kuźnicki

# Ministerial Commissioned Grants

- PolSenior "Ageing of the Polish population – medical, psychological, sociological and economic aspects" (PBZ-MEiN-9/2/2006); 12,178,420 PLN; 2007-2011; Director: P. Błędowski, coordinator M. Mossakowska

# Doctoral Degrees in 2011

- Łukasz Świech, PhD thesis: „Role of CLIP-170 and IQGAP1 in mTOR-regulated dendritogenesis of hippocampal neurons". Thesis advisor: J. Jaworski; 17.02.2011, Nencki Institute of Experimental Biology PAN, Warsaw, Poland
- Anna Urbańska, PhD thesis: „Biochemical characterization of APPL endosomes and their associated proteins". Thesis advisor: M. Miączyńska; 13.06.2011, Nencki Institute of Experimental Biology PAN, Warsaw, Poland
- Katarzyna Misztal, PhD thesis: „Mechanism of β-catenin accumulation in the cytoplasm and nucleus of mature thalamic neurons". Thesis advisor: J. Kuźnicki; 29.11.2011, Nencki Institute of Experimental Biology PAN, Warsaw, Poland

# Other publications

- Kraszewska MD, Dawidowska M, Kosmalska M, Sędek L, Grzeszczak W, Szczepański T, **Witt M**. Immunoglobulin/T-cell receptor gene rearrangements in the diagnostic paradigm of pediatric T-cell acute lymphoblastic leukemia patients. **Leukemia & Lymphoma**, 2012 Mar 16. [Epub ahead of print]
- Ziętkiewicz E, **Bukowy-Bieryłło Z**, Voelkel K, Klimek B, Dmeńska H, Pogorzelski A, Sulikowska-Rowińska A, Rutkiewicz E, **Witt M**. Mutations in radial spoke head genes and ultrastructural cilia defects in East-European cohort of primary ciliary dyskinesia patients. PLoS One, 2012 7(3), e33667
- Kraszewska MD, Dawidowska M, Larmonie NS, Kosmalska M, Sędek L, Szczepaniak M, Grzeszczak W, Langerak AW, Szczepański T, **Witt M**. DNA methylation pattern is altered in childhood T-cell acute lymphoblastic leukemia patients as compared with normal thymic subsets: insights into CpG island methylator phenotype in T-ALL. Leukemia, 2012; 26(2):367-71
- Ziętkiewicz E, Witt M, Daca P, Zebracka-Gala J, Goniewicz M, Jarząb B, **Witt M**. Current genetic methodologies in the identification of disaster victims and in forensic analysis. J Appl Genet, 2012; 53(1):41-60
- Kraszewska MD, Dawidowska M, Szczepanski T, **Witt M**. T-cell acute lymphoblastic leukaemia: recent molecular biology findings. Br J Haematol, 2012; 156(3):303-15
- Rajska-Neumann A, **Mossakowska M**, Klich-Rączka A, Życzkowska J, Grześkowiak E, Shieh S, Wieczorowska-Tobis K. Drug consumption among Polish centenarians. Arch Gerontol Geriatr, 2011; 53(1):E29-E32
- Bledowski P, **Mossakowska M**, Chudek J, Grodzicki T, Milewicz A, **Szybalska A**, Wieczorowska-Tobis K, Wiecek A, Bartoszek A, Dabrowski A, Zdrojewski T. Medical, psychological and socioeconomic aspects of aging in Poland Assumptions and objectives of the PolSenior project. Exp Gerontol, 2011; 46(12):1003-9
- **Geremek M**, Bruinenberg M, Zietkiewicz E, Pogorzelski A, **Witt M**, Wijmenga C. Gene expression studies in cells from primary ciliary dyskinesia patients identify 208 potential ciliary genes. Hum Genet, 2011; 129(3):283-93
- **Bukowy Z**, Zietkiewicz E, **Witt M**. In vitro culturing of ciliary respiratory cells-a model for studies of genetic diseases. J Appl Genet, 2011; 52(1):39-51.
- **Mossakowska M**, Więcek A, Błędowski P. (eds) Ageing of the Polish population –medical, psychological, sociological and economic aspects. Termedia 2012, pp 596 (in Polish, shown on left)

# Details of Selected Projects and Cooperation with Other Institutions

## Structural Funds

- "Centre for Pre-clinical Research and Technology" (CePT) (IE OP.02.02.00-14-024/08-00); 14,625,545 PLN; 2008–2013; J. Kuźnicki, IEOP 2.2.2
- "Biocentrum Ochota – IT infrastructure for the development of strategic directions in biology and medicine", (IE OP.02.03.00-00-003/09); 4,834,300 PLN; 2009-2013; J.M. Bujnicki and S. Filipek, IE OP 2.2.3
- WELCOME Programme "Biogenesis and turnover of mitochondrial intermembrane space proteins" (WELCOME/2009/1); 5,940,670 PLN; 2009-2014; A. Chacińska, IE OP 1.1.2
- TEAM Programme "Modeling of RNA and protein-RNA complexes: from sequence to structure to function"; PLN 2,200,000; 2010-2014; J.M. Bujnicki, IE OP 1.1.2
- TEAM Programme "Structural biology of methylation and hydroxymethylation"; 2,023,940 PLN; 2011-2015; M. Bochtler
- "Support for bio tech med scientists in technology transfer"; (UDA-POKL. 08.02.01-14-041 /09-00), PLN 2,586,221; 2010-2013; M. Powierża, PO KL 8.2.1
- International PhD Projects Programme (MPD) "PhD Programme in Molecular Biology: Studies of nucleic acids and proteins - from basic to applied research"; (MPD/2009-3/2); PLN 2,265,421; 2010-2015; M. Witt, IE OP 1.1.2

**Centre for Preclinical Research and Technology (CePT)**

The Centre for Preclinical Research and Technology (CePT) is the largest biomedical and biotechnology enterprise in Central and Eastern Europe. The project objective is to create a dynamic scientific centre in Warsaw, consisting of closely cooperating local research units, to investigate the most prevalent civilization diseases, in particular cancer, neurological and cardiovascular diseases, and diseases associated with ageing. The CePT Consortium consists of: the Medical University of Warsaw (project coordinator), the University of Warsaw, the Warsaw University of Technology and seven institutes: the Nencki Institute of Experimental Biology PAN, the Institute of Biochemistry and Biophysics PAN, the Mossakowski Medical Research Centre PAN, the Institute of Fundamental Technological Research PAN, the Institute of High Pressure Physics PAN, the Nałęcz Institute of Biocybernetics and Biomedical Engineering PAN and the International Institute of Molecular and Cell Biology. The greatest asset of the CePT project lies in bringing together the potential of outstanding scientists and the opportunities provided by the infrastructure of well equipped state-of-the-art core research facilities: physical and chemical laboratories, biomolecular and biotechnological facilities, biomedical engineering and biomaterial technology laboratories, units conducting preclinical research on animal models of diseases associated with the progress of civilization, as well as a specialized base for clinical research provided by the Medical University of Warsaw. The real value of the CePT project is an interdisciplinary and systemic approach to the issues examined: from gene and protein to cell and whole organism. The CePT project has become an integrated part of the global dynamic development of translational medicine aimed at the transformation of the latest achievements of preclinical research into new ways to diagnose and treat patients. An integral part of the CePT project concept is the creation of a technology transfer platform in accordance with the best models of such solutions in Europe, taking into account the developing and innovative pharmaceutical industry and the health needs of society.

The CePT project has finalized its purchase phase and research equipment acquired by the IIMCB within the CePT framework is worth PLN 14m. This has created the infrastructure basis for the Centre of Protein Structure and Function Analysis located at the IIMCB. The specific items of equipment purchased under the CePT framework are:

- protein crystallography platform with crystallization robot and microfocus high-flux X-ray diffractometer equipped with a CCD detector,
- mass spectrometry platform (ESI and MALDI) for the analysis of bio-macromolecules and their chemical modifications,
- laser scanner Typhoon Trio+, CCD camera system LAS 4010 and software for visualization/documentation/quantification of gel electrophoresis and blots,
- Applied Biosystems 7900HT Fast Real-Time PCR System to provide quantitative detection of mRNA using real-time analysis,
- workbench for the physico-chemical analysis of proteins consisting of Infinite microplate reader, Nanodrop spectrophotometer, ProteomeLab analytical ultracentrifuge, two preparative Avanti ultracentrifuges,
- maintenance station for laboratory rodents equipped with six individually ventilated cage systems for small rodents, air control units, four safety cabinets for animal care and surgery.

**Biocentrum Ochota – IT infrastructure for the development of strategic directions in biology and medicine (Biocentrum)**

The purpose of the Biocentrum project is the development and implementation of advanced computational methods for the prediction of biomacromolecule structure and function. The main objectives include the development of servers to provide online software for bioinformatics analyses of proteins and RNA, in particular for 3D structure modeling, and for predicting GPCRs-ligand complex structures by ligand docking. These objectives will be achieved thanks to the purchase of a high-performance

computing cluster which is part of a campus-wide computing grid. The cluster comprises 1444 cores, has 3.36 TB of RAM and brings additional 30 TB of storage space to complement the previous computational resources of the institute. The total computing power of the cluster will reach 14 TFLOP. The new server room was constructed according to the highest technical standards: raised floor, two independent power lines, water detection system, data centre grade cooling, power control with UPS, automatic fire suppression system and secure, camera controlled access.

**Welcome Programme**

The Welcome programme of the Foundation for Polish Science aims to create in Polish research institutions research teams led by internationally experienced and well recognized scientists. There have been eleven Laureates of this programme in all disciplines (bio, info, techno). One of them is Dr. Agnieszka Chacińska of the IIMCB, who joined the Institute at the end of 2009 as Head of the Laboratory of Mitochondrial Biogenesis. The team of young scientists led by Dr. Chacińska study an important aspect of cell biology, i.e. the biogenesis of mitochondrial proteins targeted to the intermembrane space. Mitochondria are essential organelles and the mitochondrial intermembrane space has remained a central focus of cell biology research since it hosts a handful of important factors involved in cellular metabolism and regulation. Biogenesis of intermembrane space proteins that lack a classical mitochondrial leader sequence is governed by a novel pathway called MIA (Mitochondrial Intermembrane space Assembly). An intriguing hallmark of this pathway is the regulated transfer of disulfide bonds. The research within the Welcome project addresses the fate of the intermembrane space proteins from their origin at the ribosome in the cytosol through mitochondrial translocation and maturation to their clearance. During the clearance process not only mitochondrial proteases but also the cellular degradation system outside mitochondria may play a role since this project puts forward an innovative idea that reduced and/or unfolded intermembrane space proteins are relocated back to the cytosol. The impact of the MIA pathway on the mitochondrial and cellular protein homeostasis and the biological consequences of mitochondrial oxidative protein biogenesis failure are also of interest in this project. In-depth understanding of the biogenesis and turnover of the mitochondrial proteins is an important step towards deciphering the human pathological conditions related to protein homeostasis and mitochondrial function.

**TEAM Projects**

***Modeling of RNA and protein-RNA complexes: from sequence to structure to function***

This project builds on the expertise of Prof. Bujnicki's group in structural bioinformatics and on their previous experience in modeling protein structure, and its purpose is to develop corresponding bioinformatic methods for RNA and apply them to biologically and medically interesting systems. The project has several objectives: First, to develop data models and ontologies to represent RNA sequences, structures, functions, as well as pathways (for biochemical reactions where RNA is a substrate and/or a product as well as for those where RNA is an enzyme or a regulatory element). Second, to develop a comprehensive database of RNA processing. Third, to develop tools for automated 3D modeling of RNA and protein-nucleic acid complexes, and for the assessment of RNA model quality. Fourth, to combine the use of the computational tools and experimental analyses with biochemical and biophysical methods to elucidate the structure and mechanism of action for biologically and medically interesting RNAs and systems involving protein-RNA interactions.

***Structural biology of methylation and hydroxymethylation***

The LSB TEAM project, leaded by Prof. M. Bochtler, centers on DNA methylation and demethylation. A particular focus is on hydroxymethylcytosine as an intermediate of active DNA demethylation. The project is divided into four parts. The first part of the project is dedicated to prokaryotic enzymes, which are dependent on methyl- or 5-hydroxymethylcytosine for their activity. It is hoped that work in this part of the project will contribute to a better basic understanding of how the presence of additional groups (methyl or hydroxymethyl) can license rather than prevent an enzymatic reaction. The second part of the project deals with a limitation of current methodology. Bisulfite sequencing, the leading technique to analyze DNA modifications at single base resolution, cannot distinguish between 5-methyl and 5-hydroxymethylcytosine. Work in this part of the project aims to overcome this limitation. The third part of the project centers on the interactions of eukaryotic DNA with methylated and hydroxymethylated DNA. In the forth and last part of the project aims, tools and insights from the other three parts of the project will be used to contribute to a better understanding of the role and interplay of currently described or suspected DNA demethylation pathways.

**Bio Tech Med Project**

The department at IIMCB which deals with technology transfer - Bio&Technology Innovations Platform (BioTech-IP) has been pursuing the "Support for bio tech med scientists in technology transfer" project since March 2010. The project is funded from the European Social Fund (OP HC 8.2.1) with the budget of PLN 2,586,221.

Thanks to this funding, BioTech-IP was able to start awareness-raising activities to encourage scientists from the Biocentrum Ochota consortium to undertake applied research projects. This is being done in three areas:

1. Training for scientists to make them familiar with technology transfer issues. Within this project, BioTech-IP has organized a series of training courses on a variety of subjects: funding paths of science-industry cooperation, negotiations in business, R&D project management, raising a company, the commercialization of R&D results, and intellectual property rights.
2. Industry internships for scientists. The unit initiated a programme for scientists from BioCentrum Ochota, encouraging them to take up 1- to 2-month internships at pharmaceutical and biotech companies in order to improve their entrepreneurial spirit and enable closer links with business partners.
3. Scholarships for PhD students who undertake applied research. The project enabled BioTech-IP to fund 23 scholarships for PhD students who are pursuing applied research projects.

**International PhD Programme (MPD)**

This programme started in 2010, based on funding from the Foundation for Polish Science. PhD projects are being carried out at the Institute of Biochemistry and Biophysics PAN and at the International Institute of Molecular and Cell Biology, in collaboration with a number of foreign partner institutions. Detailed information can be found in the "Educational Activities" section on p. 82.

# Ministerial Commissioned Grant

**PolSenior Project**

Rapid ageing of today's societies results mainly from an increasing life span and declining birth rate. To obtain proper insights into the ageing process, multi- and interdisciplinary studies are needed that frame research questions and hypotheses, based on the interplay between social, economic, medical, and genetic factors of ageing.

The PolSenior project is a multicenter, publicly funded research project, commissioned by the Ministry of Science and Higher Education and entitled "Ageing of the Polish population – medical, psychological, sociological and economic aspects". It is the largest-ever scientific research programme carried out in Poland with the focus on elderly subjects, involving 40 research groups. The IIMCB was one of the major initiators of PolSenior, with Prof. Piotr Błędowski from the Warsaw School of Economics (President of the Polish Gerontological Society) as head of the project and Dr. Małgorzata Mossakowska (IIMCB) as coordinator.

The aim of the project was to examine the medical, psychological and socioeconomic aspects of ageing in Poland. The research sample included 5695 respondents (2899 males and 2796 females). The study consisted of three visits performed by trained nurses and included a questionnaire survey, comprehensive geriatric assessment and blood and urine sampling. The questionnaire consisted of medical and specific socioeconomic questions. The comprehensive geriatric assessment included blood pressure and anthropometric measurements, as well as selected scales and tests routinely used in the examination of elderly subjects. Blood and urine samples were collected from 4737 and 4526 individuals, respectively. More than 50 biochemical parameters were measured, and DNA was isolated and banked. In a selected group of 1018 subjects, a medical examination by a physician was performed. The self-rated health was lower in females than in males in age groups 70-84, but similar in individuals of both sexes aged 65-69 and 85 years. Apart from providing data on health and functioning of the elderly population, the PolSenior project aims to analyze interrelationships between different elements of health and social status, and between genetics and health status in advanced age. The results of the PolSenior project will facilitate prioritizing the state's public health and social policies in the elderly population. Such a programme also provides an excellent starting point for longitudinal studies and a basis for comparative analysis between Poland and other European countries or regions.

In 2011, the Polish and English edition of the monthly journal "Social Policy", devoted to the PolSenior project, was published. Results of the project are presented in detail in a monograph edited by M. Mossakowska, A. Więcek and P. Błędowski (multiauthor, 35 chapters, 596 pages, published by Termedia, Poznań, 2012 – see page 14).

# Domestic Cooperation

**Biocentrum Ochota**

In January 2008, the scientific activities of the Biocentrum Ochota Consortium of the Polish Academy of Sciences were launched at the initiative of six research institutes operating at the Ochota Campus in Warsaw.

The founders and members of the Consortium are:

1. Institute of Biochemistry and Biophysics PAN
2. Nałęcz Institute of Biocybernetics and Biomedical Engineering PAN
3. Nencki Institute of Experimental Biology PAN
4. Mossakowski Medical Research Centre PAN
5. Institute of Fundamental Technological Research PAN
6. International Institute of Molecular and Cell Biology

The basic principle behind Biocentrum Ochota is that the considerable scientific potential, represented by the combined group of experts working in these six institutes, should be pooled and used for the development of large-scale research projects that go beyond the capabilities of individual units. The implementation of such projects will overlap with the statutory research areas of individual institutes in the fields of biology, medicine and bioengineering. Pooling the resources and expertise of individual institutions will also aid the acquisition of financial backing, including European Union grants under the Operational Programme – Innovative Economy and the Operational Programme – Human Capital, co-financed by the European Social Fund.

EU funds obtained by Biocentrum Ochota are not only used for research projects, but also to expand the team of researchers. The scientists from the member institutions of Biocentrum Ochota are specialists recognized on the national and international arena as experts in their fields. This is evidenced by a broad spectrum of scientific cooperation with Polish and foreign research centres, by numerous invitations to participate in projects, symposia, conferences and publications, and by the volume of scientific output. Researchers at Biocentrum Ochota have also received many awards at home and abroad, including the most prestigious awards for scientific achievements, awarded annually by the Foundation for Polish Science.

**University of Gdańsk**

In October 2011, an agreement was signed between the Intercollegiate Faculty of Biotechnology at the University of Gdańsk - Medical University of Gdańsk and the IIMCB, regarding IIMCB's

accession to Life Sciences and Mathematics Interdisciplinary Doctoral Studies (LiSMIDoS) at the University of Gdańsk. LiSMIDoS was originally an initiative of councils of four faculties at the University of Gdańsk, namely: the Intercollegiate Faculty of Biotechnology UG and MUG, the Faculty of Biology, the Faculty of Chemistry and the Faculty of Mathematics, Physics and Informatics. The major objective is to provide a programme of interdisciplinary training to PhD students that will allow them to work in today's competitive scientific environment that very often requires cross-disciplinary expertise. The studies will prepare candidates to obtain a PhD degree in the area of biological sciences (biology and biochemistry), chemical sciences (chemistry), physical sciences (physics) and mathematical sciences (mathematics). First IIMCB students will start their education in 2012. Prof. Janusz M. Bujnicki and Dr. Jacek Jaworski have been appointed members of LiSMIDoS Programme Council.

# International Cooperation

**Max Planck Society**

MAX-PLANCK-GESELLSCHAFT

This cooperation started in 2001 as an initiative of the Max Planck Society (MPG) and the Polish Academy of Sciences (PAN). According to the agreement, Junior Research Group, with Dr. Matthias Bochtler as Lab Leader was funded by the MPG and hosted at the IIMCB. The Laboratory of Cell Cortex Mechanics MPG/PAN headed by Dr. Ewa Paluch, a twin lab of Matthias Bochtler's MPG/PAN laboratory, was established in February 2006. The equipment and running costs of the lab, including personnel, are covered by Polish funds, but the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (MPI-CBG), being a host for this laboratory, is responsible for local operational costs, maintenance, and administrative support. Dr. Ewa Paluch's group focuses on biochemical and physical mechanisms of cell shape and deformations. The research concentrates on movements of the actomyosin cortex, and the involvement of spontaneous cortical ruptures and flows in cell division in particular; their recent achievement is a paper published in "Nature". The activities of the two laboratories should be considered a major scientific and organizational success. This model scenario of international cooperation between IIMCB and MPG developed over the years by both partner sides seems to be optimal for this type of projects.

In March of 2012 the cooperation agreement between our Institute and the Max-Planck Society (MPG) has been finalized and signed by Prof. Peter Gruss, President and Dr. Ludwig Kronthaler, Secretary General of MPG and by Prof. Jacek Kuźnicki, Director and Prof. Michał Witt, Scientific Director of IIMCB. The agreement concerns the establishment of two Max Planck/IIMCB Research Groups, one in IIMCB, other in the Max-Planck Institute of Heart and Lung Research in Bad Nauheim. Each of the parties will finance a research group with its own budget. On March 22, in Bad Nauheim, a competition for both positions was held. Negotiations with successful candidates are currently underway. A research group in IIMCB is to focus on studies on zebrafish model; core equipment for this facility has already been purchased. This new laboratory is planned as the first in Poland working on the zebra fish as a model of pathomechanisms of various human diseases. Existing significant experience of MPG in this area should greatly facilitate the rapid launch and research progress of such a unit.

**Institute of Molecular Biology and Genetics, Kiev, Ukraine**

The IIMCB has taken initiatives to share experiences from participating in the Research Potential programme with Ukrainian scientists and managers from the Institute of Molecular Biology and Genetics (IMBG) in Kiev. In the past, IIMCB and IMBG representatives were meeting every 2 years during Polish-Ukrainian Parnas conferences. Closer bilateral relationships were established in 2008 when IIMCB director, Prof. Jacek Kuźnicki, presented a lecture entitled "Research organisation in the 21st century: experience and achievements of the IIMCB" at the Ukrainian Ministry of Education and Science. He proposed to share IIMCB's experience with Ukrainian scientific institutions. Prof. Kuźnicki was then invited by Ukraine's State Agency for Science, Innovation and Information to participate in the International Expert Council responsible for ranking grant applications from Ukrainian scientists. Prof. Kuźnicki was one of 11 members of the Council, next to Dr. Erwin Neher from Germany (Nobel Prize Laureate) and Dr. Alan North and Dr. Ole H. Petersen from the UK. As a follow up, in 2009, the IIMCB organized a Polish-Ukrainian scientific conference accompanied by the HEALTH-PROT kick-off meeting. IMBG director, Prof. Elskaya, participated in the meeting of IIMCB's International Advisory Board and IMBG managers had meetings with their IIMCB counterparts. This intense cooperation evolved into shared participation in a three-year COMBIOM project entitled "Strengthening Cooperation in Molecular Biomedicine between EU and Ukraine" (01.12.2011-30.11.2014), supported by FP7 under the ERA-WIDE activity (Integrating Europe's neighbours into the ERA). Apart from the IMBG (coordinator) and the IIMCB, the third partner involved is the Institute Gustave-Roussy (IGR) from France. IIMCB's role is to support the Institute in Kiev by twinning with Ukrainian researchers (M. Bochtler, J. Jaworski, M. Miączyńska), providing training for IMBG researchers and administration staff, and developing IMBG's Biomed Research Strategy (J. Kuźnicki, A. Żylicz). The project kick-off meeting will take place on 16-17 May in Kiev but the first twinning visit of a Ukrainian researcher to the IIMCB is already planned for April.

# Proteins in Health and Disease HEALTH-PROT

## Coordination and support actions project financed by the 7th Framework Programme of the European Union within the Research Potential scheme

**Objectives**

HEALTH-PROT project is targeted at continuation and expansion of activities initiated as the FP5 Centre of Excellence in Molecular Bio-Medicine (CEMBM). In the past as the Centre of Excellence we developed an advanced methodology of analysis of complex protein structures with the use of cell and molecular biology techniques, biochemical methods, crystallographic analysis and computer modelling. Our goal is to become a top protein studies Centre in the region by unlocking the potential of all our research groups. This is being achieved mainly by twinning each of the Institute's groups with European groups leading in the field **(first objective)**, through joint research activities, organization of workshops and conferences and participation in consortia within FP7 and European Structural Funds. We create the place for experienced researchers to conduct research at the highest level **(second objective)**, and for junior researchers to obtain the best possible mentoring and a degree based on the top-flight theses. We also intend to reach an ultimate critical mass by completing the organisation of IIMCB's structure **(third objective)**. We aspire to be more innovative towards applications in medicine and biotechnology **(fourth objective)**. Alongside, we popularize science and raise social awareness of the benefits of modern biology and biotechnology **(fifth objective)**. Ultimately, a reinforced S&T potential of our research groups will allow us to become more visible and attractive as a collaborating partner in the European Research Area, for both academia and industry.

## Twinning partners and their projects

**Matthias Bochtler**, Laboratory of Structural Biology, IIMCB and **Ruedi Allemann**, University of Cardiff, UK. ***The structure and function of proteases and endonucleases with relevance to human medicine.***

**Janusz M. Bujnicki,** Laboratory of Bioinformatics and Protein Engineering, IIMCB and **Saulius Klimasauskas**, Laboratory of Biological DNA Modification, Institute of Biotechnology, Vilnius, Lithuania. ***Enzymes acting on nucleic acids.***

**Sławomir Filipek**, Biomodelling Laboratory, IIMCB and **Vicenza Andrisano**, Department of Pharmaceutical Sciences, University of Bologna, Italy. ***Understanding of beta-amyloid formation in Alzheimer's Disease.***

**Jacek Jaworski**, Laboratory of Molecular and Cellular Neurobiology, IIMCB and **Casper Hoogenraad**, Erasmus MC, Rotterdam, The Netherlands. ***mTOR dependent microtubule dynamics in shaping dendritic arbor in physiological and pathological brain development.***

**Jacek Kuźnicki**, Laboratory of Neurodegeneration, IIMCB and **Jochen Herms**, Ludwig-Maximilians-University of Munich, Centre for Neuropathology, Germany. ***Relationship between deregulated calcium homeostasis and synaptic pathology in Alzheimer's disease as a target for therapy.***

**Marta Miączyńska**, Laboratory of Cell Biology, IIMCB and **Harald Stenmark**, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway. ***Endosomal proteins in regulation of cell signaling and proliferation.***

**Marcin Nowotny**, Laboratory of Protein Structure, IIMCB and **Roland Marquet**, Retroviruses and RNA viruses laboratory, RNA Architecture and Reactivity Unit, Université Louis Pasteur, CNRS, Strasbourg, France. ***Structural and biochemical studies of reverse transcriptases.***

**Michał Witt**, Ciliary Proteins Function Project, IIMCB and **Heymut Omran**, Department of Pediatrics and Adolescent Medicine, University of Freiburg, Germany. ***Role of ciliary proteins in pathogenesis of cilia-related disorders.***

**Maciej Żylicz**, Department of Molecular Biology, IIMCB and **Ted Hupp**, Cancer Research of UK Cell Signalling Unit, Edinburgh Cancer Research Centre, University of Edinburgh, UK. ***Molecular chaperones in cell transformation.***

# Project progress in 2011
# Increasing scientific expertise through twinning

**Research visits of IIMCB scientists at the twinning institutions**

- Agnieszka Mamińska - laboratory of Harald Stenmark, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway
- Tomasz Sołtysiński - laboratory of Eric Westhof, Institut de Biologie Moléculaire et Cellulaire, Université Louis Pasteur, Strasbourg, France
- Dorota Latek - laboratory of Vicenza Andrisano, Department of Pharmaceutical Sciences, University of Bologna, Italy
- Irina Tuszyńska - ADAMED, Pieńków, Poland
- Emilia Białopiotrowicz - laboratory of Angeles Martin-Requero, Centro de Investigaciones Biológicas, Madrid, Spain
- Katarzyna Dębowska - laboratory of Casper Hoogenraad, Erasmus MC, Rotterdam, The Netherlands
- Malgorzata Figiel - laboratory of Roland Marquet, Université Louis Pasteur, CNRS, Strasbourg, France
- Honorata Czapińska - laboratory of Ruedi Allemann, University of Cardiff, United Kingdom
- Izabela Rutkowska-Włodarczyk - laboratory of Saulius Klimasauskas, Institute of Biotechnology, Vilnius, Lithuania
- Paweł Wiśniewski - laboratrory of Urszula Hibner, Institute of Molecular Genetics, Montpellier, France
- Zuzanna Bukowy - laboratory of Heymut Omran, Department of Pediatrics, University Hospital Münster, Germany
- Tomas Sołtysiński - laboratory of Claus Seidel, Institute of Molecular Physical Chemistry, University of Dusseldorf, Germany

**Short visits of IIMCB researchers at the twinning institutions**

- Janusz Bujnicki - Institute of Biotechnology, Vilnius, Lithuania
- Paweł Wiśniewski - Institute of Molecular Genetics, Montpellier, France
- Janusz Bujnicki - Institute of Molecular Physical Chemistry, University of Dusseldorf, Germany
- Marcin Nowotny - EMBL c/o DESY, Hamburg, Germany
- Sławomir Filipek - Facultat de Medicina, Universitat Autònoma de Barcelona, Spain
- Jacek Kuźnicki - Centre for Neuropathology at the Ludwig-Maximilians-University of Munich, Germany
- Alicja Żylicz and Maciej Żylicz - Institute of Molecular Genetics, Montpellier, France
- Elżbieta Nowak - EMBL c/o DESY, Hamburg, Germany

**Research visits of twinning partners to IIMCB**

- Arash Foroutan, Departament de Bioquímica i Biologia Molecular Universitat Autònoma de Barcelona, Spain
- Thomas Fricke, Cardiff University, United Kingdom
- Jordi Villà i Freixa, Computational Biochemistry and Biophysics lab Research Group on Biomedical Informatics (GRIB) - IMIM/UPF Parc de Recerca Biomèdica de Barcelona, Spain
- Bartosz Wawrzynów, University of Edinburgh, United Kingdom
- Henri Grosjean, Institute of Genetics and Microbiology, University Paris-South, France

**Short visits of twinning partners to IIMCB**

- Česlovas Venclovas, Institute of Biotechnology, Vilnus University, Lithuania
- Urszula Hibner, Institute of Molecular Genetics, Montpellier, France
- Mark Helm, Institute of Pharmacy and Biochemistry, University of Mainz, Germany
- Kaisa Haglund, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway
- Vinceza Andrisano, Manuela Bartolini, Department of Pharmaceutical Sciences, University of Bologna, Italy
- Esteve Padros, Tzvetana Lazarova, Facultat de Medicina, Universitat Autònoma de Barcelona, Spain
- Jorg Hoehfeld, Institute for Cell Biology, Bonn University, Germany
- Niki Tomas Loges, University Hospital, Muenster, Germany

# Expanding research capacity

To increase research capacity at IIMCB, nine experienced scientists selected through an open international competition have been recruited to the project. Additionally, an Equipment Specialist was recruited to support scientists in specialized equipment usage.

**Honorata Czapińska,** Laboratory of Structural Biology, head Matthias Bochtler.

Within the framework of the HEALTH-PROT project we have collaborated with Cardiff University on the elucidation of structures of novel endonucleases. Hpy188I belongs to the GIY-YIG nucleases, which have roles in nucleotide excision repair, Holliday junction resolution and transposon migration. The structure of Hpy188I in complex with DNA represents the first structure of a protein-DNA complex for this group of enzymes and clarifies the previously ill-defined catalytic mechanism. The structure of the TthI-DNA complex provides a rare example for deep amino acid intercalation into non-damaged DNA. DpnI is an unusual endonuclease that uses double readout mechanism to ensure specificity for the methylated target sequence. Moreover we also worked on two protein engineering projects: modified Trp tRNA synthetase of according to published data altered substrate specificity and designed protein scaffold, highly efficient in energy transfer and constructed by coupling eGFP and cyt b562 domains.

**Marcin Pawłowski**, Laboratory of Bioinformatics and Protein Engineering, head Janusz M. Bujnicki.

The MQAPmulti program was created to predict the overall global quality of protein models. The method compares structural features generated from a 3D model with those predicted from its primary sequence (secondary structure, solvent accessibility, contact maps), uses statistical potential to estimate the value of pseudo-energy for a single model, uses hydrogen bonds pseudoenergy, and takes into account information from proteins that are evolutionary related to the target protein. We evaluated the method on CASP9 targets.

The correlation between predicted and real global quality of a model (GDT_TS score) is 0.987. RecobmineIt, the second method developed during the project, is a fully automatic procedure comprising collection of 3rd-party models, local assessment of model quality by MQAPmuli, and recombination of best-scoring fragment. The method was ranked among the 10 best methods in the last CASP experiment.

**Marta Wiśniewska**, Laboratory of Neurodegeneration, head Jacek Kuźnicki.

Involvement of β-catenin in regulation of T-type Ca2+ currents in thalamic neurons.

Although Wnt/β-catenin signalling has been implicated in neurodegenerative diseases, the possible function of β-catenin in mature neurons remains elusive. We found that in the adult mouse brain β-catenin accumulates specifically in thalamic neurons and regulates expression of the Cacna1g gene that encodes a voltage-gated Ca2+ channel Cav3.1. We investigated the consequences of β catenin-induced Cacna1g expression in thalamic neurons at the functional level, in collaboration with electophysiologists from Wroclaw Medical University, and demonstrated that activation of the Wnt/β-catenin signaling pathway increases amplitude of T-type Ca2+currents in these cells. This imply the involvement of β-catenin in regulation of neuronal excitability.

Quantitive analysis of the Stim1 and Stim2 genes' expression in neurons.

The interaction between Ca2+ sensors STIM1 and STIM2 and Ca2+ channel-forming protein ORAI1 is a crucial element of store-operated calcium entry (SOCE) in non-excitable cells. However, the molecular mechanism of SOCE in neurons remains unclear. In particular, the issue of Stim1 gene expression in neurons has been controversial. Understanding the mechanism of neuronal SOCE is important, because some disturbances in calcium homeostasis has been observed in neurodegenerative diseases, including Alzheimer's disease. We performed absolute quantification of Stim1 and Stim2 expression in primary cultures of cortical and hippocampal neurons and in cortical astrocytes for comparison. We also calculated the copy number of the above transcripts in 50 laser-captured hippocampal neurons. The examination showed that cortical and hippocampal neurons express sufficient and comparable amount of both Stim mRNA, and the level of Stim1 expression is similar in neurons and astrocytes.

**Tomasz Węgierski**, Laboratory of Neurodegeneration, head Jacek Kuźnicki.

The work in the laboratory of Prof. J. Kuźnicki focuses on the role of store-operated calcium entry (SOCE) in neuronal physiology and its disturbances in neurodegenerative diseases such as Alzheimer's Disease. SOCE machinery is composed of STIM proteins, which constitute calcium sensors, and ORAI proteins, which constitute calcium channels. Using Split-Ubiquitin System (SUS), a yeast genetic screening system well suited for the interaction analysis of full-length membrane proteins, T. Wegierski performed a search for neuronal partners of STIM and ORAI proteins. In addition, individual cytosolic domains of STIM proteins are being analyzed in a classical yeast-two hybrid assay. The isolated hits are confirmed by independent methodology. The results obtained so far with SUS indicate the existence of an interesting physical link between SOCE machinery and proteins crucially involved in the development of neurodegeneration.

**Elżbieta Nowak**, Laboratory of Protein Structure, head Marcin Nowotny.

The aim of our studies is to determine a crystal structure of a monomeric reverse transcriptase (RT) in complex with the DNA/RNA hybrid substrates. We expressed and purified three monomeric RTs, but only one protein was well-behaved and suitable for crystallization trials. We obtained crystals of the protein-RNA/DNA complex, which diffracted X-rays up to 3.05 Å resolution. The structure was solved by molecular replacement method. The structure reveals the presence of polymerase domain with catalytically bound DNA/RNA hybrid. This is the first such structure for a monomeric RT. The electron density of the RNase H domain is not invisible in our structure due to its mobility. The analyses of contacts between protein and DNA/RNA show that in the polymerase domain reverse transciptases, regardless whether they are monomeric or dimeric share a very similar mode of substrate binding, as well as the details of the catalytic mechanism. Towards the RNase H domain the contacts with the substrate are markedly different and so is the trajectory of the substrate. Based on our structure we also prepared a model of full-length monomeric RT in complex with its substrate. Our results are currently being prepared for publication.

**Zuzanna Bukowy-Bieryllo**, Ciliary Proteins Function Project, head Michał Witt.

Dr Bukowy-Bieryllo visited the laboratory of the HEALTH-PTOR partner, Prof. Heymut Omran in Münster, Germany, where she has learned immunofluorescence staining of respiratory epithelial cells. The method has been introduced into the laboratory of Dr Bukowy-Bieryllo, and further improved due to experience exchange with Dr Anita Becker, a scientist from Prof. Omran's group, who has visited our laboratory this year. Moreover, a manuscript summarizing previous studies performed with Prof. Omran has been prepared by Dr Bukowy-Bieryllo and is currently under revision in Pediatric Pulmonology.

**Paweł Wiśniewski**, Department of Molecular Biology, head Maciej Żylicz.

MDM2 is an E3-ubiquitin ligase and is a major negative regulator of p53 suppressor protein. However, it also possesses chaperone like activities towards mRNA of p53 and towards WT p53 protein. In this project we showed further evidences that ATP-binding to MDM2 is a key player in many regulatory pathways on the level of gene expression involved in cancer cells development and regulation of transcription factors via the PI3/AKT signaling pathway. We found that TEK/Tie2 gene expression turned to be significantly affected by MDM2 K454A, ATP-binding deficient mutant, comparing to MDM2 WT. TEK receptor is involved in the regulation of cell motility, differentiation and angiogenesis, acting predominantly via AKT kinase pathway. Next we showed that phosphorylation of AKT is strongly down-regulated by MDM2 K454A and AKT binding to MDM2 is impaired in MDM2 K454A transfected cells. Finally, we demonstrated that mutated form of MDM2 up-regulates

the transcriptional activity of AP1 and ISRE transcription factors via the PI3/AKT pathway.In complementary study we have shown that human cells are equipped with many functionally distinct subsets of chaperones, some of which seem to be dedicated to (re)folding and some that may have evolved to dispose of non-foldable proteins. We investigated the new HSPA6 protein which lacks the generic chaperone-like properties of other HSP70s and may have evolved to maintain specific critical functions under conditions of severe stress.

**Ewelina Szymańska**, Laboratory of Cell Biology, head Marta Miączyńska.
The project was aimed to identify novel alternative functions of endocytic proteins in the regulation of gene transcription mediated by AP-1 transcription factor. During the first year of the project primary screens involving RNAi-mediated knockdown of selected endocytic genes were performed and finally both positive and negative potential regulators of AP-1 were identified. In the second year, to validate the selected candidates, their impact on AP-1 activity was further tested in the secondary assays. First, to exclude false-positives the additional siRNA targeting candidate genes were used to reproduce the effect on AP-1 from primary screens and the silencing efficiency of all used siRNA was estimated by qRT-PCR and western blotting. Finally, we analyzed the impact of knockdown of selected endocytic proteins on AP-1 activity during pathway stimulation and on expression of AP-1 target genes.

**Matylda Macias**, Laboratory of Molecular and Cellular Neurobiology, head Jacek Jaworski.
The major objective of Laboratory of Molecular and Cell Neurobiology in frame of Health-Prot grant is understanding how mTOR influences cytoskeleton dynamics with special focus on microtubule + end tracking proteins (+TIPs). Work done in LMCN, thus far, clearly demonstrates that neuronal activity and mTOR influence protein-protein interactions of these proteins as well as their spatial distribution. These proteins contribute to morphological changes of dendrites, axons and synapses. In 2011 Dr. Macias focused investigated mTOR activity and +TIPs in epileptogenesis, a pathological process characterized by abnormal neuronal activity and gross morphological changes in the brain. As one of the aims of HelthProt project was to investigate such relationship in vivo, she tested effects of in vivo inhibition of mTOR activity. Her research shows that chronic mTOR inhibition causes gross brain morphology changes, most likely due to dysfunction of ependymal cells surrounding brain ventricles, major activity of which is control of microtubule based spinocerebral fluid circulation. Currently we investigate potential link between mTOR, microtubules and ependymal cells.

**Roman Szczepanowski**, equipment specialist assists newly employed postdoctoral researchers in scientific and technical matters related to the usage of highly specialized research equipment.

