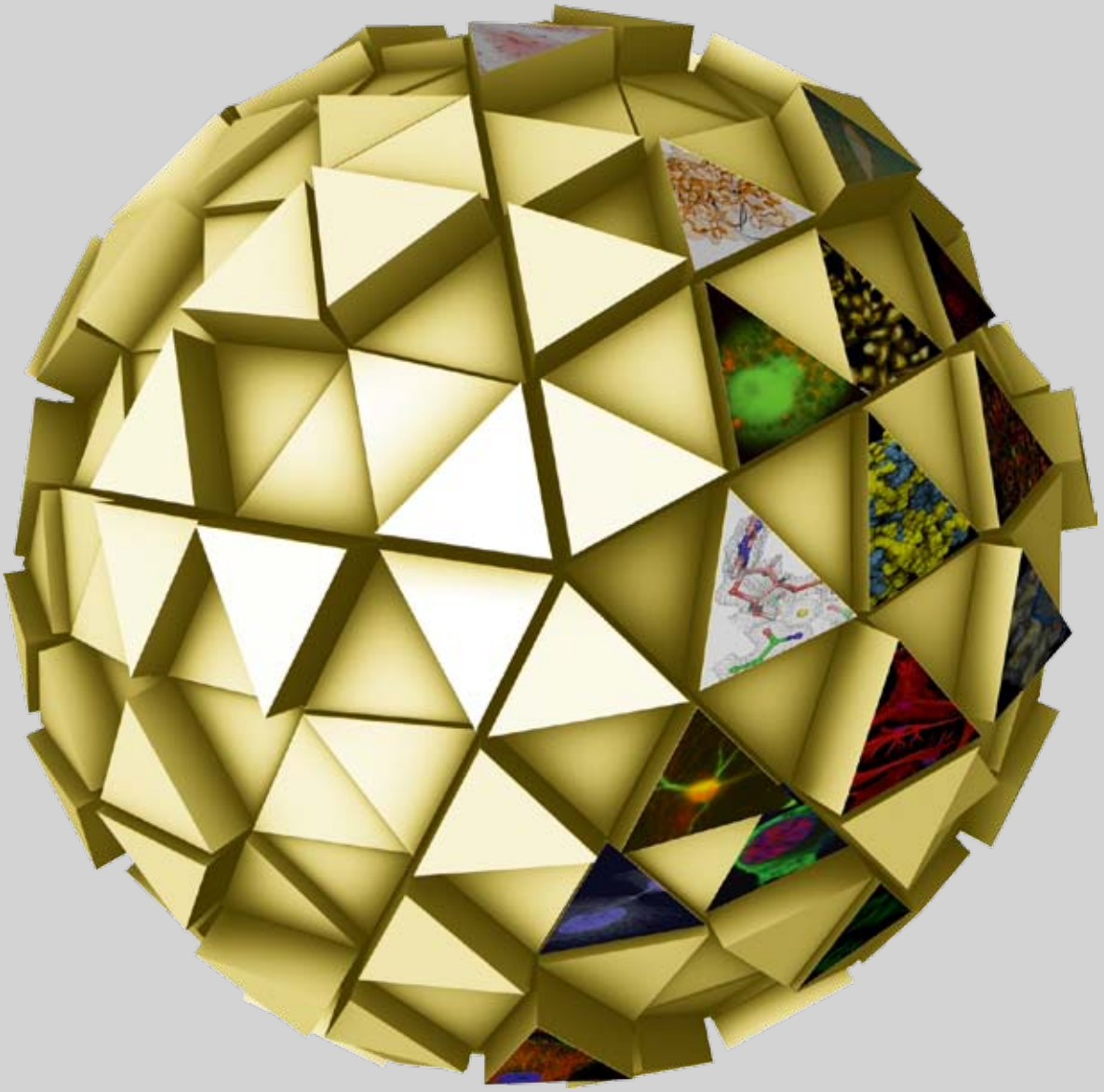
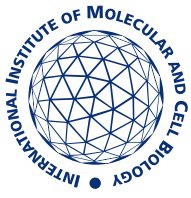
Annual Report 2010



INTERNATIONAL INSTITUTE
OF MOLECULAR AND CELL BIOLOGY



Director
Jacek Kuźnicki

Deputy Scientific Director
Michał Witt

Financial Manager
Hanna Iwaniukowicz

Chairman of the International Advisory Board
Anna Tramontano

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This Report 2010 was edited by:
Agnieszka Wagner-Ziemka and Michał Witt

ISBN 978-83-917905-3-3

Contents

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 5th 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

Directors' Note

After having written this part of our annual report for many years, it is difficult to decide what else we should consider here. Slogans such as „sustainable development", „achieving critical mass," „maturity" have already been used and some of them have even been repeated several times. And not without reason. Will the next decade of the Institute, which has only recently begun, have some distinct characteristics, differentiating it from the previous decade? The answer would appear to be thus: after the growth phase we have entered a phase of professional stability. It is a natural step in the development of the Institute, and it has been achieved thanks to a well pursued process of internal evolution. We have reached a reasonable plateau, which does not mean that the Institute is not still developing. Our recent spectacular successes are evidence of this further development – and while we enjoy them, we see them as an expected normality in this Institute: the recently awarded prestigious ERC grant, one of seven in Poland and the only one in the field of biomedical sciences (Janusz M. Bujnicki), the paper published in *Nature Structure and Molecular Biology* by authors affiliated exclusively with IIMCB (Marcin Nowotny et al), other in *Nature* (co-authored by Alicja Żylicz) and Nature Biotechnology (co- authored by Janusz M. Bujnicki). The fact that the largest part of our annual income comes from non-budgetary sources is nothing new, but this is the first time we have achieved such a high level of funding in this mechanism: in 2010 more than 40% of the Institute's funding came from grants, foreign and domestic, and another 40% from Structural Funds. With more than 50 doctoral students, only one third are financed by the Ministry, while two thirds are financed by grants. It shows not only the wide extent to which financing is generated in IIMCB using external sources, but also the ability to use European money available at the moment: the 7th Framework Programme and Structural Funds. The latter are instruments of the EU structural policy, and their task is to support the restructuring and modernization of the economies of EU countries: the co-realized CePT project at our Institute is the largest biomedical and biotechnology undertaking in Central and Eastern Europe, which in our case finances the purchases of major research equipment.

A yet unresolved, though particularly burning issue is that of the new location of the Institute. The current location limits the opportunities for growth, opening new laboratories and taking new challenges. The growth concept involves the extension of the Institute's premises simultaneously with the extension of IIMCB's area of interest towards more medical problems, with a greater focus on translational research. We plan to continue to develop our cooperation with the Max-Planck Society, led so far with such success by Matthias Bochtler and Ewa Paluch. Matthias returned from Cardiff to IIMCB as a full-time Lab Leader and the expansion of his laboratory has shown the most positive effects of the cooperation program started ten years ago.

The program of the reformation of Polish science is in progress. The package of bills prepared by the government alters many previously existing rules. Some of the new national laws are modeled on the rules that have applied at IIMCB from its very beginning. This only strengthens our conviction that these rules were well adopted, and IIMCB's years of operation have proven that sticking to them leads to positive results.

Directors and Administration

Jacek Kuźnicki
Director

Michał Witt
Deputy Scientific Director

Janusz M. Bujnicki
Deputy Director

Hanna Iwaniukowicz
Financial Manager

Scientific Office

Dominika Dubicka-Boroch
Director's Assistant

Katarzyna Dąbrowska
Domestic Grants Administrator

Agnieszka Ziemka
Director's Representative for Research Management

Administration Unit

Robert Banasiak
Maintenance Specialist

Dorota Makulska
Secretary

Agnieszka Karbowska
Director's Representative for Administrative Matters

Anna Brzezińska
Tenders Specialist

International Cooperation Unit

Dorota Libiszowska
Foreign Grants Specialist

Urszula Białek-Wyrzykowska
International Cooperation Manager

Marcin Ogonowski
International Cooperation Specialist

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

Financial & Human Resources Units

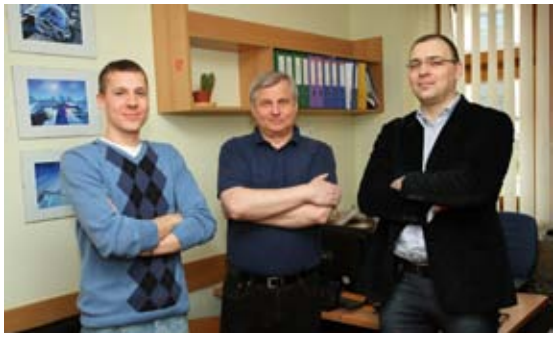
Monika Nowicka
Payroll Specialist

Beata Tkacz
Human Resources Specialist

Mariola Arkuszewska
Accounting Specialist

Hanna Iwaniukowicz
Financial Manager

Renata Knyziak
Accounting Specialist
(not on the picture)



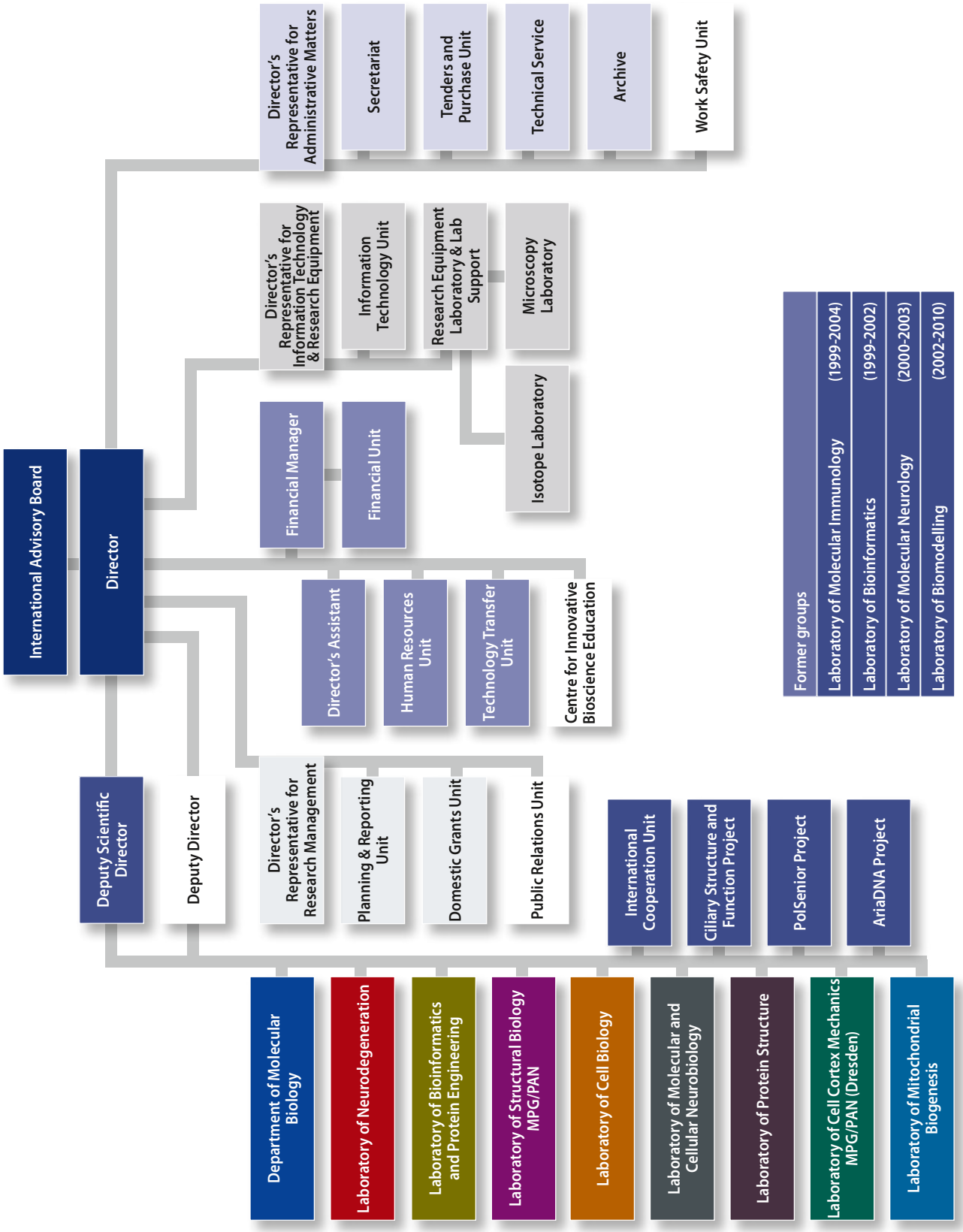
IT Unit

Jakub Skaruz
IT Specialist

Roman Szczepanowski
Director's Representative for Information Technology & Research Equipment