# Organization of scientific events

- Workshop "**Biology of cancer**" 12-13.06.2010, Warsaw, organizers A. Żylicz and M. Żylicz, 40 participants including 18 lecturers
- Session "**Calcium toolkit**" within the European Calcium Society meeting 6-9.09.2010, Warsaw, organizer J. Kuźnicki, 300 participants including 5 lecturers
- Workshop "**Proteins: structures, folding, and interactions**", 27-30.08.2010, Warsaw, organizers J. M. Bujnicki, M. Bochtler and M. Nowotny, 60 participants including 26 lecturers
- Workshop "**Mechanisms of cytoskeleton dynamics and intracellular trafficking**" 21-24.10.2010, Warsaw, organizers M. Miączyńska and J. Jaworski, 103 participants including 27 lecturers
- Conference „**The Modern Techniques for Drug Design Purposes**", 4-5.10.2011, Warsaw; organizer S. Filipek, 48 participants including 16 lectures
- „**HealthProt Symposium on Inherited Disorders of Ciliary Function**", 25.11.2011, Warsaw, organizer M. Witt, 76 participants including 14 lectures
- **IIMCB-MPS Research Group Leader Search Symposium**, 22.03.2012, Bad Nauheim, Germany
- HEALTH-PROT Workshop „**Summary of scientific results of the project and domestic actions beyond**" 26.03.2012, Sopot, Poland
- HEALTH-PROT/SMM Workshop "**From Gene to Phenotype – Interdisciplinary Research in Molecular Biology and Biomedicine**" 28-30.03.2012, Warsaw

# Participation in international events

**Matthias Bochtler**, Laboratory of Structural Biology
Biophysical chemistry, molecular biology and cybernetics of cell functions, 13-25.01.2011, Klosters, Switzerland
Oral presentation: "Symmetry and pseudosymmetry in protein DNA interactions"

**Jacek Kuźnicki**, Laboratory of Neurodegeneration
AD/PD Conference, 9-13.03.2011, Barcelona, Spain
Poster: "No correlation between levels of secreted beta amyloid, dysregulation of cell cycle and age of onset of Alzheimer's disease patients with different presenilin1 FAD mutations"

**Urszula Wojda**, Laboratory of Neurodegeneration
AD/PD Conference, 9-13.03.2011, Barcelona, Spain
Poster: "No correlation between levels of secreted beta amyloid, dysregulation of cell cycle and age of onset of Alzheimer's disease patients with different presenilin1 FAD mutations"

**Marcin Nowotny**, Laboratory of Protein Structure
Responses to DNA damage: from molecular mechanism to human disease, 3-8.04.2011, Egmond aan Zee, The Netherlands
Oral presentation: "Structural studies of bacterial NER proteins"

**Janusz M. Bujnicki**, Laboratory of Bioinformatics and Protein Engineering
Cold Spring Harbor- Asia conference: High Throughput Biolo, 19-23.04.2011, Suzhou, China
Poster: "New computational methods for RNA 3D structure prediction"

**Alicja Żylicz**, Department of Molecular Biology
The Biology of Molecular Chaperones, From basic mechanism to intervention strategies in disease and aging", 19-24.05.2011, Grundlsee, Austria
Poster: "Hsp70 in mutant p53 gain-of-function phenotypes"

**Maciej Żylicz**, Department of Molecular Biology
The Biology of Molecular Chaperones, From basic mechanism to intervention strategies in disease and aging", 19-24.05.2011, Grundlsee, Austria

**Michał Witt**, Ciliary Proteins Function Project
International Conference on Inherited Disorders of Mucociliary Clearance (Focus on PCD), 20-22.05.2011, Muenster, Germany
Oral presentation: "Symptomatic ciliary dyskinesias: does RPGR cause PCD?"

**Szymon Niewieczerzał**
Frontiers in Medicinal Chemistry, 19-21.06.2011, Stockholm, Sweden
Poster: "An approach for a new coarse grain – implicit solvent method for simulations of membrane proteins"

**Matthias Bochtler**, Laboratory of Structural Biology
Nucleic Acid Enzymes & Enzymes in Human Disease, 19-24.06.2011, Tianjin, China
Poster: "Structural variability of type II restriction endonucleases"

**Joanna Gruszczyńska-Biegała**, Laboratory of Neurodegeneration
8th IBRO World Congress on Neuroscience, 14-18.07. 2011, Florence, Italy
Poster: "Ca2+ release from ER stores in Alzheimer's disease models"

**Sławomir Filipek**
VII Joint Meeting on Medicinal Chemistry, 30.06-2.07.2011, 2011, University of Catania, Sicily, Italy
Poster: "The CTF Presenilin-1 – the first protein structure from gamma-secretase complex – numerical simulations in micelles and lipid bilayers and interactions with APP"

**Janusz M. Bujnicki**, Laboratory of Bioinformatics and Protein Engineering
9th Annual International Conference on Intelligent Systems for Molecular Biology (ISMB) and 10th European Conference on Computational Biology (ECCB), J. 17-19.07.2011, Vienna, Austria
Poster: "New computational methods for RNA 3D structure prediction"

**Grzegorz Chojnowski**, Laboratory of Bioinformatics and Protein Engineering
9th Annual International Conference on Intelligent Systems for Molecular Biology (ISMB) and 10th European Conference on Computational Biology (ECCB), J. 15-19.07.2011, Vienna, Austria
Poster: "RIBER and DIBER: a software suite for crystal content analysis in the studies of protein-nucleic acid complexes"

**Malgorzata Figiel**, Laboratory of Protein Structure
International Union of Crystallography Congress, 22-30.08.2011, Madrid, Spain
Poster: "Crystal structure of human RNase H2"

**Matthias Bochtler**, Laboratory of Structural Biology
XXII Congress and General Assembly, International Union of Crystallography, 22-30.08.2011, Madrid, Spain
Oral presentation: "Diversity of type II restriction endonucleases"

**Honorata Czapińska**, Laboratory of Structural Biology
XXII Congress and General Assembly, International Union of Crystallography, 22-30.08.2011, Madrid, Spain
Poster: "Hpy188I-DNA structures - snapshots of the GIY-YIG nuclease mediated catalysis"

**Marta Miączyńska**, Laboratory of Cell Biology
EMBO meeting advancing the life sciences, 10-13.09. 2011,Vienna, Austria
Poster: "Tracking the endocytic pathways of internalized platelet-derived growth factor (PDGF) and their impact on signaling"

**Marta Miączyńska**, Laboratory of Cell Biology
EMBO Conference Series: Dynamic endosomes: mechanisms controlling endocytosis, 24-29.09.2011, Kato Galatas, Crete, Greece
Oral presentation: "Tracking the endocytic pathways of internalized platelet-derived growth factor (PDGF) and their impact on signaling"

**Zuzanna Tracz**, Department of Molecular Biology
The 2011 NCRI Cancer Conference, Nov 6, 2011 - Nov 9, 2011 Liverpool, United Kingdom
Poster: "Stress induced mutant p53 gain-of-function phenotypes"

**Milena Wiech**, Department of Molecular Biology
Course of Microscopy and Imaging, 17-20.10.2011, Geneva, Switzerland

# Promotion and management

**Research Symposium and International Advisory Board meeting**

Scientific achievements of the project were presented by senior scientists at the Research Symposium on May, 13th, 2011:

- Zuzanna Bukowy "Use of the in vitro ciliogenesis method for analysis of cilia structure and function"
- Łukasz Świech "CLIP-170 and IQGAP1 cooperatively regulate dendrite morphology"
- Marcin Pawłowski "New computational methods for protein structure prediction and model quality estimation"
- Marta Wiśniewska "Genetic program activated by β-catenin in mature neurons"
- Elżbieta Nowak "Structural studies of monomeric reverse transcriptases"
- Paweł Wiśniewski "ATP-dependent MDM2 activity in the regulation of cellular signaling in NSCLC cells"
- Matthias Bochtler "A new CG methyltransferase"

At the annual meeting of the International Advisory Board, Dr. Urszula Białek-Wyrzykowska presented the report on the activities within the HEALTH-PROT since the start of the project. After the

presentation covering all workpackages members of the Board fully accepted a pace of progress and a quality of events organized. Increase in scientific expertise and expansion of research capacity was appreciated. Board members also positively evaluated research progress of the project presented at the Research Symposium.

**Other promotional activities**

Conference: FP7 Health Partnering Event, Brussels, Belgium, 10.06.2011, Presentation by Dr Urszula Bialek-Wyrzykowska

Conference: Multi-Pole Approach to Structural Biology, IIMCB, Warsaw, Poland, 16-19.11.2011. Lectures and posters on project results

Presentation of HEALTH-PROT as a success story on NCP website designed to highlight Polish successful projects financed under FP5, FP6 and FP7

Participation in other initiatives by EC, NCP and the Ministry of Science aiming at promotion of Polish science and shaping of FP8

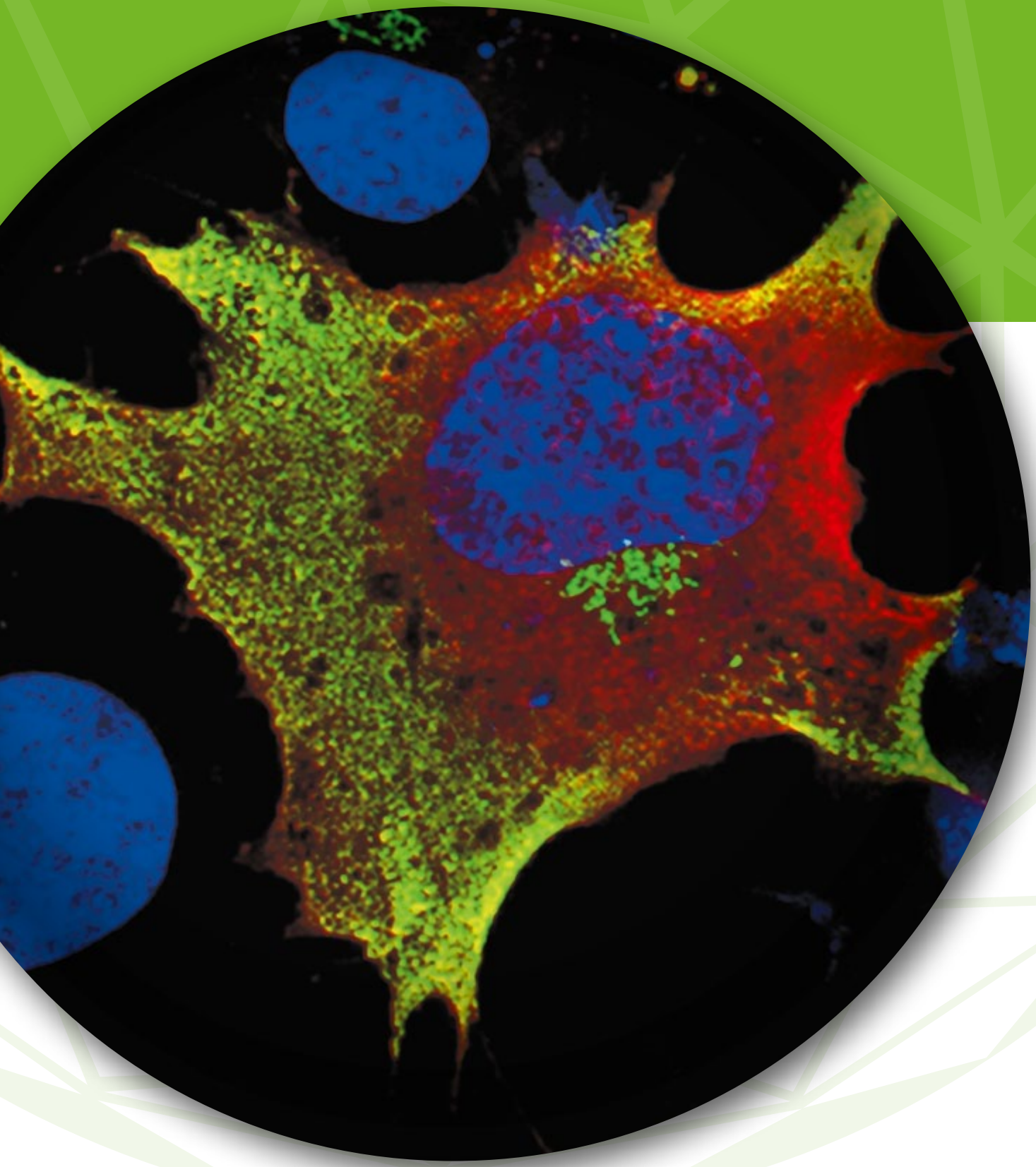
# Publications resulting from the project


- **Czerwoniec A**, Kasprzak JM, **Kaminska KH, Rother K, Purta E, Bujnicki JM**. Folds and functions of domains in RNA modification enzymes, In "DNA and RNA modification enzymes: comparative structure, mechanism, functions, cellular interactions and evolution". Editor: Grosjean H. Landes Bioscience 2009
- **Czerwoniec A, Dunin-Horkawicz S, Purta E, Kaminska KH, Kasprzak JM, Bujnicki JM**, Grosjean H, **Rother K.** MODOMICS: a database of RNA modification pathways. 2008 update. Nucleic Acids Res 2009 Jan;37(Database issue):D118-21
- **Gajda MJ, Tuszynska I**, Kaczor M, Bakulina AY, **Bujnicki JM**. FILTREST3D: discrimination of structural models using restraints from experimental data. Bioinformatics 2010 Dec 1;26(23):2986-7
- **Wisniewska M, Misztal K, Michowski W**, Szczot M, **Purta E**, Lesniak W, **Klejman M**, Dąbrowski M, Filipkowski R, **Nagalski A**, Mozrzymas J, **Kuznicki J**. LEF1/beta-catenin complex regulates transcription of the Cav3.1 calcium channel gene (Cacna1g) in thalamic neurons of the adult brain. J Neurosci. 2010 Apr 7;30(14):4957-69
- **Miaczynska M**, Bar-Sagi D. Signaling endosomes: seing is believing. Curr Opin Cell Biol. 2010 Aug;22(4):535-40
- **Kosinski J**, Hinrichsen I, **Bujnicki JM**, Friedhoff P, Plotz G. Identification of Lynch syndrome mutations in the MLH1-PMS2 interface that disturb dimerization and mismatch repair. Human Mut 2010 Aug;31(8):975-82
- **Sokolowska M, Czapinska H, Bochtler M**. Hpy188I-DNA pre- and post-cleavage complexes-snapshots of the GIY-YIG nuclease mediated catalysis. Nucleic Acids Res. 2011 Mar 1;39(4):1554-64
- Firczuk M, **Wojciechowski M, Czapinska H, Bochtler M**. DNA intercalation without flipping in the specific ThaI-DNA complex. Nucleic Acids Res. 2011 Jan;39(2):744-54
- Antonczaka A K, Simova Z, Yonemoto I T, **Bochtler M**, Piasecka A, **Czapinska H**, Brancale A, Tippmann E M. Importance of single molecular determinants in the fidelity of expanded genetic codes. Proc Natl Acad Sci USA. 2011 Jan 25;108(4):1320-5
- Hageman J, van Waarde-Verhagen M, **Zylicz A, Walerych D**, Kampinga HH. The diverse members of the mammalian HSP70 machine show distinct chaperone-like activities. Biochem J. 2011 Apr 1;435(1):127-42
- **Milanowska K**, Krwawicz J, **Papaj G, Kosinski J, Poleszak K, Lesiak J, Osinska E, Rother K, Bujnicki JM**. REPAIRtoire – a database of DNA repair pathways. Nucleic Acids Res. 2011 Jan;39(Database issue):D788-92
- Zietkiewicz E, Nitka B, Voelkel K, Skrzypczak U, **Bukowy Z**, Rutkiewicz E, Huminska K, Przystalowska H, Pogorzelski A, **Witt M**. Population specificity of the DNAI1 gene mutation spectrum in primary ciliary dyskinesia (PCD). Respir Res. 2010 Dec 8;11(1):174
- **Gajda MJ, Pawlowski M, Bujnicki JM**. Protein structure prediction: from recognition of matches with known structures to recombination of fragments. Book chapter in „Multiscale approaches to protein modeling: structure prediction, dynamics, thermodynamics and macromolecular assemblies". Editor: Kolinski A, Springer, 2010, ISBN: 978-1-4419-6888-3
- **Bukowy Z**, Zietkiewicz E, **Witt M**. In vitro culturing of ciliary respiratory cells - a model for studies of genetic diseases. J Appl Genet. 2011 Feb;52(1):39-51
- **Swiech L, Blazejczyk M, Urbanska M, Pietruszka P**, Dortland B. R, **Malik A. R**, Wulf P. S, Hoogenraad C. C, **Jaworski J**. CLIP-170 and IQGAP1 cooperatively regulate dendrite morphology. J Neurosci. 2011 Mar 23;31(12):4555-68
- **Perycz M, Urbanska AS**, Krawczyk PS, Parobczak K, **Jaworski J.** Zipcode binding protein 1 regulates the development of dendritic arbors in hippocampal neurons. J Neurosci. April 6, 2011; 31(14):5271–85
- **Pilecka I, Sadowski L**, Kalaidzidis Y, **Miaczynska M**. Recruitment of APPL1 to ubiquitin-rich aggresomes in response to proteasomal impairment. Exp Cell Res. 2011, 317:1093-107
- **Gruszczynska-Biegala J**, Pomorski P, **Wisniewska MB, Kuznicki J**. Differential roles for STIM1 and STIM2 in store-operated calcium entry in rat neurons. PLoS ONE, April 2011, Volume 6, Issue 4
- **Misztal K, Wisniewska MB, Ambrozkiewicz M, Nagalski A, Kuznicki J.** WNT-independent constitutive nuclear localization of β-catenin and its low degradation rate in thalamic neurons. J Biol Chem. 2011 Sep 9;286(36):31781-8
- **Urbanska A, Sadowski L**, Kalaidzidis Y, **Miaczynska M**. Biochemical Characterization of APPL Endosomes: The Role of Annexin A2 in APPL Membrane Recruitment. Traffic 2011 Sep;12(9):1227-41
- Kapitein L, Yau KH, Montenegro Gouveia S, van der Zwan W, Wulf P, Keijzer N, Demmers J, **Jaworski J**, Akhmanova A, Hoogenraad CC. NMDA receptor activation suppresses microtubule growth and spine entry. J Neurosci. 2011 Jun 1;31(22):8194-209.
- **Milanowska K, Rother K, Bujnicki JM**. Databases and bioinformatics tools for the study of DNA repair. Molecular Biology International 2011;2011:475718.

- **Rother K, Potrzebowski W**, Puton T, Rother M, Wywial E, **Bujnicki JM**. A toolbox for developing bioinformatics software. Briefings in Bioinformatics 2012 Mar;13(2):244-57
- **Hupalowska A, Miaczynska M**. The New Faces of Endocytosis in Signaling. Traffic 2012 Jan;13(1):9-18
- **Urbanska M, Swiech L, Jaworski J**. Developmental plasticity of the dendritic compartment: focus on the cytoskeleton' in 'Synaptic Plasticity – Dynamics, Development and Disease'. edited by Kreutz M, Sala C. Springer Wien New York, in press
- **Swiech L, Urbanska M, Macias M, Skalecka A, Jaworski J**. „Mammalian Target of Rapamycin" in Protein Kinase Technologies. edited by Mukai H., Humana Press, in press
- Drozdz M, Piekarowicz A, **Bujnicki JM**, Radlinska M. Novel nonspecific DNA adenine methyltransferases. Nucleic Acid Res 2011 Nov 18. [Epub ahead of print] doi: 10.1093/nar/gkr1039
- Mebrhatu M, **Wywial E**, Ghosh A, Michiels C, Lindner A, Taddei F, **Bujnicki JM**, van Melderen L, Aertsen A. Evidence for an evolutionary antagonism between Mrr and Type III modification systems. Nucleic Acid Res 2011 Aug 1;39(14):5991-6001
- Kozlowski L, Orlowski J, **Bujnicki JM**. Structure prediction of alternatively spliced proteins In „Alternative Pre-mRNA Splicing: Theory and Protocols". Editors: Stamm S, Smith C, Luhrmann R, Wiley-Blackwell, 2012, ISBN: 978-3-527-32606-8
- **Philips A, Milanowska K, Lach G, Boniecki M, Rother K, Bujnicki JM**. MetalionRNA: computational predictor of metal-binding sites in RNA structures. Bioinformatics 2012 Jan 15;28(2):198-205
- Nakagome S, Mano S, **Kozlowski L, Bujnicki JM**, Shibata H, Fukumaki Y, Kidd JR, Kidd KK, Kawamura S, Oota H. Crohn's disease risk alleles on the NOD2 locus have been maintained by natural selection on standing variation. Mol Biol Evol 2012 Jan [Epub ahead of print] doi: 10.1093/molbev/mss006
- **Chojnowski G, Bujnicki JM, Bochtler M**. RIBER/DIBER: a software suite for crystal content analysis in the studies of protein-nucleic acid complexes. Bioinformatics 2012 Jan 11. [Epub ahead of print] doi: 10.1093/bioinformatics/bts00
- Trzaskowski B, **Latek D, Yuan S, Ghoshdastider U**, Debinski A, Filipek S. Action of Molecular Switches in GPCRs - Theoretical and Experimental Studies. Current Medicinal Chemistry, 2012, 19, 1090-1109
- Pulawski W, **Ghoshdastider U**, Andrisano V, Filipek S. Ubiquitous Amyloids. Applied Biochemistry and Biotechnology, 2012, DOI: 10.1007/s12010-012-9549-3


# Successful grant applications prepared with HEALTH-PROT partners

- EU/FP7 - ImageNinND "Imaging Neurogenesis in Neurodegenerative Disease: In vivo imaging of dopaminergic adult-born neurons in the olfactory bulb of animal models of Parkinson's disease" (ERA-NETNEURON/03/2010); 1,085,875 PLN; 2010-2013; **J. Jaworski/J. Herms**
- Structrural Funds - IE OP 1.1.2 Programme MPD "PhD Programme in Molecular Biology: Studies of nucleic acids and proteins - from basic to applied research"; (MPD/2009-3/2); 2,265,421 PLN; 2010-2015; **J. Jaworski/C. Hoogenraad; J. Kuźnicki/J. Herms; M. Witt/H. Omran; M. Żylicz/T. Hupp**
- Structrural Funds - IE OP 1.1.2 Programme TEAM "Structural biology of methylation and hydroxymethylation"; 2,023,940 PLN; 2011-2015; **M. Bochtler/S. Klimasauskas**
- Polish Norwegian Research Fund "Screening for novel functions of endocytic and autophagic proteins in the regulation of gene expression, cell growth and carcinogenesis" (PNRF-27-AI-1/07); 672,572 EUR; 2010-2011; **M. Miączyńska/H. Stenmark**
- Ministerial Research Grant "Function of STIM proteins in capacitative calcium entry to the ER of healthy neurons and of cells with calcium dyshomeostasis in Alzheimer's disease" (NN301190039); 480,000 PLN; 2010-2013; **J. Kuźnicki/J. Herms**
- Ministerial Research Grant "Mechanism of oncogenic activities of mutated TP53" (NN302621838); 600,000 PLN; 2010-2013; **A. Żylicz/T. Hupp**
- Ministerial Research Grant "Biochemical and structural studies of lentiviral reverse transcriptases" (NN301439738); 599,800 PLN; 2010-2013; **M. Nowotny/R. Marquet**
- Polish Swiss Research Fund „The role of tumor susceptibility gene 101 (Tsg101) in transcriptional regulation in health and disease" (PSPB-094/2010); 5,365,200 PLN; 2012-2016; **M. Miączyńska; project based on HEALTH-PROT results**
- Structural Funds, IE OP 1.2. Programme POMOST "Characterization of selected endocytic proteins as novel regulators of AP-1 mediated transcription" (POMOST/2011-3/11); 430,000 PLN; 2012-2014; **E. Szymańska; continuation of research conducted in HEALTH-PROT**
- NCN, MAESTRO "New functions of ebdocytic proteins in transcriptional regulation"; 2,875,000 PLN; 2012-2017 **M. Miączyńska; project based on HEALTH-PROT results**



Egzogenic HSP70/HSPA1 in MEFs (red – HSP70, blue nucleus, green – Golgi).

# Department of **Molecular Biology**

## Lab leader: **Maciej Żylicz**, PhD, Professor

**Vice Head:**
Alicja Żylicz, PhD, Professor (until September 2011)

**Senior Researcher:**
Bartosz Wawrzynów, PhD

**Postdoctoral Fellow:**
Paweł Wiśniewski, PhD

**Junior Researchers:**
Marta Małuszek, MSc
Magdalena Pruszko, MSc
Zuzanna Tracz-Gaszewska, MSc
Milena Wiech, MSc

**Secretary:**
Grażyna Orleańska, MSc

**Technician:**
Wanda Gocal

# Head of Department of Molecular Biology

## **Maciej Żylicz**, PhD, Professor

### Degrees

| | |
|---|---|
| 1992 | Professor, nomination by the President of the Republic of Poland |
| 1986 | DSc Habil in Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland |
| 1979 | PhD in Biochemistry, Medical University of Gdansk, Poland |
| 1977 | MSc in Physics, University of Gdansk, Poland (student of physics and biology) |

### Postdoctoral Training

| | |
|---|---|
| 1982-1984 | Department of Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City, Utah, USA, and Department of Biochemistry, Stanford University, Stanford, California, USA |
| 1979-1981 | Department of Biochemistry, University of Gdansk, Poland |

### Professional Employment

| | |
|---|---|
| 2005-Present | President, Executive Director, Foundation for Polish Science |
| 1999-Present | Head, Department of Molecular Biology, IIMCB |
| 1994-1999 | Head, Department of Molecular and Cellular Biology, Faculty of Biotechnology, University of Gdansk, Poland |
| 1991-1994 | Head, Department of Molecular Biology, University of Gdansk, Poland |
| 1993-1994 | Visiting Professor, University of Utah, Medical Center, Institute of Oncology, Salt Lake City, Utah, USA |
| 1990-1993 | Vice President, University of Gdansk, Poland |
| 1988-1991 | Associate Professor, Department of Molecular Biology, University of Gdansk, Poland |
| 1981-1988 | Assistant Professor, Department of Biochemistry, University of Gdansk, Poland |

### Other Professional Activities

| | |
|---|---|
| 2010-Present | Advisor of the President of the Republic of Poland |
| 2010-Present | Member, ERC Identification Committee (appointed by European Commission) |
| 2010-Present | Chair of Selection Committee, Council of the National Science Center, Poland |
| 2008-2010 | Panel Chair, Molecular and Structural Biology and Biochemistry (LS1), ERC |
| 2000-2004 | Chair of Biology, Earth Sciences and Environmental Protection Commission, State Committee for Scientific Research, Poland |
| 2000-2001 | Chair of Basic Science Commission, State Committee for Scientific Research, Poland |

### Membership in Scientific Societies, Organizations, and Panels

- European Molecular Biology Organization (EMBO), Member
- Polish Academy of Sciences, Full Member
- German National Academy of Sciences Leopoldina, Member
- Polish Academy of Arts and Sciences, Member
- Academia Europaea, Member
- European Academy of Cancer Research, Member
- American Society of Biochemistry and Molecular Biology, Member
- Advisory Editorial Board, EMBO Journal, EMBO Reports (2004-2008), and IUBMB Life, Member
- EMBO Council (2004-2007), Member
- Selection Committee, EMBO Young Investigator Programme (2001-2003), Member
- European Molecular Biology Conference (2001-2004), Polish delegate
- European Science Foundation Life Science Committee (2003-2005), Polish Delegate
- Selection Committee, Special DFG Programmes (2001-2005), Member
- Max Planck Society, Member of Senate
- State Committee for Scientific Research (1997-2004), Member

### Honors, Prizes and Awards

| | |
|---|---|
| 2011 | Doctor Honoris Causa, University of Gdansk |
| 2008 | Officer's Cross of the Order of Polonia Restituta (awarded by the President of the Republic of Poland) |
| 2007 | Doctor Honoris Causa, University of Wrocław |
| 2002 | Prime Minister Award for Scientific Achievements |
| 2001 | Marchlewski Award, Committee of Biochemistry and Biophysics, Polish Academy of Sciences |
| 1999 | Award in biological/medical sciences, Foundation for Polish Science |

| | |
|---|---|
| 1996, 2007, 2010 | Awards for best biochemistry work performed in Polish laboratories, Polish Biochemical Society |
| 1994 | Award from Ministry of Education |
| 1993 | Heweliusz Prize for Scientific Achievements (awarded by President of Gdansk) |
| 1990 | Award from Polish Academy of Sciences |
| 1986 | Individual Award for Scientific Achievements, Polish Academy of Sciences |

### Doctorates

Liberek K, Skowyra D, Osipiuk J, Banecki B, Wojtkowiak D, Jakóbkiewicz J, Puzewicz J, Barski P, King F, Bućko-Justyna M, Kudła G, Helwak A, Lipiński L, Szymańska Z, Urbański J

### Academic Habilitations

Liberek K, Werel W, Marszałek J, Konieczny I, Wawrzynów A, Banecki B, Bieganowski P

### Professor Titles Received

Liberek K, Marszalek J, Konieczny I, Wawrzynów A

### Publications

Over 80 publications in primary scientific journals, including two papers published in *Cell*, six in *EMBO J*, six in *Proc Natl Acad Sci USA*, and more than 30 in *J Biol Chem*. These papers were cited more than 5500 times with a Hirsch index of H = 40.

## Selected publications


- Hageman J, van Waarde MA, **Zylicz A**, **Walerych D**, Kampinga HH. The diverse members of the mammalian HSP70 machine show distinct chaperone-like activities. **Biochem J**, 2011; 435:127-142
- **Walerych D, Gutkowska M, Klejman MP, Wawrzynow B, Tracz Z, Wiech M, Zylicz M, Zylicz A**. ATP binding to Hsp90 is sufficient for effective chaperoning of p53 protein. J Biol Chem, 2010; 285:32020-8
- Zubrienė A, **Gutkowska M**, Matulienė J, Chaleckis R, Michailovienė V, Voroncova A, Venclovas C, **Zylicz A, Zylicz M**, Matulis D. Thermodynamics of radicicol binding to human Hsp90 alpha and beta isoforms. Biophys Chem, 2010; 152:153-163
- **Zurawska A, Urbanski J**, Matulienė J, Baraniak J, **Klejman MP**, Filipek S, Matulis D, **Bieganowski P**. Mutations that increase both Hsp90 ATPase activity in vitro and Hsp90 drug resistance in vivo. Biochim Biophys Acta – Mol Cell Res, 2010; 1803:575-583
- Kirkegaard T, Roth AG, Petersen NH, Mahalka AK, Olsen OD, Moilanen I, **Zylicz A**, Knudsen J, Sandhoff K, Arenz C, Kinnunen PK, Nylandsted J, Jäättelä M. Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated lysosomal pathology. Nature, 2010; 463:549-553
- **Walerych D, Olszewski MB, Gutkowska M, Helwak A, Zylicz M, Zylicz A**. Hsp70 molecular chaperones are required to support p53 tumor suppressor activity under stress conditions. Oncogene, 2009; 28:4284-94
- Narayan V, Eckert M, **Zylicz A, Zylicz M**, Ball KL. Cooperative regulation of the interferon regulatory factor-1 tumor suppressor protein by core components of the molecular chaperone machinery. J Biol Chem, 2009; 284:25889-99
- Wawrzynow B, Pettersson S, **Zylicz A**, Bramham J, Worrall E, Hupp TR, Ball KL. A function for the RING finger domain in the allosteric control of MDM2 conformation and activity. J Biol Chem, 2009; 284:11517-30
- **Szymanska Z, Zylicz M**. Mathematical modeling of heat shock protein synthesis in response to temperature change. J Theor Biol, 2009; 259:562-569
- **Szymanska Z, Urbanski J**, Marciniak-Czochra A. Mathematical modelling of the influence of heat shock proteins on cancer invasion of tissue. J Math Biol, 2009; 58:819-44
- **Zurawska A, Urbanski J, Bieganowski P**. Hsp90n - An accidental product of a fortuitous chromosomal translocation rather than a regular Hsp90 family member of human proteome. Biochim Biophys Acta, 2008; 1784:1844-6
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# Summary of work

The research conducted in the Department of Molecular Biology is mainly focused on the activities of molecular chaperones in mammalian cells, including cell transformation. Using highly purified recombinant human proteins, we previously identified intermediate reactions that lead to the assembly of molecular chaperone complexes with the wildtype or mutant p53 tumor suppressor protein. We also demonstrated that the HSP90 molecular chaperone was required for the binding of wildtype p53 to the promoter sequences under a physiological temperature of 37°C and that chaperoning activity was adenosine triphosphate (ATP)-dependent. Recently, we provided *in vivo* evidence that HSP90 and HSP70/HSPA chaperone machines were required for the proper folding of wildtype p53, its specific binding to chromatin, and the transcription of p53-dependent genes (Walerych et al., *Oncogene*, 2009). p53 as an unstable protein *in vitro* likely requires stabilizing factors to act as a tumor suppressor *in vivo*. We have shown that in human cells transfected with wildtype *p53*, *HSP90*, and *HSP70*, molecular chaperones maintain the native p53 conformation under heat-shock conditions (42°C) and assist the refolding of p53 at 37°C during the recovery from heat shock. We also demonstrated that the interaction between wildtype p53 and the *WAF1* promoter in cells is sensitive to HSP70 and HSP90 inhibition at 37°C and further decreased upon heat shock. The influence of chaperones on p53 binding to the *WAF1* promoter sequence has been confirmed *in vitro* using highly purified proteins. HSP90 stabilized p53 binding to the promoter sequence at 37°C, whereas under heat shock conditions, the requirement for the HSP70-HSP40 system and its cooperation with HSP90 increased. The Hop co-chaperone additionally stimulated these reactions. Interestingly, the combined HSP90 and HSP70-HSP40 allowed for a limited *in vitro* restoration of DNA binding activity by the p53 oncogenic variant R249S and affected its conformation in cells. Our results indicated for the first time that, especially under stress conditions, not only HSP90 but also HSP70 was required for the chaperoning of wildtype and R249S p53 (Walerych et al., *Oncogene*, 2009).

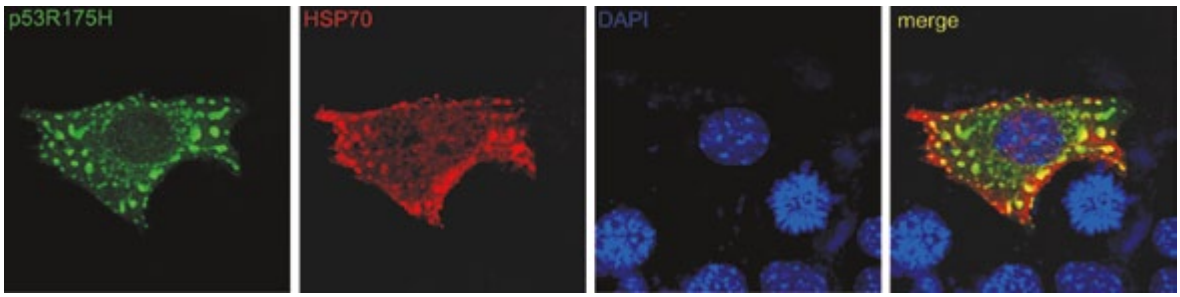
We also elucidated the role of the adenine nucleotide in the HSP90 chaperone cycle by taking advantage of a unique *in vitro* assay that measures HSP90-dependent p53 binding to the promoter sequence (Walerych et al., J Biol Chem, 2010). E42A and D88N HSP90β variants bind but do not hydrolyze ATP, whereas E42A increased and D88N decreased ATP affinity compared with wildtype HSP90β. Nevertheless, both of these mutants interact with wildtype p53 with similar affinity. Surprisingly, in the case of wildtype and also E42A HSP90β, the presence of ATP stimulated the dissociation of HSP90-p53 complexes and resulted in p53 binding to the promoter sequence. D88N HSP90β is not efficient in either of these reactions. Using a trap version of the GroEL chaperonin, which irreversibly captures unfolded proteins, we showed that the HSP90 chaperone action on wildtype p53 resulted in a partial unfolding of the substrate. The ATP-dependent dissociation of the p53-HSP90 complex allowed further folding of the p53 protein to an active conformation that is able to bind to the promoter sequence. Furthermore, supporting these results, the overproduction of wildtype or E42A HSP90β stimulated transcription from the *WAF1* gene promoter in H1299 cells. Altogether, our research indicated that ATP binding to HSP90β was a sufficient step for effective wildtype p53 client protein chaperoning (Walerych et al., *J Biol Chem*, 2010).

HSP90 inhibitors are currently tested in clinical trials as anticancer agents. We investigated whether inhibitor resistance may arise as a result of a point mutation in HSP90 (Zurawska et al., Biochim Biophys Acta – Mol Cell Res, 2010). We used yeast cells that express human HSP90β to select inhibitor-resistant mutants from the randomly mutagenized library. A single amino acid substitution, I123T, in a selected mutant was sufficient to confer inhibitor resistance. Transfection of human cells with *HSP90β* I123T and the corresponding *HSP90β* I128T yielded cell lines resistant to inhibitors of HSP90 ATPase. Unexpectedly, the mutations did not result in diminished inhibitor binding *in vitro*. Similarly, resistant cells were obtained after transfection with previously described A116N and T31I mutants of *HSP90β* that cause an increase in ATPase activity *in vitro*. Inhibitor-resistant phenotypes of the I123T and A116N mutants depended on their increased affinity for Aha1, whereas the T31I mutation did not result in increased Aha1 binding. These results reveal a possible scenario by which resistance may arise in patients treated with HspSP0 inhibitors. Additionally, our results showed that each HSP90 isoform could alone sustain cellular function (Zurawska et al., Biochim Biophys Acta – Mol Cell Res, 2010).

In collaboration with Prof. Jacek Jassem, a clinician oncologist at the Medical University of Gdańsk, we previously demonstrated that *MDM2* overexpression is a new independent factor of adverse prognosis in nonsmall cell lung cancer. We also discovered that MDM2, in addition to its E3-ubiquitin ligase activity, exhibited molecular chaperone activity. We demonstrated that a MDM2 mutant protein that is defective in ATP binding (K454A) lacked chaperone activity both *in vivo* and *in vitro*. Wildtype *MDM2* coexpressed with wildtype *p53* stimulated efficient p53 protein folding *in vivo*, and this effect was abrogated with an ATP binding-defective form of MDM2.

Recently, in collaboration with the Prof. Kathryn Ball laboratory at the University of Edinburgh, we showed that the binding affinity of MDM2's hydrophobic pocket could be regulated through the RING finger domain and that increases in pocket affinity were reflected by a gain in MDM2 transrepressor activity (Wawrzynow et al., J Biol Chem, 2009). Thus, mutations within the RING domain that affect zinc coordination but not mutations that inhibit ATP binding produce MDM2 proteins that have a higher affinity for the BOX-I transactivation domain of p53 and a reduced $I_{0.5}$ for p53 transrepression. An allosteric model of the regulation of the hydrophobic pocket was supported by differences in protein conformation and pocket accessibility between wildtype and RING domain mutant MDM2 proteins. Additionally, the data demonstrated that the complex relationship between different domains of MDM2 can impact the efficacy of anticancer drugs directed toward its hydrophobic pocket (Wawrzynow et al., J Biol Chem, 2009).

Interferon regulatory factor-1 (IRF-1), the founding member of the interferon regulatory factor family, is a transcription factor that regulates a diverse range of target genes during the response to stimuli, such as pathogen infection, DNA damage, and hypoxia. Additionally, the loss of *IRF-1* can cooperate with c-Ha-ras in cellular transformation. It becomes upregulated in cells that

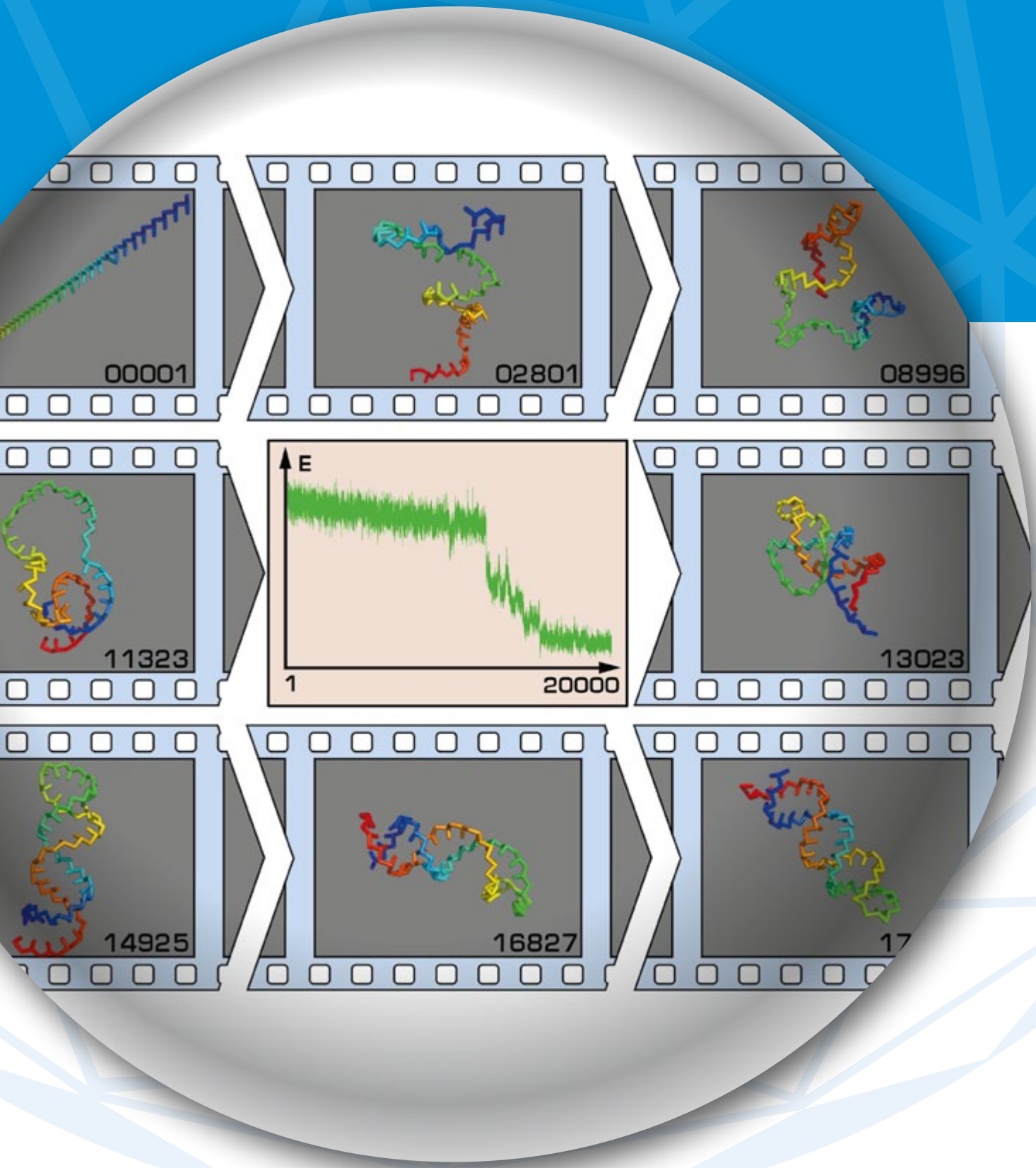



HSP70-dependent aggregation of p53 R175 protein in mouse embryonic fibroblasts (Milena Wiech)

bear oncogenic lesions, and deletions of *IRF-1* are associated with the development of gastric and esophageal tumors and some leukemias. Recently, in collaboration with the Prof. Kathryn Ball laboratory, we provided evidence that linked IRF-1 to the HSP70/HSPA1 and HSP90 families, the core components of the molecular chaperone machinery. Narayan et al. (J Biol Chem, 2009) demonstrated a requirement for the *C*-terminal multifunctional-1 (Mf1; amino acids 301-325) domain of IRF-1 in the recruitment of HSP70 proteins. Consequently, HSP70 was shown to recruit HSP90, together impacting the turnover, localization, and activity of IRF-1. The data highlight a novel IRF-1 interaction that contributes to its activation pathway, suggesting that the molecular chaperones are key components of a regulatory network that maintains IRF-1 tumor suppressor function.

Heat shock protein 70 (HSP70/HSPA1) is an evolutionarily highly conserved molecular chaperone that promotes the survival of stressed cells by inhibiting lysosomal membrane permeabilization, a hallmark of stress-induced cell death. Clues to its molecular mechanism of action may lie in the recently reported stress- and cancer-associated translocation of a small portion of HSP70 to the lysosomal compartment. Prof. Marja Jaattela's laboratory at the Denmark Cancer Institute, in collaboration with our department, showed that HSP70 stabilizes lysosomes by binding to endolysosomal anionic phospholipid bis(monoacylglycero)phosphate (BMP), an essential cofactor for lysosomal sphingomyelin metabolism (Kirkegaard et al., Nature, 2010). In acidic environments, HSP70 binds with high affinity and specificity to BMP, thereby facilitating the BMP binding and activity of acid sphingomyelinase (ASM). Inhibition of the HSP70-BMP interaction by BMP antibodies or a point mutation in HSP70 (Trp90Phe) and the pharmacological and genetic inhibition of ASM effectively reverse the HSP70-mediated stabilization of lysosomes. Notably, the reduced ASM activity in cells from patients with Niemann-Pick disease (NPD) A and B (i.e., severe lysosomal storage disorders caused by mutations in the sphingomyelin phosphodiesterase 1 [*SMPD1*] gene that encodes ASM) is also associated with a marked decrease in lysosomal stability, and this phenotype can be effectively corrected by treatment with recombinant HSP70. Altogether, these data open exciting possibilities for the development of new treatments for lysosomal storage disorders and cancer with compounds that enter the lysosomal lumen through the endocytic delivery pathway (Kirkegaard et al., Nature, 2010).

Humans contain many *HSP70/HSPA*- and *HSP40/DNAJ*-encoding genes, and most of the corresponding proteins are localized in the cytosol. To test for possible functional differences or substrate specificity, the laboratory of Prof. Kampinga (Groningen, The Netherlands) in collaboration with the Department of Molecular Biology (IIMCB) assessed the effect of overexpression of each of these HSPs on the refolding of heat-denatured luciferase and suppression of aggregation of a non-foldable polyQ (polyglutamine)-expanded Huntingtin fragment. Overexpressed chaperones that suppressed polyQ aggregation were found not to be able to stimulate luciferase refolding. Conversely, chaperones that supported luciferase refolding were poor suppressors of polyQ aggregation. This was not related to client specificity itself because the polyQ aggregation inhibitors often also suppressed the heat-induced aggregation of luciferase. Surprisingly, the exclusively heat-inducible HSPA6 lacks both luciferase refolding and polyQ aggregation-suppressing activities. Furthermore, whereas overexpression of *HSPA1A* protected cells from heat-induced cell death, overexpression of *HSPA6* did not. Conversely, siRNA (small interfering RNA)-mediated blockade of *HSPA6* did not impair the development of heat-induced thermotolerance. However, HSPA6 has a functional substrate-binding domain and possesses intrinsic ATPase activity that is as high as that of the canonical HSPA1A when stimulated by J-proteins. *In vitro* data suggest that this may be relevant to substrate specificity because purified HSPA6 could not chaperone heat-unfolded luciferase but was able to assist in the reactivation of heat-unfolded p53. Therefore, even within the highly sequence-conserved HSPA family, functional differentiation is larger than expected, with HSPA6 as an extreme example that may have evolved to maintain specific critical functions under conditions of severe stress (Hageman et al, Biochem J, 2011).




Snapshots of RNA folding simulation using a coarse-grained method SimRNA. Data and artwork by Dr. Michal Boniecki.

# Laboratory of **Bioinformatics and Protein Engineering**

## Lab leader: **Janusz M. Bujnicki**, PhD, Professor

**Postdoctoral Fellows:**
Michał Boniecki, PhD; Grzegorz Chojnowski, PhD; Bogusław Kluge, PhD (from February 2012); Marcin Pawłowski, PhD (until August 2011); Elżbieta Purta, PhD; Krzysztof J. Skowronek, PhD; Tomasz Soltysinski, PhD (until March 2012); Ewa Wywiał, PhD (until December 2011); Tomasz Waleń, PhD; Stanisław Dunin-Horkawicz, PhD; Grzegorz Łach, PhD; Izabela Rutkowska-Włodarczyk, PhD

**Junior Researchers:**
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**Undergraduate Students:**
Xavier Lucas, BSc (until June 2011); Albert Bogdanowicz, BSc; Mateusz Dobrychłop, BSc; Magdalena Byszewska, BSc

**Office Manager:**
Agnieszka Faliszewska, MSc

**Computer Administrators:**
Jan Kogut, BSc; Tomasz Jarzynka; Łukasz Munio

# Head of Laboratory of Bioinformatics and Protein Engineering

## **Janusz Bujnicki**, PhD, Professor

### Degrees

| | |
|---|---|
| 2009 | Professor of Biological Sciences, nomination by the President of the Republic of Poland |
| 2005 | DSc Habil in Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland |
| 2001 | PhD in Biology, University of Warsaw, Faculty of Biology, Poland |
| 1998 | MSc in Microbiology, University of Warsaw, Faculty of Biology, Poland |

### Professional Experience

| | |
|---|---|
| 2002-Present | Head, Laboratory of Bioinformatics and Protein Engineering, IIMCB |
| 2006-Present | Visiting Associate Professor, Bioinformatics Laboratory, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland |
| 2004-2006 | Assistant Professor, Bioinformatics Laboratory, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland |
| 2001-2002 | Group Leader, Molecular Evolution Research Group, Laboratory of Bioinformatics, IIMCB |
| 2001 | Visiting Scientist, Computational Biology Branch, National Center for Biotechnology Information, National Institutes of Health, Bethesda, Maryland, USA (with Dr. E.V. Koonin) |
| 1999-2000 | Research Scientist, Bioinformatics Laboratory, IIMCB (with Dr. L. Rychlewski) |
| 1998-2000 | Senior Research Assistant, Molecular Biology Research Program, Henry Ford Health System, Detroit, Michigan, USA (with Dr. L.C. Lutter) |

### Professional Affiliations

- Polish Bioinformatics Society, PTBI (founding member, Vice-President 2007-2010, President 2011- Present)
- Society of Bioinformatics in Northern Europe (SocBiN) (board member, 2004-Present)
- Member, International Society for Computational Biology
- Member, RNA Society
- Series Editor, *Nucleic Acids and Molecular Biology* (Springer Verlag, 2009-Present)
- Deputy Section Editor, *BMC Bioinformatics* (2010-Present)
- Editorial Board, *Nucleic Acids Research* (2005-Present), *Advances in Bioinformatics* (2008-2011), *Journal of Applied Genetics* (2004-Present), *Database Journal* (2008-Present), *Journal of Nucleic Acids* (2008-Present)

### Awards

| | |
|---|---|
| 2011 | Elected member of the Academy of Young Scientists at Polish Academy of Sciences |
| 2011 | Adam Mickiewicz University Rector Special Award for Top Performance in Publishing High Impact Research Articles in 2010 |
| 2010 | ERC Starting Grant (2011-2015) |
| 2009 | Fellowship for Outstanding Young Scientists, Ministry of Science and Higher Education |
| 2009 | Award for Research Achievements, Ministry of Science and Higher Education (Individual work) |
| 2008 | Adam Mickiewicz University Rector Award for Research Achievements (Individual work) |
| 2006 | Award from Prime Minister for habilitation thesis |
| 2006 | Young Researcher Award in Structural and Evolutionary Biology, Visegrad Group Academies of Sciences |
| 2003, 2004 | Fellowship for Young Scientists, Foundation for Polish Science |
| 2002 | EMBO/Howard Hughes Medical Institute Young Investigator Program Award |
| 2002 | Award for best Polish genetics-related publication in 2001 (*Trends Biochem Sci* 2001, Jan, 26[1]:9-11), Polish Society of Genetics |
| 2001 | Award for best Polish publication on nucleic acid biochemistry in 2000 (*FASEB J* 2000, Nov, 14[14]:2365-2368), Polish Biochemical Society |

# Publications in 2011


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- Czerwoniec A, **Bujnicki JM**. Identification and modeling of a phosphatase-like domain in a tRNA 2′-O-ribosyl phosphate transferase Rit1p. Cell Cycle, 2011; 10(20):3566-70
- Rother M, Rother K, Puton T, **Bujnicki JM**. RNA tertiary structure prediction with ModeRNA. Brief Bioinform, 2011 [Epub ahead of print] doi: 10.1093/bib/bbr050
- **Tuszynska I, Bujnicki JM**. DARS-RNP and QUASI-RNP: New statistical potentials for protein-RNA docking. BMC Bioinformatics, 2011; 12(1):348
- Bateman A, Agrawal S, Birney E, Bruford EA, **Bujnicki JM**, Cochrane G, Cole JR, Dinger ME, Enright AJ, Gardner PP, Gautheret D, Griffiths-Jones S, Harrow J, Herrero J, Holmes IH, Huang HD, Kelly KA, Kersey P, Kozomara A, Lowe TM, Marz M, Moxon S, Pruitt KD, Samuelsson T, Stadler PF, Vilella AJ, Vogel JH, Williams KP, Wright MW, Zwieb C. RNAcentral: a vision for an international database of RNA sequences. RNA, 2011; 17(11):1941-6
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- **Rother M**, **Milanowska K**, **Puton T**, Jeleniewicz J, **Rother K**, **Bujnicki JM**. ModeRNA server: an online tool for modeling RNA 3D structures. Bioinformatics, 2011;27(17):2441-2
- **Milanowska K**, **Rother K**, **Bujnicki JM**. Databases and bioinformatics tools for the study of DNA repair. Mol Biol Int, 2011, 475718 [Epub 2011 Jul 14] doi:10.4061/2011/475718
- **Rother K, Potrzebowski W, Puton T, Rother M**, **Wywial E, Bujnicki JM**. A toolbox for developing bioinformatics software. Brief Bioinform, 2011 Jul 29 [Epub ahead of print] doi:10.1093/bib/bbr035
- Sikorski K, Czerwoniec A, **Bujnicki JM**, Wesoly J, Bluyssen HAR. STAT1 as a novel therapeutical target in pro-atherogenic signal integration of IFNg, TLR4 and IL-6 in vascular disease. Cytokine Growth Factor Rev, 2011; 22(4):211-9
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- Mebrhatu M, **Wywial E**, Ghosh A, Michiels C, Lindner A, Taddei F, **Bujnicki JM**, van Melderen L, Aertsen A. Evidence for an evolutionary antagonism between Mrr and Type III modification systems. Nucleic Acid Res, 2011; 39(14):5991-6001
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- **Rother K, Rother M, Boniecki M, Puton T, Bujnicki JM**. RNA and protein 3D structure modeling: similarities and differences. J Mol Model, 2011; 17(9):2325-36
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# Current Research

The Laboratory of Bioinformatics and Protein Engineering is involved in theoretical and experimental research on sequence-structure-function relationships in proteins and nucleic acids and macromolecular complexes.

Theoretical research involves the development of computer software for the analysis of biological macromolecules. Currently, the focus is on the development of software for the structural prediction and modeling of RNA and RNA-protein complexes. Thus far, we have developed and made publicly available one of the first methods for the automated comparative modeling of RNA (ModeRNA; http://iimcb.genesilico.pl/moderna/), a method for the structure-based prediction of metal ion binding sites (MetalionRNA; http://metalionrna.genesilico.pl/), and statistical potentials for predicting the structure of RNA-protein complexes (DARS-RNP and QUASI-RNP; http://iimcb.genesilico.pl/RNP/).

Our suite of programs for protein structure prediction and analysis include the GeneSilico MetaServer for primary, secondary, and tertiary structure prediction (https://www.genesilico.pl/meta2/), a method for the quality assessment of protein models (MetaMQAP; https://genesilico.pl/toolkit/unimod?method=MetaMQAPII), and a method for the discrimination of models according to their agreement with experimental data (FILTREST3D; http://filtrest3d.genesilico.pl/). We also developed methods for the prediction of order/disorder in protein structures (http://iimcb.genesilico.pl/metadisorder/) and protein localization in Gram-negative bacterial cells (MetaLocGramN; http://genesilico.pl/MetaLocGramN/). We also developed a system of nucleic acid metabolism databases. Published elements of this system include MODOMICS, a database for the systems biology of RNA modification (http://modomics.genesilico.pl/), and the REPAIRtoire database for the systems biology of DNA repair (http://repairtoire.genesilico.pl/).

Our experimental research is focused on the elucidation of sequence-structure-function relationships in proteins and nucleic acids using biophysics, biochemistry, molecular biology, and cell biology methods. Three principal types of analyses are performed by researchers in our "wet lab":

- Experimental testing of functional predictions by gene cloning, protein expression, purification, development of *in vitro* and *in vivo* functional assays, and biochemical and cellular characterization.
- Experimental testing of structural predictions by application of low-resolution structural probing methods, such as mutagenesis, chemical modification, crosslinking, mass spectrometry, circular dichroism, and limited proteolysis.
- Protein engineering to obtain enzymes with new, useful features, particularly altered substrate specificity (e.g., nucleases that recognize and cut new sequences in DNA or RNA).

Our theoretical and experimental research is tightly integrated, demonstrated by the publication of articles that comprise a combination of theoretical and experimental analyses (e.g., prediction and characterization of new enzymes). Protein engineering involves iterative protein structure model building, model-based experimental planning, a series of experimental analyses, and experiment-based improvement of the models and tools used for model building.

# Recent highlights

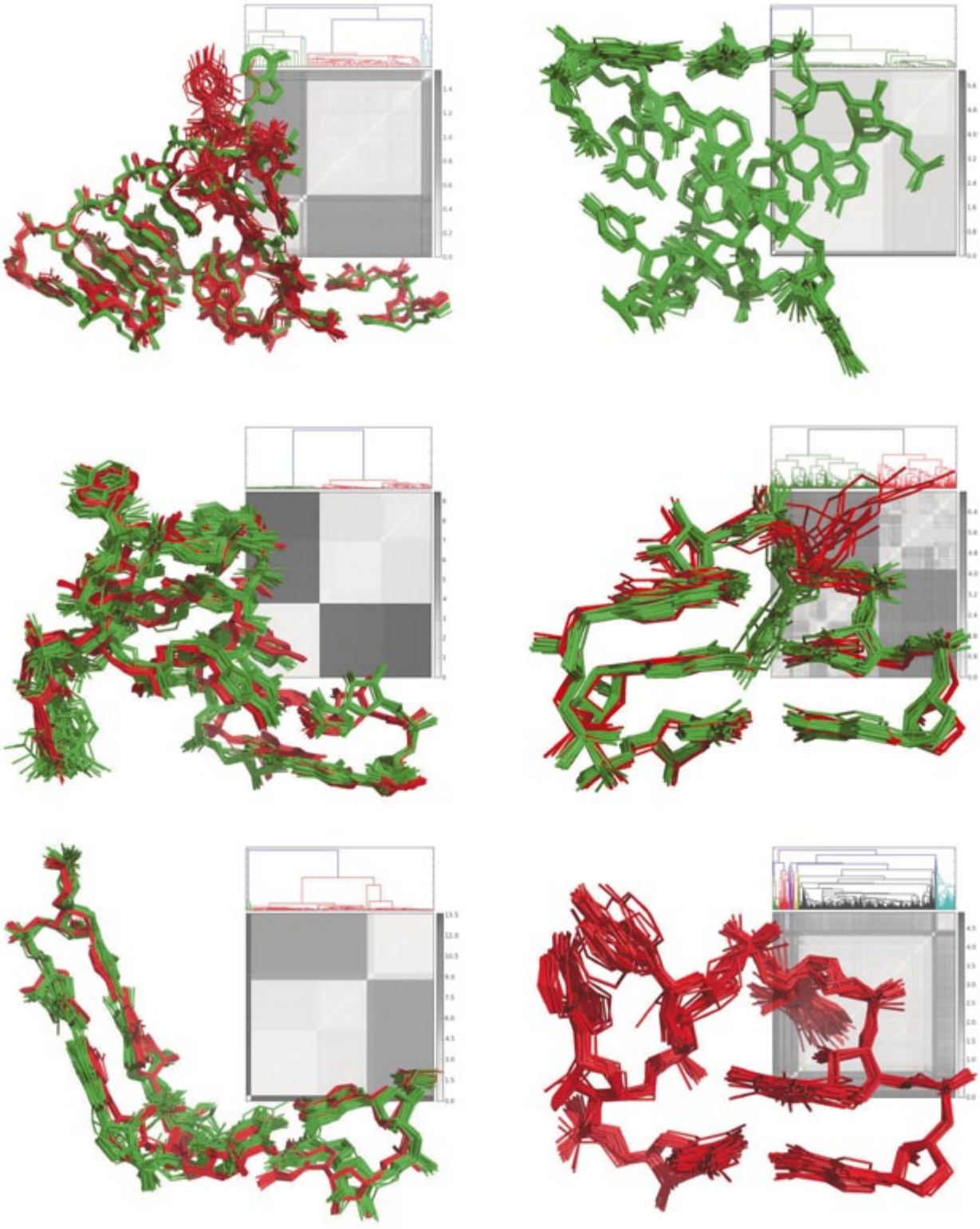
**Experimental characterization of new enzymes – a case study:**

The 5′ cap of human messenger RNA consists of an inverted 7-methylguanosine linked to the first transcribed nucleotide by a unique 5′-5′-triphosphate bond followed by 2′-*O*-ribose methylation of the first and often second transcribed nucleotides, likely serving to modify the efficiency of transcript processing, translation, and stability. Researchers in the Bujnicki laboratory bioinformatically predicted and experimentally verified human genes that encode the enzymes that methylate the ribose of the first and second transcribed nucleotide (hMTr1 and hMTr2, respectively). Neither N(7) methylation of the guanosine cap nor 2′-*O*-ribose methylation of the first transcribed nucleotide are required for hMTr2, but the presence of cap1 methylation (introduced by hMTr1) increases hMTr2 activity. The hMTr2 protein is distributed throughout the nucleus and cytosol, in contrast to nuclear hMTr1. The 2′-*O*-ribose RNA cap methyltransferases are present in various combinations in most eukaryotic and many viral genomes. An article that describes this analysis was published by Werner et al. (*Nucleic Acids Research*, 2011, Jun, 39(11):4756-68).

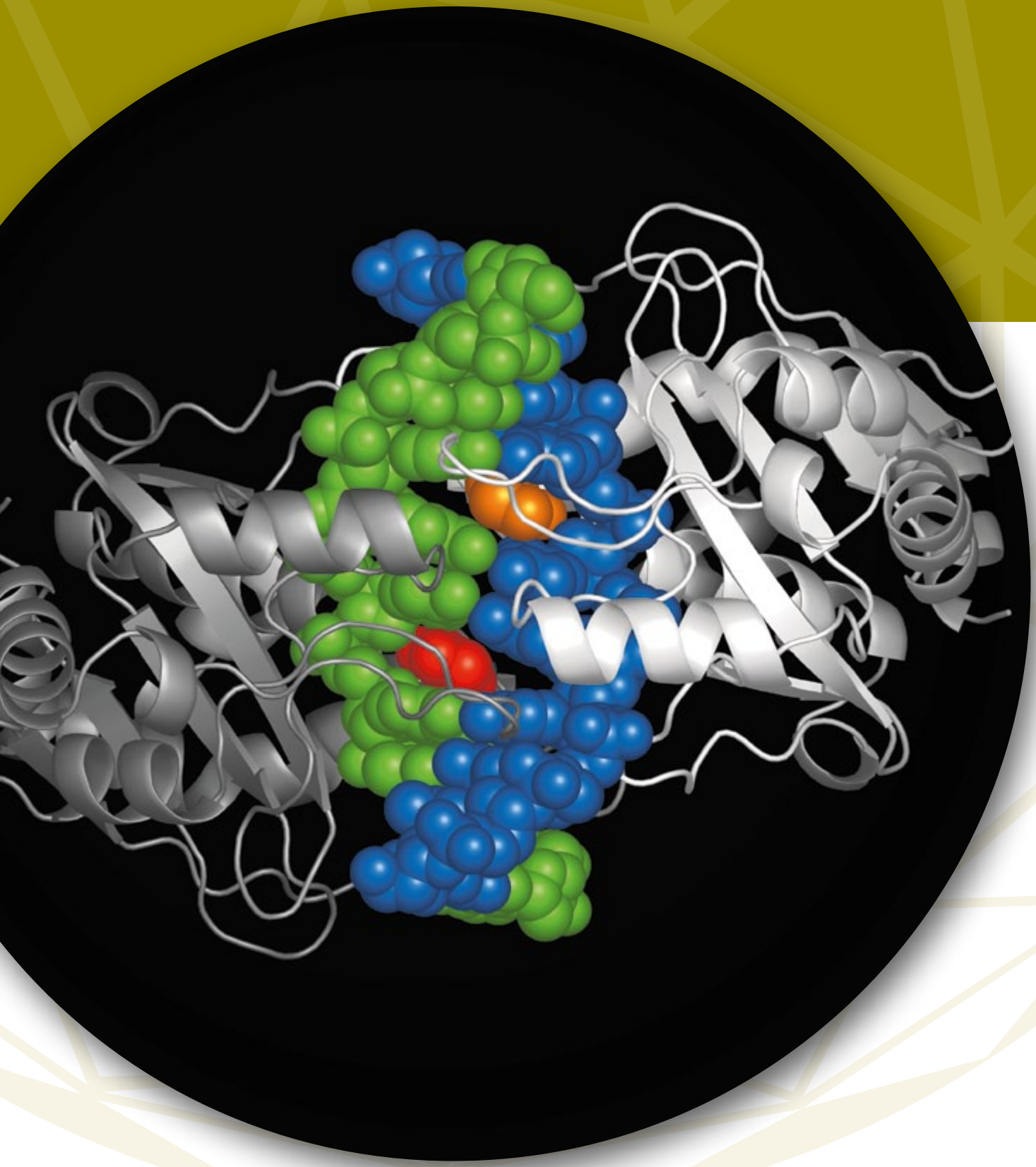
**Software development – a case study:**

The collaborative effort of the Bujnicki and Bochtler laboratories in IIMCB, particularly the work of Dr. Grzegorz Chojnowski (now in the Bujnicki laboratory), has led to the development of the RIBER method for crystal content analysis in studies of protein-RNA complexes. This method has been integrated into the RIBER/DIBER suite (http://diber.iimcb.gov.pl). The program provides an easy method of judging the DNA/RNA content of a crystal based on diffraction data only before the crystal structure is solved. The method may help avoid a laborious phasing procedure when the component or complex of interest is not present in the crystal. An article that describes the RIBER method and RIBER/DIBER server has been accepted for publication and appears as an electronic version in *Bioinformatics* (Chojnowski et al., doi: 10.1093/bioinformatics/bts003). Earlier estimates of very a high performance DIBER in judging the DNA content of a crystal has been confirmed. The performance of RIBER with double-stranded RNA has been shown to be much better than DIBER. Therefore, RIBER complements DIBER in the analysis of crystal content in crystallization studies of protein-nucleic acid complexes.

Currently, the expertise of the Bujnicki laboratory in structural bioinformatics is exploited in the development of new software that automatically builds RNA models into experimental electron density maps. The existing polynucleotide model-building tools require all phosphate and base positions within a continuous chain fragment to accurately determine the backbone conformer. This is a serious limitation because the detection of bases is generally much more difficult than the detection of phosphates alone. In our laboratory, we address this problem by implementing a new method of fitting recurrent RNA structural motifs into electron density maps based on phosphate positions only with new algorithms for RNA structure comparisons. A few such motifs, extracted by Dr. Chojnowski from known RNA structures, are depicted in the figure below.



Clusters of 3D motifs in RNA structures - building blocks for a new RNA modeling tool. Data and artwork by Dr. Grzegorz Chojnowski.



Probing of the weak purine-pyrimidine CpG step by ThaI restriction endonuclease.

# Laboratory of
# **Structural Biology**

## Lab leader: **Matthias Bochtler**, PhD, Professor

**Postdoctoral Fellows:**
Honorata Czapińska, PhD
Monika Sokołowska, PhD
Roman Szczepanowski, PhD (until Fall 2011)

**Junior Researchers:**
Patrycja Haniewicz, MSc (on maternity leave)
Asgar Abbas Kachrani, MSc (since December 2011)
Karolina Kolak, MSc (since November 2011)
Dominik Rafalski, MSc (since December 2011)
Karthik Shanmuganandam, Msc (since December 2011)
Wojciech Siwek, MSc (since October 2011)
Marek Wojciechowski, MSc
Michał Pastor, BSc (since September 2011)

**Technician:**
Elżbieta Grzelak (part-time)

# Head of Laboratory of Structural Biology

## **Matthias Bochtler**, PhD, Professor

### Degrees

| | |
|---|---|
| 2009 | Professor of Biological Sciences, nomination by the President of the Republic of Poland |
| 2006 | DSc Habil, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland |
| 1999 | PhD in Biochemistry, Technical University of Munich, Germany |
| 1995 | MSc in Experimental Physics, Munich University, Germany |

### Research Training

| | |
|---|---|
| 1996-1999 | Research Assistant, Max Planck Institute of Biochemistry, Martinsried, Germany |
| 1995-1996 | Internship, Medical Microbiology, University of Regensburg, Germany |
| 1992-1993 | Guest Student, Cambridge University, United Kingdom |
| 1990-1992 | Studies in physics, Munich University, Germany |

### Professional Employment

| | |
|---|---|
| 2011-Present | Head, Structural Biology Laboratory, International Institute of Molecular and Cell Biology and Institute of Biochemistry and Biophysics, Warsaw, Poland |
| 2007-2011 | Part-time Director of Structural Biology, Cardiff University, United Kingdom |
| 2001-2010 | Head, Joint MPG-PAN Junior Research Group, IIMCB, Warsaw, Poland |
| 2000 | Patent training, Weickmann & Weickmann |
| 1999-2000 | Postdoctoral Fellow, Max Planck Institute of Biochemistry, Martinsried, Germany |

### Honors, Prizes, Awards

| | |
|---|---|
| 2011 | Full Professor, Institute of Biochemistry and Biophysics, Warsaw |
| 2005 | Pieńkowski Award |
| 2004 | EMBO/HHMI Young Investigator Award |
| 2000 | Crystal Award, Germany |
| 1998 | Crystal Award, Germany |
| 1990-1992 | Scholarship from Deutsche Studienstiftung and Bavarian State |

# Selected publications

**Protein-nucleic acid interactions**

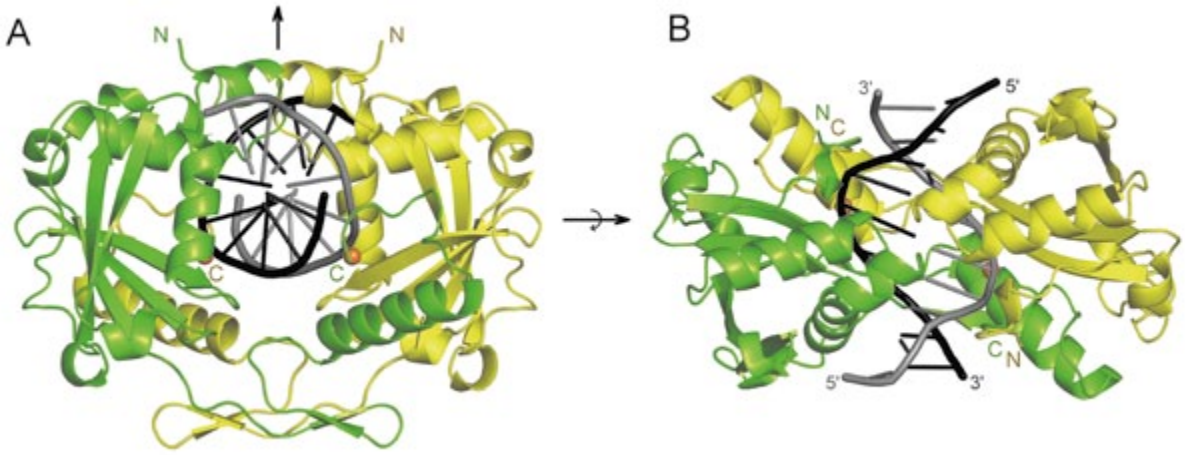

- Antonczak AK, Simova Z, Yonemoto IT, **Bochtler M**, Piasecka A, **Czapinska H**, Brancale A, Tippmann EM. Importance of single molecular determinants in the fidelity of expanded genetic codes. Proc Natl Acad Sci USA, 2011; 108:1320-5
- Braun S, Humphreys C, Fraser E, Brancale A, **Bochtler M**, Dale TC. Amyloid-Associated Nucleic Acid Hybridisation. PLoS One, 2011; 6:e19125
- **Sokolowska M, Czapinska H, Bochtler M.** Hpy188I-DNA pre- and post-cleavage complexes - snapshots of the GIYYIG nuclease mediated catalysis. Nucleic Acid Res, 2011; 39:1554-64
- **Firczuk M, Wojciechowski M, Czapinska H, Bochtler M**. DNA intercalation without flipping in the specific ThaIDNA complex. Nucleic Acid Res, 2011 39:744-754
- **Sokolowska M, Czapinska H, Bochtler M**. Crystal structure of the ββα-Me type II restriction endonuclease Hpy99I with target DNA. Nucleic Acid Res, 2009; 37:3799-810
- **Szczepanowski RH**, Carpenter MA, **Czapinska H**, Zaremba M, Tamulaitis G, Siksnys V, Bhagwat AS, **Bochtler M**. Central base pair flipping and discrimination by PspGI. Nucleic Acids Res, 2008; 36:6109-17
- Tamulaitis G, Zaremba M, **Szczepanowski RH**, **Bochtler M**, Siksnys V. Central base pair flipping and discrimination by PspGI. How PspGI, catalytic domain of EcoRII and Ecl18kI acquire specificities for different DNA targets. Nucleic Acids Res, 2008; 36:6101-8
- Sukackaite R, Grazulis S, **Bochtler M**, Siksnys V. The recognition domain of the BpuJI restriction endonuclease in complex with cognate DNA at 1.3-A resolution. J Mol Biol, 2008; 378:1084-93
- Tamulaitis G, Zaremba M, **Szczepanowski RH**, **Bochtler M**, Siksnys V. Nucleotide flipping by restriction enzymes analyzed by 2-aminopurine steady-state fluorescence. Nucleic Acids Res, 2007; 35:4792-9
- **Sokolowska M, Kaus-Drobek M, Czapinska H**, Tamulaitis G, **Szczepanowski RH**, Urbanke C, Siksnys V, **Bochtler M**. Monomeric restriction endonuclease BcnI in the apo form and in an asymmetric complex with target DNA. J Mol Biol, 2007; 369:722-34
- **Kaus-Drobek M, Czapinska H, Sokolowska M**, Tamulaitis G, Szczepanowski RH, Urbanke C, Siksnys V, **Bochtler M**. Restriction endonuclease MvaI is a monomer that recognizes its target sequence asymmetrically. Nucleic Acids Res, 2007; 35:2035-46
- **Bochtler M, Szczepanowski RH**, Tamulaitis G, Grazulis S, **Czapinska H**, Manakova E, Siksnys V. Nucleotide flips determine the specificity of the Ecl18kI restriction endonuclease. EMBO J, 2006; 25:2219-29
- Grazulis S, Manakova E, Rössle M, **Bochtler M**, Tamulaitiene G, Huber R, Siksnys V. Structure of the metal-independent restriction enzyme BfiI reveals fusion of a specific DNAbinding domain with a nonspecific nuclease. Proc Natl Acad Sci USA, 2005; 102:15797-802

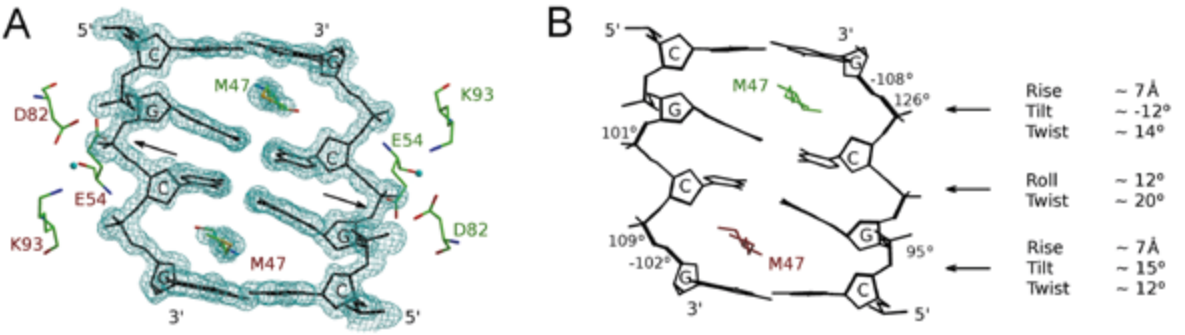

**Other**


- **Chojnowski G, Bochtler M**. DIBER: protein, DNA or both? Acta Crystallogr D, 2010; 66:643-653
- Gentsch M, **Kaczmarczyk A**, van Leeuwen K, de Boer M, **Kaus-Drobek M**, Dagher MC, Kaiser P, Arkwright PD, Gahr M, Rösen-Wolff A, **Bochtler M**, Secord E, Britto-Williams P, Saifi GM, Maddalena A, Dbaibo G, Bustamante J, Casanova JL, Roos D, Roesler J. Alu-repeat-induced deletions within the NCF2 gene causing p67-phoxdeficient chronic granulomatous disease (CGD). Hum Mutat, 2010; 31:151-158
- **Chojnowski G**, Breer K, Narczyk M, Wielgus-Kutrowska B, **Czapinska H**, Hashimoto M, Hikishima S, Yokomatsu T, **Bochtler M**, Girstun A, Staron K, Bzowska A. 1.45 A resolution crystal structure of recombinant PNP in complex with a pM multisubstrate analogue inhibitor bearing one feature of the postulated transition state. Biochem Biophys Res Commun, 2010; 391:703-708
- **Piano D, El Alaoui S, Korza HJ, Filipek R, Sabala I, Haniewicz P**, Buechel C, De Sanctis D, **Bochtler M**. Crystallization of the photosystem II core complex and its chlorophyll binding subunit CP43 from transplastomic plants of Nicotiana tabacum. Photosyn. Res, 2010; 106:221-226

# Scientific Report

The group focuses on sequence- and modification-specific protein-nucleic acid interactions. The work on sequence recognition is still largely concentrated on type II restriction endonucleases. In this area, we have aimed to elucidate representative structures of almost all classes (PD-[D/E]XK, HNH, GIY-YIG, phospholipase-like) of enzymes that cut DNA with different staggers. The former has led to the illustration of a few catalytic strategies to cleave phosphodiester bonds. The latter has shown how different oligomerization states and subunit arrangements define the distance between cleavage sites. In this context, we found that the repertoire of molecular solutions to adjust cleavage stagger is surprisingly diverse and includes nucleotide flipping (e.g., Ecl18kI, PspGI) and the sequential cleavage of DNA strands (e.g., MvaI, BcnI). If the "spacer" between the cleavage sites of the two DNA strands is odd, then sequence recognition of the central base pair is inevitably degenerate (i.e., only W and S pairs can be distinguished). Our structures have demonstrated various ways of specific semi-degenerate sequence recognition, including the perhaps most "logical" and long postulated strategy of readout in the central minor groove. Last year, we expanded our work to "programmable" nucleases. Fusions of zinc fingers with the nuclease domain of the FokI restriction enzyme have long been used for targeted genome deletion. More recently, transcription activator-like effector (TALE) domains have begun to replace zinc finger domains for targeting. Our group has been involved in structural and modeling studies to explain the bases of the "cipher" that relates the protein and specifically recognized nucleic acid sequences. We are still engaged in attempts to solve the first structure of a TALE nuclease (TALEN). In addition to the work on sequence-specific DNA recognition, our group is now also delving into the field of DNA modifications and modification-specific DNA recognition. We have started structural studies of restriction endonucleases that cleave DNA only in the presence of a particular DNA modification. In collaboration with Prof. Bujnicki's group at IIMCB, we have determined the structure of the $N^6$-methyladenine-dependent restriction endonuclease DpnI. The structure and corresponding biochemical results show that DpnI is a two-domain enzyme with a winged helix and an endonuclease domain that are separately specific for both the DNA sequence and the modification. We are continuing the work on modification-specific endonucleases, with a particular focus on enzymes that require a methyl– or hydroxymethylcytosine modification for their activity. Such proteins have potential and are partially already used as tools for mechanistic studies of epigenetic phenomena in eukaryotes.