Michał Romiszewski
IT Specialist

Structure of the International Institute of Molecular and Cell Biology



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 2010
From left (first row): H. Saibil, A.A. Bogdanov, A. Tramontano, A. Wlodawer, M.J. Nałęcz;
(second row) I. Dikič, N. Blin, J. Kuźnicki, F. van Leuven, R. Przewłocki, R.P. Erickson, F.E. Baralle, D. Picard, K. Hahlbrock, W. Filipowicz, J. Mallet, A. Azzi, M. Witt.

Chairman: Anna Tramontano

Deputy Chairman: Ineke Braakman

Members:

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

Nicolaus 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, who supervises the organization and activities of the Institute. The President of PAN nominates members of IAB and the Institute's Directors.

The Organization of Research at IIMCB

Ten research groups comprised the structure of IIMCB in 2010: Department of Molecular Biology (Żylicz), Laboratory of Bioinformatics and Protein Engineering (Bujnicki), Laboratory of Structural Biology MPG/PAN (Bochtler), Laboratory of Neurodegeneration (Kuźnicki), Laboratory of Biomodelling (Filipek), 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 p53 and molecular chaperones and oncogenic activity of MDM2 and mutated forms of p53 (Żylicz group).
2. Development and application of computer software for structural bioinformatics of proteins and nucleic acids and theoretical and experimental studies on enzymes that act on nucleic acids (protein structure prediction, evolutionary analyses, functional characterization, protein engineering) (Bujnicki group).
3. Crystallographic structure determination of biological macromolecules (Bochtler group).
4. Studies of calcium and beta-catenin signalling in the brain and of molecular mechanisms of neurodegeneration (Kuźnicki group).
5. Molecular modeling of the structure and function (molecular switches) of proteins and their oligomerization and complexes, with a focus on rhodopsin and other G-protein-coupled receptors; molecular role of presenilin mutations in neurodegenerative diseases (Filipek group; activity discontinued since June 2010).
6. Interdependence between intracellular endocytic transport and nuclear signal transduction (Miączyńska group).
7. Molecular processes, including gene transcription, kinase-dependent cell signaling, cytoskeleton dynamics, intracellular trafficking underlying neuronal development and plasticity, and central nervous system pathologies (tuberous sclerosis, epilepsy, and neurodegenerative disorders) (Jaworski group).
8. Mechanics of the actomyosin cortex, study of cortical contractility and the role of cortical mechanics during cytokinesis and migration (Paluch group).
9. Structural and biochemical studies of nucleic acid enzymes (Nowotny group).
10. Biogenesis of mitochondrial proteins (Chacińska group).

Awards and Honors

- Maciej Żylicz was nominated the Scientific Advisor to the President of the Republic of Poland
- Jacek Kuźnicki was nominated a member of the Scientific Policy Committee, the advisory body of the Minister of Research and Higher Education
- 2010 Award from the Polish Biochemical Society for the best biochemistry work performed in Polish laboratories to Dawid Walerych and Alicja Żylicz
- The Prime Minister Award for a Doctoral thesis "Structural and biochemical studies of restriction endonucleases BcnI i Hpy99I" to Monika Sokołowska
- The Prime Minister Award for a Doctoral thesis (defended in 2009), and a prize from the Polish Bioinformatics Society to Jan Kosiński
- Awards from Biocentrum Ochota PAN for the best PhD theses defended in 2009/2010 to Monika Sokołowska and Roman Szczepanowski
- An award of distinction for a PhD thesis by Henryk Korza
- An award of distinction from the Polish Bioinformatics Society for a MSc thesis by Paweł Łukasz (defended in 2009)
- A tenure-track position for Jean-Philippe Borges in France (a former postdoctoral fellow of Bochtler Lab)
- A tenure-track position for Dario Piano in Italy (a former postdoctoral fellow of Bochtler Lab)

Bio-Technology Innovations Platform

In April 2010, at the IIMCB, a Technology Transfer Unit – the Bio-Technology Innovations Platform (BioTech-IP) was established. The foundation of the BioTech-IP was possible thanks to two received grants: one from the Operational Programme Human Capital 8.2.1 and the other from the Ministry of Science and Higher Education.

The BioTech-IP provides services to all six bio-tech-med institutes forming the Biocentrum Ochota. Its main goal is to support entrepreneurial spirit in researchers by offering stipends for applied research projects, internships in innovative bio-tech companies, practical training and legal advice in IPR and tech transfer, and assistance in applying for applied research projects.

In 2010 BioTech-IP managed to:

- award 14 PhD stipends to students carrying out applied research projects in Ochota institutes
- organize seminars and workshops on IPR and tech transfer
- organize free-of-charge individual consultations with patent attorneys for scientists developing patentable research
- conduct a number of individual meetings with researchers aimed to assess the market potential of technologies developed in Ochota.

Publishing NEWSKO

Since 2000 the e-bulletin NEWSKO has been providing the Ochota Campus community with current information on seminars, symposia, conferences, job opportunities and other essential events. This information is currently available exclusively at www.iimcb.gov.pl/seminars.php.

Computer Network

The IIMCB network has recently been upgraded to Category 7 and contains more than 150 interconnected personal computers and computers designated to control scientific equipment. We are also currently in the process of upgrading the Institute's file servers and computer clusters operated by the Laboratory of Bioinformatics and Protein Engineering: the computing power of our cluster will increase from 1,2 TFLOP (110CPUs/ 120 cores, 200GB RAM and 30TB of HD) to 14 TFLOP (224 CPUs / 1344 cores), 3,36 TB of RAM memory and an additional 30 TB of HD.

Last year our IT Unit was outsourced to New Business Technologies – a company with extensive experience in computer systems integration. All performance reports and measurements show greater efficiency and lower operating costs than in the past.

Furthermore, a significant number of improvements were made concerning the network itself:

- the possibility of creating back-up copies in the case of the Institute's "critical computer services"
- a special test environment to safely update our accounting software
- a security program (equipped with an e-mail notification system) in order to closely monitor our servers.

Thanks to an initiative of the Polish Ministry of Science and Higher Education we were allowed direct access (on every computer) to the most important biomedical journals through the Polish Virtual Library of Science – Nature, Science, Elsevier Journals, Springer Journals and the Web of Knowledge database.

Scientific Degrees in 2010

Academic Habilitation:

- **Jacek Jaworski**, habilitation thesis: „Selected mechanisms of regulation of dendritic arbor and dendritic spine morphology of hippocampal neurons." Warsaw University, Faculty of Biology, Warsaw, 27.09.2010

PhD:

- **Michał Koliński**, PhD thesis: „Modeling of structures of GPCRs and mechanisms of their activation in case of opioid receptors". Thesis advisor: S. Filipek; 02.02.2010, Institute of Biochemistry and Biophysics PAN, Warsaw, Poland
- **Agnieszka Obarska-Kosińska**, PhD thesis: „Structural modeling of multi-domain Type I, III, and IIC restriction-modification systems in combination with experimental data". Thesis advisor: J.M. Bujnicki; 02.02.2010, Institute of Biochemistry and Biophysics PAN, Warsaw, Poland
- **Sebastian Pawlak**, PhD thesis: „Functional analysis and engineering of novel substrate specificity of Bsp6I restriction endonuclease". Thesis advisor: J.M. Bujnicki; 20.04.2010, Institute of Biochemistry and Biophysics PAN, Warsaw, Poland
- **Grzegorz Chojnowski**, PhD thesis: „Random and non-random features of protein crystal diffraction patterns". Thesis advisor: M. Bochtler; 17.05.2010, Institute of Experimantal Physics, Faculty of Physics, Warsaw University, Poland
- **Jakub Urbański**, PhD thesis: „HSP90 molecular chaperone. The effect on breast cancer cell invasion and functional interactions with Aha1 co-chaperone". Thesis advisors: M. Żylicz, A. Żylicz, I. Braakman; 02.09.2010, Utrecht University, Netherlands
- **Zuzanna Szymańska**, PhD thesis: „Mathematical modelling of the heat shock response and the involvement of heat shock proteins in cancer development". Thesis advisors: M. Żylicz, J. Willi; 29.09.2010, Institute of Biochemistry and Biophysics PAN, Warsaw, Poland
- **Elżbieta Purta**, PhD thesis: „New enzymes involved in RNA modification - identification and characterization". Thesis advisors: J.M. Bujnicki; 19.10.2010, Institute of Biochemistry and Biophysics PAN, Warsaw, Poland
- **Karolina Tkaczuk**, PhD thesis: „Sequence, structure, function analysis of SAM dependent methyltransferases". Thesis advisors: J.M. Bujnicki; 19.10.2010, Technical Uniwersity of Łódź, Poland
- **Marta Olchowik**, PhD thesis: „Endocytic cargo transport via APPL-positive endosomes". Thesis advisor: M. Miączyńska; 15.11.2010, Nencki Institute of Experimental Biology PAN, Warsaw, Poland
- **Henryk Korza**, PhD thesis: „Structural and biochemical studies of LD-carboxypeptidase". Thesis advisor: M. Bochtler; 23.11.2010, Warsaw, Institute of Biochemistry and Biophysics PAN, Poland

Scientific Meetings and Lectures

The European Calcium Society (ECS) Symposia are biannual events, with average participation of about 300 people, renown as an international forum for presentation of the latest achievements and breakthroughs in the field of calcium biology. The first meeting devoted to this subject was organized in 1973 in Jabłonna near Warsaw at the initiative of Professor Witold Drabikowski from the Nencki Institiute of Experimental Biology. During the 11th ECS Meeting 2010 held in Warsaw, the Calcium Toolkit Workshop was organized, supported by the EC FP7 grant *Proteins in Health and Disease*, HEALTH-PROT, GA No 229676. Invited lectures were delivered by Mitsuhiko Ikura (University of Toronto, Canada), Olga Moroz (University of York in Heslington, UK) and David B. Sacks (Harvard Medical School, USA); oral presentations were given by Guenter Fritz (University of Konstanz, Germany) and by Hannah V. McCue (University of Liverpool, UK). Discussion was moderated by Anna Filipek from the Nencki Institute of Experimental Biology in Warsaw and Walter Chazin from the Vanderbilt University in Nashville, USA. The poster session accumulated 112 poster communications.

Seminars of invited speakers

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

Andrzej Koliński (Laboratory of Theory of Biopolymers Department of Chemistry, University of Warsaw, Poland, Laureate of FNP Prize 2009) "Multiscale modeling of protein structure, dynamics and interactions", 21.01.2010

Tomasz Guzik (Laboratory of Translational Medicine, Department of Internal and Agricultural Medicine J Dietl Hospital, Jagiellonian University, Collegium Medicum, Kraków, Poland, Recipient of the EMBO Installation and the FNP WELCOME Grants "Immune mechanisms of hypertensions – it is time for new therapies", 04.02.2010

Joanna Trylska (Interdisciplinary Centre for Mathematical and Computational Modeling, University of Warsaw, Laureate of FNP TEAM grant 2009) "Ribosomal RNA as an antibiotic target", 08.04.2010

Ewa Zuba-Surma (Department of Medical Biotechnology Jagiellonian University, Kraków), Poland „Missed pearls"- the evidence for the presence of stem cells smaller than erythrocytes in animal and human tissues", 10.06.2010

Krystian Jażdżewski (Genomic Medicine, Medical University of Warsaw and Comprehensive Cancer Center, Ohio State University, Columbus, USA) „The role of microRNA in thyroid cancer", 09.12.2010

- **Research Symposium (14th Lab Leader Competition), 14.05.2010**

Małgorzata Borowiak (Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, USA) "How to cure disease? Lessons from diabetes and embryonic stem cell biology"

Aleksandra Glavaski-Joksimovic (Neurobiology Program, Children's Memorial Research Center, Chicago, USA) "Genetically modified mesenchymal stem cells for Parkinson's disease"

Milan Joksimovic (Department of Neurology, Northwestern University, Chicago, USA) "Wnt antagonism of Shh facilitates midbrain floor plate neurogenesis"

Hui Ma (Molecular and Cell Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, USA) "PP4c-R3/SMEK protein phosphatase complex, a universal regulator of cellular stress responses"

Dietbert Neumann (Institute of Cell Biology, ETH Zurich, Zurich, Switzerland) "Molecular approaches to the signaling of AMP-activated protein kinase"

- **Regular IIMCB seminars**

Joanna I. Sułkowska (Center for Theoretical Biological Physics, University of California San Diego, La Jolla, USA and Institute of Physics, PAN, Warsaw, Poland) "Dodging the crisis of folding proteins with knots", 07.01.2010

Raul E. Alcantra (Computational Biochemistry and Biophysics Lab, Universitst Pompeu Fabra Barcelona, Spain) "Going from sequence to structure to dynamics. How can one enrich a sequence alignment?", 12.01.2010

Zbigniew Dauter (Synchrotron Radiation Research Section, Macromolecular Crystallography Laboratory, National Cancer Institute, Argonne National Laboratory, Argonne, USA) "Nobel Prize winners with relations to crystallography", 04.03.2010

Szymon Świeżewski (Institute of Biochemistry and Biophysics, PAN, Warsaw, Poland) "The HOT and COOL side of AIR – Antisense Intragenic RNA", 18.03.2010

Agnieszka Chęcińska (Melanoma Group, Spanish National Cancer Research Center, Madrid, Spain), "Autophagy in melanoma maintenance and chemoresistance", 30.03.2010

Karol Kozak (Institute for Biochemistry, ETH, Zürich, Switzerland) „Data mining of large-scale RNAi silencing as a route to gene function in mammalian cells", 24.04.2010

Mikołaj Słabicki (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany) „From bench to disease: a DNA repair screen identifies a novel gene associated with HSP", 06.05.2010

Robert Szoszkiewicz (Department of Physics, Kansas State University, Manhattan, USA) "Some applications and basic research in bio-nano-technology: from pulling single protein molecules to thermochemical nanolithography", 27.05.2010

Johannes Söding (Gene Center, Ludwig-Maximilians-University of Munich, Germany) "Evolution of protein domains from modular fragments", 09.06.2010

*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.

Jordi Villá Freixa (Research Group on Biomedical Informatics, Universitat Pompeu Fabra Barcelona, Spain) "Molecular simulations got a wider scope: going multiscale", 09.06.2010

Kristian Vlahoviček (Division of Biology, Faculty of Science, University of Zagreb, Croatia) "Do microbial communities behave as metagenomes?", 14.06.2010

Gordana Maravić-Vlahovicek (Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia) "From antibiotics to signals: bacterial small talk", 14.06.2010

Bettina Warscheid (Medical Proteome-Center, Ruhr-University, Bochum, Germany) "Revealing the intricate membrane protein interaction networks of yeast organelles: new insight through quantitative proteomics" 01.07.2010

Martin Krzywinski (Canada's Michael Smith Genome Sciences Centre, Vancouver, Canada) "Infographing genomics: creating information-rich, informative and appealing genome data graphics", 09.07.2010

Andrzej Wierzbicki (University of Michigan, Ann Arbor, USA) "Non-coding RNA in transcriptional gene silencing", 15.07.2010

Nikolaus Pfanner (Institute for Biochemistry and Molecular Biology University of Freiburg, Germany) "The mitochondrial machinery for import and assembly of proteins", 19.07.2010

Ichizo Kobayashi (University of Tokyo, Graduate School of Frontier Sciences, Department of Medical Genome Sciences, Tokyo, Japan) "TBA", 30.07.2010

Magdalena Jonikas (Stanford University, Stanford, USA) "Modeling the structure of large RNAs, and modeling the health of large populations", 23.08.2010

Krystof Bankiewicz (Neurosurgery and Neurology Kinetics Foundation, Translational Research, Neurological Surgery, University of California, San Francisco, USA) „Translational studies in neurodegeneration and neurooncology using viral vectors and nanoparticles", 07.10.2010

Per Larrson (Centre for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, Sweden) "Methods to create higher quality protein structures using homology modeling and stimulations", 26.10.2010

Antoni Wiedlocha (Department of Biochemistry, Institute for Cancer Research, Oslo, Norway) „Activation and termination of fibroblast growth factor cellular signaling" 28.10.2010

Thomas Sørensen (Macromolecular Crystallography, Diamond Light Source Ltd., Didcot, United Kingdom) "How to get ions across the biological membrane", 29.10.2010

Kristian Rother (Institute of Molecular Biology and Biotechnology, Laboratory of Structural Bioinformatics, Adam Mickiewicz University in Poznań, Poland), Seminar: „Tools for RNA 3D structure modeling", Workshop: „Getting started with ModeRNA", 04.11.2010

Bogdan Podwysocki (The National Centre for Research and Development, Warsaw, Poland) "Innovative medicines initiative joint undertaking (IMI-JU)", 22.11.2010

Patrycja Wizińska (Department of Histology and Embryology, Wroclaw Medical University, Wrocław, Poland) "Laser microdissection in molecular biology" (in Polish), 24.11.2010

Dominika Nowis (Department of Immunology, Center of Biostructure Medical University of Warsaw, Poland) „Cardiotoxicity of anticancer therapeutic bortezomib", 26.12.2010

IIMCB researchers' seminars

Małgorzata Urbańska (Laboratory of Molecular and Cellular Neurobiology) "GSK3 and mTOR in neurons – friends or enemies", 14.01.2010

Anna Hupałowska (Laboratory of Cell Biology) "TRAF2 & APPL1 – players of the NF-kB team", 28.01.2010

Jean-Philippe Borges (Laboratory of Structural Biology) "Porins of Campylobacter: more than a simple channel in the membrane", 11.02.2010

Jacek Jaworski (Laboratory of Molecular and Cellular Neurobiology) "Dynamic microtubules shape neurons", 18.02.2010

Jerzy Orłowski (Laboratory of Bioinformatics and Protein Engineering) "Bioinformatics for everyone: Why not every biologist does bioinformatics and how to change it", 25.02.2010

Karolina Górecka (Laboratory of Protein Structure) "Structural studies of RuvC Holliday junction resolvase", 11.03.2010

Anna Toruń (Laboratory of Cell Biology) "Personal guide to RNAi screening: how to perform a medium-scale screen", 25.03.2010

Dorota Latek (Laboratory of Biomodelling) "Structure modeling of proteins and molecular docking using coarse grain and atomistic approaches", 22.04.2010

Katarzyna Dębowska (Laboratory of Neurodegeneration) „Calmyrin 2 - neuronal mission", 29.04.2010

Małgorzata Figiel (Laboratory of Protein Structure) "Crystal structure of human RNase H2", 25.05.2010

Marcin Klejman (Department of Molecular Biology) "NudC – a novel Hsp90 cochaperone", 24.06.2010

Maciej Żylicz (Department of Molecular Biology) "Hsp90 inhibitors in anti-cancer therapy", 14.10.2010

Marcin Nowotny (Laboratory of Protein Structure) "Structural studies of DNA repair", 21.10.2010

Agnieszka Mamińska (Laboratory of Cell Biology) "Are endocytic proteins involved in NF-kappaB signaling?", 25.11.2010

IIMCB Annual Report Session, 28.05.2010, Jachranka, Poland

Martin Bergert (Laboratory of Cell Cortex Mechanics MPG/PAN in Dresden) "Mechanisms of bleb and lamellipodium formation in migrating cells"

Emilia Białopiotrowicz (Laboratory of Neurodegeneration) "Cell cycle and amyloid in Alzheimer's disease: interacting or independent?"

Magdalena Błażejczyk (Laboratory of Molecular and Cellular Neurobiology) "Analysis of mTOR kinase effects on mRNA and protein levels in in vitro model of epilepsy"

Grzegorz Chojnowski (Laboratory of Structural Biology MPG/PAN) "DIBER: protein, DNA, or both?"

Renata Filipek (Laboratory of Structural Biology MPG/PAN) "Structural studies of membrane proteases - antibody fragment-mediated crystallization"

Umesh Ghoshdastider (Laboratory of Biomodelling) "Structural investigation of the C-terminal catalytic fragment of presenilin 1"

Marcin Jaciuk (Laboratory of Protein Structure) "Crystal structure of UvrA – insights into DNA damage recognition"

Jacek Jaworski (Laboratory of Molecular and Cellular Neurobiology) "Interfering dendritic growth - shRNA library screen for dendritc arbor morphology regulators"

Iga Korneta (Laboratory of Bioinformatics and Protein Engineering) "Spliceosomal proteome: the good, the bad, and the disordered"

Sonja Kroschwald (Laboratory of Cell Cortex Mechanics MPG/PAN in Dresden) "Novel assay for analyzing de novo cell cortex assembly in blebs"

Marta Małuszek (Department of Molecular Biology) "Mdm2-dependent modulation of genomic stability: is ATP-binding involved?"

Andrzej Nagalski (Laboratory of Neurodegeneration) "Context dependent gene expression regulation by beta-catenin in thalamus"

Ewelina Szymańska (Laboratory of Cell Biology) "Alternative functions of endocytic proteins in the regulation of AP-1 transcriptional activity"

Zuzanna Tracz (Department of Molecular Biology) "TP53 gene family on the road to cancer"

Anna Urbańska (Laboratory of Cell Biology) "APPL, ANNEXIN and ACTIN - The A threesome?"

Maria Werner (Laboratory of Bioinformatics and Protein Engineering) "New human mRNA modification enzymes".

Lab Leaders 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 is supposed to come with the binding decision based on this recommendation.