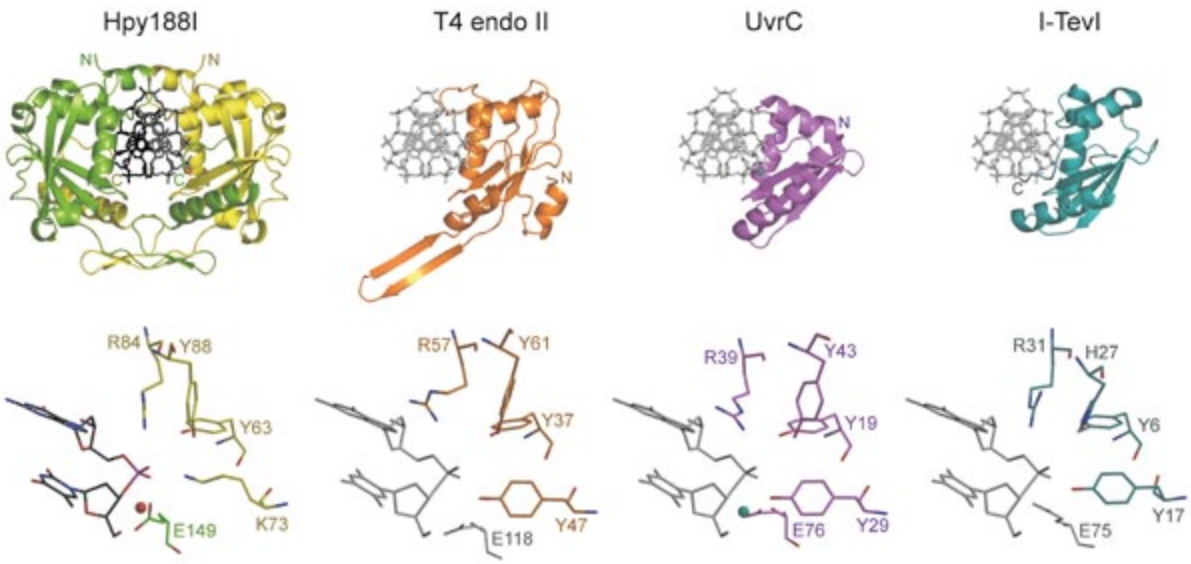



**Fig. 1. Observed and predicted GIY-YIG nuclease-DNA complexes:** GIY-YIG nucleases with different cellular functions have been crystallized previously (T4 endo II, UvrC and I-TevI). However, because the nuclease domains of these enzymes are not sequence specific, the structures of their DNA complexes were not obtained. We have superimposed these structures on Hpy188I in complex with substrate DNA. The top row panels show the composite overall models, the bottom row panels - details upon zooming into the active sites. The catalytic residues in the DNA-free structures are found in correct or nearly correct conformations. Apart from suggesting a fairly rigid active site, this result supports our belief that the catalytic mechanism that we have described for Hpy188I is general for GIY-YIG nucleases *(Figure taken from Sokolowska et al., 2010).*

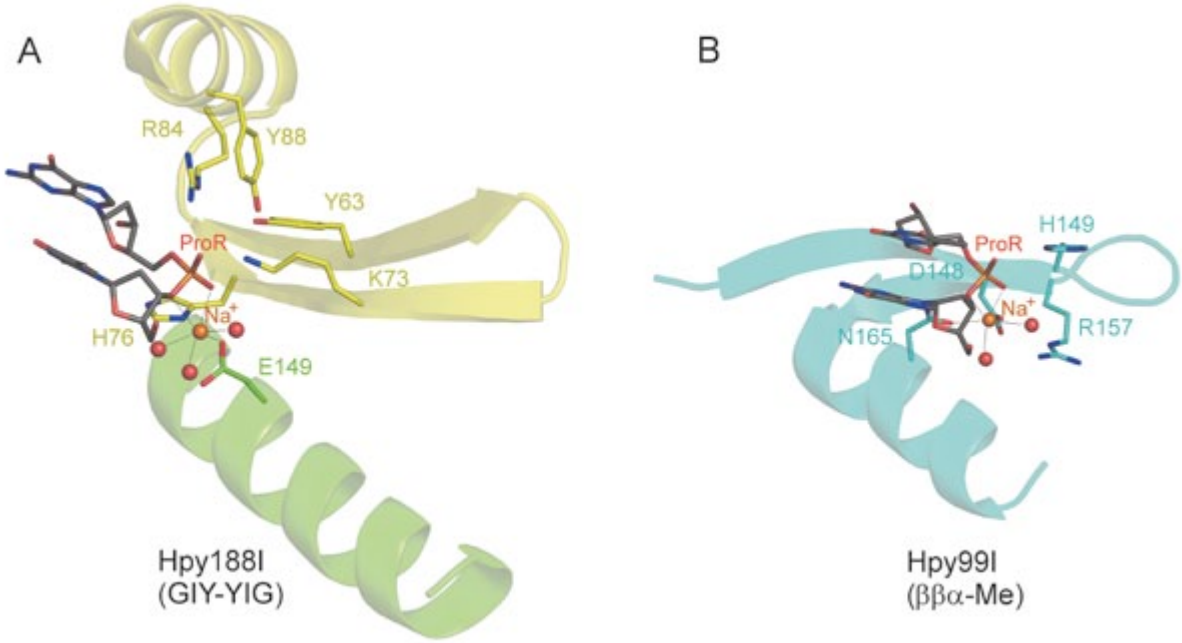
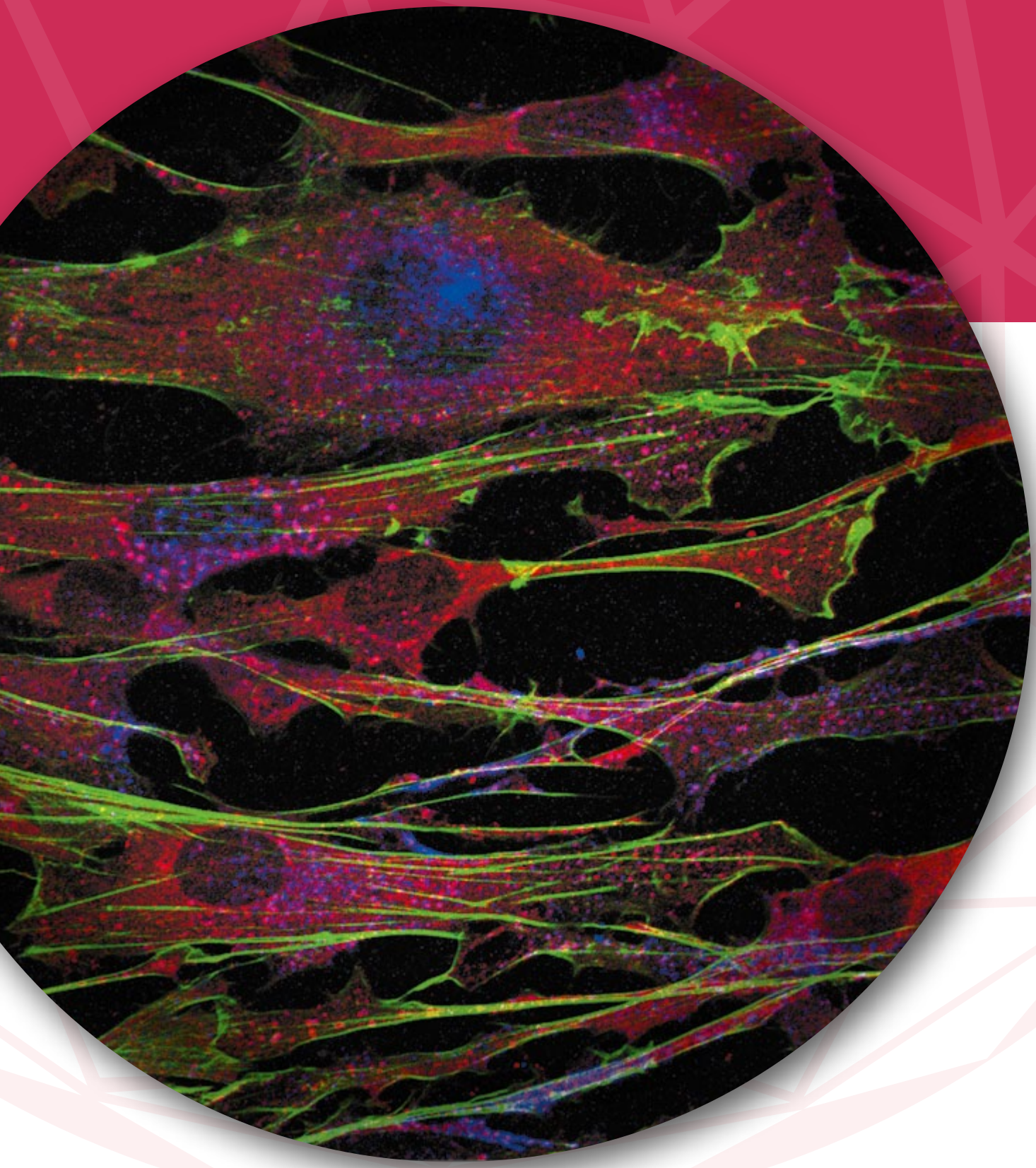


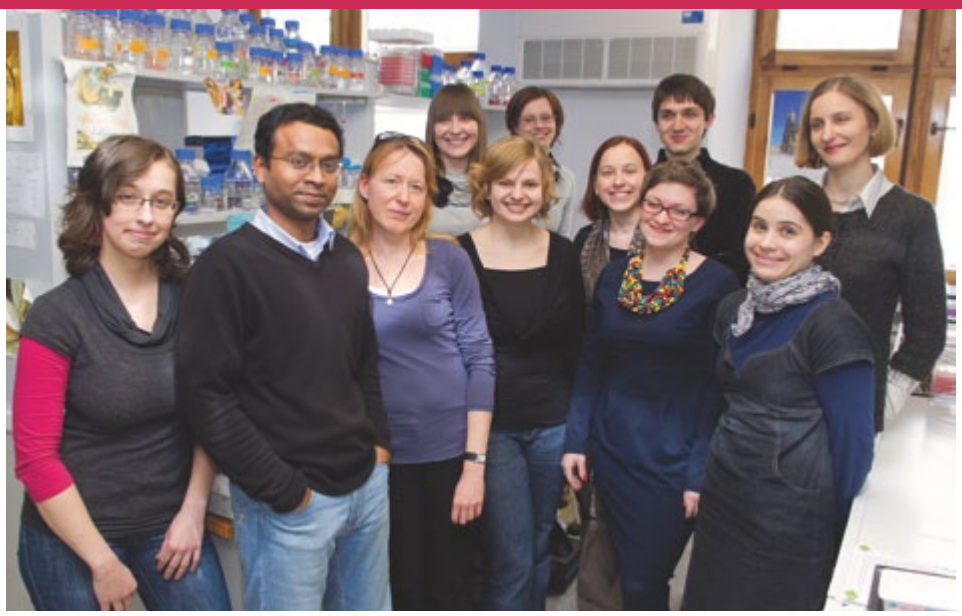

**Fig. 2. Striking similarities of the GIY-YIG and ββα-Me active sites:** GIY-YIG and ββα-Me nucleases represent an example of convergent evolution. Both groups bind only a single metal ion per active site and both can accept a wide array of metal cations that support DNA cleavage. In both cases, the place of the divalent metal ion can be occupied by a $Na^+$ ion from the buffer if no suitable divalent cation is available. The metal ion is anchored by an acidic residue (Glu149 in Hpy188I and Asp148 in Hpy99I), which however need not be the only amino acid ligand. The metal ion contacts the proS oxygen atom of the scissile bond phosphate, and the leaving group 3'-oxygen atom. In both cases, a water molecule attacks the scissile bond phosphate from the back, most likely in a single substitution reaction. The water molecule is activated by a basic residue in spatially conserved position (Tyr63 in Hpy188I and His149 in Hpy99I). The secondary structure elements that anchor key catalytic residues are analogous. The general base for activating the water molecule is located in a β-hairpin, and the metal ligand is found in an α-helix that immediately follows the β-hairpin in sequence *(Figure taken from Sokolowska et al., 2010).*



Human fibroblasts stained for actin (green), PDGFRβ (red) and transferrin (blue) (author: Kamil Jastrzębski).

# Laboratory of **Cell Biology**

## Lab leader: **Marta Miączyńska**, PhD, DSc Habil



**Postdoctoral Fellows:**
Magdalena Banach-Orłowska, PhD
Maciej Lipko, PhD (joint with Department of Molecular Biology)
Iwona Pilecka, PhD
Beata Pyrzyńska, PhD
Ewelina Szymańska, PhD

**Junior Researchers:**
Anna Hupałowska, Msc
Kamil Jastrzębski, MSc
Agnieszka Mamińska, MSc
Łukasz Sadowski, MSc
Sam D. Stephen, MSc (since July 2011)
Anna Toruń, MSc

**Trainees:**
Daniela Chmiest, MSc (until June 2011)
Magdalena Miętkowska, MSc (until September 2011)

**Grant Administrator and Lab Manager:**
Izabela Zacharek, MSc

**Technician:**
Monika Dudek

# Head of Laboratory of Cell Biology

## **Marta Miączyńska**, PhD, DSc Habil

### Degrees

| | |
|---|---|
| 2008 | DSc Habil in Cell Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |
| 1997 | PhD in Genetics, University of Vienna, Austria |
| 1993 | MSc in Molecular Biology, Jagiellonian University, Cracow, Poland |
| 1991 | BSc in Biological Sciences, University of Wolverhampton, UK |

### Research Training

| | |
|---|---|
| 2001-2005 | Senior Postdoctoral Fellow, Max Planck Institute for Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany |
| 1997-2000 | Postdoctoral training, European Molecular Biology Laboratory, Heidelberg, Germany |
| 1993-1996 | PhD studies, Institute of Microbiology and Genetics, University of Vienna, Austria |
| 1990-1991 | Exchange Student, University of Wolverhampton, UK |

### Fellowships and Awards

| | |
|---|---|
| 2007 | Habilitation Fellowship of L'Oreal Poland for Women in Science |
| 2005 | International Research Scholar, Howard Hughes Medical Institute, USA (2006-2010) |
| 2005 | International Senior Research Fellowship, Wellcome Trust, UK (2006-2011) |
| 2005 | Partner Group grant, Max Planck Society, Germany (2006-2010) |
| 2001-2004 | Postdoctoral Fellowship, Max Planck Society, Germany |
| 1999-2000 | Long-Term Postdoctoral Fellowship, Human Frontier Science Program Organization (HFSPO) |
| 1998-1999 | Erwin Schrödinger Postdoctoral Fellowship, Austrian Science Fund (FWF) |
| 1993-1996 | Bertha von Suttner PhD Scholarship, Austrian Ministry of Science |
| 1990-1991 | Studentship, European Community Tempus Scheme |

# Selected publications


- **Hupalowska A**, **Miaczynska M**. The new faces of endocytosis in signaling. (Review) Traffic, 2012; 13:9-18
- **Urbanska A**, **Sadowski L**, Kalaidzidis Y, **Miaczynska M**. Biochemical Characterization of APPL Endosomes: The Role of Annexin A2 in APPL Membrane Recruitment. Traffic, 2011; 12:1227-41
- **Pilecka I**, **Sadowski L**, Kalaidzidis Y, **Miaczynska M**. Recruitment of APPL1 to ubiquitin-rich aggresomes in response to proteasomal impairment. Exp Cell Res, 2011; 317:1093-107
- **Miaczynska M**, Bar-Sagi D; Signaling endosomes: seeing is believing. (Review) Curr Opin Cell Biol, 2010; 22:535-540
- **Banach-Orlowska M, Pilecka I, Torun A, Pyrzynska B, Miaczynska M**. Functional characterization of the interactions between endosomal adaptor protein APPL1 and the NuRD co-repressor complex. Biochem J, 2009; 423:389–400
- **Pyrzynska B, Pilecka I, Miaczynska M**. Endocytic proteins in the regulation of nuclear signaling, transcription and tumorigenesis. (Review) Mol Oncol, 2009; 3: 321-338
- **Rashid S, Pilecka I, Torun A, Olchowik M, Bielinska B, Miaczynska M**. Endosomal Adaptor Proteins APPL1 and APPL2 Are Novel Activators of beta-Catenin/TCF-mediated Transcription. J Biol Chem, 2009; 284:18115-28
- **Sadowski L, Pilecka I, Miaczynska M**. Signaling from endosomes: Location makes a difference. (Review) Exp Cell Res, 2009; 315:1601-09
- *Ohya T, **Miaczynska M**, Coskun U, Lommer B, Runge A, Drechsel D, Kalaidzidis Y, Zerial M. Reconstitution of Rab and SNARE-dependent membrane fusion by synthetic endosomes. Nature, 2009; 459:1091-97
- **Miaczynska M**, Stenmark H. Mechanisms and functions of endocytosis. J Cell Biol, 2008; 80:7-11
- **Pilecka I, Banach-Orlowska M, Miaczynska M**. Nuclear functions of endocytic proteins. Eur J Cell Biol, 2007; 86:533-547
- *Mace G, **Miaczynska M**, Zerial M, Nebreda AR. Phosphorylation of EEA1 by p38 MAP kinase regulates μ opioid receptor endocytosis. EMBO J, 2005; 24:3235-46
- ***Miaczynska M**, Pelkmans L, Zerial M. Not just a sink: endosomes in control of signal transduction. (Review) Curr Opin Cell Biol, 2004; 16:400-406
- ***Miaczynska M**, Christoforidis S, Giner A, Shevchenko A, Uttenweiler-Joseph S, Habermann B, Wilm M, Parton RG, Zerial M. APPL proteins link Rab5 to signal transduction via an endosomal compartment. Cell, 2004; 116:445-56


* no IIMCB affiliation

# Description of Current Research

Our major research interest concerns the mutual relationship between the processes of intracellular signal transduction and membrane trafficking. We study the molecular mechanisms by which endocytic transport regulates intracellular signal transmission and affects final signaling output. The specific projects developed by our group follow two general lines of investigation, with the aim of clarifying the following:

**I. Role of endosomal compartments in the trafficking and signaling of growth factors.**

**II. Involvement of endocytic proteins in the regulation of gene expression in the nucleus.**

The intracellular compartmentalization of signal transduction processes may play an important role in modulating the overall cellular response. Endocytosis was first viewed simply as a mechanism of signal termination by the downregulation and degradation of surface receptors. However, more recent data strongly argue that endosomal compartments and their resident proteins play an important role in transmitting intracellular signals by transporting ligand-receptor complexes and affecting their activity inside the cell (Hupalowska and Miaczynska, *Traffic*, 2012; Miaczynska and Bar-Sagi, *Curr Opin Cell Biol*, 2010). The proposal that endosomes serve as signaling compartments, which was initially postulated in the mid-1990s, has gained increasing experimental support in the past few years (Sadowski et al., *Exp Cell Res*, 2009).

Moreover, the relaying of signals from the plasma membrane via endosomes to the nucleus requires signal mediators to be transported between different cellular locations. Intriguingly, a growing number of clathrin adaptors and endosomal proteins are reported to undergo nucleocytoplasmic shuttling. Endocytic proteins can interact with nuclear molecules involved in transcription or chromatin remodeling, changing their localization or activity, and thus may directly modulate the levels or specificity of gene transcription. Certain endocytic proteins translocate to the nucleus in response to extracellular signals to exert a specific biological effect, thus serving as a vehicle for molecular communication between intracellular organelles. In most other cases, however, unclear is the extent to which endocytic and nuclear functions are related or represent disparate tasks (so-called moonlighting; Pilecka et al., *Eur J Cell Biol*, 2007). Importantly, some such dual-function endocytic and nuclear proteins affect cell proliferation or act as tumor suppressors, or their expression is changed in human cancers (Pyrzynska et al., *Mol Oncol*, 2009).

Our direct links to both lines of research were previous studies of adaptor proteins APPL1 and APPL2. These homologous proteins are localized to a particular subpopulation of endosomes but can also act as signal transducers capable of nuclear translocation. As such, they provide examples of both the involvement of endosomes in signaling and the activity of endocytic proteins

in the nucleus (Miaczynska et al., *Cell*, 2004). Our initial research efforts concentrated on APPL1 and APPL2 as exemplary proteins involved in endocytic trafficking and nuclear signaling, whereas more recently we have been extending our studies toward exploring other dual-function endocytic proteins.

**Role of endosomal compartments in the trafficking and signaling of growth factors**

Within this general theme, our efforts were concentrated on the characterization of APPL endosomes and studies of the endocytic trafficking of platelet-derived growth factor (PDGF).

APPL endosomes are a recently identified subpopulation of early endosomes characterized by the presence of two homologous proteins, APPL1 and APPL2, that are effectors of the small guanosine triphosphatase (GTPase) Rab5 (Fig. 1). APPL endosomes exhibit only limited colocalization with EEA1, another Rab5 effector and marker of canonical early endosomes. Although APPL endosomes appear to play important roles in cargo trafficking and signal transduction, no specific markers of this compartment, other than APPL proteins, have been described. To characterize APPL endosomes biochemically, various cell fractionation and gradient purification techniques were established to separate different populations of endosomal vesicles. We compared the distribution of APPL endosomes with canonical EEA1-positive early endosomes during density gradient ultracentrifugation. Although APPL endosomes appear to consist of heterogeneous membrane structures of various densities, they can be partially separated from canonical early endosomes by biochemical fractionation, arguing that the two populations are physically distinct. Membrane preparations enriched in APPL endosomes were further used to determine their protein content and identify other resident markers. As a result of such research, Annexin A2 was identified as a protein localized on APPL endosomes and an interacting partner of both APPL1 and APPL2. Annexin A2 is a $Ca^{2+}$ and phosphatidylinositol 4,5-bisphosphate binding protein, previously implicated in several endocytic steps. Although Annexin A2 is not an exclusive marker of APPL endosomes, it cofractionated and colocalized with this compartment. Importantly, Annexin A2 turned out to be essential for the membrane recruitment of APPL2, because silencing of its expression caused solubilization of APPL2 from the endosomes. Membrane recruitment of APPL proteins was previously shown to depend on the active, GTP-bound form of Rab5 (Miaczynska et al., *Cell*, 2004). Interestingly, high levels of Annexin A2 prevented the loss of APPL2 from the endosomes caused by the inactive, guanosine diphosphate-bound Rab5 mutant. These data argue that Annexin A2 acts independently of Rab5 and can at least partially compensate for Rab5 deficiency in mediating the membrane association of APPL proteins. Cumulatively, these results indicate that the presence of APPL proteins on endosomes is determined by at least two factors, such as active Rab5 and the levels of Annexin A2 (Urbanska et al., *Traffic*, 2011).

Intriguingly, the inhibition of proteasomes by drugs, such as MG132, ALLN, and bortezomib, leads to the solubilization of APPL1 protein from APPL endosomes and its clustering in the perinuclear region, as we found out in another line of research. Such treatment specifically affects APPL endosomes but not the canonical early endosomes marked by EEA1. The redistribution of APPL1 reflects its localization to aggresomes, which are large, insoluble, nonmembranous protein deposits where misfolded proteins become sequestered. Typical for aggresomes, perinuclear APPL1 clusters are encapsulated within a vimentin cage and colocalize with aggregates positive for ubiquitin. We showed that APPL1 itself was polyubiquitinated via lysine-63 linkages, and this modification decreased its solubility and correlated with the redistribution to aggresomes (Pilecka et al., *Exp Cell Res*, 2011).

In another project, we focused on investigating the endocytic routes of internalized PDGF. The ultimate goal of these studies, performed in collaboration with Dr. Carina Hellberg (University of Birmingham), Prof. Carl-Henrik Heldin (Ludwig Institute for Cancer Research, Uppsala), and Dr. Yannis Kalaidzidis (Max Planck Institute, Dresden), was to evaluate the impact of endocytosis on PDGF-dependent signaling events. We established methods to label PDGF molecules for microscopic detection and subsequently characterized the colocalization of internalized PDGF with markers of various endocytic routes and compartments (Fig. 2). Using chemical inhibitors and RNAi-mediated knockdown of endocytic components, the endocytic routes of PDGF could be altered. We showed that such changes affected the activation of certain signaling molecules, arguing that PDGF endocytosis directly impacts intracellular signal transduction downstream of this growth factor. Our data support the general view that the components that govern endocytic trafficking may selectively regulate signaling effectors activated by a growth factor (Sadowski et al., submitted).

**Involvement of endocytic proteins in the regulation of gene expression in the nucleus**

In the projects that investigate this general topic, we uncovered novel roles and interactions of the APPL proteins with nuclear factors, and we performed RNAi-based screens to find new functions of endocytic proteins in the regulation of transcription.

Regarding the nuclear functions of APPL proteins, we discovered that they act as positive regulators of β-catenin/TCF-mediated transcription in the canonical Wnt signaling pathway. Both APPL proteins interact with transcriptional repressor Reptin and are found in an endogenous complex that contains Reptin, β-catenin, and histone deacetylases HDAC1/HDAC2. The overexpression of either APPL protein attenuates Reptin-dependent transcriptional repression and correlates with the reduced amounts of HDACs and β-catenin associated with Reptin and with the lower levels of Reptin and HDAC1 on the promoters of β-catenin target genes. We proposed that APPL proteins exert their stimulatory effects on β-catenin/TCF-dependent transcription by decreasing the repressive activity of a Reptin- and HDAC-containing complex (Rashid et al., *J Biol Chem*, 2009). Intriguingly, the ability of APPL proteins to affect gene expression is not limited to the Wnt pathway (Hupalowska et al., submitted).

APPL1 was previously shown to interact with the nucleosome remodeling and deacetylase (NuRD) complex (Miaczynska et al., *Cell*, 2004). More recently, we identified HDAC2 as the key NuRD subunit responsible for this association. However, the extent of APPL1-NuRD interactions is regulated by the cellular

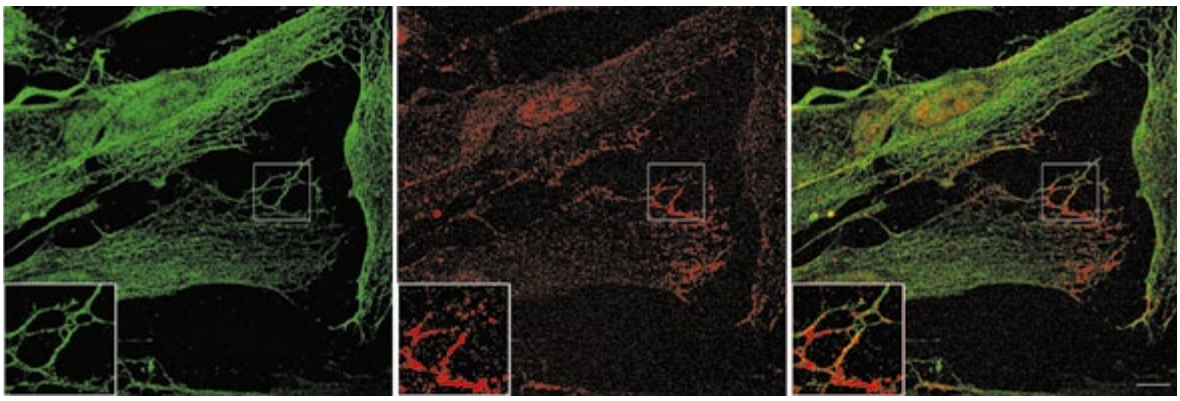


**Fig. 1.** Human CCD-1070Sk fibroblasts stained for Annexin A2 (green) and APPL2 (red). In order to remove the cytoplasmic pool of Annexin A2, cells were permeabilized prior to fixation. Scale bar 10 μm (author: Łukasz Sadowski).

levels of HDAC1, concomitantly affecting the nucleocytoplasmic distribution of APPL1. Moreover, we uncovered a NuRD-independent interaction of APPL1 with HDAC1 and showed that APPL1 overexpression affects the expression of the HDAC1 target p21WAF1/CIP1. These data revealed the surprising complexity of APPL1 interactions with histone deacetylases, with functional consequences for the modulation of gene expression (Banach-Orlowska et al., *Biochem J*, 2009).

Multiple functions of APPL proteins make them important players in the regulation of various cellular processes, such as proliferation and survival. These processes are frequently dysregulated in cancer, and we studied the function of APPL proteins in oncogenesis, particularly with respect to glioblastoma multiforme, the most common and aggressive cancer of the central nervous system. We demonstrated that the levels of APPL2 protein can affect gene expression patterns in glioblastoma cells (Pyrzynska et al., submitted).

Our discoveries of new roles of APPL proteins related to the regulation of gene expression and chromatin remodeling have prompted us to extend our studies to other proteins implicated in endocytosis and capable of nucleocytoplasmic shuttling. We performed systematic RNAi-based screens for the involvement of candidate proteins in transcriptional regulation mediated by different transcription factors. These screens resulted in the identification of several candidate hit proteins, and we are currently investigating their molecular mechanisms of action in the Wnt, AP-1, and NF-κB signaling pathways.

With regard to the methodology used in our laboratory, our main experimental systems involve cultured mammalian cells, but we have also initiated collaborative studies performed in primary cells and in model organisms to broaden the impact of our cell-based observations. In our research, we use various methods, including cell fractionation and purification of endosomal compartments, confocal microscopy followed by quantitative image analyses, biochemical characterization of proteins and their post-translational modifications, identification of protein interacting partners, cell-based assays for endocytosis, proliferation, and apoptosis, and RNAi-based screens that use transcriptional reporters.

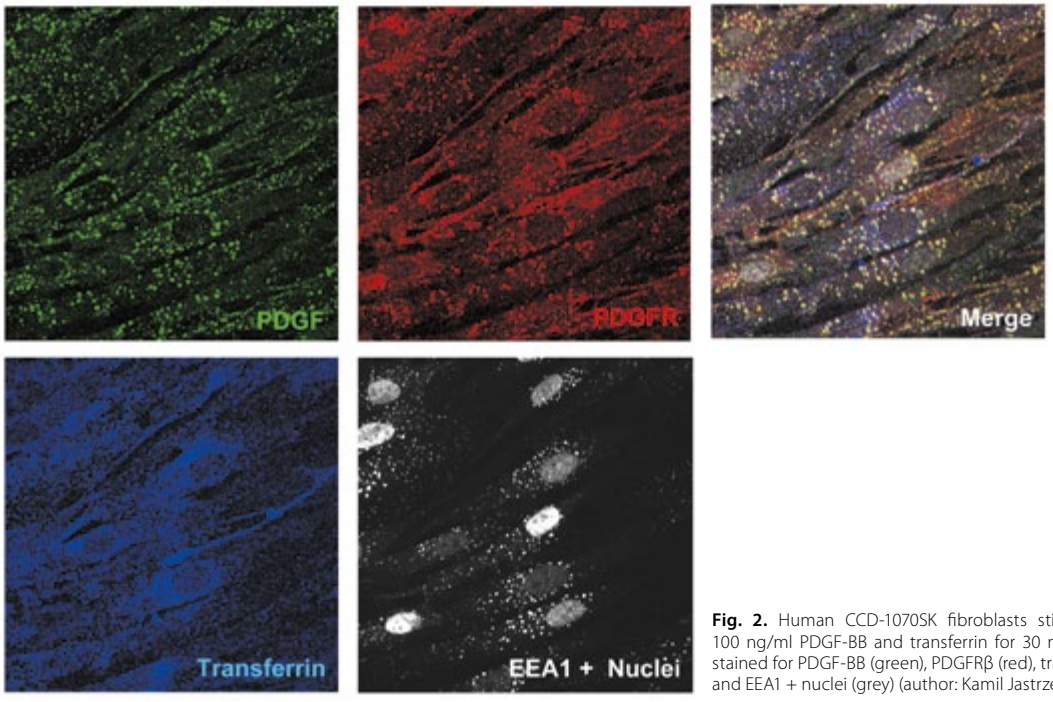
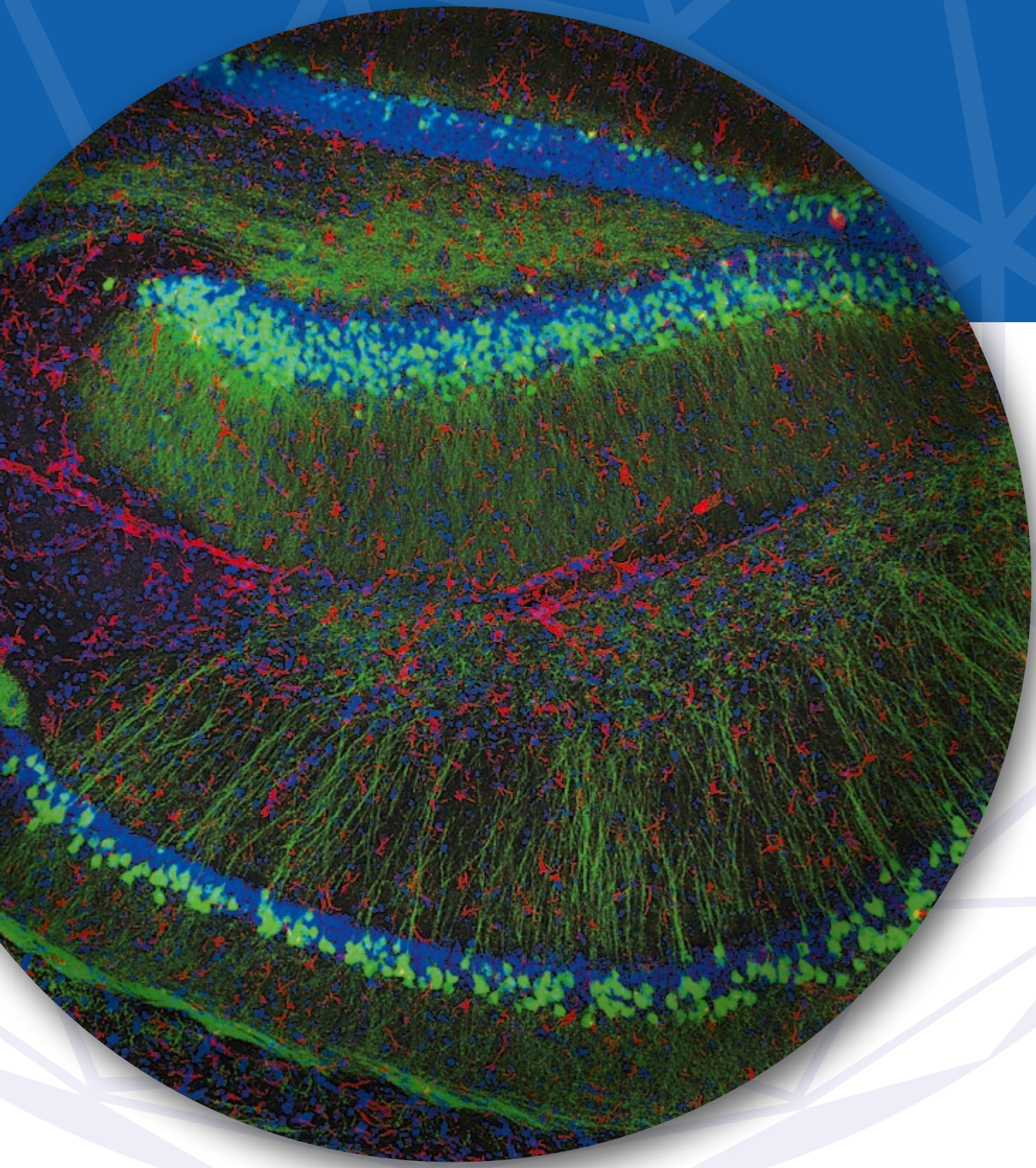


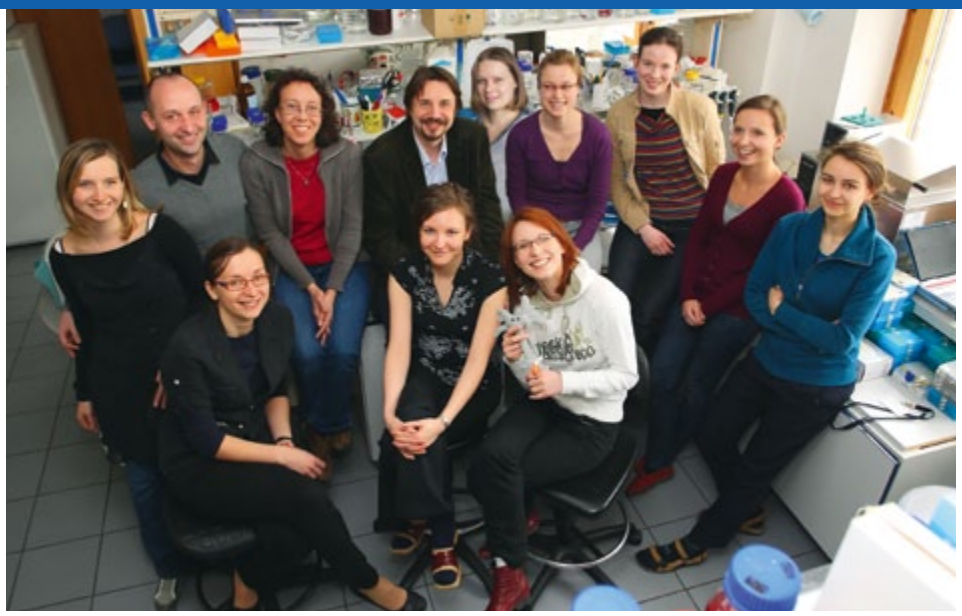

**Fig. 2.** Human CCD-1070SK fibroblasts stimulated with: 100 ng/ml PDGF-BB and transferrin for 30 min. Cells were stained for PDGF-BB (green), PDGFRβ (red), transferrin (blue) and EEA1 + nuclei (grey) (author: Kamil Jastrzębski).



Picture of a section of hippocampal formation of Thy-GFP transgenic mice expressing GFP in neurons (green) immunofluorescently stained for GFAP (red) to detect astrocytes and counterstained with DAPI (blue) to visualize cell nuclei. Such mice can be used for visualization of single neuron morphology, also in a living brain. Author: Agnieszka Skałecka, Thy-GFP brains obtained thanks to a courtesy of Prof. Jochen Herms.