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	-

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

Grants

7th Framework Programme

- 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); 973,080 PLN; 2011-2014; J. Kuźnicki
- AMPREPCELL "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); 1,419,075 PLN; 2011-2014; J. Jaworski
- RNA+P=123D "Breaking the code of RNA sequence-structure-function relationships: New strategies and tools for modelling and engineering of RNA and RNA-protein complexes" (ERC, 261351); 1,500,000 EUR; 2011-2015; J.M. Bujnicki
- 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; 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
- MemProt "Structural studies of membrane proteases" (MTKD-CT-2006-042486); 626,800 EUR, matching funds 1,453,851 PLN; 2006-2010; M. Bochtler
- EndoTrack "Tracking the endocytic routes of polypeptide growth factor receptor complexes and their modulatory role on signalling" (LSHG-CT-2006-019050); 428,400 EUR; matching funds 1,011,709 PLN; 2006-2010; M. Miączyńska
- EUROGENTEST "Genetic Testing in Europe - Network for test development harmonization, validation and standardization of services" (LSHB-CT-2004-512148); 30,000 EUR; matching funds 60,002 PLN; 2005-2010; M. Witt

Other International Funds

- 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
- International Visegrad Fund "Organisation of the 11th Symposium of the ECS" (20920140); 13,000 EUR; 2010; J. Kuźnicki
- 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
- Polish Norwegian Research Fund "Aberrant synaptic plasticity in epilepsy" (PNRF-96-AI-1/07); 362,200 EUR; 2008-2010; J. Jaworski
- 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
- NIH Grant "High-accuracy protein models derived from lower resolution data" subcontract (430-46-22 B) within a collaborative grant coordinated by A. Kloczkowski, Iowa State University, USA; 60,000 USD; 2007-2010; J.M. Bujnicki
- 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
- The MPI-CBG/IIMCB Partner Group at the IIMCB "Biochemical and microscopial characterization of APPL-positive endosomes"; 109,000 EUR; 2006-2010; 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; 2005-2011; M. Miączyńska

Structural Funds

- IE OP 1.1.2 Programme TEAM "Structural biology of methylation and hydroxymethylation"; 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 1.1.2 Programme 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 1.2. Programme POMOST „Post-translational modifications and nuclear functions of endosomal APPL proteins" (HOM/ed2007/126); 120,000 PLN; 2007-2010; I. Pilecka

Ministerial Research Grants

- "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
- "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
- Polish-German Special Grant "Relationship between dysregulated calcium homeostasis and synaptic pathology in Alzheimer's disease as a target for therapy" (P-N/001/2006); 1,050,000 PLN; 2007-2010; J. Kuźnicki
- Polish-Spanish Special Grant "Computer prediction and simulation of RNA tertiary structure formation" (HISZPANIA/152/2006); 553,600 PLN; 2007-2010; J.M. Bujnicki
- "Experimental characterization of the complete set of RNA methyltransferases in the model organisms and identification of their counterparts in sequenced genomes" (NN301239633); 460,000 PLN; 2007-2010; J.M. Bujnicki
- "Investigation of the mechanisms regulating expression of calmyrin2, a novel EF-hand Ca2+-binding protein, and elucidation of its role in Ca2+-signal transduction in physiology and in death of neurons" (N30110932/3854); 303,000 PLN; 2007-2010; U. Wojda

- "Role of dendritic mRNA transport and local protein synthesis in development of dendritic arbor of neurons" (N N301 314733); 300,000 PLN; 2007-2010; J. Jaworski
- "Investigations of activation of GPCRs by theoretical methods" (N N301 203833); 205,000 PLN; 2007-2010; S. Filipek
- "Proteins S-nitrosylation and Cdk5 kinase-dependent phosphorylation. Proteomic studies of synaptosomal fractions from transgenic mice - Alzheimer's disease models" (NN301254333); 70,000 PLN; 2007-2010; A. Szybinska (subcontractor). Coordinator: M. Dadlez, IBB

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 Ca^{2+}-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 Commissioned Grants

- "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
- "Novel computer programs for homology modeling and fold recognition of RNA" (PBZ/MNiSW/07/2006/ 04POLPOSTDOC III); 240,000 PLN; 2007-2010; M. Boniecki
- "From patterns of gene expression and regulatory motifs towards prediction and modeling of global gene expression in brain physiology and pathology", Director: B. Kamińska-Kaczmarek from Nencki Institute of Experimental Biology; within the commissioned grant: „Application of functional genomics and bioinformatics for characterization and modeling of biological processes of critical importance for medicine and agriculture" (3/0-PBZMNiI-2/1/2005); 375,000 PLN; 2006-2010; J. Jaworski

Ministerial Research-and-Development Grant

- AriaDNA 2010 Project (OR00002712); 9,904,670 PLN; 2010-2012; M. Witt
- "New tools for analysis and manipulations of nucleic acids: restriction enzymes acting on RNA and DNA-RNA hybrids" (R1200202); 1,000,000 PLN; 2007-2010; J.M. Bujnicki

Publications resulting from grants (not affiliated to IIMCB research groups)

- **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):283-293
- **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
- 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; 11(1):174
- Czarnecka AM, Krawczyk T, Plak K, Klemba A, Zdrozny M, Arnold RS, Kofler B, Golik P, **Szybinska A**, Lubinski J, **Mossakowska M**, Bartnik E, Petros JA. Mitochondrial genotype and breast cancer predisposition. Oncol Rep. 2010; 24(6):1521-34
- Rajska-Neumann A, **Mossakowska M**, Klich-Rączka A, Zyczkowska J, Grześkowiak E, Shieh S, Wieczorowska-Tobis K. Drug consumption among Polish centenarians. Arch Gerontol Geriatr. 2010 Oct 30. doi:10.1016
- Klemba A, Kowalewska M, Kukwa W, Tonska K, **Szybinska A, Mossakowska M**, Scinska A, Golik P, Koper K, Radziszewski J, Kukwa A, Czarnecka AM, Bartnik E. Mitochondria genotype in vulvar carcinoma - cuckoo in the nest. J Biomed Sci, 2010; 17:73
- Polosak J, Roszkowska-Gancarz M, Kurylowicz A, Owczarz M, Dobosz P, **Mossakowska M, Szybinska A**, Puzianowska-Kuznicka M. Decreased expression and the Lys751Gln polymorphism of the XPD gene are associated with extreme longevity. Biogerontology, 2010; 11(3):287-297
- Adler G, Parczewski M, Czerska E, Łoniewska B, Kaczmarczyk M, Gumprecht J, Grzeszczak W, **Szybinska A, Mossakowska M**, Ciechanowicz A. An age-related decrease in factor V Leiden frequency among Polish subjects, J Appl Genet 2010, 51(3): 337–341

Cooperation with Other Institutions

Domestic Cooperation

Biocentrum Ochota (www.biocentrumochota.gov.pl)

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

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 the concentration and use of considerable scientific potential, represented by a large group of experts working in these six institutes, for the preparation of large-scale research projects that go beyond the capabilities of the activity of individual units. The implementation of such projects will overlap with the statutory research area of the institutes based on the subjects of biology, medicine and bioengineering.

The unification of expertise in each Institute will also aid the acquisition of grants, including European Union grants under the Innovative Economy and the European Social Fund, the Human Capital Operational Programme, which allows more significant results in research to be achieved. Biocentrum Ochota obtained EU funds not only for research projects, but also to expand the team of researchers.

The experts from the Institutes comprising Biocentrum Ochota are specialists renowned on the national and international arena. This is evidenced by broad scientific cooperation with Polish and foreign research centers and 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.

Bio-Technology Innovations Platform (www.biotechip.pl)

The Bio-Technology Innovations Platform, at the International Institute of Molecular and Cell Biology, was created as a Technology Transfer unit which under the project „Support for bio-tech-med entrepreneurship researchers, through scholarships, internships and training (2010-2014)", began the implementation of a scholarship programmme for graduate students pursuing application projects (see also page 82).

Centre of Preclinical Research and Technology (CePT)

The Centre of Preclinical Research and Technology (CePT) is the largest biomedical and biotechnology enterprise in Central and Eastern Europe. The project aims to create a dynamic scientific center in Warsaw, consisting of closely cooperating local research units, investigating the most common civilizational diseases, in particular cancer, neurological and cardiovascular diseases, and diseases associated with aging. The CePT Consortium consists of: the Medical University of Warsaw, which is the project coordinator, the University of Warsaw, Warsaw University of Technology and seven institutes: Nencki Institute of Experimental Biology PAN, the Institute of Biochemistry and Biophysics PAN, 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 main goal and at the same time the asset of the CePT project is to bring together the potential of outstanding scientists and the opportunities provided by an infrastructure of well equipped state-of-the-art core-facility research laboratories: 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 becomes an integrated part of the dynamic development of world translational medicine, aimed at the introduction of the latest achievements of preclinical research to practical medicine. 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.

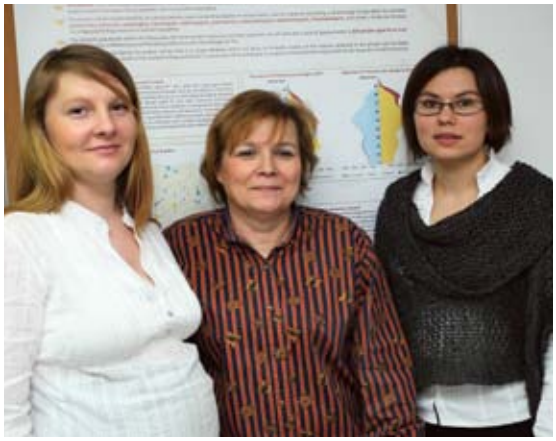
Responding to the needs of the scientific community and with reference to ESFRI recommendations regarding the creation of national research infrastructure maps, the Ministry of Science and Higher Education created a **Polish Roadmap for Research Infrastructure**. It was assumed that the Roadmap, in its first version, exemplifies the needs and aspirations of Polish science with regard to equipment and research tools. The list of 33 projects covered by the Polish Roadmap for Research Infrastructures comprises various scientific fields, including most of the major research centers

in the country. The Centre of Preclinical Research and Technology (CePT) has been placed on this list in the area of "High-performance health care and increased effectiveness of health promotion", as one out of five bio-med projects.

The total cost of the project is 388,444,071 PLN. The Centre of Preclinical Research and Technology is implemented under the Operational Program - Innovative Economy for 2007 - 2013, Priority 2 area infrastructure R & D, Activity 2.2 Support for the creation of infrastructure for joint scientific research.

PolSenior Project

The IIMCB was one of the major initiators of multidisciplinary projects on ageing and is currently a coordinator of the Ministry Commissioned grant entitled "Ageing of the Polish population – medical, psychological, sociological and economic aspects – PolSenior". The project is being conducted by the International Institute of Molecular and Cell Biology in Warsaw with Prof. Piotr Błędowski from Warsaw School of Economics (President of the Polish Gerontological Society) as head of the project and Dr. Małgorzata Mossakowska (IIMCB) as coordinator.



The country's largest project in this area of research, with a budget of over 12 million PLN, aims to conduct an interdisciplinary study of various ageing-related problems encompassing diverse research disciplines concerning the ageing process in Polish society. Specialists involved range from sociologists, psychologists, economists and demographers to geriatricians, cardiologists, nephrologists, neurologists, epidemiologists and molecular biologists from research centers in Białystok, Bydgoszcz, Gdańsk, Lublin, Łódź, Katowice, Kraków, Poznań, Szczecin, Wrocław and Warsaw. About 40 research groups are involved in the project.

The field work was completed in June 2010. The total number of participants was 5695 (2899 males and 2796 females). Until the end of 2010, genomic DNA was isolated from 2,500 blood samples. A self-completed questionnaire including WHOQOL-BREF and nutritional habits was filled out by 3667 participants. More than 600 patients were examined by geriatricians (out of 1,000 planned). The Institute also provides the whole Consortium with samples of biological material and with a database constructed specifically for the project. Biological material from all respondents is deposited at IIMCB.

AriaDNA2010 Project

The major purpose of forensic investigation is the identification of the person who left a trail of biological material at a crime scene. Unfortunately, in recent years, a trend has been noted of perpetrators leaving decreasing amounts of biological material. The minute amount of biological material remaining at a crime scene as a mix of material originating from different people can be assumed as a model of very scarce study material available to a researcher. The aim of this project is to adapt modern molecular biology techniques for analyzing the genetic material in single human cells to forensic purposes (identification of evidence of the perpetrator on the basis of trace amounts of heterogeneous biological substance). This objective will be achieved through:

- a comparative analysis of genetic material belonging to representatives of various populations,
- the identification of genetic differences between pairs of populations at the level of gene expression (transcriptome)
- the design of fluorescent molecular probes to enable reliable identification of cells based on differences in gene expression using in situ hybridization and fluorescence microscopy,
- developing a system of laser microdissection (LCM) allowing the physical segregation of individual cells.

International Cooperation

Max Planck Society

This cooperation started in 2001 as an initiative of Max Planck Society and Polish Academy of Sciences. According to the agreement Junior Research Group, with Dr. Matthias Bochtler as a Lab Leader was funded by MPS and located at IIMCB. Research activity of this lab turned to be one of the most successful step in the history of 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 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.

Dr. Marta Miączyńska, a leader of the Laboratory of Cell Biology at IIMCB in Warsaw, was heading a Partner Group of the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden. The MPI CBG/IIMCB Partner Group was established based on a 3-year contract between the two institutions in January 2006, further extended until December 2010. From the side of MPI-CBG, Prof. Marino Zerial (Managing Director of MPI-CBG) was the cooperation partner and a scientific mentor of the Partner Group. Dr. Miączyńska was working in Prof. Zerial's group in Dresden as a senior

postdoctoral fellow in the years 2001-2005, before her return to Poland in April 2005. The scientific project of the Partner Group, dealing with the characterization of APPL-positive endosomes, is a continuation of the work that Dr. Miączyńska carried out in the laboratory of Prof. Zerial in Dresden.

Institute of Molecular Biology and Genetics, Kiev, Ukraine

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. IIMCB and IMBG representatives meet every 2 years during Polish-Ukrainian Parnas conferences. Closer bilateral relations between IIMCB and IMBG were established in 2008 thanks to an initiative of IIMCB director Prof. Jacek Kuźnicki. During the round table "Research organization in the 21st century: experience and achievements of IIMCB", organized by the Ukrainian Ministry of Education and Science, it was proposed that IIMCB's experience could be used by Ukrainian scientific institutions. In 2009 IIMCB organized a Polish-Ukrainian scientific conference accompanied by the HEALTH-PROT kick-off meeting. The IMBG director, Prof. Elskaya, participated in the IIMCB International Advisory Board meeting and the IMBG managers had meetings with their IIMCB counterparts. One of the results of this cooperation is the submission of a shared proposal "Strengthening cooperation in molecular biomedicine between the EU and Ukraine" (FP7-INCO-2011-6, ERA-WIDE).

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**, PhD (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

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 (April 2010 – March 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
- **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
- **Marek Wojciechowski** - laboratory of **Ruedi Allemann**, University of Cardiff, United Kingdom
- **Szymon Niewieczerzał** - laboratory of **Esteve Padros**, Universitat Autònoma de Barcelona, Spain
- **Zuzanna Bukowy** - laboratory of **Heymut Omran**, Department of Pediatrics, University Hospital Münster, Germany
- **Aleksandra Szybińska** - laboratory of **Jochen Herms**, Centre for Neuropathology, Ludwig-Maximilians-University of Munich, Germany

Short visits of IIMCB researchers at the twinning institutions

- **Małgorzata Durawa** - Institute of Biotechnology, Vilnius, Lithuania
- **Sławomir Filipek** – Department of Pharmaceutical Sciences, University of Bologna, Italy
- **Jacek Kuźnicki** - Centre for Neuropathology at the Ludwig-Maximilians-University of Munich, Germany
- **Alicja Żylicz** and **Maciej Żylicz** - Cancer Research of UK Cell Signalling Unit, Edinburgh Cancer Research Centre, University of Edinburgh, UK

Visits of twinning partners to IIMCB

- **Casper Hoogenraad** and **Lukas Kapitein**, Erasmus MC, Rotterdam, The Netherlands
- **Harald Stenmark, Andreas Brech, Kristi Bache, Hilde Abrahamsen, Antoni Wedlocha**, The Norwegian Radium Hospital, Oslo

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.
Dr. Czapińska has 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 ThaI-DNA complex provides a rare example for deep amino acid intercalation into non-damaged DNA.

Marcin Pawłowski, Laboratory of Bioinformatics and Protein Engineering, head Janusz M. Bujnicki.
Prediction of protein structure from its sequence is a challenging problem in structural bioinformatics. An important stage, if not the most important, in the structure prediction process is to evaluate the quality of a structure model. Dr. Pawłowski addressed the problem by cooperative development of two computer programs:

– MQAPmulti compares structural features generated from a 3D model with those predicted from its primary sequence (secondary structure, solvent accessibility, contact maps), uses a series of statistical potentials 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. Moreover, MQAPmulti compares conformations of many alternative models of the protein of interest to enhance the quality estimation process
– MQAPsingle, is a variant of MQAPmulti, but designed to estimate the quality of a single model. Tested in the latest CASP (Critical Assessment of protein Structure Prediction) experiment, both methods have been ranked among the best model quality assessment programs.

Marta Wiśniewska, Laboratory of Neurodegeneration, head Jacek Kuźnicki.
Dr. Wiśniewska participated in a project on the role of Ca^{2+} sensors STIM1 and STIM2 in neuronal cells. The question of Stim1 expression in neurons has been controversial. She employed quantitative PCR with TaqMan primers and probes to calculate the exact number of Stim1 and Stim2 mRNA copies in primary neuronal and astrocytic cultures (per 1 ng of total RNA), as well as in laser-dissected hippocampal neurons (per cell). Then, the involvement of STIM1 and STIM2 in store operated Ca^{2+} was investigated.

Tomasz Węgierski, Laboratory of Neurodegeneration, head Jacek Kuźnicki.
Dr. Węgierski participated in a project on the mechanism of store-operated calcium entry (SOCE) in neurons. Using yeast genetic screens, he is searching for novel neuronal proteins interacting with key molecular players of SOCE, such as STIM1 protein. He is also involved in the search for repair mechanisms of disturbed calcium homeostasis in cells from Alzheimer's disease patients, using Fura2-based calcium imaging.

Elżbieta Nowak, Laboratory of Protein Structure, head Marcin Nowotny.
The project was aimed at structural studies of the different monomeric reverse transcriptases (RT) in complex with the DNA/RNA hybrids. Dr. Nowak expressed and purified three RTs, but only one was in the monomeric form. The protein was co-crystallized with different combination of DNA/RNA hybrids. Crystals of the protein-nucleic acid complex were obtained and tested at the synchrotron radiation showing diffraction up to 4 Å. Currently she is in the process of optimizing crystallization condition and crystallization of the SeMet derivative protein in order to obtain phases for solving the structure.

Zuzanna Bukowy, Ciliary Proteins Function Project, head Michał Witt.
Dr. Bukowy introduced to the laboratory the in vitro ciliogenesis technique, a method of culturing the respiratory epithelial (RE) cells and prepared a review article on the techniques of culturing differentiated RE cells. From September 2010 until March 2011, she has been staying in

the collaborating laboratory of Professor Heymut Omran at the Department of Pediatrics at the University Hospital in Műnster, Germany. In order to gain more experience in the in vitro ciliogenesis and in immunofluorescence (IF) staining of cilia components, she was involved in a project on the role of the Notch signaling pathway on the differentiation of the RE cells. Moreover, she has also been working on a manuscript summarizing the results of another project, performed in collaboration with the Professor Omran's laboratory, investigating the role of the RPGR protein in the primary ciliary dyskinesia.

Paweł Wiśniewski, Department of Molecular Biology, head Maciej Żylicz.
MDM2 is commonly considered as a E3 ligase that plays a crucial role in p53 ubiquitin-dependent degradation. Dr. Wiśniewski addressed a question whether MDM2 possesses another function or activity apart from its E3 ligase activity. In order to answer this question MDM2 ATP-binding mutant (MDM2 K454A) was created assuming that the nucleotide can modulate MDM2 function or activity. Using H1299 cells (non-small cell lung cancer, p53-/-) the influence of MDM2 and the mutant on cell functions, gene expression profiles as well as signaling pathways was tested.

Ewelina Szymańska, Laboratory of Cell Biology, head Marta Miączyńska.
The aim of the project is to characterize the novel molecular mechanisms by which selected proteins, acting in endocytosis participate in signaling and transcriptional regulation mediated by AP-1 transcription factor. For this purpose, a small-scale screening platform was established. The basic principle of the screens was to carry out RNAi-mediated knockdown of selected candidate genes and to test their impact on AP-1–dependent transcription using a luciferase-based reporter assay. As a result, several candidate regulators acting in a positive or negative manner in the AP-1 pathway were identified. In further studies, the mechanisms of action of identified regulators in the AP-1 pathway will be investigated. In particular, in the focus is on mapping the stage of the AP-1 pathway at which the candidate proteins act, identification of domains involved in the AP-1 regulation and analysis of the impact of selected endocytic proteins on AP-1 target gene expression.

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. Work done by Dr. Macias focused on proteomics identification of microtubule binding proteins binding to mTOR as well as on analysis of their spatial distribution with EM. Moreover, Dr. Macias investigated mTOR activity and +TIPs in epileptogenesis, a pathological process characterized by abnormal neuronal activity and gross morphological changes in the brain.

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.

Participation in international events

Michał Witt, Ciliary Proteins Function Project
European Human Genetics Conference 2010, June 12 - 15, 2010, Gothenburg, Sweden
Poster: "Genetic background of PCD in Polish patients: search for mutations in candidate genes"

Marta Miączyńska, Laboratory of Cell Biology
Gordon Research Conference "Lysosomes & Endocytosis", June 20-25, 2010, Andover, New Hampshire, USA
Invited lecture: "Endocytic proteins in the regulation of transcription"

Łukasz Świech, Laboratory of Molecular and Cellular Neurobiology
7th FENS Forum of European Neuroscience, July 3-7, 2010, Amsterdam, The Netherlands
Poster: "mTOR, CLIP-170 and IQGAP1 proteins: team players in the cytoskeleton dependent dendritogenesis"

Małgorzata Perycz, Laboratory of Molecular an Cellular Neurobiology
7th FENS Forum of European Neuroscience, July 3-7, 2010, Amsterdam, The Netherlands
Poster: "Tyrosine phosphorylation of RNA-binding proteins: a common regulation of dendritogenesis?"