# Laboratory of **Molecular and Cellular Neurobiology**

Lab leader: **Jacek Jaworski**, PhD, DSc Habil



**Postdoctoral Fellows:**
Magda Błażejczyk, PhD
Iwona Cymerman, PhD
Agata Góźdź, PhD
Matylda Macias, PhD
Ewa Liszewska, PhD (starting Oct 2011)
Łukasz Świech, PhD (until Sep 2011, currently Broad Institute MIT)

**Junior Researchers:**
Joanna Lipka, MSc
Anna Malik, MSc
Małgorzata Perycz, MSc
Agnieszka Skałecka, MSc
Anna Urbańska, MSc
Małgorzata Urbańska, MSc

**Technicians:**
Monika Dudek
Marcelina Pieprzyk, MSc

# Head of Laboratory of Molecular and Cellular Neurobiology

## **Jacek Jaworski**, PhD, DSc Habil

### Degrees

| | |
|---|---|
| 2010 | DSc Habil in Molecular Biology, Warsaw University, Poland |
| 2001 | PhD in Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |
| 1996 | MSc in Biology, Department of Genetics, Warsaw University, Poland |

### Research Training

| | |
|---|---|
| 2011 | Research visit (2 weeks) with Dr. Carlo Sala, CNR Institute of Neuroscience & Instituto Neurologico Carlo Besta, Milan, Italy |
| 2006 | Research visit (1 month) with Dr. C.C. Hoogenraad, Erasmus Medical Center, Rotterdam, Holland |
| 2002-2005 | Postdoctoral Associate with Prof. Morgan Sheng, Picower Center for Learning and Memory, Massachusetts Institute of Technology and Howard Hughes Medical Institute, Cambridge, MA, USA |
| 2000 | Research training (1 month) with Dr. J. Guzowski, ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, USA |
| 1997-2001 | Research training (7 months) with Prof. J. Mallet, Laboratoire de Genetique Moleculaire de la Neurotransmission et des Processus Neurodegeneratifs (LGN), UMR 9923, Centre National de la Recherche Scientifique, Paris, France |
| 1996-2002 | PhD student (until 2001) and Postdoctoral Associate (until May 2002) with Prof. L. Kaczmarek, Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |
| 1995-1996 | Master's degree, Prof. P. Węgleński, Department of Genetics, Warsaw University, Poland |

### Fellowships and Awards

| | |
|---|---|
| 2011 | Prime Minister Award for habilitation thesis |
| 2009 | 2nd Division (Biological Sciences) of Polish Academy of Sciences Award for series of publications on MMP9 (together with teams of Prof. Kaczmarek and Dr. Wilczynski) |
| 2005 | Konorski Award for best publication of 2004 in the field of neuroscience (Kowalczyk et al., J Cell Biol, 2004, 167:209-213), Polish Neuroscience Society and Polish Academy of Sciences |
| 2002 | Prime Minister Award for PhD thesis |
| 2001 | Foundation for Polish Science National Scholarship for Young Investigators (1 year scholarship) |
| 2000 | EMBO Short-Term Fellowship |
| 1999 | Polish Network for Cell and Molecular Biology UNESCO/PAN Scholarship |
| 1997 | French Government Scholarship |

### Membership in Scientific Societies, Organizations, and Panels

| | |
|---|---|
| 2011 | Neurobiology Committee of the Polish Academy of Sciences, Member |

### Awards, Honors and Titles (Lab members - 2011)

| | |
|---|---|
| 2011 | EMBO Long-Term Scholarship for postdoctoral training at Broad Institute, Ł. Świech |
| 2011 | Selection for "Top Innovator 500" Ministerial Program, I. Cymerman |
| 2011 | The Nencki Institute Scientific Council distinction for PhD thesis, Ł. Świech |
| 2011 | PhD in Molecular Biology, Nencki Institute, Ł. Świech |
| 2011 | Mazovia 1-year PhD Scholarship, M. Urbanska |

# Selected publications

**Publications in 2010-2011**

- **Urbanska M**, **Swiech L**, **Jaworski J**. Developmental plasticity of the dendritic compartment: focus on the cytoskeleton. *Synaptic plasticity,* eds. Kreutz M., Sala C., Springer, 2012 in press
- Kapitein LC, Yau KW, Gouveia SM, van der Zwan WA, Wulf PS, Keijzer N, Demmers J, **Jaworski J**, Akhmanova A, Hoogenraad CC. NMDA Receptor Activation Suppresses Microtubule Growth and Spine Entry. J Neurosci, 2011; 31(22):8194-209
- Werner M, Purta E, Kaminska KH, **Cymerman IA**, Campbell DA, Mittra B, Zamudio JR, Sturm NR, **Jaworski J**, Bujnicki JM. 2'-O-ribose methylation of cap2 in human: function and evolution in a horizontally mobile family. Nucleic Acids Res. 2011; 39(11):4756-68
- **Perycz M**, **Urbanska AS**, **Krawczyk PS**, **Parobczak K**, **Jaworski J.** Zipcode Binding Protein 1 Regulates the Development of Dendritic Arbors in Hippocampal Neurons. J Neurosci, 2011; 31(14):5271–85
- **Swiech L**, **Blazejczyk M, Urbanska M, Pietruszka P**, Dortland B. R, **Malik AR**, Wulf P. S, Hoogenraad C. C, **Jaworski J**. CLIP-170 and IQGAP1 Cooperatively Regulate Dendrite Morphology. J Neurosci, 2011; 31(12):4555-68 • Azoulay-Alfaguter I, Yaffe Y, Licht-Murava A, **Urbanska M, Jaworski J**, Pietrokovski S, Hirschberg K, Eldar-Finkelman H. Distinct molecular regulation of GSK-3alpha isozyme controlled by its N-terminal region. Functional role in calcium/calpain signaling. J Biol Chem, 2011; 286(15):13470-80
- Piechota M, Korostynski M, Solecki W, Gieryk A, Slezak M, Bilecki W, Ziolkowska B, Kostrzewa E, **Cymerman I, Swiech L, Jaworski J**, Przewlocki R. The dissection of transcriptional modules regulated by various drugs of abuse in the mouse striatum. Genome Biol, 2010; 11(5):R48
- Stefaniuk M, **Swiech L**, Dzwonek J, Lukasiuk K. Expression of Ttyh1, a member of the Tweety family in neurons *in vitro* and in vivo and its potential role in brain pathology. J Neurochem, 2010; 115:1183-94
- Kieper J, Lauber C, Gimadutdinow O, **Urbańska A, Cymerman I**. Ghosh M, Szczesny B, Meiss G. Production and characterization of recombinant protein preparations of Endonuclease G-homologs from yeast, C. *elegans and humans*. Protein Expr Purif, 2010; 73:99-106

**Other selected publications**

- **Jaworski J**, Kapitein LC, Montenegro Gouveia S, Dortland BR, Wulf PS, Grigoriev I, Camera P, Spangler SA, di Stefano P, Demmers J, Krugers H, Defilippi P, Akhmanova A, Hoogenraad CC. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity, Neuron, 2009; 61:85-100
- **Swiech L, Perycz M, Malik A, Jaworski J**. Role of mTOR In physiology and pathology of the nervous system. Biochim Biophys Acta, 2008; 1784:116-132
- ***Jaworski J**, Spangler S, Seeburg DP, Hoogenraad CC, Sheng M. Control of dendritic arborization by the PI3-kinase – Akt – mTOR pathway. J Neurosci, 2005; 25:11300-12
- *Dunah AW, Hueske E, Wyszynski M, Hoogenraad CC, **Jaworski J**, Pak DT, Simonetta A, Liu G, Sheng M. LAR receptor protein tyrosine phosphatases in the development and maintenance of excitatory synapses in hippocampal neurons. Nat Neurosci, 2005; 8:458-467
- *Chang CJ, **Jaworski J**, Nolan EM, Sheng M, Lippard SJ. A tautomeric zinc sensor for ratiometric fluorescence imaging: Application to nitric oxide-induced release of intracellular zinc. Proc Natl Acad Sci USA, 2004; 101:1129-34
- ***Jaworski J**, Mioduszewska B, Sanchez-Capelo A, Figiel I, Habas A, Gozdz A, Proszynki T, Hetman H, Mallet J, Kaczmarek L. Inducible cAMP Early Repressor (ICER), an endogenous antagonist of cAMP responsive element binding protein (CREB) evokes neuronal apoptosis *in vitro*. J Neurosci, 2003; 23:4519-26
- ***Jaworski J**, Biederman I, Lapinska J, Szklarczyk AW, Figiel I, Konopka D, Nowicka D, Filipkowski RK, Hetman M, Kowalczyk A, Kaczmarek L. Neuronal excitation driven and AP-1-dependent activation of timp1 gene expression in rodent hippocampus. J Biol Chem, 1999; 274: 28106-12

*no IIMCB affiliation

# Description of Current Research

The research of our team concentrates on the role of protein kinase mammalian target of rapamycin (mTOR) in the control of proper neuronal morphology in health and disease. Establishing proper neuronal morphology is required for proper brain function. Therefore, the mechanisms of axon targeting, dendritic arbor patterning, proper cell contact formation, and the maintenance of plasticity of neuronal connectivity are at the center of interest of molecular neurobiology. Dendrites are the main site of information input into neurons, and dendritic arbor shape is one of the crucial factors that determine how signals that originate from individual synapses are integrated. In fact, several neurodevelopmental pathologies are characterized by abnormalities in dendritic tree structure. Dendritic arbor development is a multistep process that depends on, among other factors, mTOR, a serine/threonine protein kinase known to merge extracellular instructions with information about cellular metabolic resources and control the rate of anabolic and catabolic processes accordingly. In neurons, mTOR has also been implicated in neuronal differentiation, axon elongation and directional movements, spinogenesis, long-term synaptic plasticity, and learning and memory. In neurons, mTOR is hypothesized to act primarily by controlling protein translation, including local protein synthesis in dendrites. Studies in different model systems (e.g., yeast, fruit flies, and cultured non-neuronal mammalian cells) strongly imply the involvement of mTOR in additional cellular processes, such as transcription, membrane trafficking, mitochondrial function, lipid metabolism, autophagy, and cytoskeleton dynamics. Thus, considering the key role that mTOR plays in cell physiology, unsurprising is that mTOR signaling is disturbed under various neuropathological conditions. Altered mTOR activity has been reported in brain tumors, tuberous

sclerosis (TSC), cortical dysplasia, and neurodegenerative disorders. However, in cases of either physiological processes or neuropathology, our knowledge of the molecular events downstream of mTOR, other than protein translation, is rather limited. We believe that expanding such knowledge is crucial for understanding the molecular biology of neurons and assessing the benefits and risks of the clinical use of mTOR inhibitors. Thus, our goal is to determine the mTOR-dependent proteins and cellular processes involved in neuronal development. For the past few years, our research has developed in two main areas:

1. Identifying mTOR partners and regulated proteins involved in the processes of dendritic branching and synapse formation and stabilization.
2. Establishing a link between local protein translation and physiological dendritic arbor development.

In 2011, we continued our work within areas 1 and 2, focusing on potential mTOR targets such as CLIP-170, β-adaptin, ESCRT proteins, and ZBP1. We also investigated links between mTOR kinase and GSK3. However, the major progress we made in 2011 was in a third area of interest, namely mTOR-related brain diseases. Our plan for 2011 was to test our basic findings and scientific questions in two new, clinically relevant models: the *in vivo* development of adult-born neurons and development of iPS cells reprogrammed to neurons. Both of these research directions are financed by ERA-NET projects. Below we discuss our advances in these two areas.

**Status epilepticus and mTOR**

The role of the mTOR pathway has also been proposed in brain pathology, including epileptogenesis and epilepsy. Epilepsy is a chronic neurological disorder with a complex pathogenesis. Triggers of epileptogenesis still remain largely unexplored, but this process is accompanied by reactive gliosis, neuronal loss, and neuronal circuitry rearrangements. Genetic disorders characterized by mTOR hyperactivity (e.g., TSC) are often associated with a high probability of epilepsy. In several animal models of epileptogenesis (e.g., kainic acid [KA]- or pilocarpine-induced status epilepticus), increased mTOR activity was biochemically proven. Consequently, mTOR inhibitors have been proposed as a potential antiepileptogenic and antiepileptic treatment. Rapamycin is one of the best known and widely used mTOR inhibitors. Rapamycin and its derivatives (e.g. RAD001) were recently tested in animal models of epilepsy for their potential to prevent various aspects of epileptogenesis. For example, Zeng et al. (2009) and Buckamster et al. (2009) found that the prolonged administration of rapamycin suppressed mossy fiber sprouting. However, the effects of rapalogs on epileptogenesis or the reduction of seizure frequency were equivocal (Zeng et al., 2009; Buckmaster and Lew, 2011; Sliwa et al., 2011) and required further investigation. Considering the potential importance of mTOR in epileptogenesis and epilepsy and our rudimentary knowledge of the spatiotemporal pattern of mTOR activation induced by proconvulsive agents, we systematically investigated this issue in a model of KA-induced status epilepticus. We found that mTOR signaling was activated by KA injection in several brain areas, including the hippocampus, cortex, and amygdala. One phenomenon we observed was very consistent 2 h post-injection and did not occur either in control brains or at later time-points after KA treatment. At this time-point, we noticed

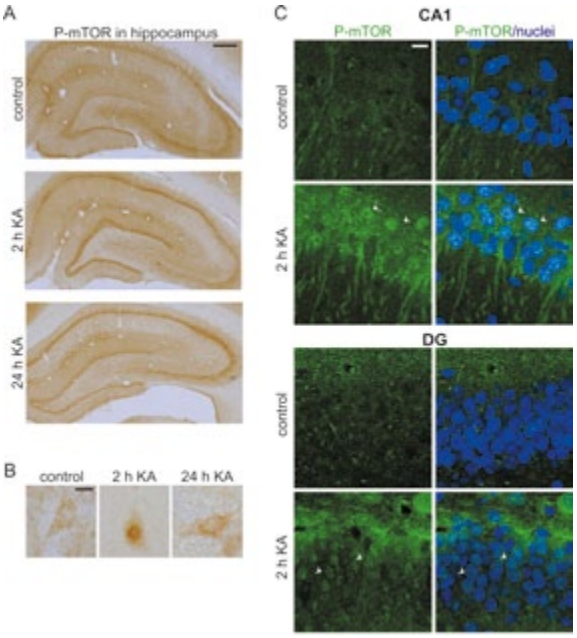



**Fig. 1. Kainic acid-induced changes in the subcellular distribution of mTOR phosphorylation at Ser2448. (*A*)** Immunohistochemical analysis of P-mTOR expression in the hippocampus in control rats and in rats 2 and 24 h after kainic acid (KA)-induced status epilepticus. Scale bar = 200 µm. **(*B*)** Images of single cells of the DG hilus of the animals described in *A*. Scale bar = 10 µm. **(*C*)** Representative confocal images of double fluorescence staining with antibodies against P-mTOR (green) and nuclear dye Hoechst 33258 (blue) of the CA1 and DG regions of the hippocampus in control rats and in rats 2 h after KA administration. Arrowheads indicate double-stained nuclei. Scale bar = 10 µm. Author: M. Macias.

the nuclear presence of P-mTOR in several neuron-like cells in the hippocampus and layer VI of the somatosensory cortex (Fig. 1B). Although such cells were very clearly visible because of the deeply dark-stained nuclei and relatively bright cytoplasm (Fig. 1B), we further confirmed the nuclear localization of P-mTOR by combined fluorescence staining with anti-P-mTOR antibody and nuclear dye Hoechst 33285 (Fig. 1C). Although the nuclear localization of mTOR was previously reported for non-neuronal

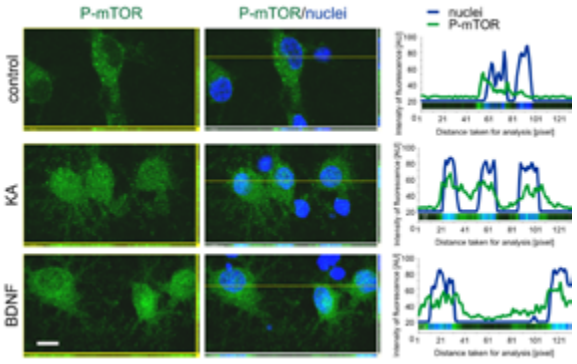



**Fig. 2. Kainic acid induces the nuclear presence of P-mTOR and total mTOR in neurons cultured *in vitro*.** Representative confocal images of cultured cortical neurons ***in vitro*** stained immunofluorescently for P-mTOR (green) and nuclear dye Hoechst 33258 (blue). Cortical neurons obtained from E18 rat embryos were cultured *in vitro* for 14 days. After the silencing of basal network activity (see Materials and Methods), the cells were treated for 15 min with either KA or BDNF. Single Z-sections are presented. The line graph shows the fluorescence intensity of P-mTOR (green) and Hoechst 33258 (blue) along the line running through the main image. The image of the analyzed area is placed at the bottom of each chart. AU, arbitrary units. Scale bar = 10 µm. Author: M. Macias.

cells (Park et al., 2002; Bachmann et al., 2006), we report it for the first time in neurons. Our additional experiments indeed confirmed that both neuronal activity and trophic factors can induce the nuclear appearance of active mTOR (Fig. 2A, B). In addition to changes in the subcellular distribution of mTOR with KA treatment, we also discovered two waves of mTOR activation: an early wave (2 h) that occurs in neurons and a late wave that predominantly occurs in astrocytes. However, the most surprising observation

concerned chronic rapamycin treatment in animals. We found that long-term pretreatment with rapamycin sensitized animals to KA-induced seizures and induced gross anatomical changes in the brain and death of ependymal cells (Fig. 3). These very striking observations undermine the safety of chronic rapalog use at doses that allow blood-brain barrier penetration.

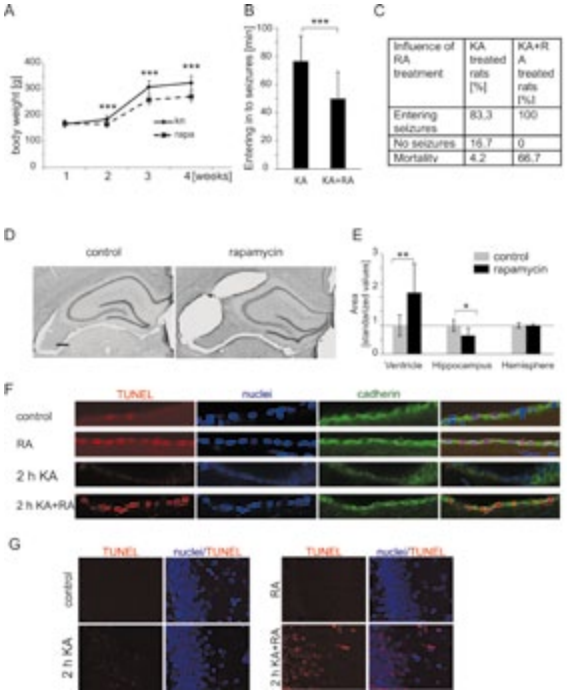



**Fig. 3. Chronic rapamycin treatment influences body weight, seizure susceptibility, and gross brain morphology. (*A*)** Analysis of mean body weight changes in vehicle-treated (solid line) and rapamycin (RA)-treated (dotted line) rats. **(*B, C*)** Analysis of mean latency from KA injection to status epilepticus onset and severity of status epilepticus induced by KA in vehicle-treated ($n$ = 14) and RA-treated ($n$ = 36) rats. **(*D*)** Representative images of Nissl-stained hippocampal sections in animals chronically treated with vehicle ($n$ = 7) or RA ($n$ = 8). Scale bar = 200 µm. **(*E*)** Analysis of changes in the hippocampus, ventricular and entire hemisphere areas in vehicle- and RA-treated rats. Error bars represent the standard deviation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Mann Whitney U-test). **(*F*)** Representative confocal images of ependymal cells surrounding lateral ventricle, stained for presence of apoptotic cells (TUNEL staining, red) and counterstained with cadherin antibody to visualize ependymal cells green) and nuclear dye Hoechst 33258 (blue), obtained from animals treated as in *F*. **(*G*)** Representative confocal images of hippocampal sections stained for presence of apoptotic cells (TUNEL staining, red) and counterstained with nuclear dye Hoechst 33258 (blue), obtained from control animals, animals treated with rapamycin, animals treated with kainic acid and evaluated at 2 h, animals treated with kainic acid and rapamycin and evaluated at 2 h. Author: M. Macias, A. Skałecka.

## Development of animal model to study dendritogenesis and spinogenesis *in vivo*

During the past few years, we have identified several potential mechanisms through which mTOR can contribute to neuronal development, including protein synthesis (Jaworski et al., 2005) and cytoskeleton dynamics control (Swiech et al., 2011). Nevertheless, all of them urgently require confirmation *in vivo*. We decided to introduce a new research model in our laboratory, specifically the integration of newly born neurons to the olfactory bulb (OB) in rodents. The OB is one of two regions in the adult brain where new functional neurons are continuously incorporated into preexisting neuronal circuits. The OB is a destination for neuronal progenitors born in the subventricular zone (SVZ) that then migrate through the rostral migratory stream (RMS). Therefore, SVZ-RMS-OB is a unique system to study the molecular mechanisms of neurogenesis, neuronal development, and neuronal network reconstruction *in vivo*. Owing to novel technology called *in vivo* electroporation (Fig. 4), the SVZ-RMS-OB system is easy for fast genetic modification. In 2011, we successfully established *in vivo* electroporation conditions in our laboratory and with the use of newly purchased two photon lasers we can currently perform deep tissue imaging of the dendritic arbors of neurons that are integrated in the OB (Fig. 4). In 2012, with the use of this new model, we will focus on analyzing the importance of mTOR and its selected targets for dendritogenesis and spinogenesis *in vivo*.

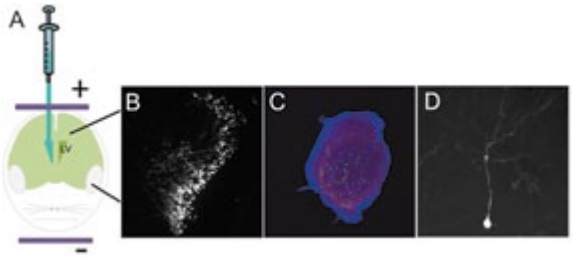



**Fig. 4. Neuroprecursors in subventricular zone can be electroporated *in vivo* postnataly and develop into mature neurons in olfactory bulb. (*A*)** Scheme presenting principles of *in vivo* electroporation of postnatal rodent brain. Cells in a ventricle wall expressing GFP 24 hrs after in vivo electroporation of P1 rat. **(*C*)** Elecroporated cells (green) that reached olfactory bulb (blue) 14 days after transfection **(*D*)** Example of fully developed neuron in the olfactory bulb (P14 rat), which developed from electroporated in the subventricular zone at P1 rat. Author: A. Skałecka.

## iPS cells: development of personalized models of human diseases

One of the obstacles in cell biology research on brain disease mechanisms is the lack of precise models of specific human pathologies. Typically, mouse models of diseases are used but they do not precisely recapitulate a disease for many reasons (e.g., the need for conditional knockouts excludes studies of some developmental aspects). Tuberous sclerosis is a genetic disease characterized by mTOR overactivation, perfectly exemplifying such a situation. Mouse models of TSC have been developed and revealed perturbations in some cellular mechanisms, but they do not fully recapitulate the disease. Recently, the technology of reprogramming human somatic cells into induced pluripotent stem (iPS) cells offers a unique opportunity to model the specific pathologies seen in genetically inherited diseases and represents a valuable tool to study disease mechanisms. Therefore, by overexpression of OCT4, SOX2, KLF4, and c-MYC we generated human iPS cells from fibroblasts of TSC patients. The TSC-iPS cell lines morphologically resembled human embryonic stem cell-like colonies (Fig. 5). The colonies were positive for alkaline phosphatase and the pluripotency markers Nanog and Tra1-81 (Fig. 5). Moreover, TSC-iPS cells formed embryoid bodies that expressed markers of all three germ layers. Finally, embryoid bodies could be differentiated to neuronal precursor cells. In 2012, we will use these lines to address issues regarding developmental and synaptic plasticity problems at the cellular level in TSC. To gain a boarder perspective of this issue, we plan to compare iPS cells derived from several patients.

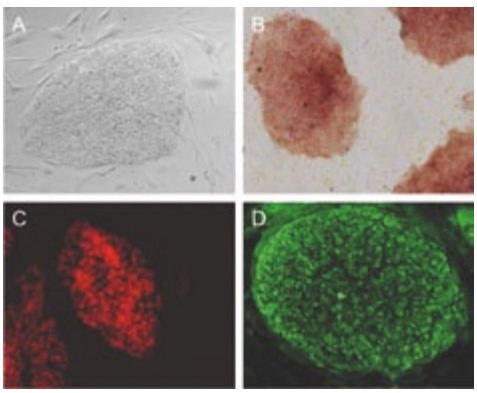
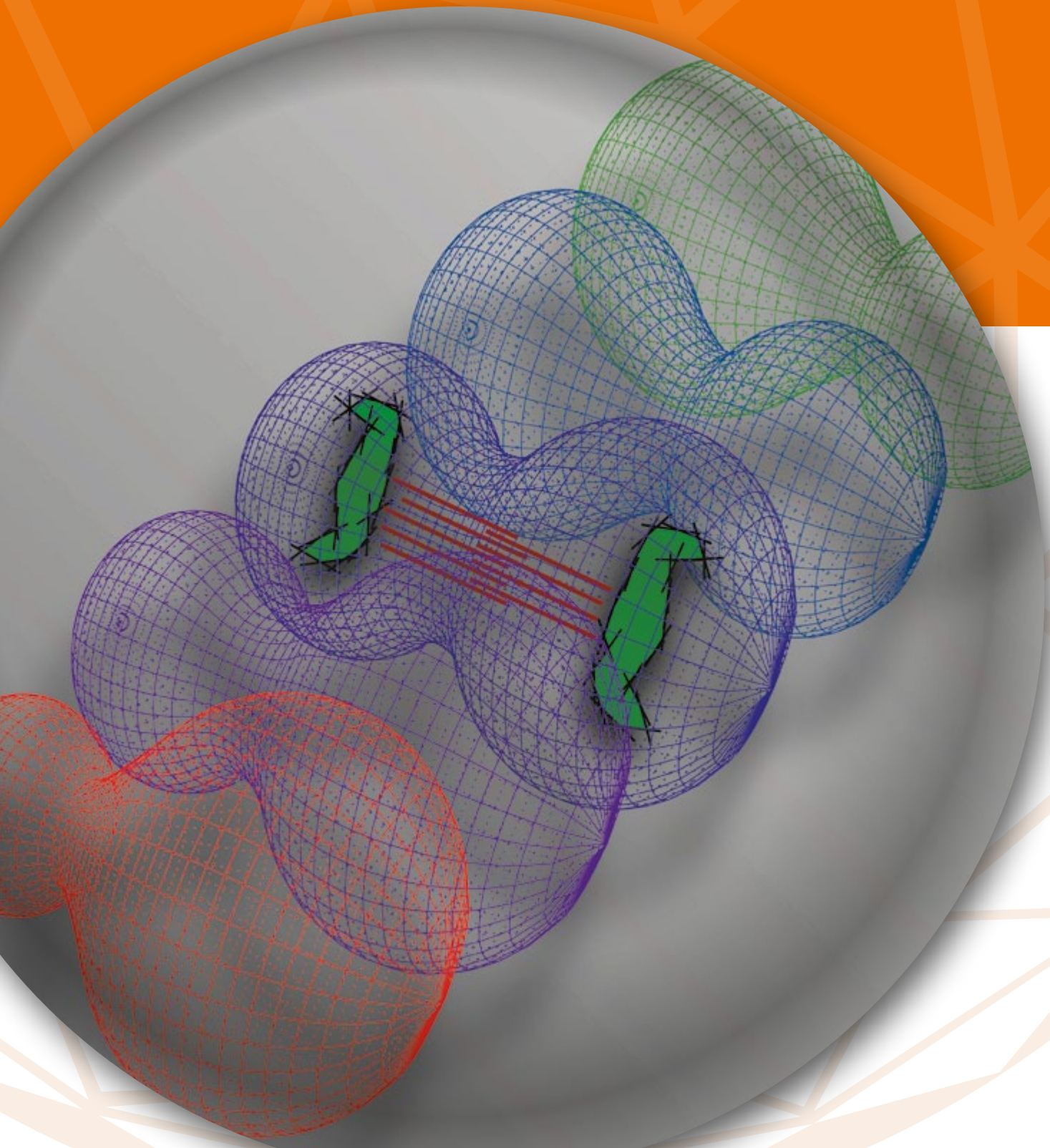



**Fig. 5. Characterization of human induced pluripotent stem (iPS) cells generated from fibroblasts of TSC patients**. (A) human iPS cell colony on mouse embryonic fibroblast feeder layer, (B) alkaline phosphatase staining, (C) and (D) expression of pluripotency markers Nanog and Tra1-81, respectively. Author: E. Liszewska.



Artist view of cell shape oscillations in cytokinesis.
Autors: Maté Biro and Jakub Sedzinski

# Laboratory of
# **Cell Cortex Mechanics MPG/PAN**

(located at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden)

Lab leader: **Ewa Paluch**, PhD

**Senior Researchers:**
Alba Diz Muñoz, PhD
Andrea Pereira, PhD

**Junior Researchers:**
Andrew G. Clark, BSc
Martin Bergert, MSc
Priyamvada Chugh, MSc

**MSc Student:**
Annett Boden, BSc

**Technician:**
Stephanie von Kannen, MSc

# Head of Laboratory of Cell Cortex Mechanics MPG/PAN

## **Ewa Paluch**, PhD

**Degrees:**

| | |
|---|---|
| 2005 | PhD in Biophysics, University Paris 7, Paris, France |
| | 2001 DEA (Master's degree) "Interfaces Physique-Biologie," University Paris 7 (rank: 1st), Paris, France |
| 2000 | Agrégation of Physics |
| 1999 | Maîtrise (equivalent to BSc) in Physics, Ecole Normale Supérieure de Lyon, France |
| 1998 | License in Physics, Ecole Normale Supérieure de Lyon, France |

**Research Training:**

| | |
|---|---|
| 2001-2005 | PhD studies at the Institut Curie, Paris, France |
| 2000-2001 | DEA (equivalent to Master's) research project in Biophysics, Institut Curie, Paris, France |
| 1999 | Maîtrise (BSc) research project in Particle Physics, CERN, Geneva, Switzerland |
| 1998 | Licence (part of BSc) research project in Relativistic Astrophysics, Paris-Meudon Observatory, France |

**Professional Employment:**

| | |
|---|---|
| 2006 - Present | Joint MPI-CBG/PAN group leader at IIMCB, located at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden |
| 2005 | Scientist position at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany |

**Honors and Fellowships :**

| | |
|---|---|
| 2005 | Joint MPI-CBG/PAN group leader at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany |
| 2004-2005 | PhD scholarship, Ligue Nationale Contre le Cancer, France |
| 2001-2004 | PhD scholarship, CNRS, France |
| 2000 | Agrégation in Physics (French national competition, rank: 6th) |
| 1998-2001 | Full salary from Ecole Normale Supérieure de Lyon, France (recruitment by national competition) |
| 1995 | Prize of Scientific and Technical Vocation of Girls, awarded by the Regional Delegation for Women Rights, region of Paris, France |

**Grants**

- 2009-2012 Polish Ministry of Science and Higher Education, International Project Grant (MPG Program), "The role of cell cortex mechanics in cell motility" (454/N-MPG/2009/0); PLN 4,692,929
- 2009-2010 *Deutsche Forschungsgemeinschaft (DFG) grant to Carl-Philipp Heisenberg (MPI-CBG, Dresden) and Ewa Paluch, "Analysis of the formation and function of different cell protrusion types during cell migration in vivo" (PA 1590/-1); EUR 70,600 + 1 PhD position/team
- 2008-2011 *Human Frontier Science Program (HFSP) Young Investigators' Grant to Guillaume Charras (UCL, London, UK), Guillaume Romet-Lemonne (CNRS, Gif-sur-Yvette, France), Philippe Roux (IRIC, Montreal, Canada), and Ewa Paluch, "Interplay between mechanical and biological mechanisms during cell cortex assembly" (RGY 67/2008); $337,500/team
- 2006-2009 Polish-German Special Grant, "The role of cell cortex contractility in the establishment and positioning of the cleavage furrow" (JRGP/37/2005), Max Planck Society (MPG) – Polish Academy of Sciences (PAN) MPI-CBG Junior Research Program – Laboratory of Cortex Movement and Cell Division MGP/PAN; PLN 3,024,200

*no IIMCB affiliation

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- **Sedzinski J**, **Biro M**, Oswald A, **Tinevez JY**, Salbreux G, **Paluch E**. Polar actomyosin contractility destabilizes the position of the cytokinetic furrow. Nature, 2011; 476(7361):462-466
- **Clark AG**, **Paluch E**. Mechanics and regulation of cell shape during the cell cycle. Book chapter in "Cell Cycle in Development", Ed. JZ Kubiak, Results Probl Cell Differ (Springer-Verlag), 2011; 53:31-73
- **Diz-Munoz A**, Krieg M (1), **Bergert M**, Ibarlucea-Benitez I, Muller DJ, **Paluch E** (1), Heisenberg CP (1). Control of directed cell migration in vivo by membrane-to-cortex attachment. PLoS Biol. 2010 Nov 30;8(11):e1000544 (1) co-corresponding
- **Tinevez JY, Schulze U**, Salbreux G, **Roensch J**, Joanny J-F, **Paluch E**. Role of cortical tension in bleb growth. Proc Natl Acad Sci USA, 2009; 106:18581-86
- **Paluch E**, Heisenberg CP. Biology and Physics of Cell Shape Changes in Development (review). Curr Biol, 2009; 19:R790-799
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- **Paluch E**, Van der Gucht J, Sykes C. Cracking up: symmetry breaking in cellular systems. J Cell Biol, 2006; 175:687-692
- ***Paluch E** (1), van der Gucht J (1), Joanny J-F, Sykes C. Deformations in actin comets from rocketing beads. Biophys J, 2006; 91:3113-22 (1) shared authorship
- ***Paluch E**, Sykes C, Prost J, Bornens M. Dynamic modes of the cortical actomyosin gel during cell locomotion and division. Trends Cell Biol, 2006; 16:5-10
- ***Paluch E**, Piel M, Prost J, Bornens M, Sykes C. Cortical actomyosin breakage triggers shape oscillation in cells and cell fragments, Biophys J, 2005; 89:724-33
- *van der Gucht J, **Paluch E**, Plastino J, Sykes C. Stress release drives symmetry breaking for actin-based movement, Proc Natl Acad Sci USA, 2005; 102:7847-52.

**no IIMCB affiliation*


**Publications (other than scientific articles)**

- Articles about the history of words related to physics and biology for Dictionnaire Culturel de la Langue Francaise (2005) directed by Alain Rey, publisher: le Robert (informations: http://www.lerobert-dictionnaireculturel.com/)
- Paluch E, Ramspacher A. (1998) Electromagnetisme, 2eme annee, collection Puissance Prepas, publisher: Breal (methods and corrected exercises for 2nd year Physics students)

# Research

The main focus of the group's research is to investigate the principles underlying cellular morphogenesis. Since cell shape is ultimately defined by cellular mechanical properties and by the cell's physical interactions with its environment, biophysical approaches are essential to understand cell shape control (Clark and Paluch, 2011; Paluch and Heisenberg, Curr Biol, 2009). We combine biology, quantitative image analysis and physical modeling to investigate the molecular regulation of cellular mechanical properties, and the contribution of these properties to cellular deformations.

Cell shape is determined to a great extent by the actin cortex, a network of actin filaments, myosin, and associated proteins lying immediately beneath the plasma membrane of most animal cells. The cortex enables the cell to resist externally applied forces and to exert mechanical work. As such, it plays a central role during events involving cell deformation, such as cell division and cell locomotion, and in the patho-physiology of diseases such as cancer, in which cortical contractility is often upregulated. Despite its importance, very little is known about cortex composition, assembly, regulation, and mechanics. Our main focus is on investigating how cortical mechanical properties are determined by the molecular components of the cortex and how these properties are regulated, locally and globally, to allow the cell to undergo deformations during cell division and migration. We are particularly interested in blebs, spherical membrane protrusions driven by contractions of the actomyosin cortex. Although blebs are commonly observed during apoptosis, cell spreading, cytokinesis, and migration, their growth and physiological functions are still poorly understood. We investigate the physical and biological mechanisms of bleb formation and study their function during cytokinesis and migration. Our main lines of research are the following:

**1. Regulation of cortex assembly and cortex mechanics**

Our aim is to understand the mechanisms and regulation of cortex assembly and steady-state turnover. Despite the physiological importance of the cortex, basic properties, such as cortex thickness, the spatial organization of the network, and its dynamical behavior (turnover), are very poorly understood. One reason for this is that the thickness of the cortical network is less than 1 µm, which makes it difficult to observe using conventional optical microscopy. Over the past few years, we have developed a method of measuring cortex thickness and monitoring the dynamics of cortex turnover with sufficient spatial and temporal resolution. We are currently using these tools to investigate the molecular regulation and physical mechanisms that underlie cortex turnover. In parallel, we are investigating *de novo* assembly of the cortex using cellular blebs as a model system. Indeed, blebs are initially devoid of filamentous actin and reassemble a contractile cortex prior to retraction. Thus, they present an ideal system for the study of cortex growth. We have developed an assay, in which cortex assembly at the surface of blebs induced by laser ablation (Tinevez et al., PNAS, 2009) can be precisely monitored in a semi-automated manner (Biro et al., submitted). In collaboration with the labs of G. Charras (UCL, London, UK), G. Romet-Lemonne (CNRS, Gif-sur-Yvette, France) and P. Roux (IRIC, Montreal, Canada), we have used this assay to investigate the nature of cortical actin nucleators (Bovellan et al, submitted).

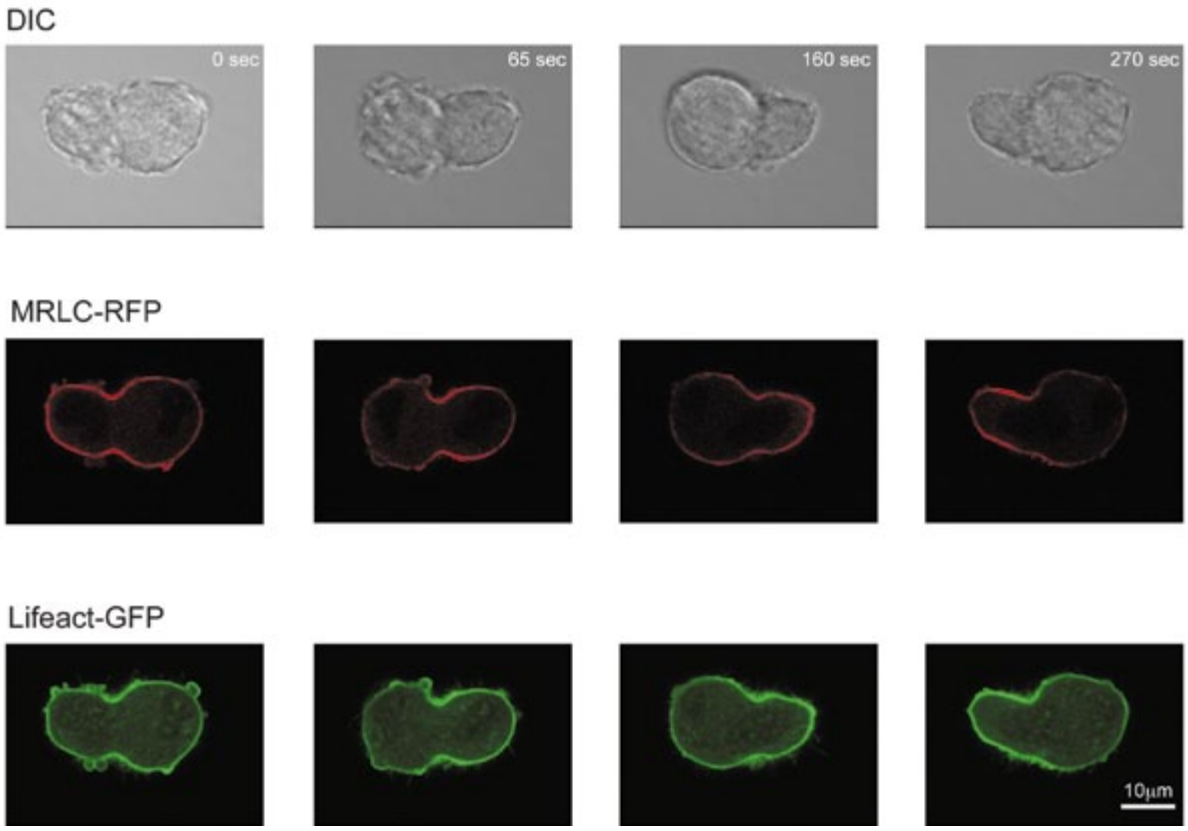



**Fig. 1.** Shape oscillations in a cytokinetic L929 fibroblast after depletion of the scaffolding protein anillin. The cell co-expresses Myosin Regulatory Light Chain coupled to tandem-dimer red fluorescent protein (MRLC-RFP) and the actin-binding peptide Lifeact coupled to green fluorescent protein (Lifeact-GFP). Anillin depletion leads to enhanced tension at the poles of the dividing cell, which results in shape oscillations, displacement of the cleavage furrow from its equatorial position and division failure. (Author: Andrea Pereira).

**2. Mechanics of cytokinesis**

Cytokinesis relies on a controlled reorganisation of the actin cortex. Most previous studies of cytokinetic mechanics have focused on force generation in the contractile acto-myosin ring at the cell equator. However, a significant amount of actin and myosin remains at the poles of a dividing cell throughout cytokinesis. We have investigated the contribution of this polar cortex to cytokinesis and revealed that polar contractility makes the symmetric shape of the dividing cell intrinsically unstable. Indeed, an imbalance in contractile forces between the two poles can displace the cleavage furrow from its equatorial position. We have shown that such instabilities can be observed during cytokinesis, and can be amplified by treatments affecting the cortex, leading to shape oscillations and division failure (Figure 1). We proposed a theoretical model coupling cortex tension, turnover and cell elasticity, which quantitatively accounts for the oscillations. Finally, we showed that blebs, which are commonly observed at the poles of dividing cells, stabilise cell shape by acting as valves releasing polar tension. By combining quantitative imaging with physical modelling, this study demonstrated that the shape of a dividing cell is inherently unstable, and that polar contractility must be tightly controlled to avoid shape asymmetries and division failure (Sedzinski et al., Nature, 2011).

**3. Formation and function of blebs and lamellipodia during cell migration**

In three-dimensional environments, bleb-based migration is a widespread alternative to lamellipodial migration, and is commonly used by cancer cells and during development (Charras and Paluch, Nature Rev Mol Cell Biol, 2008). What determines the type of protrusion formed by a migrating cell and how the various protrusions contribute to migration remains elusive. We have developed two model systems to address these questions:

- We are studying cell migration *in vivo*, during *Danio rerio* [zebrafish] embryonic development (collaboration with the laboratory of C.P. Heisenberg, IST, Austria). We have shown that mesendoderm progenitor cells migrate during gastrulation using a combination of blebs, lamellipodia and filopodia. Therefore, they constitute an ideal system for investigating the respective contributions of the different protrusion types to cell migration. We have used a variety of methods to increase the proportion of blebs at the expense of the other protrusion types and have shown that increasing bleb formation has the effect of slowing down migration by reducing the directional persistence of the migrating cells (Diz-Muñoz et al., PLoS Biol, 2010). We are currently further investigating the contribution of blebs and lamellipodia to migration by analysing the dynamics and orientation of the different protrusions with respect to migration direction (Figure 2).
- We are also investigating the mechanisms leading to bleb or lamellipodium formation using cultured Walker carcinosarcoma cells. These cells can be induced to form either blebs or lamellipodia by varying culture conditions (Figure 3). We have compared the cells forming lamellipodia to those forming blebs and have characterised the mechanical and molecular requirements for the formation of one or the other protrusion type (Bergert et al., in revision).

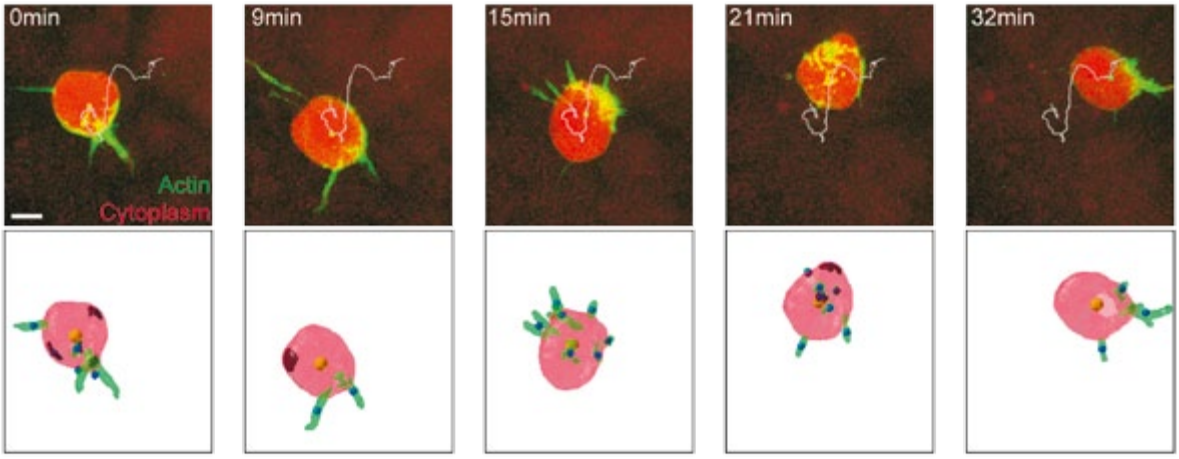



**Fig. 2.** Protrusion formation during migration of a zebrafish mesendoderm progenitor. The mesendoderm progenitor cell, expressing the actin-binding peptide Lifeact-GFP and injected with red fluorescent dextran (cytoplasmic marker), was transplanted in a host embryo lacking mesendoderm progenitors (Mzoep mutant). The cell displays single cell migration in the host embryo and forms lamellipodia (actin-filled protrusions) and blebs (actin-free spherical protrusions). An image analysis software (developed in collaboration with Weimiao Yu A-star, Singapore) allows for automated segmentation of the cell body and identification of the protrusions (bottom line). Scale bar: 10 µm. (Authors: Martin Bergert and Alba Diz Muñoz).