Andrzej Nagalski, Laboratory of Neurodegeneration
7th FENS Forum of European Neuroscience, July 3-7, 2010, Amsterdam, The Netherlands
Poster: "Beta-catenin transcriptional activity can influence membrane conductance of thalamic neurons"

Tomasz Węgierski, Laboratory of Neurodegeneration
7th FENS Forum of European Neuroscience, July 3-7, 2010, Amsterdam, The Netherlands

Poster: "Beta-catenin transcriptional activity can influence membrane conductance of thalamic neurons"

Bartosz Trzaskowski, Laboratory of Biomodelling
21st International Symposium on Medicinal Chemistry, September 5-9, 2010, Brussels, Belgium
Poster: "Key Interactions of Anti--HIV Drug Candidate PF-232798 with CCR5 Receptor"

Łukasz Świech, Laboratory of Molecular and Cellular Neurobiology
40th Annual Meeting - Neuroscience 2010, November 13-17, 2010, San Diego, USA
Poster: "mTOR regulates dendrite morphology by coordinating microtubule-actin interactions"

Matthias Bochtler, Laboratory of Structural Biology
Biophysical chemistry, molecular biology and cybernetics of cell functions, January 15-25, 2011, Klosters, Switzerland
Invited lecture: "Symmetry and pseudosymmetry in protein DNA interactions"

Jacek Kuźnicki, Laboratory of Neurodegeneration
AD/PD Conference, March 9-13, 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, March 9-13, 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"

Promotion and management

Research Symposium and International Advisory Board meeting

Scientific achievements of the project were summarized on nine posters prepared by all laboratories involved. They were presented at the Research Symposium on May, 14th and during the meeting of the International Advisory Board on May, 15th. Following posters were presented:

- RAGs and the origin of the specific immune system. H. Czapińska, R. Szczepanowski, M. Bochtler, R. Rehlich, L. Wiedemann, M. Ostertag, R. Allemann
- The Bujnicki lab: scientific achievements during the first year of the project implementation. M. Pawłowski, Ł. Kozłowski, M. Durawa, J. M. Bujnicki
- Understanding of β-amyloid formation in Alzheimer's Disease. Sławomir Filipek's group and Vincenza Andrisano, Department of Pharmaceutical Sciences, University of Bologna, Italy
- mTOR regulates dendrite morphology by coordinating microtubule-actin interactions. Ł. Świech, M. Błażejczyk, M. Urbańska, P. Pietruszka, B. Dortland, A. Malik, P. Wulf, C. Hoogenraad, J. Jaworski
- Transcriptional regulation of voltage-gated Ca^{2+} channel Cav3.1 by LEF1/beta-catenin in the adult brain. M. Wisniewska, W. Michowski, E. Purta, W. Leśniak, K. Misztal, M. Dąbrowski, J. Kuźnicki
- Endosomal proteins in the regulation of cell signaling and proliferation. Establishing middle - throughput RNAi screening platforms for identifications of transcriptional regulators. E. Szymańska, A. Toruń, I. Pilecka, M. Miączyńska
- Structural and biochemical studies of reverse transcriptases. E. Nowak, M. Figiel, J. Jurkowski, M. Nowotny
- Method for culturing nasal epithelial cells – introduction into laboratory. Z. Bukowy, E. Ziętkiewicz, M. Witt
- MDM2 regulates Beclin-1 gene expression in H1299 lung cancer cells. P. Wiśniewski, A. Żylicz, M. Żylicz

At the annual meeting of the International Advisory Board, Dr. Urszula Białek-Wyrzykowska presented the report on the activities within the HEALTH-PROT during the first year of project implementation. 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 nine posters prepared by all research groups involved.

Other promotional activities

Presentation: Dr. Urszula Białek-Wyrzykowska, Symposium organized by the National Council of Coordinators of Research Projects UE (in Polish), 12-13.05.2010, Institute of Genetics and Animal Breeding, Jastrzębiec n/Warsaw, Poland

Presentation: Dorota Libiszowska, BioForum 2010 - Central European Business Forum of Biotechnology & Innovatoive BioEconomy, 20.05.2010, Łódź, Poland

Publication: WIRE 2010, Week of Innovative Regions in Europe, Taking Stock and Moving Forward.

Publications resulting from the project

- **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

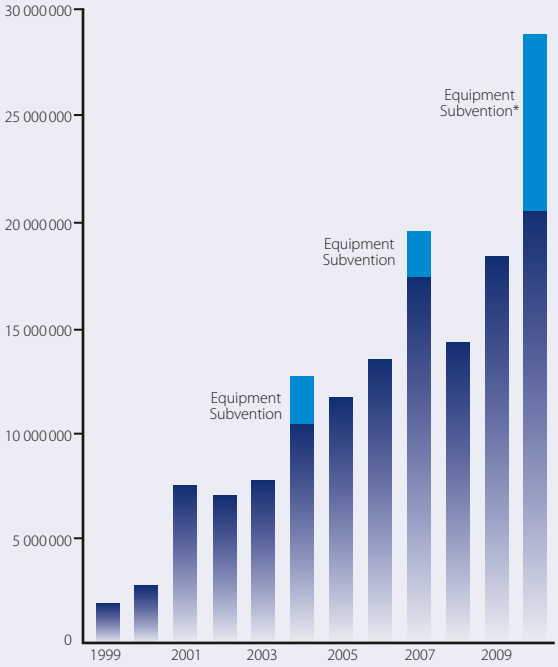
- Firczuk M, **Wojciechowski M, Czapinska H, Bochtler M**. DNA intercalation without flipping in the specific ThaI-DNA complex. Nucleic Acids Res. 2011 Jan;39(2):744-54
- Antonczaka A K, Simova Z, Yonemoto I T, **Bochtler M**, Piasecka A, **Czapinska H**, Brancale A, Tippmann E M. Importance of single 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.

Successful common 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**

Diversity of Funding IIMCB'2010

Annual income (in PLN)



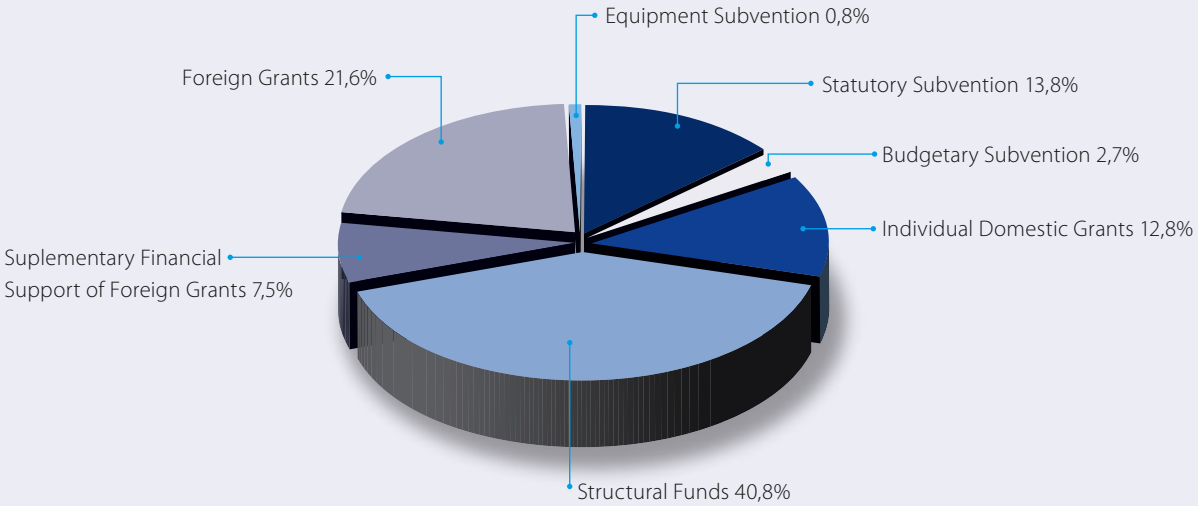
* 9,161,225 PLN from Structural Project CePT + 233,500 PLN Equipment Subvention (Ministerial)

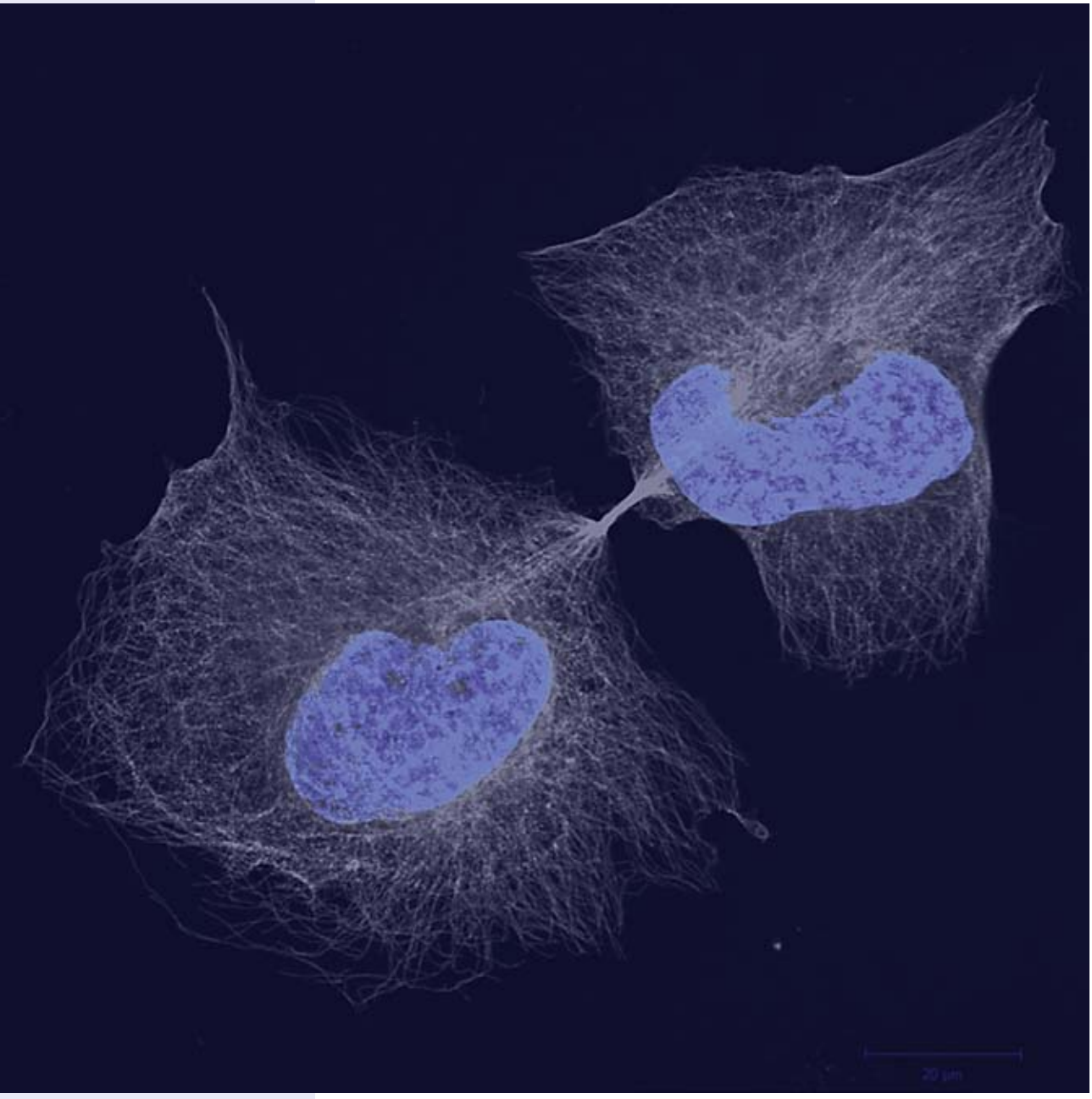
Sources of Funding	amounts in PLN	amounts in EUR[1]
Statutory Subvention	3 956 995	999 165
Budgetary Subvention	779 000	196 702
Individual Domestic Grants	3 687 081	931 011
Structural Funds	11 737 604	2 963 817
Supplementary Financial Support of Foreign Grants	2 169 823	547 894
Foreign Grants	6 204 011	1 566 551
Equipment Subvention (Ministerial)	233 500	58 960
Total	**28 768 014**	**7 264 100**