**4. Mechanisms of bleb formation**

Despite increasing evidence showing that blebs are instrumental during cytokinesis (Sedzinski et al., Nature, 2011) and cell migration (Charras and Paluch, Nature Rev Mol Cell Biol 2008), very little is known about the mechanisms behind their formation. By using laser ablation to induce blebs in combination with cortex tension measurements, we could show that bleb growth is directly driven by, and considerably reduces, the pressure generated in the cell body by the actomyosin cortex. In combination with a physical model (collaboration with the theory group of J.F. Joanny, Institut Curie, Paris), these experiments have allowed us to identify the mechanical factors determining bleb size (Tinevez et al., PNAS, 2009). More recently, we have investigated the dynamics of bleb expansion and characterised the mechanical properties controlling the speed of bleb growth. These studies open new avenues of research for the understanding of the regulation of bleb expansion during cell motility.

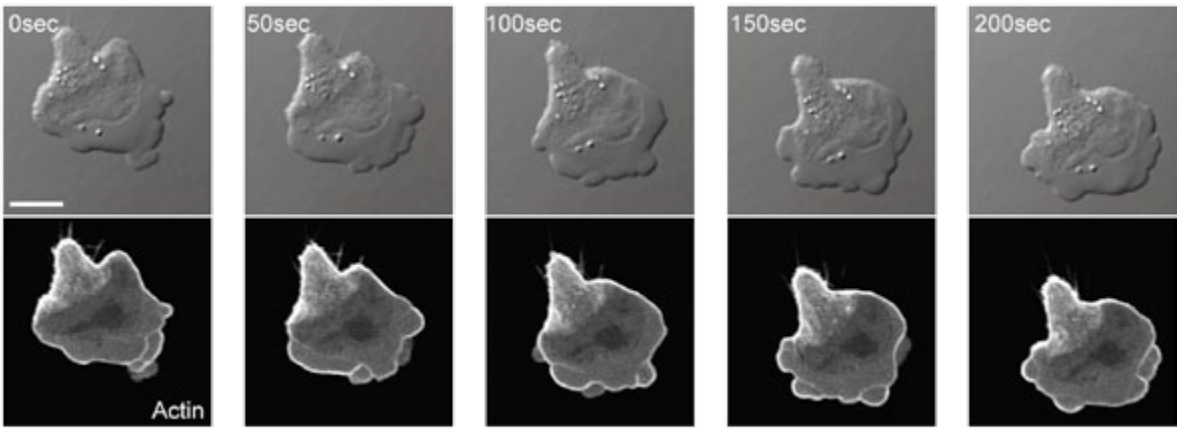
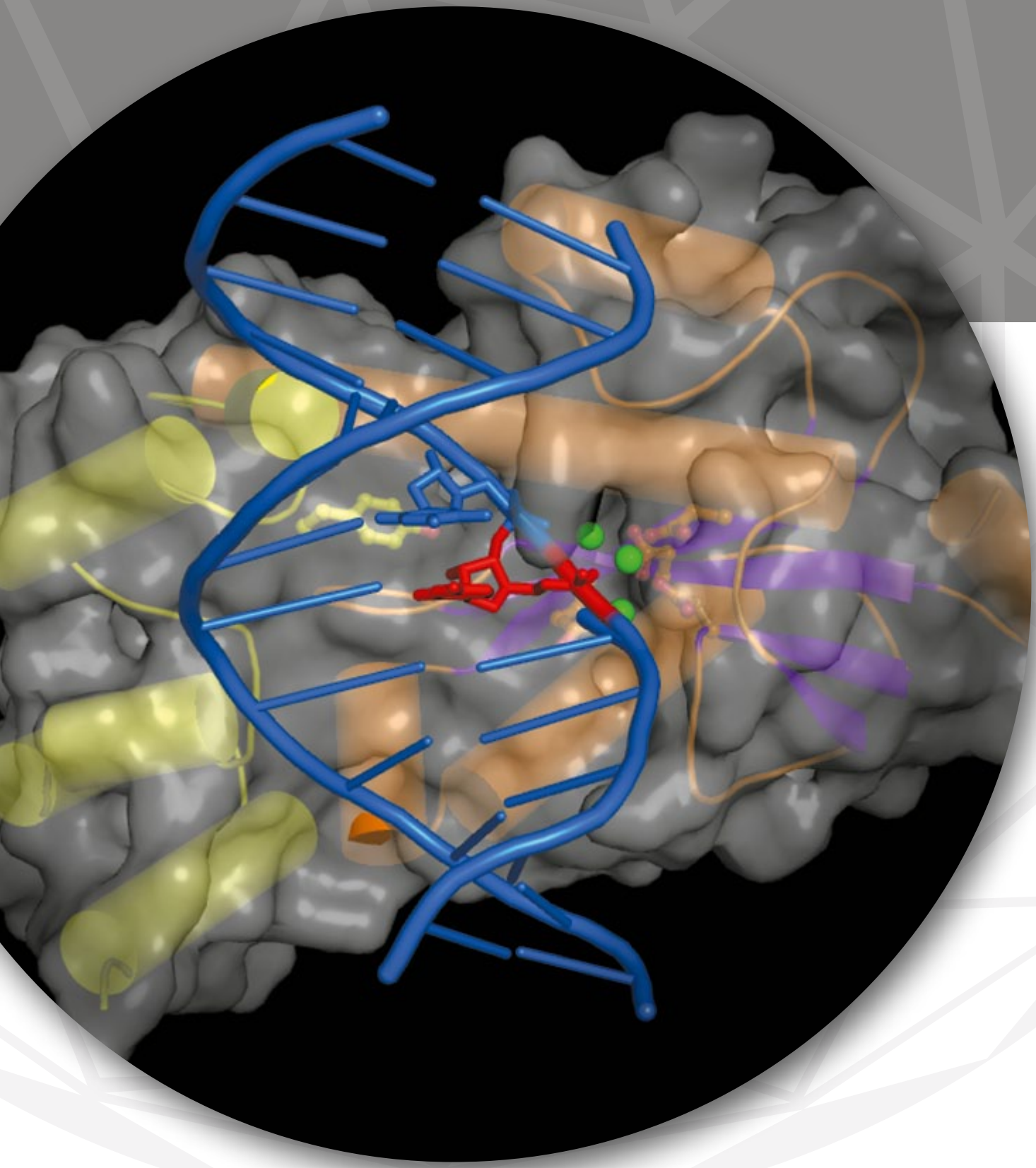



**Fig. 3.** Bleb formation in a migrating Walker carcinosarcoma cell. The cell, expressing the actin-binding peptide Lifeact-GFP, has been placed between a glass substrate and an agarose pad and thus migrates in a confined environment. The formation of blebs, actin-free spherical protrusions, can be observed at the leading edge of the cell. Scale bar: 10 µm. (Author: Martin Bergert)



The structure of T. *maritima* RNase H2 in complex with nucleic acid substrate solved at 2.0 Á resolution. The protein is shown in cartoon representation and colored by the domains (yellow and orange). The DNA is shown in ladder representation (blue for DNA and red for the single ribonucleotide located at the active site). The calcium ions are shown as green spheres.

# Laboratory of **Protein Structure**

## Lab leader: **Marcin Nowotny**, PhD

**Postdoctoral Fellows:**
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Karolina Górecka, PhD

**Junior Researchers:**
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Marcin Jaciuk, MSc
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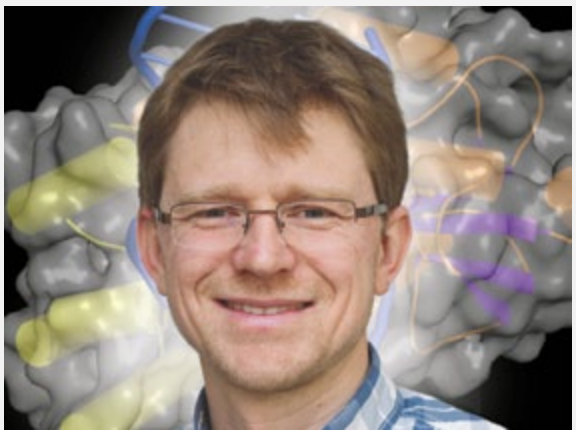
Michał Miętus, MSc

**Lab Managers:**
Magdalena Łazęcka, MSc
Monika Rychlik, MSc

**Technicians:**
Marzena Nowacka, BSc
Iwona Ptasiewicz



# Head of Laboratory of Protein Structure

## **Marcin Nowotny**, PhD

**Degrees**

| | |
|---|---|
| 2002 | PhD *magna cum laude* in Biochemistry, under the supervision of Prof. dr hab. Jacek Kuźnicki, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw |
| 1998 | MSc in Organic Chemistry and Biochemistry, Department of Chemistry, Warsaw University |

**Postdoctoral Training**

| | |
|---|---|
| 2003-2008 | Postdoctoral Fellow, Wei Yang Laboratory, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA |

**Professional Employment**

| | |
|---|---|
| 2008-Present | Head, Laboratory of Protein Structure, IIMCB |

**Honors, Prizes, Awards**

| | |
|---|---|
| 2012 | HHMI Early Career Scientist Award |
| 2011 | ERC Starting Grant |
| 2007 | EMBO Installation Grant |
| 2007 | Wellcome Trust Senior Research Fellowship |
| 2003 | Prime Minister's Award for PhD thesis |
| 2001, 2002 | Annual Stipend for Young Scientists, Foundation for Polish Science |

# Selected publications


- Rosta E, **Nowotny M**, Yang W, Hummer G. Catalytic mechanism of RNA backbone cleavage by ribonuclease h from quantum mechanics/molecular mechanics simulations. J Am Chem Soc, 2011; 133(23):8934-41
- **Figiel M**, Chon H, Cerritelli SM, **Cybulska M**, Crouch RJ, **Nowotny M**. The structural and biochemical characterization of human RNase H2 complex reveals the molecular basis for substrate recognition and Aicardi-Goutieres syndrome defects. J Biol Chem, 2011; 286:10540-50
- **Jaciuk M, Nowak E, Skowronek K, Tanska A, Nowotny M**. Structure of UvrA nucleotide excision repair protein in complex with modified DNA. Nat Struct Mol Biol, 2011; 18:191-197
- **Rychlik MP**, Chon H, Cerritelli SM, **Klimek P**, Crouch RJ, **Nowotny M**. Crystal Structures of RNase H2 in Complex with Nucleic Acid Reveal the Mechanism of RNA-DNA Junction Recognition and Cleavage. Mol Cell, 2010; 40:658-670
- **Nowotny M**. Retroviral integrase superfamily: the structural perspective (review). EMBO Rep, 2009; 10:144-51
- **Nowotny M**, Yang W. Structural and functional modules in RNA interference. Curr Opin Struct Biol. 2009;19:286-293. Review
- ***Nowotny M**, Cerritelli SM, Ghirlando R, Gaidamakov SA, Crouch RJ, Yang W. Specific recognition of RNA/DNA hybrid and enhancement of human RNase H1 activity by HBD. EMBO J, 2008; 27:1172-81
- ***Nowotny M**, Gaidamakov SA, Ghirlando R, Cerritelli SM, Crouch RJ, Yang W. Structure of human RNase H1 complexed with an RNA/DNA hybrid: Insight into HIV Reverse Transcription. Mol Cell, 2007; 28:264-276
- ***Nowotny M**, Yang W. Stepwise analyses of metal Ions in RNase H catalysis: From substrate destabilization to product release. EMBO J, 2006; 25:1924-33
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- ***Nowotny M**, Gaidamakov SA, Crouch RJ, Yang W. Crystal structures of RNase H bound to an RNA/DNA hybrid: substrate specificity and metal-dependent catalysis. Cell, 2005; 121:1005-16
- *Lee YT, Jacob J, Michowski W, **Nowotny M**, Kuznicki J, Chazin WJ. Human Sgt1 binds HSP90 through the CHORDSgt1 domain and not the tetratricopeptide repeat domain. J Biol Chem, 2004; 279:16511-7
- ***Nowotny M**, Spiechowicz M, Jastrzebska B, Filipek A, Kitagawa K, Kuznicki J. Calcium-regulated interaction of Sgt1 with S100A6 (calcyclin) and other S100 proteins. J Biol Chem, 2003; 278:26923-8
- *Filipek A, Jastrzebska B, **Nowotny M**, Kuznicki J. CacyBP/SIP, a calcyclin and Siah-1-interacting protein, binds EFhand proteins of the S100 family. J Biol Chem, 2002; 277:28848-52
- *Filipek A, Jastrzebska B, **Nowotny M**, Kwiatkowska K, Hetman M, Surmacz L, Wyroba E, Kuznicki J. Ca2+-dependent translocation of the calcyclin-binding protein in neurons and neuroblastoma NB-2a cells. J Biol Chem, 2002; 277:21103-9
- ***Nowotny M**, Bhattacharya S, Filipek A, Krezel AM, Chazin W, Kuznicki J. Characterization of the interaction of calcyclin (S100A6) and calcyclin-binding protein. J Biol Chem, 2000; 275:31178-82.


*no IIMCB affiliation

# Description of Current Research

Our laboratory focuses on structural and biochemical studies of nucleic acid enzymes using protein crystallography as a primary method. The key results obtained recently in our laboratory concern three proteins: bacterial and human RNases H2 and UvrA.

**1. Structural studies of bacterial RNases H2**

RNases H are small nucleases that specifically hydrolyze RNA in RNA/DNA hybrids. They are divided into two types—RNases H1 and RNases H2—which have a similar structure of the catalytic core but have different domain organization and biochemical properties. The most important feature of RNases H2, differentiating them from type 1 enzymes, is their ability to cleave single ribonucleotides embedded in the DNA. Such single ribonucleotides occur quite frequently in genomic DNA and most often result from misincorporation by DNA polymerases. They must be removed to maintain genomic stability, and RNase H2 is the only enzyme that can initiate this process by cleaving the phosphate linkage on the 5′ side of the ribonucleotide. The removal is completed by the second cut on the 3′ side of the RNA by FEN-1 endonuclease.

The mechanism of the specific cleavage of single ribonucleotides by RNase H2 was previously unknown. To elucidate this, we solved the crystal structures of *Thermotoga maritima* RNase H2 in complex with the nucleic acid substrate. The results showed that the nucleic acid is bound in a cleft between the *N*-terminal, catalytic domain, and *C*-terminal helical domain (cover figure). The key element that ensures the substrate specificity of the enzyme is the recognition mechanism for a (5′)RNA-DNA(3′) junction. The RNA residue of the junction forms a network of interactions between its 2′-OH and the backbone of three protein residues: two glycines and an arginine that forms an element we called the "GRG motif." The hydroxyl group of an absolutely conserved tyrosine residue from the *C*-terminal domain also forms a hydrogen bond with the 2′-OH group. This tyrosine also interacts with the second group of the junction, forming a stacking interaction with its ribose ring. This interaction can be efficient only if a 2′-OH group is absent from the ring and therefore is selective for deoxyribonucleotide in the second position of the junction. The stacking interaction leads to a deformation of the nucleic acid, changing the conformation of the phosphodiester backbone of the RNA-DNA junction. Because of this deformation, the phosphate group of the junction can participate in the coordination of a metal ion at the active site. This mechanism ensures very stringent substrate specificity. Only when a correct substrate is present (e.g., an RNA-DNA junction)

that can be properly deformed, the metal ion is coordinated at the active site, and the reaction can proceed.

The active site of RNase H2 is formed by four conserved carboxylate residues. In the wildtype structure solved in the presence of $Ca^{2+}$ ions, we observed three ions at the active site. Two of them occupy positions very similar to the two catalytic metal ions in related enzymes, and we assume that RNase H2 uses a canonical two-metal ion mechanism. In this mechanism, one metal ion activates the attacking nucleophile, and the second ion stabilizes the transition state and reaction product.

**2. Structural studies of human RNase H2**

Eukaryotic RNases H2 are complexes of three proteins. In addition to the catalytic subunit (RNase H2A), they contain two auxiliary subunits (RNases H2B and H2C). The function of the additional subunits is unknown, but they are required for activity. Mutations of human RNase H2 lead to a severe genetic disease called Aicardi-Goutieres syndrome (AGS), the symptoms of which are observed already in newborns and involve a massive autoimmune response that leads to calcification of brain tissue. This response is probably triggered by the accumulation of RNA/DNA hybrids or single ribonucleotides.

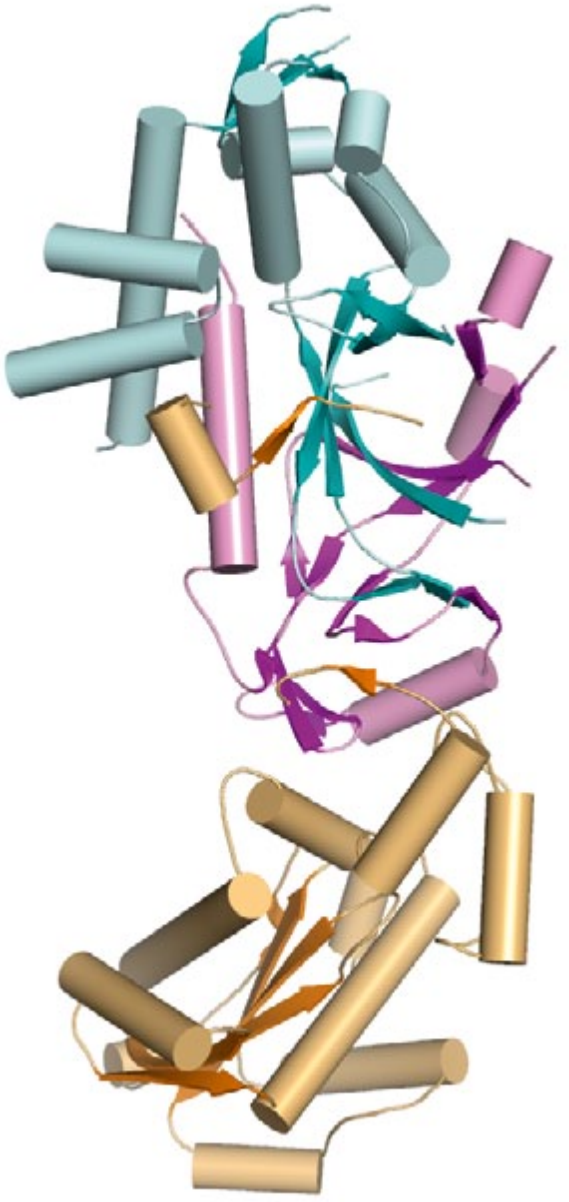

**Fig. 1.** Crystal structure of human RNase H2 solved at 3.1 Å resolution. The catalytic subunit is shown in orange, and the auxiliary subunits are shown in pink (subunit C) and cyan (subunit B).

We sought to solve a crystal structure of the human RNase H2 complex to determine the architecture of the complex and shed some light on the possible functions of the auxiliary subunits. For crystallization, we used an RNase H2 complex that contains a truncated version of one of the subunits. We obtained crystals that diffracted X-rays to 3.1 Å resolution. While this work was underway, a structure of mouse RNase H2 was published. We used its coordinates to solve our structure of human protein based on our native 3.1 Å dataset. After the first rounds of our structural refinement, the initial model apparently did not fit well into our density maps, indicating that the starting mouse structure contained tracing errors. We subsequently rebuilt several regions of the initial model.

The human RNase H2 complex forms an oblong molecule with A and B subunits in its ends and the C subunit in the middle (Fig. 1). The catalytic subunit closely resembles the monomeric bacterial and archaeal RNase H2. The B and C subunits form a highly intertwined dimer that adopts a triple-barrel fold. The interactions of this dimer with the A subunit are mediated by several hydrophobic regions of the C protein. An additional interaction is formed by the last 15 residues of the A subunit that add a strand to the central β-sheet the B/C dimer.

Our tracing corrections allowed us to map the positions of all 29 reported mutations from AGS patients, whereas only 20 could be correctly placed in the mouse structure. Based on the possible effect of these mutations on RNase H2 structure and function, we divided the mutations into three groups: (*i*) mutations that affect substrate binding and cleavage, (*ii*) mutations that affect the structure of the individual subunits or the structure of the complex, and (*iii*) mutations that affect the interactions with putative target proteins. An example of the first group of residues is G37, which is mutated to serine in some AGS patients. This residue is a part of the GRG 2′-OH-sensing module. The G37S mutation likely affects substrate recognition. Mutations that belong to the second group are often located in the hydrophobic core of the protein or at the interfaces between subunits. The third group of mutations encompasses residues that are located on the surface of the protein and can potentially interact with yet unidentified target proteins.

We next used our substrate complex structure of bacterial RNase H2 to prepare a model of the human enzyme that interacts with the nucleic acid and is corroborated by directed mutagenesis studies. Bacterial and eukaryotic RNases H2 show significant differences in substrate specificity. In the presence of $Mg^{2+}$ ions, bacterial enzymes only cleave RNA-DNA junctions and are not able to hydrolyze regular RNA/DNA hybrids. Eukaryotic enzymes also prefer to cleave at the junctions but can efficiently cleave regular hybrids. Our model of the substrate complex of human RNase H2 offers an explanation of this difference in substrate preference. The tyrosine residue critical for RNA-DNA junction recognition is positioned differently in human and *T. maritima* RNases H2. In human enzyme, it is shifted away from the ribose ring of the second residue of the junction, possibly leading to less discrimination against the presence of the 2′-OH group in this position. This would allow the enzyme to bind and cleave regular RNA/DNA hybrids, in which all of the residues of the cleaved strand contain 2′-OH groups and do not contain RNA-DNA junctions.

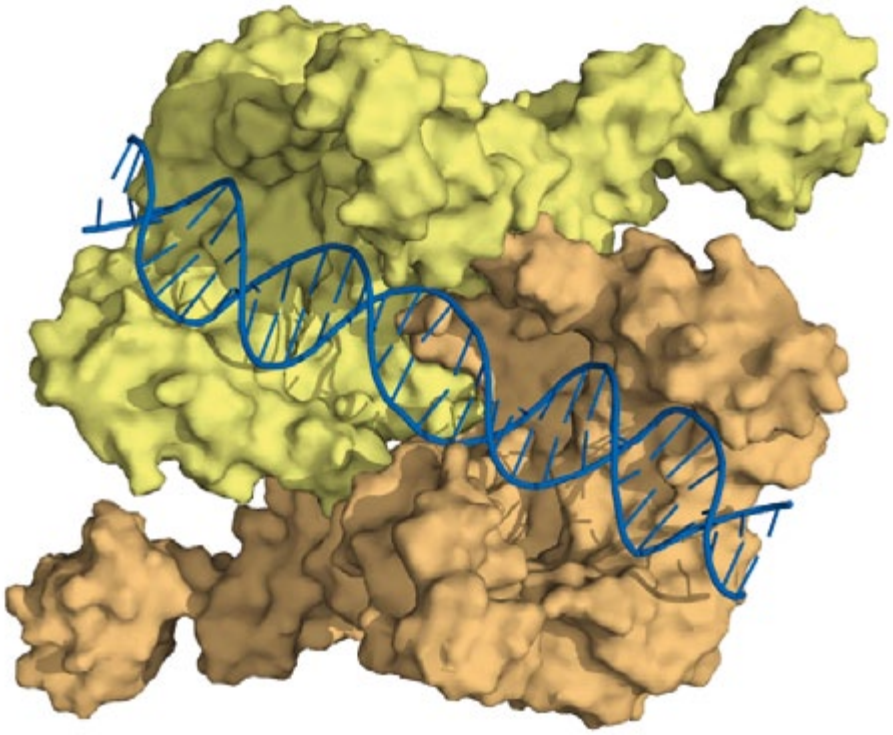

**Fig. 2.** Structure of *T. maritima* UvrA in complex with DNA solved at 2.9 Å resolution. The protein is shown in surface representation (the dimer subunits are colored in yellow and orange), and the DNA is shown in blue cartoon representation.

### 3. Structural and biochemical studies of UvrA DNA repair protein

DNA constantly undergoes detrimental chemical modifications (also called DNA damage) that occur spontaneously or are caused by physical and chemical factors. To maintain the genetic stability of the cell and protect the organism, these modifications need to be corrected. One of the primary pathways to achieve this is nucleotide excision repair (NER). The most important feature of NER is its ability to recognize a wide variety of DNA lesions of unrelated chemical structures. Different proteins are involved in NER in bacteria and eukaryotes, but the principle is the same. The site of damage is located, its presence is verified, and the DNA is incised on both sites of the lesion. The DNA fragment that contains the lesion is removed by a helicase, and the gap is filled by DNA polymerase. In bacteria, the first component of the pathway, which locates the lesion, is UvrA protein. It is a dimeric adenosine triphosphatase (ATPase) from the ATP-binding cassette (ABC) family. After the damage is found, the DNA is handed over to UvrB, which possesses weak helicase activity and verifies the presence of the lesion. UvrC nuclease executes the two cuts on the two sides of the modification.

The key unanswered question in NER is how its remarkably wide specificity is achieved. To elucidate this, we sought to solve the crystal structure(s) of a UvrA protein in complex with modified DNA. In our extensive crystallization trials, we used UvrA proteins from two bacterial species and DNA oligonucleotides that contained a single thymine residue with a fluorescein moiety attached through a flexible tether. We used DNA duplexes with a modified thymine residue in one of the DNA strands and duplexes that consisted of palindromic oligonucleotides that contain symmetrically placed modified thymines in both strands. We reasoned that the symmetry of such DNAs would reflect the two-fold symmetry of the UvrA dimer and hence promote crystallization. Indeed, we only obtained crystals with the palindromic oligonucleotides. We then verified, using biochemical assays, that each of the strands of the palindromic substrates can be independently processed by the NER machinery that consisted of UvrA, UvrB, and UvrC. The crystals diffracted X-rays up to 2.9 Å resolution, and the structure was solved using molecular replacement. In the structure, the DNA is bound in a cleft that runs across the UvrA dimer. The interactions between the protein and nucleic acid are formed almost exclusively with the terminal regions of the DNA duplex. We identified a conserved, positively charged patch on the surface of the protein that forms extensive contacts with the DNA backbone.

The key to DNA damage recognition by UvrA is the conformation of the DNA. The duplex is bent by approximately 15 degrees, stretched in the middle, and unwound. Only this deformed conformation is complementary with the protein surface. The DNA deformations we observe are also often seen in various modified DNAs in free, unbound form. Unwinding is a particularly common feature of many damaged DNAs. We therefore proposed that UvrA uses an indirect readout mechanism to detect the presence of the damage. The protein senses the deformations of the DNA caused by the lesion. At the same time, it may also adjust those deformations so that the duplex fits to the protein surface. Modified DNA duplexes are known to be more flexible and easier to deform. UvrA probes the conformation of the DNA symmetrically on both sides of the lesion without directly interacting with the site of modification itself. Its dimeric structure is ideally suited for this purpose, but the symmetrical damage detection does not provide information about which strand is damaged and needs to be incised. This is most likely the role of the UvrB protein, which is recruited to the DNA after UvrA finds the damage site.

The mechanism of indirect readout we described is unique. Eukaryotic NER proteins, for which crystal structures are available, such as UV-DDB and XPC/HR25 complexes, form specific contacts with the site of lesion and use base flipping to probe the strength of the base pair hydrogen bonds to detect the damage.


Collage by Agata Trojanowska

# Laboratory of
# **Mitochondrial Biogenesis**

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Magdalena Kaus-Drobek, PhD (until October 2011)
Adrianna Łoniewska-Lwowska, PhD (until March 2011)
Małgorzata Sztolsztener, PhD

**PhD Students:**
Tomasz Czerwik, MSc
Agnieszka Górnicka, MSc
Paulina Kwiatkowska, MScEng
Aksana Varabyova, MSc
Lidia Wróbel, MSc

**Research Assistant:**
Anita Brewińska, MSc

**Technician:**
Elżbieta Grzelak

**Undergraduate Students:**
Kamila Ornoch
Magdalena Stankiewicz
Agata Trojanowska

# Head of Laboratory of Mitochondrial Biogenesis

## **Agnieszka Chacińska**, PhD, DSc Habil

### Education and Degrees

| | |
|---|---|
| 2008 | DSc Habil, Institute of Biochemistry and Biophysics, Warsaw, Poland |
| 2000 | PhD in Biochemistry, Institute of Biochemistry and Biophysics, Warsaw, Poland |
| 1993 | MSc in Molecular Biology, University of Warsaw |
| 1988-1993 | Biology, University of Warsaw, Poland |

### Awards

| | |
|---|---|
| 2010 | EMBO Installation Grant |
| 2009 | Welcome Programme, Foundation for Polish Science |
| 2008 | Eugen-Graetz Prize for Research, University of Freiburg, Germany |
| 2001-2003 | Long-term FEBS fellowship |
| 2001 | Award for PhD thesis, Institute of Biochemistry and Biophysics, Warsaw, Poland |
| 1997 | Grant for Young Scientists, Polish State Committee for Scientific Research |
| 1996 | Short-term FEBS fellowship |

### Research experience and Appointments

| | |
|---|---|
| 2009-Present | Professor and Head of Laboratory of Mitochondrial Biogenesis, International Institute of Molecular and Cell Biology, Warsaw, Poland |
| 2008-2009 | Associate Member of Excellence Cluster BIOSS, Centre for Biological Signalling Studies, University of Freiburg, Germany |
| 2007-2009 | Member of the Board, Collaborative Research Centre (SFB 746) |
| 2007-2010 | Project Leader in Collaborative Research Centre (SFB 746) |
| 2004-2009 | Group Leader (German equivalent of Assistant Professor), Institute for Biochemistry and Molecular Biology, University of Freiburg, Germany |
| 2001-2004 | Postdoctoral Fellow, Laboratory of Prof. Nikolaus Pfanner, University of Freiburg, Germany |
| 1999 | Visiting Scientist, Laboratory of Prof. Sabine Rospert, Max Planck Research Unit, Halle, Germany |
| 1997 | Visiting Scientist, Laboratory of Prof. Gottfried Schatz, Biozentrum, University of Basel, Switzerland |
| 1994-2000 | Doctoral research with Prof. Magdalena Boguta, Institute of Biochemistry and Biophysics, Warsaw, Poland |

# Publications

**2011**

- von der Malsburg K, Müller JM, Bohnert M, Oeljeklaus S, **Kwiatkowska P**, Becker T, **Loniewska-Lwowska A**, Wiese S, Rao S, Milenkovic D, Hutu DP, Zerbes RM, Schulze-Specking A, Meyer HE, Martinou JC, Rospert S, Rehling P, Meisinger C, Veenhuis M, Warscheid B, van der Klei IJ, Pfanner N*, **Chacinska A***, van der Laan M. Dual Role of mitofilin in mitochondrial membrane organization and protein biogenesis. Developmental Cell, 2011; 21:694-707 (*corresponding authors)
- Schulz C, Lytovchenko O, Melin J, **Chacinska A**, Guiard B, Neumann P, Ficner R, Jahn O, Schmidt B, Rehling P. Tim50's presequence receptor domain is essential for signal driven transport across the TIM23 complex. J Cell Biol, 2011; 195:643-56
- Becker T, Wenz LS, Krüger V, Lehmann W, Müller JM, Goroncy L, Zufall N, Lithgow T, Guiard B, **Chacinska A**, Wagner R, Meisinger C, Pfanner N. The mitochondrial import protein Mim1 promotes biogenesis of multispanning outer membrane proteins. J Cell Biol, 2011; 194:387-95.

**Other selected publications**

- Chacinska A, Koehler CM, Milenkovic D, Lithgow T, Pfanner N. Importing mitochondrial proteins: machineries and mechanisms. Cell, 2009; 138:628-644
- Milenkovic D, Ramming T, Müller JM, Wenz LS, Gebert N, Schulze-Specking A, Stojanovski D, Rospert S, Chacinska A. Identification of the signal directing Tim9 and Tim10 into the intermembrane space of mitochondria. Mol Biol Cell, 2009; 20:2530-9
- Chacinska A*, Guiard B*, Müller JM, Schulze-Specking A, Gabriel K, Kutik S, Pfanner N. Mitochondrial biogenesis: switching the sorting pathways of the intermembrane space receptor Mia40. J Biol Chem, 2008; 283:29723-9 (*equal contribution)
- Stojanovski D, Milenkovic D, Müller JM, Gabriel K, Schulze-Specking A, Baker MJ, Ryan MT, Guiard B, Pfanner N, Chacinska A. Mitochondrial protein import: precursor oxidation in a ternary complex with disulfide carrier and sulfhydryl oxidase. J Cell Biol, 2008; 183:195-202
- Müller JM, Milenkovic D, Guiard B, Pfanner N, Chacinska A. Precursor oxidation by Mia40 and Erv1 promotes vectorial transport of proteins into the mitochondrial intermembrane space. Mol Biol Cell, 2008; 19:226-236
- Milenkovic D, Gabriel K, Guiard B, Schulze-Specking A, Pfanner N, Chacinska A. Biogenesis of the essential Tim9-Tim10 chaperone complex of mitochondria: site-specific recognition of cysteine residues by the intermemembrane space receptor Mia40. J Biol Chem, 2007; 282:22472-80
- Meinecke M, Wagner R, Kovermann P, Guiard B, Mick DU, Hutu DP, Voos W, Truscott KN, Chacinska A, Pfanner N, Rehling P. Tim50 maintains the permeability barrier of the mitochondrial inner membrane. Science 2006; 312:1523-1526
- Wiedemann N, Pfanner N, Chacinska A. Chaperoning through the mitochondrial intermembrane space. Mol Cell, 2006; 21:145-148
- Chacinska A*, Lind M*, Frazier AE, Dudek J, Meisinger C, Geissler A, Sickmann A, Meyer HE, Truscott KN, Guiard B, Pfanner N, Rehling P. Mitochondrial presequence translocase: switching between TOM tethering and motor recruitment involves Tim21 and Tim17. Cell, 2005; 120:817-829 (*equal contribution)
- Rissler M, Wiedemann N, Pfannschmidt S, Gabriel K, Guiard B, Pfanner N, Chacinska A. The essential mitochondrial protein Erv1 cooperates with Mia40 in biogenesis of intermembrane space proteins. J Mol Biol, 2005; 353:485-492
- Chacinska A, Pfannschmidt S, Wiedemann N, Kozjak V, Sanjuán Szklarz LK, Schulze-Specking A, Truscott KN, Guiard B, Meisinger C, Pfanner N. Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins. EMBO J, 2004; 23:3735-46
- Meisinger C, Rissler M, Chacinska A, Sanjuán Szklarz LK, Milenkovic D, Kozjak V, Schönfisch B, Lohaus C., Meyer, H.E., Yaffe, M.P., Guiard, B., Wiedemann, N., and Pfanner, N. The mitochondrial morphology protein Mdm10 functions in assembly of the preprotein translocase of the outer membrane. Dev Cell. 2004; 7:61-71
- Sickmann A, Reinders J, Wagner Y, Joppich C, Zahedi R, Meyer HE, Schönfisch B, Perschil I, Chacinska A, Guiard B, Rehling P, Pfanner N, Meisinger C. The proteome of Saccharomyces cerevisiae mitochondria. Proc Natl Acad Sci USA, 2003; 100:13207-13212
- Wiedemann N, Kozjak V, Chacinska A, Schönfisch B, Rospert S, Ryan MT, Pfanner N, Meisinger C. Machinery for protein sorting and assembly in the mitochondrial outer membrane. Nature, 2003; 424:565-571
- Chacinska A, Rehling P, Guiard B, Frazier AE, Schulze-Specking A, Pfanner N, Voos W, Meisinger C. Mitochondrial translocation contact sites: separation of dynamic and stabilizing elements in formation of a TOM-TIM-preprotein supercomplex. EMBO J, 2003; 22:5370-5381
- Geissler A*, Chacinska A*, Truscott KN, Wiedemann N, Brandner K, Sickmann A, Meyer HE, Meisinger C, Pfanner N, Rehling P. The mitochondrial presequence translocase: an essential role of Tim50 in directing preproteins to the import channel. Cell, 2002; 111:507-518 (*equal contribution).

(Publications until 2010 have no IIMCB affiliation)

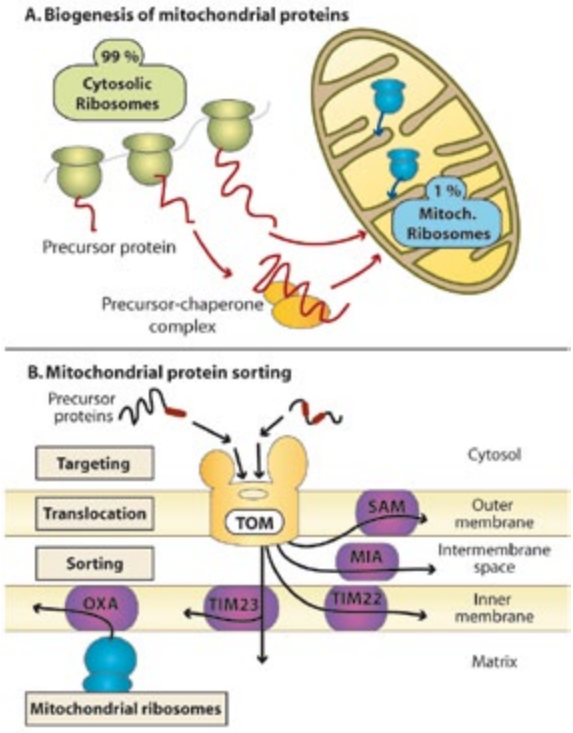



**Fig. 1.** A large majority of mitochondrial proteins are synthesized on cytosolic ribosomes and enter mitochondria via the entry formed by the TOM complex. After crossing the TOM complex, mitochondrial precursor proteins are sorted inside mitochondria into their final destinations (i.e., one of two mitochondrial membranes, the matrix or intermembrane space). A small number of hydrophobic proteins are encoded by mitochondrial DNA, synthesized by mitochondrial ribosomes and enter the inner mitochondrial membrane in a cotranslational process. Figure adopted from Chacinska et al., Cell 2009 (138:628).

Mitochondria play an important role in metabolism and regulatory processes in the cell. Thus, the formation of mitochondria is essential for cellular function in the entire eukaryotic kingdom, from unicellular organisms to mammals. Mitochondria comprise 1000-1500 cellular proteins that are synthesized outside the mitochondria in the cytosol and must be imported into mitochondria (Fig. 1). The biogenesis of mitochondria relies on the efficient import, sorting, and maturation of proteins governed by conserved protein translocases and other complex machineries. In the course of earlier work at the University of Freiburg, we made a surprising discovery that contradicted the dogma on the absence of disulfide bonds in reducing cellular compartments, such as mitochondria. We identified and characterized a novel mitochondrial intermembrane space assembly (MIA) pathway that utilizes the transfer of disulfide bonds and is dedicated to the import and biogenesis of intermembrane space proteins that lack a classical mitochondrial leader sequence (Fig. 2).

In our current research, supported by a Welcome Grant from the Foundation for Polish Science, an EMBO Installation Grant, and a grant from the Ministry of Science and Higher Education, our group seeks to understand the complex and dynamic processes involved in the formation of functional mitochondria, the maintenance of mitochondrial protein homeostasis, and their failure that results in pathology. Our major interests are related to redox-dependent processes involved in mitochondrial protein biogenesis. We concentrate on the following issues:

- Redox-related biogenesis events of mitochondrial proteins in yeast and higher eukaryotes.
- Impact of the MIA pathway on mitochondrial and cellular protein homeostasis.
- Biological consequences of oxidative protein biogenesis failure.

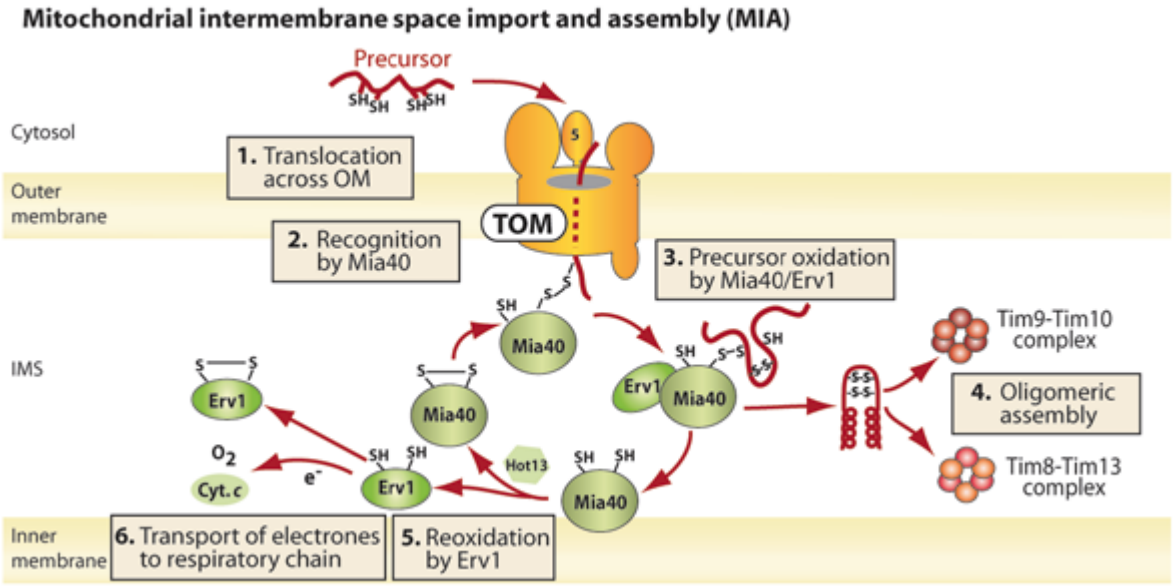



**Fig. 2.** Intermembrane space precursor proteins are posttranslationally transferred to mitochondria via TOM. The possibility exists that protein synthesis is coupled to protein transport. After arriving on the *trans* side of the TOM complex, intermembrane space proteins enter the MIA pathway, which drives their import completion and maturation by catalyzing disulfide bond formation and folding. Figure adopted from Chacinska et al., Cell 2009 (138:628).