[1] 1EUR – 3,9603 @ 31st Dec' 2010

Profit & loss statement

	amounts in PLN
A. net revenue on sales and equivalents*	29 585 102
B. operational activity costs:	30 501 819
Depreciation (equipment)	1 325 866
Research materials	13 233 696
Utilities	449 778
Services	2 952 885
Fees and taxes	2 032 178
Salaries and wages	6 802 890
Social and health insurance	1 673 788
Other operational expenses, in this:	2 030 739
business trips	1 024 520
property insurance	30 297
fellowships	973 041
others	2 881
C. other operational income (subventions)	923 017
D. other operational expenses:	167 665
E. financial income (interest):	130 953
F. financial expenses (other):	1 350
Profit/Loss on business activity (A-B+C-D+E-F)	**-31 763**





H1299- Non Small Lung Cancer Cells just after cell division

Department of **Molecular Biology**

Lab Leader:

Maciej Żylicz, PhD, Professor

Vice Head:

Alicja Żylicz, PhD, Professor

Research Associates:

Marcin Klejman, PhD

Dawid Walerych, PhD

Paweł Wiśniewski, PhD

Junior Researchers:

Marta Małuszek, MSc

Magdalena Pruszko, MSc (started Oct 2010)

Zuzanna Szymańska, PhD (thesis defense Sep 2010)

Zuzanna Tracz, MSc

Jakub Urbański, PhD (thesis defense Sep 2010)

Milena Wiech, MSc

Secretary:

Grażyna Orleańska, MSc

Technician:

Wanda Gocal

Maciej Żylicz, PhD, Professor

DEGREES:

1992	Professor
1986	DSc Habil in Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
1979	PhD in Biochemistry, Medical University of Gdańsk, Poland
1977	MSc in Physics, University of Gdańsk, 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 Gdańsk, 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 Gdańsk, Poland
1991-1994	Head, Department of Molecular Biology, University of Gdańsk, Poland
1993-1994	Visiting Professor, University of Utah, Medical Center, Institute of Oncology, Salt Lake City, Utah, USA
1990-1993	Vice President, University of Gdańsk, Poland
1988-1991	Associate Professor, Department of Molecular Biology, University of Gdańsk, Poland
1981-1988	Assistant Professor, Department of Biochemistry, University of Gdańsk, Poland

OTHER PROFESSIONAL ACTIVITIES:

2010 - Present	Advisor of the President of Polish Republic
2010 - Present	Member, European Academy of Cancer Research
2010 - Present	Member, ERC Identification Committee (appointed by European Commission)
2010 - Present	Chair of Selection Committee, National Resarch Center Council, 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:

- Member, EMBO Full Member, Polish Academy of Sciences
- Member, Polish Academy of Arts and Sciences
- Member, Academia Europaea
- 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 YIP (2001-2003)
- Polish delegate to EMBC (2001-2004)
- Member, State Committee for Scientific Research (1997-2004)
- Polish Delegate, ESF Life Science Committee (2003-2005)
- Member, Selection Committee, Special DFG Programmes (2001-2005)

HONORS, PRIZES, AND AWARDS:

1. 2008 Officer's Cross of the Order of Polonia Restituta (awarded by the President of the Republic of Poland)
2. 2007 Doctor Honoris Causa, University of Wrocław
3. 2002 Prime Minister Award for Scientific Achievements
4. 2001 Marchlewski Award, Committee of Biochemistry and Biophysics, Polish Academy of Sciences
5. 1999 Award in biological/medical sciences, Foundation for Polish Science
6. 1996, 2007, 2010 Awards for best biochemistry work performed in Polish laboratories, Polish Biochemical Society
7. 1994 Award from Ministry of Education
8. 1993 Heweliusz Prize for Scientific Achievements (awarded by President of Gdańsk)
9. 1990 Award from Polish Academy of Sciences
10. 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 *PNAS*, and more than 30 in *J Biol Chem*. These papers were cited approximately 5500 times with a Hirsch index of H = 40.

Selected publications

- **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. (2009) 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
- Stevens C, Pettersson S, **Wawrzynow B**, Wallace M, Ball K, **Zylicz A**, Hupp TR. ATP stimulates MDM2-mediated inhibition of the DNA-binding function of E2F1. FEBS J, 2008; 275:4875-86
- **Wawrzynow B**, **Zylicz A**, Wallace M, Hupp T, **Zylicz M**. MDM2 chaperones the p53 tumor suppressor. J Biol Chem, 2007; 282:32603-12
- Issat T, Nowis D, Legat M, Makowski M, **Klejman MP**, **Urbanski J**, Skierski J, Koronkiewicz M, Stoklosa T, Brzezinska A, Bil J, Gietka J, Jakobisiak M, Golab J. Potentiated antitumor effects of the combination treatment with statins and pamidronate *in vitro* and *in vivo*. Int J Oncol, 2007; 30:1413-25
- Spiechowicz M, **Zylicz A**, **Bieganowski P**, Kuznicki J, Filipek A. Hsp70 is a new target of Sgt1- an interaction modulated by S100A6A. Biochem Biophys Res Commun, 2007; 357:1148-53
- **Kudla G**, **Lipinski L**, **Caffin F, Helwak A**, **Zylicz M**. High guanine and cytosine content increases mRNA levels in mammalian cells. PLoS Biology, 2006; 4:0933-42
- **Walerych D**, **Kudla G**, **Gutkowska M**, **Wawrzynow B**, Muller L, **King FW**, **Helwak A**, **Boros J**, **Zylicz A**, **Zylicz M**. Hsp90 chaperones wild-type p53 tumor suppressor protein. J Biol Chem, 2004; 279: 48836-45
- Dworakowska D, Jassem E, Jassem J, Peters B, Dziadziuszko R, **Zylicz M**, Jakobkiewicz-Banecka J, Kobierska-Gulida G, Szymanowska A, Skokowski J, Roessner A, Schneider-Stock R. MDM2 gene amplification: a new independent factor of adverse prognosis in non-small cell lung cancer (NSCLC) Lung Cancer, 2004; 43:285-295
- **Kudla G**, **Helwak A**, **Lipinski L**. Gene conversion and GC-content evolution in mammalian Hsp70. Mol Biol Evol, 2004; 21:1438-44
- **Zylicz M**, **King FW**, **Wawrzynow A**. Hsp70 interactions with the p53 tumour suppressor protein. EMBO J, 2001; 20:4634-8
- **King FW**, **Wawrzynow A**, Hohfeld J, **Zylicz M**. Cochaperones Bag-1, Hop and Hsp40 regulate Hsc70 and Hsp90 interactions with wild-type or mutant p53. EMBO J, 2001; 20:6297-305.

Summary of work

The research conducted in the Department of Molecular Biology is mainly focused on 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 wild-type or mutant p53 tumor suppressor protein. We also demonstrated that the Hsp90 molecular chaperone was required for binding of wild-type p53 to the promoter sequences under a physiological temperature of 37°C and that chaperoning activity was ATP-dependent.

Recently, we provided *in vivo* evidence that Hsp90 and Hsp70 chaperone machines were required for 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 p53 refolding at 37°C during the recovery from heat shock. We also demonstrated that the interaction of wildtype p53 with 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., 2010). E42A and D88N Hsp90β variants bind but do not hydrolyze ATP, whereas E42A increased and D88N decreased ATP affinity compared with WT Hsp90β. Nevertheless, both of these mutants interact with wild-type p53 with similar affinity. Surprisingly, in the case of wild-type 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 wild-type 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 able to bind to the promoter sequence. Furthermore, supporting these results, the overproduction of wild-type 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 wild-type 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., 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

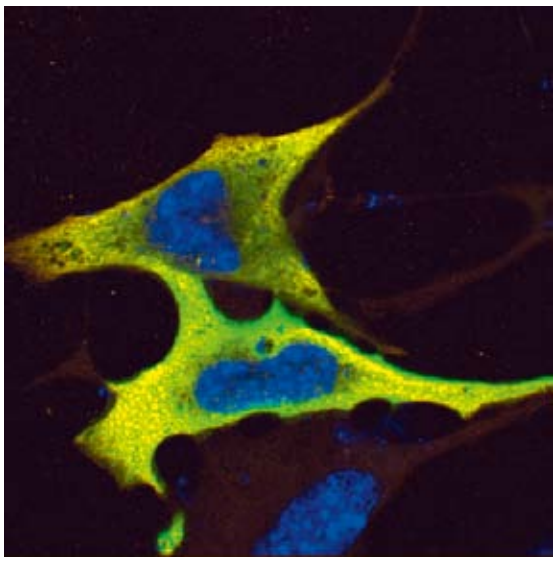


Fig. 1. Co-localization of p53R175H and Hsp70 in protein aggregates in MEFs (mouse embryonic fibroblasts). MEFs were transfected with plasmids coding for p53R175H (orange) and Hsp70 (green). 48 hours post-transfection cells were fixed, stained with fluorescent antibodies (AlexaFluor 488 and 555) and analyzed under the confocal microscope. MG132 proteasome inhibitor has been applied for 12 hours before staining; blue – DAPI, yellow–merge.

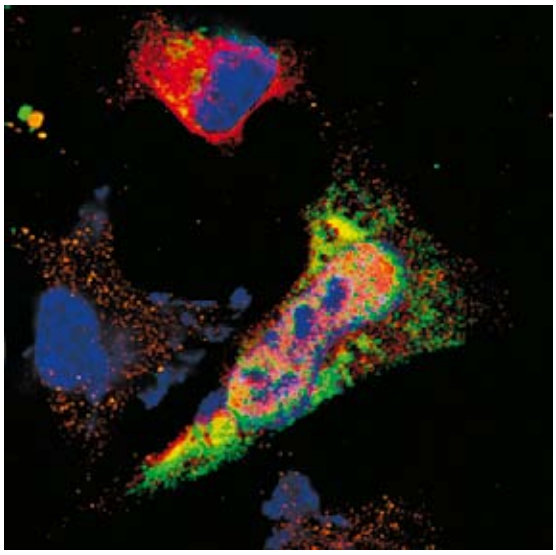


Fig. 2. p53R175H protein aggregates in MEFs (mouse embryonic fibroblasts). MEFs were transfected with plasmids coding for p53R175H (green), MDM2 (orange) and Hsp70 (red). 48 hours post-transfection cells were fixed, stained with fluorescent antibodies (AlexaFluor 488, 555 and 633) and analyzed under the confocal microscope. MG132 proteasome inhibitor has been applied for 12 hours before staining; blue – DAPI.

scenario by which resistance may arise in patients treated with Hsp90 inhibitors. Additionally, our results showed that each Hsp90 isoform could alone sustain cellular function (Zurawska et al., 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 non-small 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, 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 I0.5 for p53 transrepression. An allosteric model for 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 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 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, which together impact 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) 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 the collaboration with our department, showed that Hsp70 stabilizes lysosomes by binding to endolysosomal anionic phospholipid bis(monoacylglycero)phosphate (BMP), an essential co-factor 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), as well as the pharmacological and genetic inhibition of ASM, effectively reverses 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 encoding 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 by the endocytic delivery pathway (Kirkegaard et al., *Nature*, 2010).