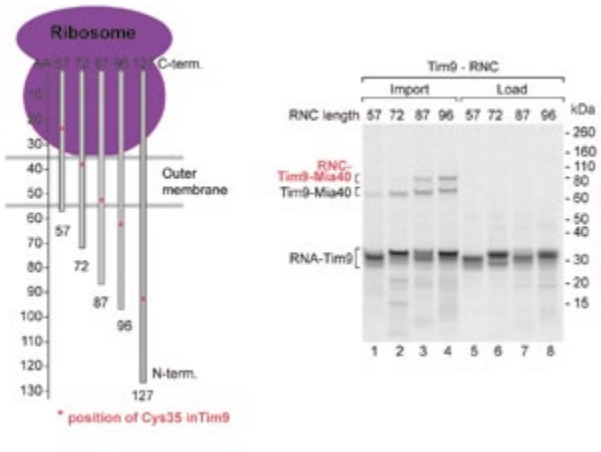



**Fig. 3.** Mia40, a *trans* receptor for intermembrane space proteins, is located in close proximity to the TOM complex. Tim9-ribosome nascent chain complexes (Tim9-RNC) were incubated with the isolated mitochondria and analyzed under non-reducing conditions. Figure adopted from von der Malsburg et al. Dev. Cell 2011 (21:694).

**Redox-related biogenesis events of mitochondrial proteins in yeast and higher eukaryotes**

We previously demonstrated (Milenkovic et al. 2007; Milenkovic et al., 2009) that mitochondrial precursor proteins are specifically recognized by Mia40, the major component of the MIA pathway, after they pass a main entry gate into mitochondria formed by the TOM complex (Fig. 2). Thus, Mia40 acts a receptor for intermembrane space proteins. Subsequently to the recognition event, Mia40 engages with precursors via formation of the intermolecular disulfide bond. However, Mia40 is located in the mitochondrial inner membrane, and this membrane is folded in structures called cristae. To address the issue of the spatial organization of the MIA pathway, we established a novel approach that is based on the generation of ribosome nascent chain complexes (RNC) with stalled precursor proteins targeted to the intermembrane space of mitochondria. Using this approach, we can manipulate and test various precursor lengths for the covalent disulfide-mediated interaction of their first cysteine residue, which arises on the *trans* side of the outer membrane, with Mia40 (Fig. 3). These data led us to conclude that Mia40 is located in close proximity to the TOM complex. In the search for factors that determine the localization of Mia40, we performed a comprehensive study of protein interactions. We identified the new interaction partner of Mia40, Fcj1 (Formation of Crista Junctions; mitofilin in higher eukaryotes), and also demonstrated that Fcj1 interacts with the TOM complex. Thus, Fcj1 is a regulatory factor that spatially organizes the biogenesis of mitochondria by positioning Mia40 in close proximity to the TOM complex. Moreover, consistent with this general function of Fcj1 in the spatial organization of mitochondria, we also characterized a large complex formed by Fcj1 that we named MINOS for its critical role in mitochondrial inner membrane organization (von der Malsburg et al., 2011). Currently, we are continuing to study the relationship between the MIA pathway and Fcj1, and its role in membrane organization.

We aim to determine the impact of the MIA pathway on mitochondrial and cellular protein homeostasis. Our studies involve approaches directed at understanding the role of the MIA pathway in biogenesis of non-canonical substrates localized to compartments other than the intermembrane space. We also set up unbiased proteomic approaches that will deliver a comprehensive and quantitative view of protein level changes in response to MIA dysfunction. These studies will provide a more complete picture of the role of MIA in mitochondrial biology and will lead to a better understanding of the biological consequences of oxidative protein biogenesis failure in cell function.

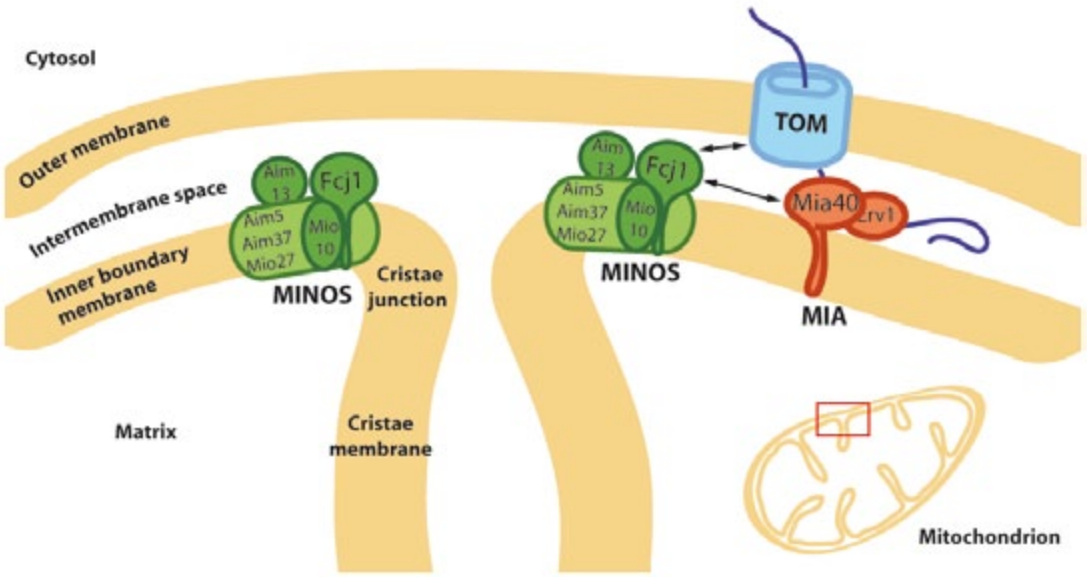
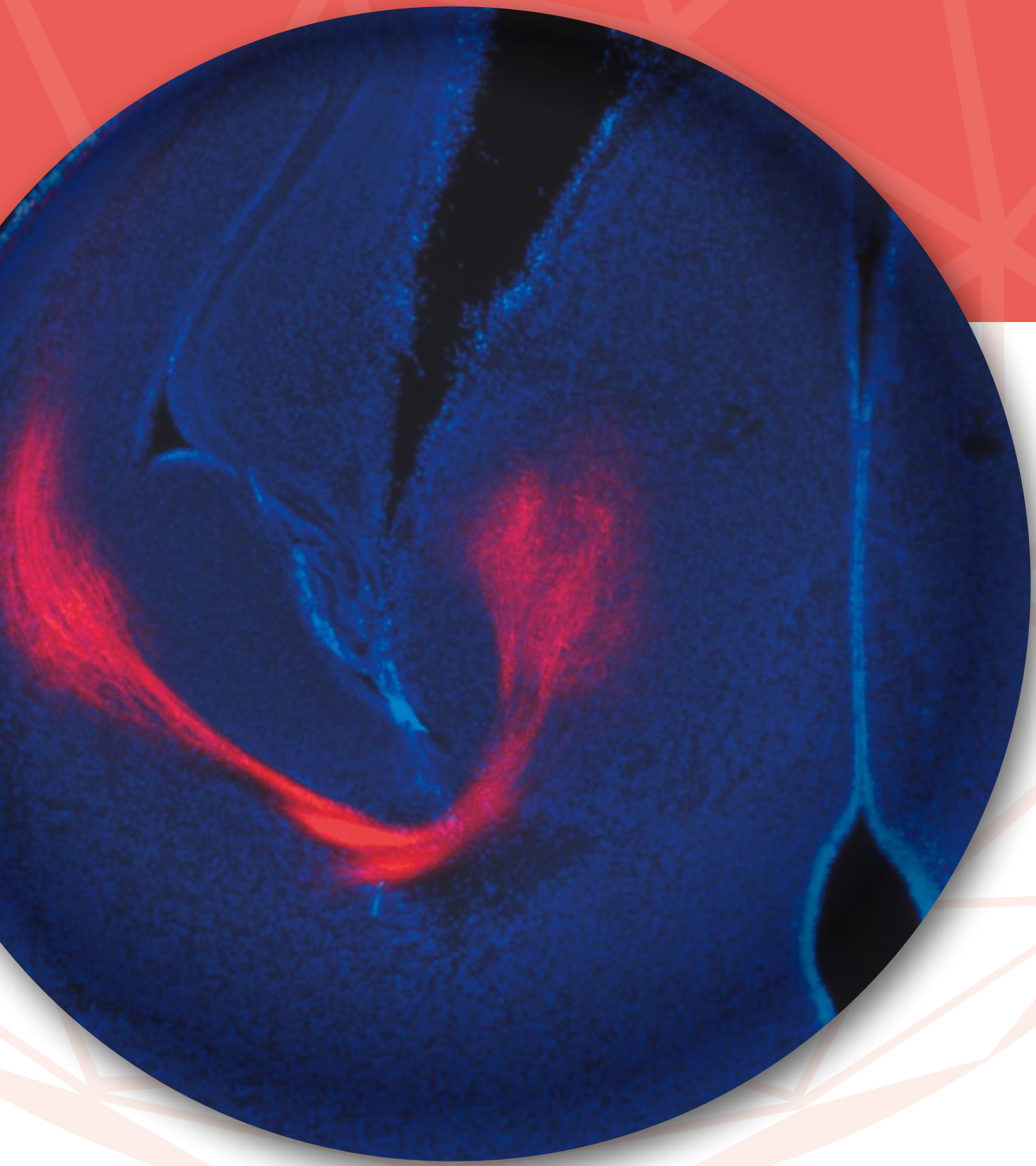



**Fig. 4.** Fcj1 is a component of the MINOS complex that is critical to the maintenance of the unique architecture of the inner mitochondrial membrane folded in cristae. It is also involved in the spatial organization of mitochondrial protein biogenesis by positioning Mia40, the receptor for intermembrane space proteins, in direct contact with precursors that arise on the *trans* side of the TOM complex.



Coronal section from the developing mouse brain (E16.5) with DiI crystals implanted in the cortex showing computer-generated overlays of DiI-labeled cortical axons reaching the thalamus (red) and cell nuclei counterstaining (blue).

# Laboratory of **Neurodegeneration**

## Lab leader: **Jacek Kuźnicki**, PhD, Professor


**Associate Professor, Vice Head:**
Urszula Wojda, PhD, DSc Habil

**Senior Scientists:**
Tomasz Węgierski, PhD
Marta Wiśniewska, PhD

**Senior Postdoctoral Fellow:**
Joanna Gruszczyńska-Biegała, PhD

**Junior Researchers:**
Emilia Białopiotrowicz, MSc
Katarzyna Dębowska, MSc
Anna Jaworska, MSc
Katarzyna Misztal, MSc
Andrzej Nagalski, MSc
Łukasz Szewczyk, MSc
Aleksandra Szybińska, MSc

**MSc Students:**
Nikola Brożko
Danuta Korona (until July 2011)

**Technician:**
Elżbieta Grzelak

**Current affiliations of former PhD and MSc students:**

- Mateusz Ambrożkiewicz, MSc/PhD Program in Neurosciences, Georg-August University and European Neurosciences Institute at Göttingen/ International Max Planck Research School
- Magdalena Błażejczyk, postdoctoral research fellow, Laboratory of Molecular and Cellular Neurobiology, IIMCB
- Łukasz Bojarski, research group leader, New Therapies of Neurological Diseases, Celon Pharma (www.celonresearch.com)
- Bożena Kuźniewska, PhD student in the laboratory of Prof. Leszek Kaczmarek, Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology
- Wojciech Michowski, postdoctoral research fellow, laboratory of Dr. Piotr Siciński, Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA
- Adam Sobczak, postdoctoral research fellow, Institute of Genetics and Biotechnology, Warsaw University, and Technology Transfer Unit of BioCentrum Ochota – Biotech-IP
- Danuta Korona, student at Deutsches Krebsforschungszentrum (DKFZ), Studying Cancer Biology at University of Heidelberg, Germany

# Head of Laboratory of Neurodegeneration

## **Jacek Kuźnicki**, PhD, Professor

**Degrees:**

| | |
|---|---|
| 1993 | Professor, nomination by the President of the Republic of Poland |
| 1987 | DSc Habil, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |
| 1980 | PhD in Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |
| 1976 | MSc in Biochemistry, Warsaw University, Poland |

Postdoctoral Training

| | |
|---|---|
| 1981-1984 | Visiting Fellow, Laboratory of Cell Biology (Head: E.D. Korn), National Institutes of Health, Bethesda, Maryland, USA |

**Professional Employment:**

| | |
|---|---|
| 2002-Present | Director of the Institute and Head of the Laboratory of Neurodegeneration, IIMCB |
| 2000-2001 | Director, Centre of Excellence for Studies on Mechanisms of Neurodegeneration Phare Sci-Tech II, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |
| 1999-2001 | Acting Director, IIMCB; Organizer and Director, Centenarian Program |
| 1996-2002 | Head, Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |
| 1992-1995 | Visiting Professor, National Institute of Mental Health, Laboratory of Clinical Science, Bethesda, Maryland, USA |
| 1991-1992 | Deputy Director (Scientific Director), Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |
| 1986-1992 | Associate Professor and Head, Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |
| 1984-1985 | Research Associate, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |
| 1981-1984 | Visiting Fellow, National Institutes of Health, Laboratory of Cell Biology, Bethesda, Maryland, USA |
| 1980-1981 | Postdoctoral Fellow, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |
| 1976-1980 | PhD Student, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland |

**Membership in Scientific Societies, Organizations, and Panels:**

| | |
|---|---|
| 2011-Present | Member, International Expert Council of the Research and Education Center, State Key Laboratory of Molecular and Cellular Biology (SKL) in Ukraine |
| Oct-Nov 2011 | Chairman of the Commission for the Assessment of Property and Legal and Organizational Joined PAN Scientific Units (units operating under the name of the Department of Antarctic Biology Polish Academy of Sciences and Institute of Biochemistry and Biophysics) |
| 2011-Present | Member, BIO-IMAGINE Steering Committee, 7th Framework Program at the Nencki Institute of Experimental Biology |
| 2011-Present | Member, Science Policy Committee, Ministry of Science and Higher Education |
| Jul 1, 2010 – | Dec 31, 2010 President, Consortium Biocentrum Ochota (rotating presidency) |
| 2010-Present | Member, Society for Neuroscience |
| 2008-2010 | Head, Scientific and Organizing Committees, 11th Meeting of the European Calcium Society |
| 2009-Present | Member, Polish Alzheimer's Society |
| 2008-Present | Board Member, European Calcium Society |
| 2006-Present | Member, Health Research Advisory Group, 7th Framework Program European Commission |
| 2004-Present | Member, Polish Academy of Sciences |
| 2003-Present | Member, American Society for Biochemistry and Molecular Biology |
| 2002-Present | Head, Advisory Board, Centre for Innovative Bioscience Education |
| 1991-Present | Member, Polish Neuroscience Society |
| 1991-2009 | Member, Polish Society for the Advancement of Science and Arts |
| 1996-1999, 2000-2002 | Vice-President, Polish Biotechnology Committee |
| 1990-2002 | Member, Polish Biotechnology Committee |
| 1989-1992 | Co-Editor, *Advances in Biochemistry* (published in Polish) |
| 1989-1991 | General Secretary, Polish Biochemical Society |
| 1977-Present | Member, Polish Biochemical Society |

**Honors, Prizes, and Awards:**

2011 Konorski Award for the best Polish research work in neurobiology (awarded by the Polish Neuroscience Society and Committee on Neurobiology of PAN)

2008 Officer's Cross of the Order of Polonia Restituta (awarded by the President of the Republic of Poland)

2004-2008 Professorial Subsidy Program Award, Foundation for Polish Science

2003 Prime Minister Award for Scientific Achievement

2001 Award from the Division of Biological Sciences, Polish Academy of Sciences (for work on calcium binding proteins)

1998 Knight's Cross of the Order of Polonia Restituta (awarded by the President of the Republic of Poland)

1987 Polish Anatomical Society Award for article on calcium binding proteins (*Advances in Cell Biology*)

1986 Skarżyński Award, Polish Biochemical Society (for best review article in *Advances in Biochemistry*)

1977 Parnas Award, Polish Biochemical Society (for publishing the best paper in biochemical research)

1977 Mozołowski Award, Polish Biochemical Society (for outstanding young Polish biochemists)

1976 MSc, Magna cum laude, University of Warsaw, Poland

# Selected publications


- Buizza L, Cenini G, Lanni C, Ferrari-Toninelli G, Prandelli C, Govoni S, Buoso E, Racchi M, Barcikowska M, Styczynska M, **Szybinska A**, Butterfield DA, Memo M, Uberti D. Conformational Altered p53 as an Early Marker of Oxidative Stress in Alzheimer's Disease. PLoS One, 2012; 7(1):e29789
- Hokkanen S, Feldmann HM, Ding H, Jung CK, **Bojarski L**, Renner-Müller I, Schüller U, Kretzschmar H, Wolf E, Herms J. Lack of Pur-alpha alters postnatal brain development and causes megalencephaly. Hum Mol Genet, 2012; 21(3):473-484
- **Bialopiotrowicz E**, **Kuzniewska B**, **Kachamakova-Trojanowska N**, Barcikowska M, **Kuznicki J**, **Wojda U**. Cell cycle regulation distinguishes lymphocytes from sporadic and familial AD patients. Neurobiol Aging, 2011; 32(12):2319.e13-26
- **Gruszczynska-Biegala J**, Pomorski P, **Wisniewska MB, Kuznicki J**. Differential Roles for STIM1 and STIM2 in Store-Operated Calcium Entry in Rat Neurons. PLOS One, 2011; 6(4):e19285.n
- **Misztal K**, **Wisniewska MB**, **Ambrozkiewicz M**, **Nagalski A**, **Kuznicki J**. Wnt-independent constitutive nuclear localization of b-catenin and its low degradation rate in thalamic neurons. J Biol Chem, 2011, 286(36):31781-8
- **Sobczak A, Debowska K, Blazejczyk M**, Kreutz MR, **Kuznicki J, Wojda U**. Calmyrin1 binds to SCG10 protein (stathmin2) to modulate neurite outgrowth. BBA – Mol Cell Res, 2011; 1813(5):1025- 37
- Steinbeck JA, Henke N, Opatz J, **Gruszczynska-Biegala J**, Schneider L, Theiss S, Hamacher N, Steinfarz B, Golz S, Brüstle O, **Kuznicki J**, Methner A. Store-operated calcium entry modulates neuronal network activity in a model of chronic epilepsy. Exp Neurol, 2011; 232(2):185-194
- **Wisniewska M, Misztal K, Michowski W**, Szczot M, Purta E, Lesniak W, **Klejman M**, Dąbrowski M, Filipkowski R, **Nagalski A**, Mozrzymas J, **Kuznicki J**. LEF1/beta-catenin complex regulates transcription of the Cav3.1 calcium channel gene (Cacna1g) in thalamic neurons of the adult brain. J Neurosci, 2010; 30:4957-69
- **Michowski W**, Ferretti R, **Wisniewska MB, Ambrozkiewicz M**, Beresewicz M, Fusella F, **Skibinska- Kijek A**, Zablocka B, Brancaccio M, Tarone G, **Kuznicki J**. Morgana/CHP-1 is a novel chaperone able to protect cells from stress. BBA - Mol Cell Res, 2010; 1803:1043-9
- **Bojarski L, Debowska K, Wojda U**. In vitro findings of alterations in intracellular calcium homeostasis in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry, 2010; 34:1367-74
- Uberti D, Cenini G, Bonini SA, Barcikowska M, Styczynska M, **Szybinska A**, Memo M. Increased CD44 Gene Expression in Lymphocytes Derived from Alzheimer Disease Patients. Neurodegener Dis, 2010; 7:143-7
- **Nagalski A**, Kiersztan A. Fizjologia i molekularny mechanizm działania glikokortykoidów (Physiology and molecular mechanism of glucocorticoid action). Postępy Hig Med Dośw (Online), 2010; 64:133-145. Review (in Polish)
- Spooren A, Rondou P, **Debowska K**, Lintermans B, Vermeulen L, Samyn B, Skieterska K, Debyser G, Devreese B, Vanhoenacker P, **Wojda U**, Haegeman G, Van Craenenbroeck K. Resistance of the dopamine D4 receptor to agonist-induced internalization and degradation. Cell Signal, 2010; 22:600-609
- **Blazejczyk M, Sobczak A, Debowska K, Wisniewska MB**, Kirilenko A, Pikula S, Jaworski J, **Kuznicki J, Wojda U**. Biochemical characterization and expression analysis of a novel EF hand Ca2+ binding protein calmyrin2 (Cib2) in brain indicates its function in NMDA receptor mediated Ca22+ signaling. Arch Biochem Biophys, 2009; 487:66-78
- **Bojarski L**, Pomorski P, **Szybinska A**, **Drab M**, **Skibinska-Kijek A, Gruszczynska-Biegala J, Kuznicki J**. Presenilindependent expression of STIM proteins and dysregulation of capacitative Ca2+ entry in familial Alzheimer's disease. Biochim Biophys Acta, 2009; 1793:1050-7
- **Klejman M, Gruszczynska-Biegala J, Skibinska-Kijek A, Wisniewska MB, Misztal K, Blazejczyk M, Bojarski L, Kuznicki J.** Expression of STIM1 in brain and puncta-like colocalization of STIM1 and ORAI1 upon depletion of Ca2+ store in neurons. Neurochem Int, 2009; 54:49-55
- **Skibinska-Kijek A, Wisniewska MB, Gruszczynska-Biegala J**, Methner A, **Kuznicki J**. Immunolocalization of STIM1 in the mouse brain. Acta Neurobiol Exp (Wars). 2009;69(4):413-28. Erratum in: Acta Neurobiol Exp (Wars), 2010; 70:115
- **Zekanowski C, Wojda U**. Aneuploidy, chromosomal missegregation, and cell cycle reentry in Alzheimer's disease. Acta Neurobiol Exp (Wars), 2009; 69:232-53. Review.

- Puzianowska-Kuznicka M, **Kuznicki J**. The ER and ageing II: calcium homeostasis. Ageing Res Rev, 2009; 8:160-72. Review
- *Peng H, Lewandrowski U, Müller B, Sickmann A, Walz G, **Wegierski T**. Identification of a Protein Kinase C-dependent phosphorylation site involved in sensitization of TRPV4 channel. Biochem Biophys Res Commun, 2010; 391:1721-5
- *Gao H, Wang Y, **Wegierski T**, Skouloudaki K, Pütz M, Fu X, Engel C, Boehlke C, Peng H, Kuehn EW, Kim E, Kramer- Zucker A, Walz G. PRKCSH/80K-H, the protein mutated in polycystic liver disease, protects polycystin-2/TRPP2 against HERP-mediated degradation. Hum Mol Genet, 2010; 19:16-24
- *Ganner A, Lienkamp S, Schäfer T, Romaker D, **Wegierski T**, Park TJ, Spreitzer S, Simons M, Gloy J, Kim E, Wallingford JB, Walz G. Regulation of ciliary polarity by the APC/C. Proc Natl Acad Sci USA, 2009; 106:17799-804
- • ***Wegierski T**, Steffl D, Kopp C, Tauber R, Buchholz B, Nitschke R, Kuehn EW, Walz G, Köttgen M. TRPP2 channels regulate apoptosis through the Ca2+ concentration in the endoplasmic reticulum. EMBO J, 2009; 28:490-499
- ***Wegierski T**, Lewandrowski U, Müller B, Sickmann A, Walz G. Tyrosine phosphorylation modulates the activity of TRPV4 in response to defined stimuli. J Biol Chem, 2009; 284: 2923-33

* no IIMCB affiliation


# Current Projects

We are interested in the molecular mechanisms involved in neurodegeneration and memory formation, with a special emphasis on the role of calcium homeostasis and signaling. These processes are being studied at the genomic, proteomic, and cellular levels. Our major projects focus on the following:

1. Calcium homeostasis and calcium signaling

1.1. Role of STIM proteins in store-operated calcium entry in neurons

1.2. Function of calmyrins in neuronal physiology and pathology

1.3 Dysregulation of calcium homeostasis in Alzheimer's disease

2. Search for biomarkers and potential therapeutic targets in lymphocytes from Alzheimer's disease patients

2.1. Calcium measurements

2.2. Cell cycle analyses

3. Role and regulation of β-catenin/Lef1 complex in mature neurons

## 1. Calcium homeostasis and calcium signaling

### 1.1. Role of STIM proteins in store-operated calcium entry in neurons (*Joanna Gruszczyńska-Biegała*)

The calcium sensors STIM1 and STIM2, located in the endothelial reticulum (ER), and calcium channel-forming protein ORAI1 are involved in store-operated calcium entry (SOCE). The process relies on extracellular calcium influx through plasma membrane channels. In non-excitable cells, the STIM interaction with ORAI1 is a crucial element of SOCE, but in neurons its mechanism remains unclear. We showed earlier that STIM1 is likely involved in thapsigargin-induced SOCE, whereas STIM2 is mostly active after the EGTA-driven depletion of extracellular calcium (Gruszczynska-Biegala et al., *PLoS One*, 2011). The depletion of calcium from the ER increased the number of puncta-like colocalization of YFP-STIM1 and ORAI1 but not YFP-STIM2 and ORAI1. In contrast, a reduction of extracellular calcium levels triggered puncta formation for both YFP-STIM1/ORAI1 and YFP-STIM2/ORAI1. In the next step, we focused on detecting complexes that contain endogenous STIM2 and ORAI1. We showed that STIM2 can interact with ORAI1 in cultured rat cortical neurons, revealed by the proximity ligation assay (PLA). Using PLA, we were able to visualize fluorescent dots that represent the site where two antibodies are bound: one against ORAI1 and another against STIM2. These dots identify the complexes between STIM2 and ORAI1. We showed that the number of these complexes increased by chelating calcium ions in the medium by EGTA treatment. The interaction was quantified and found to correlate well with the number of exogenous complexes formed under the same conditions. To confirm that the observed PLA dots represent authentic STIM2/ORAI1 complexes, we used different pairs of anti-STIM2 and anti-ORAI1 antibodies. Our results indicate that the interaction between endogenous STIM2 and ORAI1 occurs in neurons *in vivo* and can be detected by removing extracellular calcium.

### 1.2. Interaction between ORAI proteins and neuronal partners (*Tomasz Węgierski, Danuta Korona*)

We performed a search for neuronal partners of STIM and ORAI proteins. Additionally, the individual cytosolic domains of STIM proteins are being analyzed in a classical yeast two-hybrid assay. The isolated hits are confirmed by independent methodology. The results obtained so far indicate the existence of an interesting physical link between SOCE machinery and proteins crucially involved in the development of neurodegeneration.

### 1.3. Function of calmyrins in neuronal physiology and pathology (*Katarzyna Dębowska; supervisor: Urszula Wojda*)

Neuronal $Ca^{2+}$ signaling regulates multiple cellular functions. Therefore, disturbances in $Ca^{2+}$ signaling pathways can result in neuronal pathologies. We study the neuronal function of a novel family of $Ca^{2+}$-signaling proteins called calmyrins (CaMy; known also as KIP or CIB proteins). We characterized the biochemical properties, localization, and protein ligands of CaMy1 and CaMy2 in the brain and showed that CaMy1 is involved in Alzheimer's disease *(BBA-Mol Cell Res* 2011; *Arch Biochem Biophys*, 2009, *Calcium Binding Proteins*, 2008; *BBA-Mol Mech Diseases*, 2006; *Neuropathol Appl Neurobiol*, 2005; *Acta Biochim Pol*, 2005). Recently, using a yeast two-hybrid system, we identified the SCG10 protein stathmin2 as a novel CaMy1 ligand in the developing human brain. SCG10 is a microtubule-destabilizing factor that plays a role in neuronal growth during brain development. Our data indicate that CaMy1, via SCG10, couples $Ca^{2+}$ signals with the dynamics of microtubules during neuronal outgrowth in the developing brain (*BBA-Mol Cell Res*, 2011). We also continued the search for the neuronal localization and function of CaMy2. We found that CaMy2 is preferentially expressed in neurons in the hippocampus and cortex.

Endogenous CaMy2 is present in neurites and the Golgi apparatus and is found in the membranous fraction. Our search for CaMy2 protein ligands in neurons using affinity chromatography, mass spectrometry, and coimmunoprecipitation approaches revealed that CaMy2 interacts *in vitro* and *in vivo* with key proteins involved in vesicular trafficking, consistent with the subcellular localization in neurons. The involvement of CaMy2 in intracellular trafficking has been analyzed by colocalization studies that involve immunocytochemistry and PLA. We also employ functional assays in primary neuronal cultures and nerve-growth factor-stimulated PC12 cells.

### 1.3. Calcium homeostasis in Alzheimer's disease (*Aleksandra Szybińska, Anna Jaworska, and Tomasz Węgierski; collaboration: Honarnejad Kamran and Jochen Herms, Munich Center for Neurosciences*)

Calcium dyshomeostasis is an early event in Alzheimer's disease pathogenesis that precedes other disease symptoms and can affect many cellular processes. Drugs with the ability to restore calcium homeostasis to values observed in healthy cells could be good therapeutics for Alzheimer's disease treatment. In collaboration with Prof. Jochen Herms, we screened approximately 20,000 chemical compounds to check their ability to influence intracellular calcium concentrations. The screen revealed over 300 compounds that decreased calcium levels. To address their putative mechanism of action, almost 160 of the best compounds were chosen for an ELISA assay for γ-secretase activity whose gain of function is believed to be a major factor in familial Alzheimer's disease pathology. Using ELISA, we measured β-amyloid 1-42 levels in HEK 293 cells that overexpress the wildtype or mutated presenilin 1 gene. Only a few compounds decreased β-amyloid 1-42 to control levels; thus, the majority of the compounds that influenced calcium signaling did not affect γ-secretase activity.

## 2. Search for biomarkers and potential therapeutic targets in lymphocytes from Alzheimer's disease patients

Some molecular changes in Alzheimer's can be observed not only in neurons but also in peripheral cells, such as lymphocytes. Because of difficulties in studying dynamic processes in postmortem material, such peripheral cells have been used as a model to study the molecular mechanisms of Alzheimer's disease. Additionally, human lymphocytes have potential diagnostic value. In our studies, we use B-lymphocytes from Alzheimer's disease patients.

### 2.1. Calcium measurements (*Anna Jaworska*)

Many studies showed that disturbed cellular calcium homeostasis is one of the features of Alzheimer's disease. Calcium dyshomeostasis is an early event in Alzheimer's disease pathogenesis that precedes other disease symptoms and can affect many cellular processes. Calcium changes can be observed not only in neurons but also in peripheral cells, such as skin fibroblasts and lymphocytes. Lymphocytes, in contrast to other cell types, can be easily obtained and therefore have great diagnostic potential. Disturbed calcium handling was shown by many research groups in immortalized human B-lymphocytes derived from patients with an inherited form of Alzheimer's disease, namely familial Alzheimer's disease, but observations of similar

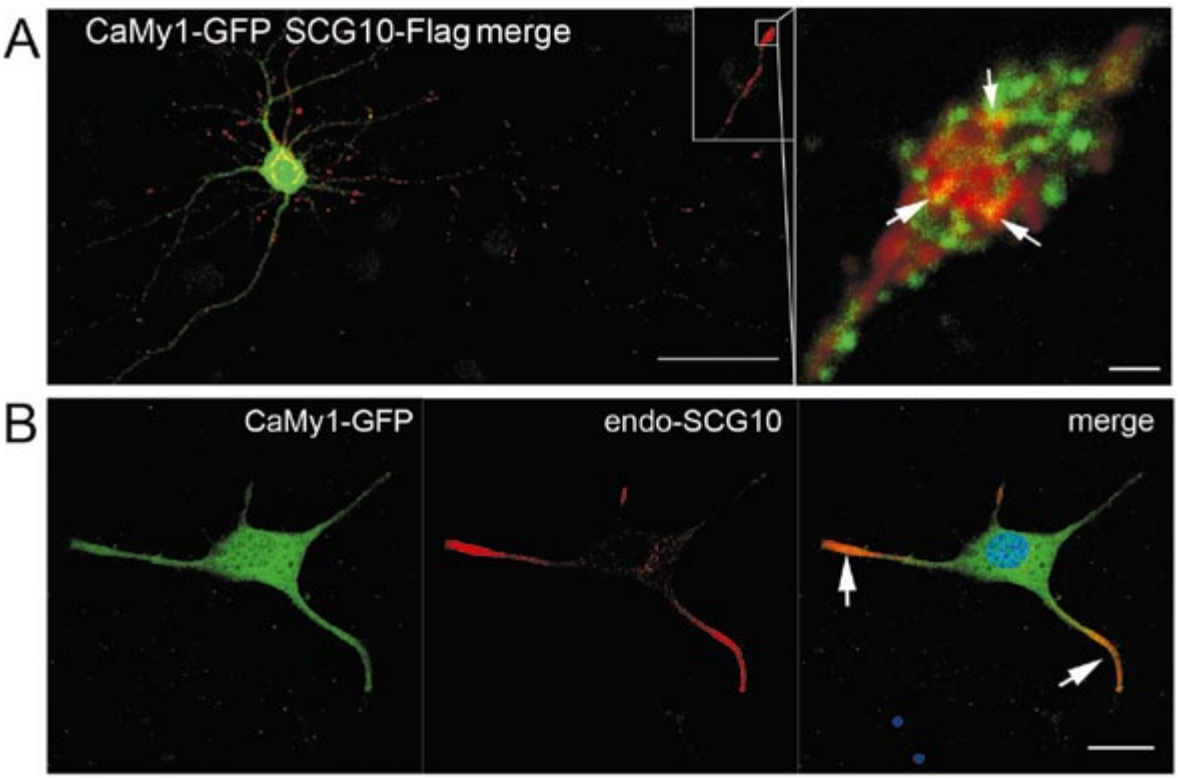



**Fig. 1. CaMy1 co-localizes with SCG10 in developing hippocampal neurons and differentiating PC12 cells.** (A) Colocalization of CaMy1-GFP and SCG10-Flag overexpressed in primary cultured hippocampal neurons (6-9 days *in vitro*). (*Left*) Maximal projection of entire neuron with marked growth cone position. Scale bar = 50 µm. (*Right*) Magnification of the neuronal growth cone. Arrows indicate the colocalization sites of both signals. *Z*-stack, scale bar = 1 µm. (B) PC12 cells transfected with CaMy1-GFP and stimulated by NGF for differentiation (75 ng/ml) were stained for endogenous SCG10. Arrows indicate the colocalization sites of CaMy1-GFP with endogenous SCG10 in proximity to neurite tips. Scale bar = 20 µm.

changes observed in cells derived from patients with the sporadic form of Alzheimer's disease (SAD) are limited. The process of immortalization could potentially change some features of cell metabolism. Therefore, these results should be confirmed with fresh, unmodified cells. Our goal is to elucidate calcium changes in unmodified B-lymphocytes from SAD patients and compare these changes with data obtained from patients with other types of cognitive deficits and healthy, age-matched controls. This type of analysis will allow us to determine whether the potential differences could be used as a diagnostic marker of Alzheimer's disease.

**2.2. Cell cycle analyses (*Emilia Białopiotrowicz; supervisor: Urszula Wojda*)**

According to the so-called cell cycle hypothesis, an important factor that contributes to the pathogenesis of Alzheimer's disease is the failure to regulate the G1/S cell cycle phases and cell cycle reentry in differentiated, postmitotic neurons. Cell cycle reentry in neurons precedes the formation of amyloid β (Aβ) plaques and neuronal death in Alzheimer's disease. Recently, we and others also detected cell cycle alterations in lymphocytes from SAD patients induced to proliferate with EB-virus (Bialopiotrowicz et al., *Neurobiol Aging*, 2011). The results of our experiments that used real-time polymerase chain reaction arrays, immunoblotting, and flow cytometry demonstrated differences in the regulation of G1/S phases between SAD B-lymphocytes and cells from non-demented subjects. Furthermore, we analyzed whether similar cell cycle changes also occur in the familial form of Alzheimer's disease linked to mutations in presenilin 1 (PS1). PS1 sustains the active site of γ-secretase, a membranous protein complex that cleaves transmembrane amyloid protein precursor (APP) to generate Aβ40 and Aβ42 peptides that in turn exert toxic effects in neurons. Mutations in PS1 that cause the familial form of Alzheimer's disease increase the γ-secretase-mediated release of Aβ from APP. To shed light on the complex role of PS1 mutations in familial Alzheimer's disease pathology, we investigated the influence of nine different PS1 mutations on cell cycle regulation and Aβ production in immortalized lymphocytes from familial Alzheimer's disease patients and in stably transfected human embryonic kidney cells. We found that both cell cycle regulation and γ-secretase activity differentiate PS1 mutations. These studies are relevant to the development of new diagnostic approaches and personalized therapeutic strategies in Alzheimer's disease and the construction of a transgenic mouse model suitable for studies of the cell cycle hypothesis of Alzheimer's disease.

**3. Role and regulation of nuclear β-catenin in mature neurons (*Katarzyna Misztal, Andrzej Nagalski, Łukasz Szewczyk, and Nikola Brożko; supervisor: Marta B. Wiśniewska*)**

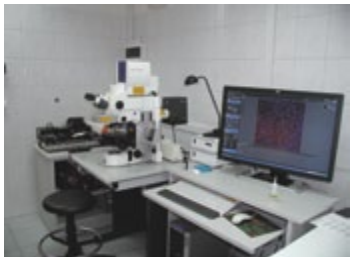
β-catenin is a gene expression regulator in the canonical Wnt pathway that is involved in early brain patterning and neurogenesis. Growing evidence implicates Wnt/β-catenin signaling also in the proper functioning of the adult central nervous system. Aberrant regulation of β-catenin has been associated with psychotic and affective disorders (e.g., major depression, bipolar disorder, and schizophrenia) and neurodegenerative diseases (e.g., Alzheimer's disease, Huntington's disease, and Parkinson's disease). However, the physiological role of Wnt/β-catenin in the adult brain is far from understood. Pioneering research by our group demonstrated that β-catenin is constitutively and specifically present in the nuclei of thalamic neurons, independent of Wnt signaling activation and associated with low levels of the proteins involved in β-catenin degradation (i.e., APC, AXIN1, and GSK3β; Misztal et al., *J Biol Chem*, 2011). Moreover, we demonstrated that β-catenin, together with LEF/TCF transcription factors, regulate the transcription of the *Cacna1g* gene that encodes Cav3.1 voltage-gated calcium channels, contributing to electrical signal propagation in thalamic neurons (Wisniewska et al., *J Neurosci*, 2010). Our current research focuses on the identification of new β-catenin target genes in thalamic neurons to provide further insights into the role of β-catenin in the adult brain. We combine bioinformatics and experimental methods to propose and validate new β-catenin-LEF1/TCF targets. Our techniques include *in silico* screenings and gene ontology analyses, gene profiling in the brain, gene delivery in primary neuronal cultures, real-time polymerase chain reaction, luciferase assays, footprinting, and chromatin immunoprecipitation. The second goal of our present research is to develop new mouse models suitable to study the involvement of β-catenin in thalamic pathologies using pharmacological and genetic approaches.

# Core Facility Laboratory

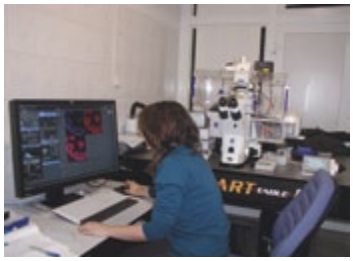
**Piotr Brągoszewski** Radiation Safety Specialist
**Krzysztof Skowronek** Research Equipment Specialist
**Alicja Żylicz** Head
**Roman Szczepanowski** Research Equipment Specialist
**Tomasz Węgierski** Research Equipment Specialist

The Core Facility Laboratory was created in October 2011with the goal of supporting innovative research at IIMCB, giving investigators access to a broad spectrum of cutting edge research technology platforms extensively used in such diverse areas as structural biology, molecular biology, bioinformatics and cell biology. More than 200 items of the equipment are grouped according to applications for biophysical and biochemical methods of protein and nucleic acids structure and function determination (e.g. bioreactor, chromatography stations – 13 of FPLC and 4 of HPLC, centrifuges, ultracentrifuges, analytical ultracentrifuge, crystallization hotels and robots, X-ray generator, spectrophotometers, spectrofluorometers with stopped flow, BIACORE 3000, circular dichroism spectrometer, FTIR, MassSpec, DNA synthetizer, light scattering detector, plate readers, RT PCR and others), cell biology techniques (FACS, confocal and fluorescence microscopes, high throughput live cell imaging, multi-photon microscopes) and isotope methods (imaging systems, scintillation counter). All equipment is staffed and maintained by experienced scientists.

The Laboratory provides sufficient assistance, from initial training through all the procedures needed for an experiment to the final interpretation and data analysis. The use of the equipment is free of charge to all faculty members and students.

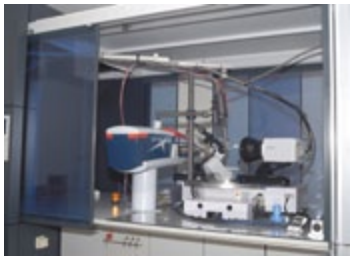


Confocal microscope Zeiss LSM5 Exciter



Confocal microscope Zeiss LSM710

Microscope Olympus ScanR Station



X-ray generator (microfocus)

Crystallographic Robot Phoenix

Crystallographic Hotel Rigaku Minstrel

Mass Spectrometry Laboratory

FT-IR Spectrometer

FPLC ACTA Avant

# Educational Activities

IIMCB continues its doctoral program in partnership with other research and educational institutes of the Ochota Campus. Currently 54 PhD students are on board within the doctoral programs of the Institute of Biochemistry and Biophysics, of the Nencki Institute, of the University of Poznań, and of the Foundation for Polish Science (FNP).

The PhD students of IIMCB are self-organized as a group with the representative Marcin Magnus. They have regular working seminars every two months. Similarly, Institute's postdocs have their open seminars; the representative of this group is Iwona Cymerman. The "Postdoc's seminars" are devoted to the presentation of personal experience of lecturer, being complementary to regular IIMCB seminars. Both groups representatives participate in meetings with Directors, Lab Leader's meetings etc.

**International PhD Programme**

This program started in 2010 based on funds of the Foundation for Polish Science. PhD projects are being realized in the Institute of Biochemistry and Biophysics PAN and in the International Institute of Molecular and Cell Biology, in collaboration with a number of foreign partner institutions. PhD projects were made available in the major areas of molecular biology, like DNA metabolism, RNA biogenesis and its control, mechanisms of cellular signalling and trafficking and in applied molecular biology field; seven of them were affiliated with IIMCB:

1. *Identification and characterization of novel nucleases*
   Supervisor: Janusz Bujnicki
   Foreign partner: Ichizo Kobayashi (Japan)
2. *mTor regulated cellular trafficking in neuronal development*
   Supervisor: Jacek Jaworski
   Foreign partner: Casper Hoogenraad (The Netherlands)
3. *High throughput detection of calcium homeostasis for AD diagnosis and drug discovery based on interaction between STIM protein and plasma membrane calcium channels*
   Supervisor: Jacek Kuźnicki
   Foreign partner: Jochen Herms (Germany)
4. *Endocytic trafficking and intracellular signaling of PDGF ligands and receptors*
   Supervisor: Marta Miączyńska
   Foreign partner: Carl-Henrik Heldin (Sweden)
5. *Structural studies of DNA substrate binding by the GYI-IYG domain*
   Supervisor: Marcin Nowotny
   Foreign partner: Titia K. Sixma (The Netherlands)
6. *Studies of genetic basis of ciliopathies*
   Supervisor: Michał Witt
   Foreign partner: Heymut Omran (Germany)
7. *Molecular mechanism of oncogenic activity of p53 gain of function cancer mutants*
   Supervisor: Alicja Żylicz
   Foreign partner: Ted Hupp (UK)

**Support for bio tech med scientists in technology transfer**

In 2010 IIMCB started two grants to support technology transfer in Biocentrum Ochota consortium. The grants are sponsored by Operational Program: Human Capital and the programme of the Minister of Science and Higher Education and Kreator Innowacyjności. Several activities were made possible within these two mechanisms:

- 14 research stipends for innovative projects for PhD students working in Biocentrum Ochota institutes
- 12 two-month practices for Biocentrum Ochota scientists at industrial sites
- training courses on issues such as: R&D project management, raising a company, commercialization of R&D results, IPR, negotiations in business.

Details are described in: Details of selected projects and cooperation with other institutions, Bio Tech Med Project (page 16).

**Life Sciences and Mathematics Interdisciplinary Doctoral Studies (LiSMIDoS) at the University of Gdańsk**

IIMCB has recently joined LiSMIDoS program with a major goal to participate in activities of independent Doctoral School, with real influence on its educational curriculum: in this framework IIMCB faculty will run their own courses, summer schools, etc. Some of them will be performed in a teleconference format. The major objective is to provide a programme of interdisciplinary training to PhD students that will allow them to work in today's competitive scientific environment that very often requires cross-disciplinary expertise. First IIMCB students will start their education in 2012 (see page 17).

# Centre for Innovative Bioscience Education (BioCEN)


The aim of the Center for Innovative Bioscience Education (BioCEN), is to reduce the gap between science and society in Poland by conducting educational activities popularizing biology: open lectures, workshops for students and courses for biology teachers. All activities are focused on improving biology education and the awareness of biology in society. The co-founders of the Center for Innovative Bioscience Education are: the International Institute of Molecular and Cell Biology (IIMCB), Nencki Institute of Experimental Biology PAN (IBD), the Institute of Biochemistry and Biophysics PAN (IBB), Faculty of Biology University of Warsaw, the BioEducation Foundation. IIMCB houses the BioCEN laboratory, office and administration. BioCEN also coordinates a second laboratory at Warsaw University of Life Sciences. In 2011, over 1450 young participants attended laboratory workshops. At the same time, over 150 biology teachers participated in laboratory workshops and courses and over 200 children performed hands-on practice experiments. During 10 years of BioCEN activity 10361 students attended all kind of workshops.

**Laboratory workshops**

The participants of workshops use laboratory equipment and techniques for real life experiments. The practical experiments are supported by lectures presenting the theoretical basis and techniques of molecular biology and genetics. Each workshop lasts four hours over the course of one day. We offered the following topics:

- Explore your own DNA – examining DNA by PCR methods
- Let's play with bacteria – a plasmid isolation and restriction map
- Green bacteria – bacteria transformation with the GFP gene
- Protein fingerprints of different tissues
- Miracles of biotechnology – purification of jellyfish protein from bacteria
- Investigate signs of evolution in your DNA – methods of molecular evolution
- Yeast – the living micro-factory
- Do you know what you eat?
- Biotechnology of antibodies in clinical practice.

**Courses for biology teachers**

Since we strongly encourage teachers to implement practical protocols at schools, we equip them with classroom scenarios and affordable experimental kits that can be used in school laboratories. The proposed teaching materials exemplify a state-of-the-art approach towards innovative biology education. They allow for the development of practical skills and introduce a teaching approach based on project development by a team of students. Last but not least, our educational procedures improve the ability of analytical thinking.

During our workshops we popularize the method known as Inquiry Based Science Education. "Inquiry" is defined as „a search for truth, information, or knowledge" – seeking information by questioning. Although Inquiry Based Science Education can be applied to all disciplines, it is especially important in science education.

In 2011, as part of teacher training, the following events were organized:

- Course for teachers "Volvox – let's teach how to experiment!"
- 10th BioCEN and Nencki Institute Symposium for teachers.

**15th Science Picnic (May 28, 2011)**

As in previous years, the BioEducation Foundation and BioCEN organized an exhibition and science show during the 15th Science Picnic in Warsaw. The motto for 2011 was "Freedom". Our demonstrations were related to DNA and the variety of methods used in molecular biology research:

- Necklaces with your own DNA – isolation of DNA from the cheek
- Let's make the experiment! – how to make the scientific experiment.

**15th Science Festival (September 17-25, 2011)**

The Warsaw Science Festival is aimed at enhancing public awareness of science and technology. Over 500 activities take place in different formats (seminars, debates, guided tours, workshops, performances, contests, films), representing various fields of science. They are aimed at different target groups (young children, primary school, high school, general public) and are run for two weeks in various universities, scientific research institutions and museums. In 2011 BioCEN organized open laboratory workshops for the public:

- "Explore your own DNA"
- "Do you know what you eat?" a workshop for students
- "Yeast – the living micro-factory" a workshop for students

**5th Children's Science Festival (September 24, 2011)**

For the first time BioCEN participated in the Children's Science Festival. During several hours of workshops, children produced artificial seeds, learned how yeast respire and investigated the properties of the juice of red cabbage. Several hundreds of children took part in the workshop.

**Science Festival in Sierpc (October 2011)**

As a part of the project (Nr 3/POKL/9.5/2011) BioCEN helped in organization of the first Festival of Science in Sierpc. During the eight-hour demonstrations we organized the 10 stands at which all visitors could independently perform simple experiments. We also presented laboratory equipment routinely used in modern molecular biology laboratories. Participants of the Festival had the opportunity to isolate their own DNA, to examine the activity of various enzymes, to watch fluorescent bacteria and to learn the evolution playing a board game "Retracing the history of evolution". Over a thousand of people participated in the event.

**The brochure „Experimenting is fun! How to change a home kitchen into laboratory"**

The brochure "Experimenting is fun! Little Scientist explores the world" subsidized by the city of Warsaw, was edited. The booklet contains 10 protocols of simple experiments for children aged 8-12 years, which can be performed at home. Each protocol additionally contains a brief theoretical introduction and summary and explanation of the results, comprehensible for children. The booklet was produced in 2500 copies and was distributed among school children in Warsaw. Exemplary titles of experiments are following:


- What cracks sugars into small particles?
- Why does the dough rise?
- Rootlets up? Rootlets down? Or how it really works.

**Family laboratory workshops**

In 2011 we developed laboratory workshops for younger children accompanied by their guardians. Many years of working with children has enabled us to develop a unique program of educational workshops tailored to their age. During our workshop little scientists perform each experiment themselves, under the supervision of an experienced tutor. Guardians accompany the children and take part in carrying out experiments. Workshop topics include:

- On DNA trail - become a detective and solve the crime mystery!
- We all eat genes, that is talking about DNA in a funny way!

**The Center for Innovative Bioscience Education – partner of the Center for Citizenship Education in the Project "The Students Academy" co-founded by the European Coherence Fund (EFS)**

"The Students Academy" is an initiative gathering 300 junior high schools and 35 thousand pupils from five regions of Poland. During workshops pupils plan and perform experiments and team projects and make observations, in accordance with scientific procedures. Teachers from schools attending "The Students Academy" participate in web-based, internet-coaching which entails professional training focusing on (*i*) the preparation of scientific observations and experiments for pupils, (*ii*) guidance for pupils' projects and (*iii*) approaches to motivate learning.

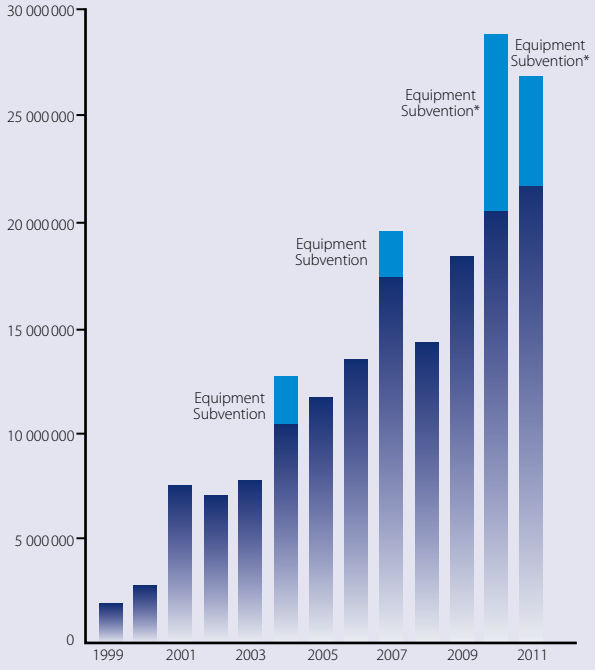
The project started in 2010 and will last for 4 years. BioCEN, monitors and verifies the accuracy of biology teachers' ideas and experimental scenarios as well as overseeing the accuracy of the biological experiments conducted by pupils.

**Staff and co-workers**

Persons who coordinate and administrate BioCEN are: Agnieszka Chołuj, Karolina Ciosek, Aleksandra Kot-Horodyńska, Marcin Wiśniewski (as a coordinator at Warsaw University of Life Sciences) and formely Joanna Lilpop and Marta Badurek. Animators and co-workers: Kamil Koper, Michał Mlącki, Marek Kulka, Bartosz Zapisek, Katarzyna Laskowska, Marta Strumiłło, Piotr Horodyński, Ewa Podobas, Zuzanna Sobańska, Maciej Lirski, Aleksandra Piechnik, Paulina Mrozek, Marek Krzyżanowski, Michał Spanier, Aleksandra Kwiatkowska, Jakub Kruszewski, Monika Ostaszewska, Katarzyna Chomiela, Anna Fogtman, Andrzej Foik, Aleksandra Skrajna, Piotr Gerlach.

# Diversity of Funding IIMCB'2011

## Annual income (in PLN)



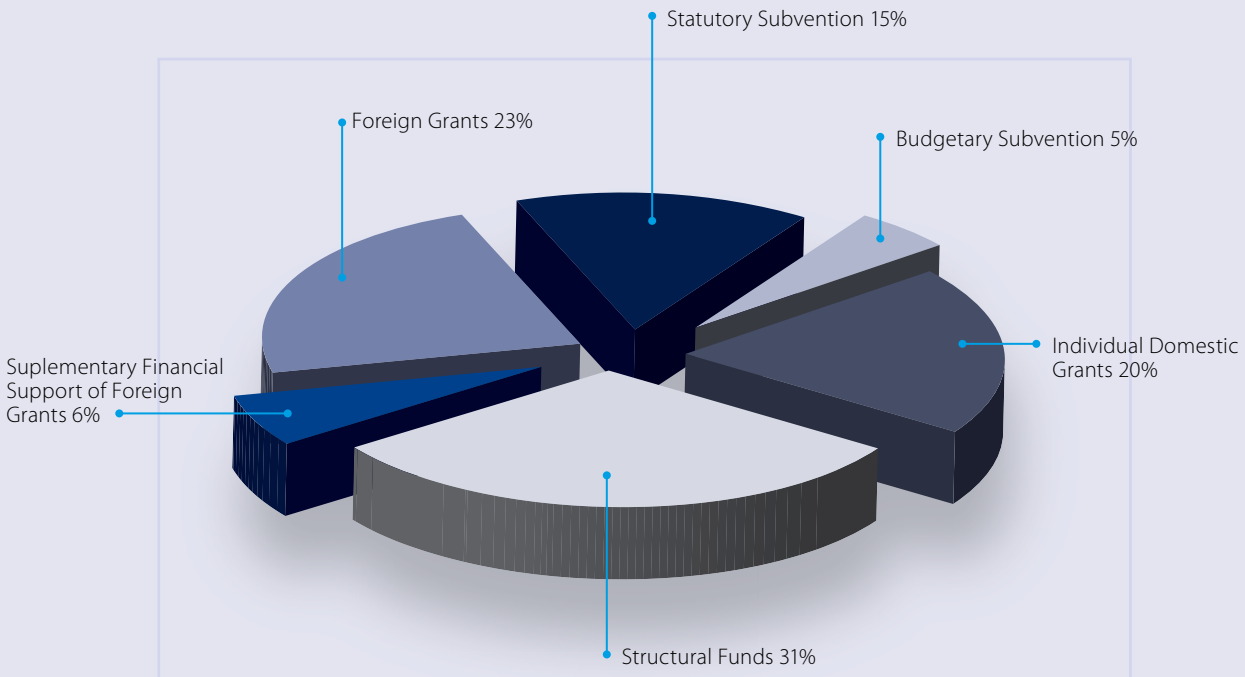

* 5,000,000 PLN from Structural Project CePT (Ministerial)

## Profit & loss statement

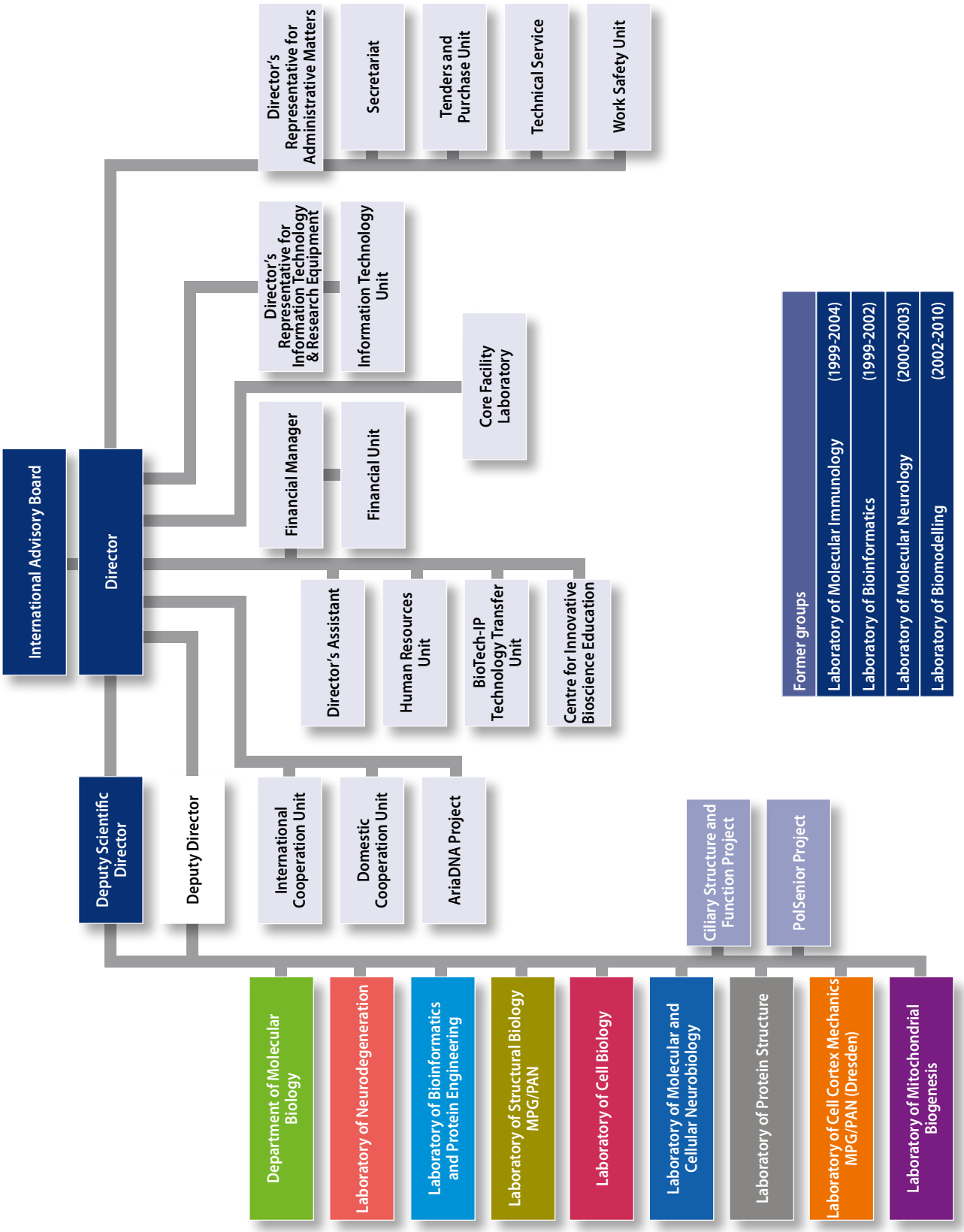
| | amounts in PLN |
|---|---|
| **A. net revenue on sales and equivalents*** | 32 517 529 |
| **B. operational activity costs:** | 33 352 725 |
| Depreciation (equipment) | 1 162 906 |
| Research materials | 16 551 250 |
| Utilities | 453 373 |
| Services | 2 277 620 |
| Fees and taxes | 1 499 393 |
| Salaries and wages | 7 489 201 |
| Social and health insurance | 1 707 142 |
| Other operational expenses, in this: | 2 211 840 |
| business trips | 1 159 726 |
| property insurance | 18 461 |
| fellowships | 1 021 630 |
| others | 12 024 |
| **C. other operational income** (subventions) | 853 785 |
| **D. other operational expenses:** | 15 590 |
| **E. financial income (interests):** | 287 648 |
| **F. financial expenses (others):** | 1 412 |
| **Profit/Loss on business activity** (A-B+C-D+E-F) | **289 234** |

| Sources of Funding | amounts in PLN | amounts in EUR[1] |
|---|---|---|
| Statutory Subvention | 3 905 743 | 884 292 |
| Budgetary Subvention | 1 274 000 | 288 444 |
| Individual Domestic Grants | 5 455 956 | 1 235 273 |
| Structural Funds | 8 380 563 | 1 897 429 |
| Supplementary Financial Support of Foreign Grants | 1 728 218 | 391 283 |
| Foreign Grants | 6 212 364 | 1 406 531 |
| **Total** | **26 956 844** | **6 103 252** |

[1] 1EUR – 4,4168 @ 31st Dec'2011



# Structure of the International Institute of Molecular and Cell Biology




Since October 2011

# Staff at IIMCB (as of 31 March 2012)

| Administration | | Funding |
|---|---|---|
| Jacek Kuźnicki | Director | IIMCB |
| Michał Witt | Deputy Scientific Director | IIMCB (1/2) |
| Hanna Iwaniukowicz | Financial Manager | IIMCB |
| Monika Nowicka | Payroll Specialist | IIMCB |
| Renata Knyziak | Accounting Specialist | IIMCB |
| Agnieszka Kuna | Accounting Specialist | IIMCB/Structural Funds |
| Mariola Arkuszewska | Accounting Specialist | IIMCB/Structural Funds |
| Beata Tkacz | Human Resources Specialist | IIMCB |
| Monika Domańska-Paśko | Human Resources Specialist | IIMCB (1/2) |
| Urszula Białek-Wyrzykowska | International Cooperation Manager | IIMCB (1/2) |
| Dorota Wasiak-Libiszowska | Foreign Grants Manager | IIMCB/EC grant/Structural Funds |
| Magdalena Powierża | International Cooperation Specialist | IIMCB/EC grant |
| Marcin Ogonowski | International Cooperation Specialist | IIMCB/Structural Funds |
| Aleksandra Nałęcz-Tolak | International Cooperation Specialist | IIMCB/EC grant (1/2) |
| Agnieszka Wagner-Ziemka | Domestic Cooperation Manager | IIMCB/EC grant |
| Katarzyna Dąbrowska | Domestic Grants Administrator | Ministerial grant |
| Agnieszka Karbowska | Director's Representative for Administrative Matters | IIMCB |
| Roman Szczepanowski | Director's Representative for Information Technology and Research Equipment | IIMCB (1/2) |
| Dominika Dubicka | Director's Assistant | IIMCB |
| Anna Brzezińska | Tenders Specialist | IIMCB |
| Dorota Makulska | Secretary | IIMCB |
| Robert Banasiak | Maintenance Specialist | IIMCB |

| Core Facility Laboratory | | |
|---|---|---|
| Alicja Żylicz | Head | IIMCB |
| Roman Szczepanowski | Research Equipment Specialist | EC grant (1/2) |
| Krzysztof Skowronek | Research Equipment Specialist | IIMCB |
| Tomasz Węgierski | Research Equipment Specialist | EC grant |
| Piotr Brągoszewski | Radiation Safety Specialist | IIMCB |

| Department of Molecular Biology | | |
|---|---|---|
| Maciej Żylicz | Head | IIMCB |
| Bartosz Wawrzynów | Senior Researcher | EC grant |
| Paweł Wiśniewski | Postdoctoral Fellow | EC grant |
| Marta Małuszek | Junior Researcher | IBB PhD School/Ministerial grant |
| Magdalena Pruszko | Junior Researcher | IBB PhD School/Structural Funds (MPD Project) |
| Zuzanna Tracz-Gaszewska | Junior Researcher | IBB PhD School/FNP Ventures Programme |
| Milena Wiech | Junior Researcher | Nencki PhD School/NCN Preludium Programme |
| Grażyna Orleańska | Secretary | IIMCB (1/2) |

| Laboratory of Cell Biology | | |
|---|---|---|
| Marta Miączyńska | Head | Wellcome Trust |
| Magdalena Banach-Orłowska | Post-doctoral Fellow | Structural Funds/FNP POMOST |
| Iwona Pilecka | Post-doctoral Fellow | Wellcome Trust/Polish-Norwegian Research Fund |
| Beata Pyrzyńska | Postdoctoral Fellow | HHMI/Ministerial grant |
| Ewelina Szymańska | Postdoctoral Fellow | EC grant |
| Anna Hupałowska | Junior Researcher | EC grant/Nencki PhD School |
| Kamil Jastrzębski | Junior Researcher | Structural Funds (MPD Project)/ IBB PhD School |
| Agnieszka Mamińska | Junior Researcher | IIMCB/Nencki PhD School |
| Sam D. Stephen | Junior Researcher | EU/Nencki PhD School |
| Anna Toruń | Junior Researcher | Ministerial grant/ Nencki PhD School |
| Izabela Sępowicz | Grant Administrator and Lab Manager | Polish-Norwegian Research Fund |

| Laboratory of Bioinformatics and Protein Engineering | | |
|---|---|---|
| Janusz M. Bujnicki | Head | IIMCB/EC grant |
| Michał Boniecki | Post-doctoral Fellow | DFG (International funds) |
| Grzegorz Chojnowski | Post-doctoral Fellow | IIMCB/ TEAM FNP |
| Elżbieta Purta | Post-doctoral Fellow | IIMCB/ TEAM FNP |
| Krzysztof Skowronek | Post-doctoral Fellow | EC grant |
| Tomasz Waleń | Post-doctoral Fellow | EC grant |
| Stanisław Dunin-Horkawicz | Post-doctoral Fellow | EC grant |
| Grzegorz Łach | Post-doctoral Fellow | IIMCB/ TEAM FNP |
| Izabela Rutkowska-Włodarczyk | Post-doctoral Fellow | EC grant |
| Bogusław Kluge | Post-doctoral Fellow | Structural Funds |
| Ilona Domagała | Junior Researcher | Structural Funds |
| Małgorzata Durawa | Junior Researcher | Structural Funds |
| Agata Kamaszewska | Junior Researcher | Structural Funds |
| Katarzyna H. Kamińska | Junior Researcher | Ministerial Funds |
| Łukasz Kozłowski | Junior Researcher | IIMCB |
| Marcin Magnus | Junior Researcher | Structural Funds |
| Dorota Matelska | Junior Researcher | IIMCB |
| Magdalena Mika | Junior Researcher | Structural Funds |
| Anna Olchowik | Junior Researcher | Structural Funds (MPD Project) |
| Marcin Pawłowski | Junior Researcher | EC grant |
| Dariusz Pianka | Junior Researcher | Structural Funds |
| Anna Philips | Junior Researcher | Structural Funds |
| Michał Piętal | Junior Researcher | Scholarship of Marshal of Podkarpackie Voivodship |
| Katarzyna Poleszak | Junior Researcher | Structural Funds |
| Wojciech Potrzebowski | Junior Researcher | Ministerial funds |
| Jakub Jopek | Junior Researcher | Structural Funds |
| Juliusz Stasiewicz | Junior Researcher | Structural Funds |
| Irina Truszyńska | Junior Researcher | Scholarship of Marshal of Mazovia Voivodship |
| Maria Werner | Junior Researcher | Ministerial funds |
| Albert Bogdanowicz | MSc Student | Structural Funds |
| Mateusz Dobrychłop | MSc Student | Structural Funds |
| Magdalena Byszewska | MSc Student | Volunteer |
| Agnieszka Faliszewska | Office Manager | IIMCB/ Structural Funds |
| Jan Kogut | Computer Administrator/Programmer | Structural Funds |
| Tomasz Jarzynka | Computer Administrator/Programmer | Structural Funds |
| Łukasz Munio | Computer Administrator | Structural Funds |

| Laboratory of Structural Biology | | |
|---|---|---|
| Matthias Bochtler | Head | IIMCB/EC grant/Structural Funds |
| Honorata Czapińska | PostDoctoral Fellow | IIMCB/EC grant/Structural Funds |
| Monika Sokołowska | PostDoctoral Fellow | NCN/Ministerial funds |
| Patrycja Haniewicz | Junior Researcher | Nencki Fellowship/NCN/ Ministerial funds |
| Asgar Abbas Kachrani | Junior Researcher | Structural Funds |
| Karolina Kolak | Junior Researcher | Structural Funds |
| Dominik Rafalski | Junior Researcher | Structural Funds |
| Karthik Shanmuganandam | Junior Researcher | Structural Funds |
| Wojciech Siwek | Junior Researcher | NCN/MNiSW |
| Marek Wojciechowski | Junior Researcher | IIMCB/Nencki Fellowship/NCN/ Ministerial funds |
| Michał Pastor | MSc Student | Structural Funds |

| Laboratory of Neurodegeneration | | |
|---|---|---|
| Jacek Kuźnicki | Head | IIMCB |
| Urszula Wojda | Associate Professor, Vice Head | IIMCB |
| Tomasz Węgierski | Senior Researcher | EC grant |
| Marta Wiśniewska | Senior Researcher | EC grant |
| Joanna Gruszczyńska-Biegała | Postdoctoral Fellow | Ministerial grant |
| Katarzyna Misztal | Postdoctoral Fellow | Ministerial grant |
| Emilia Białopiotrowicz | Junior Researcher | Ministerial grant |
| Katarzyna Dębowska | Junior Researcher | Ministerial grant |
| Anna Jaworska | Junior Researcher | IBB PhD School/Structural Funds |
| Andrzej Nagalski | Junior Researcher | IIMCB |
| Aleksandra Szybińska | Junior Researcher | IIMCB |
| Nikola Brożko | MSc Student | Volunteer |

| Laboratory of Molecular and Cellular Neurobiology | | |
|---|---|---|
| Jacek Jaworski | Head | IIMCB |
| Magda Błażejczyk | Postdoctoral Fellow | Era-Net Neuron grant |
| Iwona Cymerman | Postdoctoral Fellow | EC grant/NCN grant |
| Agata Góźdź | Postdoctoral Fellow | EC grant/Era-Net Neuron grant |
| Matylda Macias | Postdoctoral Fellow | EC grant |
| Ewa Liszewska | Postdoctoral Fellow | Era-Net Neuron grant |
| Joanna Lipka | Junior Researcher | IBB PhD School/Structural Funds |
| Anna Malik | Junior Researcher | IIMCB/Nencki PhD School |
| Agnieszka Skałecka | Junior Researcher | Era-Net Neuron grant/ Nencki PhD School |
| Anna Urbańska | Junior Researcher | IIMCB/Nencki PhD School |
| Malgorzata Urbańska | Junior Researcher | Ministerial grant/ Nencki PhD School |
| Marcelina Pieprzyk | Technician | Era-Net Neuron grant |

| Laboratory of Protein Structure | | |
|---|---|---|
| Marcin Nowotny | Head | Wellcome Trust |
| Elżbieta Nowak | Postdoctoral Fellow | EC grant |
| Karolina Górecka | Postdoctoral Fellow | Wellcome Trust |
| Agata Jacewicz | Postdoctoral Fellow | HHMI |
| Małgorzata Figiel | Junior Researcher | IIMCB |
| Marcin Jaciuk | Junior Researcher | IIMCB |
| Mirosław Śmietański | Junior Researcher | Ministerial grant |
| Michał Miętus | Junior Researcher | IBB PhD School/Structural Funds |
| Magdalena Łazęcka | Lab Manager | Wellcome Trust |
| Marzena Nowacka | Technician | EC grant |
| Iwona Ptasiewicz | Technician | IIMCB |
| Justyna Studnicka | Technician | External company |

| Laboratory of Cell Cortex Mechanics MPG/ PAN | | |
|---|---|---|
| Ewa Paluch | Head | IIMCB |
| Jakub Sędzinski | Senior Researcher | Ministerial grant |
| Maté Biro | Junior Researcher | HFSP grant |
| Alba Diz Muñoz | Junior Researcher | Ministerial grant |
| Andrew G. Clark | Junior Researcher | Ministerial grant |
| Martin Bergert | Junior Researcher | DFG grant |
| Steve Simmert | MSc Student | Volunteer |
| Julia Roensch | Technician | Ministerial grant |
| Annett Boden | MSc Student | Volunteer |

| Laboratory of Mitochondrial Biogenesis | | |
|---|---|---|
| Agnieszka Chacińska | Head | IIMCB |
| Piotr Brągoszewski | Postdoctoral Fellow | Structural Funds/IIMCB |
| Małgorzata Sztolsztener | Postdoctoral Fellow | IIMCB/Structural Funds |
| Ulrike Topf | Postdoctoral Fellow | Swiss National Science Foundation fellowship |
| Michal Wasilewski | Postdoctoral Fellow | EMBO IG/Structural Funds |
| Tomasz Czerwik | PhD Student | Structural Funds |
| Agnieszka Górnicka | PhD Student | Structural Funds |
| Paulina Kwiatkowska | PhD Student | EMBO IG |
| Aksana Varabyova | PhD Student | Ministerial grant |
| Lidia Wróbel | PhD Student | Structural Funds |
| Anita Brewińska | Research Assistant | Structural Funds |
| Elżbieta Grzelak | Technician | IIMCB |
| Magdalena Stankiewicz | Undergraduate Student | Structural Funds |
| Kamila Ornoch | Undergraduate Student | Structural Funds |
| Agata Trojanowska | Undergraduate Student | Structural Funds |

| Ministerial Projects | | |
|---|---|---|
| Małgorzata Mossakowska | Coordinator of PolSenior Project | IIMCB |
| Aleksandra Szybalska | Project Assistant | Ministerial grant (PolSenior) |
| Przemysław Ślusarczyk | IT Specjalist | Ministerial grant (PolSenior) |
| Marta Świech | Technician | Ministerial grant (PolSenior) |
| Małgorzata Szwed | Technician | Ministerial grant (PolSenior) |
| Izabela Sabała | Senior Researcher | Ministerial grant |

| Research Equipment Laboratory | | |
|---|---|---|
| Wanda Gocal | Technician | IIMCB |
| Elżbieta Grzelak | Technician | IIMCB |
| Monika Matuszczyk | Technician | IIMCB |
| Iwona Ptasiewicz | Technician | IIMCB |

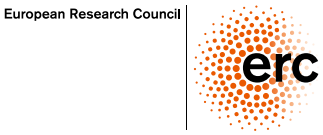
| Technology Transfer Unit (Biotech-IP) | | |
|---|---|---|
| Magdalena Powierża | Head | Structural Funds |
| Adam Sobczak | Project Manager | Structural Funds |
| Leszek Lipiński | Industrial Cooperation Manager | Structural Funds |

| Centre for Innovative Bioscience Education | | |
|---|---|---|
| Agnieszka Chołuj | Head | CEO (projekt) |
| Karolina Ciosek | Coordinator | IBB/Warsaw Univ./IIMCB |
| Aleksandra Kot-Horodyńska | Coordinator | Nencki Institute |
| Marcin Wiśniewski | Coordinator | SGGW |
| Kamil Koper | | Volunteer |
| Michał Mlącki | | Volunteer |
| Marek Kulka | | Volunteer |
| Bartosz Zapisek | | Volunteer |
| Katarzyna Laskowska | | Volunteer |
| Marta Strumiłło | | Volunteer |
| Piotr Horodyński | | Volunteer |
| Ewa Podobas | | Volunteer |
| Zuzanna Sobańska | | Volunteer |
| Maciej Lirski | | Volunteer |
| Aleksandra Piechnik | | Volunteer |
| Paulina Mrozek | | Volunteer |
| Marek Krzyżanowski | | Volunteer |
| Michał Spanier | | Volunteer |
| Aleksandra Kwiatkowska | | Volunteer |
| Jakub Kruszewski | | Volunteer |
| Monika Ostaszewska | | Volunteer |
| Katarzyna Chomiela | | Volunteer |
| Anna Fogtman | | Volunteer |
| Andrzej Foik | | Volunteer |
| Aleksandra Skrajna | | Volunteer |
| Piotr Gerlach | | Volunteer |

# Important Dates in the Institute's History

| | |
|---|---|
| **Sept. 1991** | The proposal to create the Institute was published in the UNESCO Bulletin of MCBN |
| **June 1994** | State Committee for Scientific Research (KBN) accepts the activities aimed at establishing the Institute |
| **Oct. 1994** | Presidium of Polish Academy of Sciences (PAN) votes to support the Institute |
| **May 1995** | An agreement between Poland and UNESCO to establish the Institute |
| **June 1996** | The Molecular and Cell Biology Department is created by PAN with Prof. M.J. Nałęcz as the Head |
| **June 1997** | Polish Parliament passes a bill to found the Institute |
| **May 1998** | Prof. A. Azzi is nominated as the Director of IIMCB |
| **Jan. 1999** | The Institute commences its independent activities; Prof. J. Kuźnicki appointed as Acting Director |
| **July 1999** | Dr. J. Dastych is appointed as a first Lab Leader at IIMCB |
| **Oct. 1999** | Prof. M. Żylicz is appointed as Chair of the Department of Molecular Biology |
| **April 2000** | An agreement between the Max Planck Society (MPG) and the Polish Academy of Sciences (PAN) to launch a Joint MPG-PAN Junior Research Group |
| **Jan. 2001** | The MPG-PAN Junior Research Group commences its activities with Dr. M. Bochtler as a Lab Leader |
| **June 2001** | Prof. J. Kuźnicki is elected by the International Advisory Board as Director of the Institute, begins to complete the Laboratory of Neurodegeneration. After consultation with UNESCO, the official nomination was signed by the President of PAN on February 1, 2002 |
| **Nov. 2002** | New members of the International Advisory Board nominated for 2002-2006 term |
| **Jan. 2003** | Status of the Centre of Excellence in Molecular Bio-Medicine is granted by the European Commission within 5$^{th}$ Framework Programme |
| **June 2005** | Professor J. Kuźnicki re-elected as Director of the Institute (term 2006-2010) |
| **May 2006** | New members of the International Advisory Board nominated for 2006-2010 term |
| **Feb. 2006** | Twin MPG-PAN laboratory established at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden with Dr. Ewa Paluch as a Lab Leader |
| **May 2009** | Professor J. Kuźnicki re-elected as Director of the Institute (term 2010-2014) |
| **Jan. 2010** | New members of the International Advisory Board nominated for 2010-2014 term |

# Funding Institutions





















# Health Prot Proteins in Health and Disease

## WP1 - Increasing scientific expertise through twinning

1. **Matthias Bochtler**, Laboratory of Structural Biology, IIMCB and **Ruedi Allemann**, University of Cardiff, UK
2. **Janusz M. Bujnicki**, Laboratory of Bioinformatics and Protein Engineering, IIMCB and **Saulius Klimasauskas**, Laboratory of Biological DNA Modification, Institute of Biotechnology, Vilnius, Lithuania
3. **Sławomir Filipek**, Biomodelling Laboratory, IIMCB and **Vicenza Andrisano**, Department of Pharmaceutical Sciences, University of Bologna, Italy
4. **Jacek Jaworski**, Laboratory of Molecular and Cellular Neurobiology, IIMCB and **Casper Hoogenraad**, Erasmus MC, Rotterdam, The Netherlands
5. **Jacek Kuźnicki**, Laboratory of Neurodegeneration, IIMCB and **Jochen Herms**, Ludwig-Maximilians-University of Munich, Centre for Neuropathology, Germany
6. **Marta Miączyńska**, Laboratory of Cell Biology, IIMCB and **Harald Stenmark**, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway
7. **Marcin Nowotny**, Laboratory of Protein Structure, IIMCB and **Roland Marquet**, Retroviruses and RNA viruses laboratory, RNA Architecture and Reactivity Unit, Université Louis Pasteur, CNRS, Strasbourg, France
8. **Michal Witt's** group, Ciliary Proteins Function Project, IIMCB and **Heimut Omran**, Department of Pediatrics and Adolescent Medicine, University of Freiburg, Germany
9. **Maciej Żylicz**, Department of Molecular Biology, IIMCB and **Ted Hupp**, Cancer Research of UK Cell Signalling Unit, Edinburgh Cancer Research Centre, University of Edinburgh, UK

## WP2 - Expanding research capacity

Employment of **9 experienced scientists** for 2 years each and an Equipment Specialist.

## WP3 - Organization of scientific events

Workshops, courses, seminars related to: ciliary disorders, cancer biology, DNA repair, neurobiology and neurodegenerative disorders, finding pathways between proteins, biology of antibiotic resistance, heat shock proteins in molecular medicine, cell biology of endocytosis. Most of the topics are within special interest of EC: rare disorders, cancer, neurodegenerative disorders, HIV.

## WP4 - Participation in international events

Participation of Centre's staff in international conferences and courses

## WP5 - Promotion

Project's website, annual reports on HEALTH-PROT activities, leaflets, posters, organization of public events, open days.

## WP6 - Management

SEVENTH FRAMEWORK PROGRAMME

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY

International Institute of Molecular and Cell Biology, Ks. Trojdena 4, 02-109 Warsaw, Poland
http://www.iimcb.gov.pl/hp, tel.: +48 22 59 70 700, e-mail: secretariat@iimcb.gov.pl

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