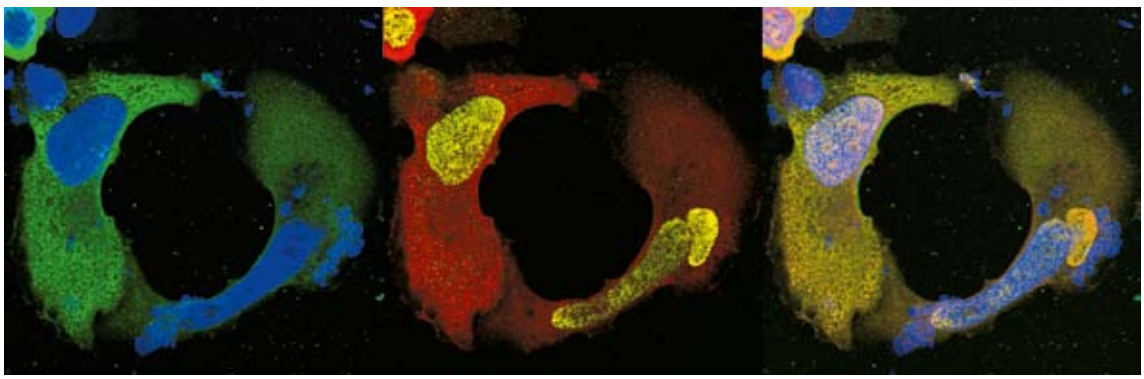
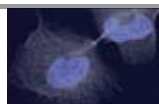
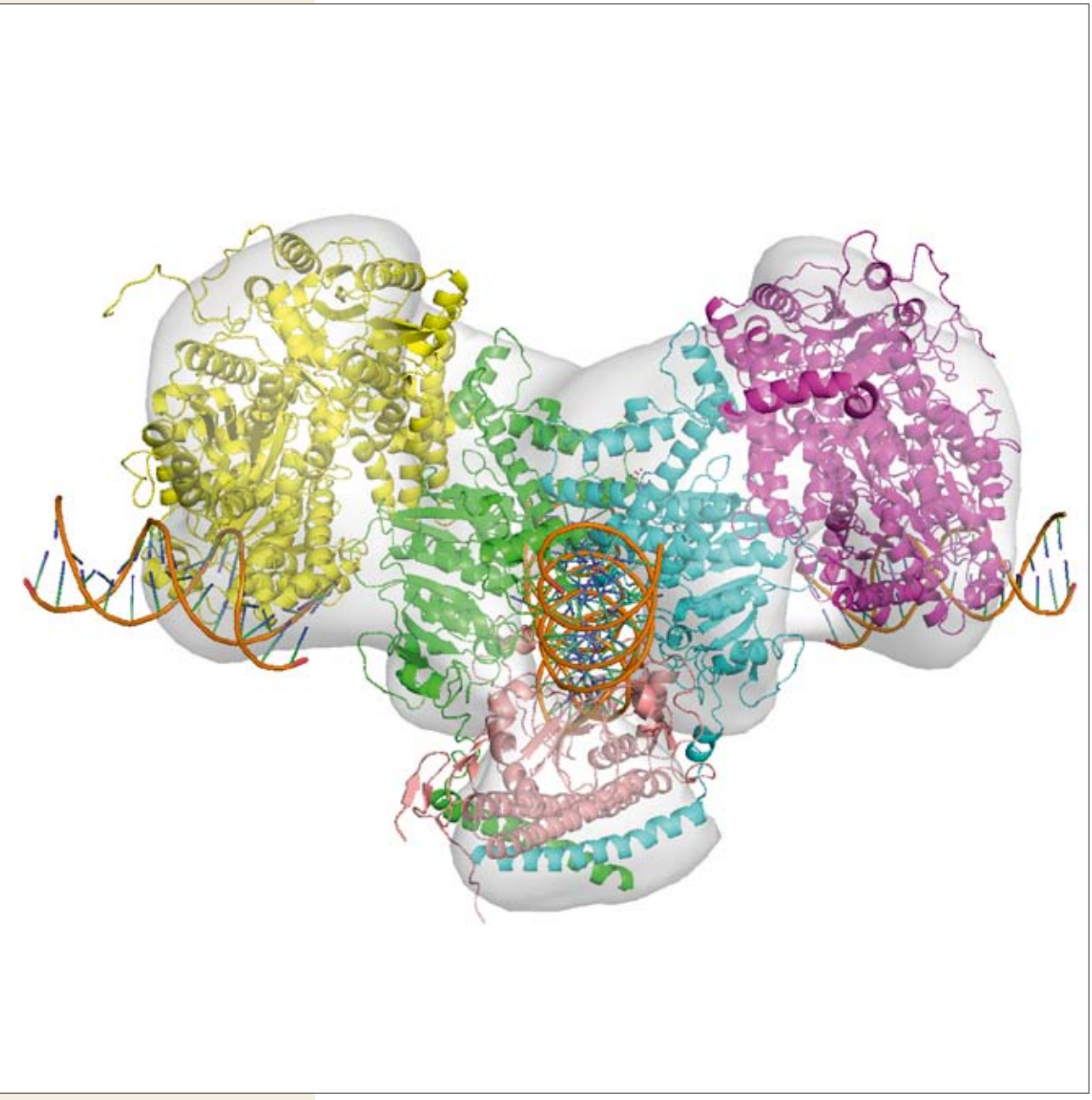


Fig. 3. Localization of p53R175H (endogenous, green), MDM2 and Hsp70 in SK-BR-3 cell line (breast cancer, adenocarcinoma). Cells were transfected with plasmids coding for MDM2 (yellow) and Hsp70 (red). 48 hours post-transfection cells were fixed, stained with fluorescent antibodies (AlexaFluor 488, 555 and 633) and analyzed under the confocal microscope. MG132 proteasome inhibitor has been applied for 12 hours before staining.





A structural model of a Type I restriction enzyme

Laboratory of **Bioinformatics and Protein Engineering**

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Postdoctoral Fellows:

Michał Boniecki, PhD; Grzegorz Chojnowski, PhD; Marcin Pawłowski, PhD; Elżbieta Purta, PhD; Krzysztof J. Skowronek, PhD; Tomasz Soltysinski, PhD; Ewa Wywiał, PhD

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Office Manager:

Agnieszka Faliszewska, MSc

Computer Administrators:

Jan Kogut, BSc; Tomasz Jarzynka; Łukasz Munio

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 Society for Bioinformatics, 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-Present), *Journal of Applied Genetics* (2004-Present), *Database Journal* (2008-Present), *Journal of Nucleic Acids* (2008-Present)

AWARDS:

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 of the 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 2010

- **Gajda MJ, Tuszynska I, Kaczor M**, Bakulina AY, **Bujnicki JM.** FILTREST3D: discrimination of structural models using restraints from experimental data. Bioinformatics, 2010; 26:2986-7
- Abrahams JP, Apweiler R, Balling R, Bertero MG, **Bujnicki JM**, Chayen NE, Chène P, Corthals GL, Dyląg T, Förster F, Heck AJ, Henderson PJ, Herwig R, Jehenson P, Kokalj SJ, Laue E, Legrain P, Martens L, Migliorini C, Musacchio A, Podobnik M, Schertler GF, Schreiber G, Sixma TK, Smit AB, Stuart D, Svergun D, Taussig MJ. 4D Biology for Health and Disease. Workshop Report Nature Biotechnol, 2010, doi:10.1016/j.nbt.2010.10.003
- Chua TK, Seetharaman J, **Kasprzak JM**, Ng C, Patel BK, Love C, **Bujnicki JM**, Sivaraman J. Crystal structure of a fructokinase homolog from Halothermothrix orenii. J Struct Biol, 2010; 171:397-401
- **Gajda MJ, Pawlowski M, Bujnicki JM**. Protein structure prediction: from recognition of matches with known structures to recombination of fragments, In „Multiscale approaches to protein modeling: structure prediction, dynamics, thermodynamics and macromolecular assemblies". Editor: Kolinski A, Springer, 2010, ISBN: 978-1-4419-6888-3
- **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. Hum Mutat. 2010; 31:975-82
- Husain N, **Tkaczuk KL**, Tulsidas SR, **Kaminska KH**, Čubrilo S, Maravić-Vlahoviček G, **Bujnicki JM**, Sivaraman J. Structural basis for the methylation of G1405 in 16S rRNA by aminoglycoside resistance methyltransferase Sgm from an antibiotic producer: A diversity of active sites in m7G methyltransferases, Nucleic Acids Res, 2010; 38:4120-32
- Kempenaers M, Roovers M, Oudjama Y, **Tkaczuk KL, Bujnicki JM**, Droogmans L. New archaeal methyltransferases forming 1-methyladenosine or 1-methyladenosine and 1-methylguanosine at position 9 of tRNA. Nucleic Acids Res, 2010; doi:10.1093/nar/gkq451
- Pukszta S, Schilke B, Dutkiewicz R, Moczulska K, Stepien B, Kominek J, Reitenga KG, **Bujnicki JM**, Williams B, Craig EA, Marszalek J. Co-evolution driven switch of J-protein specificity toward an Hsp70 partner. EMBO Rep;11:360-5
- Zhang Z, Theler D, **Kaminska KH**, Hiller M, de la Grange P, Pudimat R, Rafalska I, Heinrich B, **Bujnicki JM**, Allain FHT, Stamm S. The YTH domain is a novel RNA binding domain. J Biol Chem, 2010; 285:14701-10
- Pukancsik M, Bekesi A, Klement Eva, Hunyadi-Gulyas E, Medzihradszky K, **Kosinski J, Bujnicki JM**, Alfonso C, Rivas G, Vertessy B. Physiological truncation and domain organization of a novel uracil-DNA degrading factor. FEBS J, 2010; 277:1245-59
- Zhou X, Khiang TC, **Tkaczuk KL, Bujnicki JM**, Sivaraman J. Crystal structure of Escherichia coli spermidine synthase SpeE reveals a unique substrate binding pocket, J Struct Biol, 2010; 169:277-285
- **Kaminska KH, Purta E**, Hansen LH, **Bujnicki JM**, Vester B, Long KS. Insights into the structure, function, and evolution of the Radical-SAM 23S rRNA methyltransferase Cfr that confers antibiotic resistance in bacteria, Nucleic Acids Res, 2010; 38:1652-63
- **Tuszynska I, Bujnicki JM**. Predicting atomic details of the unfolding pathway for YibK, a knotted protein from the SPOUT superfamily J Biomol Struct Dyn, 2010; 27:511-520
- Khan F, Furuta Y, Kawai M, **Kaminska KH**, Ishikawa K, **Bujnicki JM**, Kobayashi I. A mobile genetic element of a novel type carrying a Type IIF restriction-modification system (PluTI) Nucleic Acids Res, 2010; 38:3019-30

Other selected publications

- Pena V, Jovin SM, Fabrizio P, **Orlowski J, Bujnicki JM**, Lührmann R, Wahl MC. Common design principles in the spliceosomal RNA helicase Brr2 and in the Hel308 DNA helicase. Mol Cell, 2009; 35:454-466
- **Purta E**, O'Connor M, **Bujnicki JM**, Douthwaite S. YgdE is the 2'-O-ribose methyltransferase RlmM specific for nucleotide C2498 in bacterial 23S rRNA, Mol Microbiol, 2009; 72:1147-58
- Kennaway CK J, **Obarska-Kosinska A**, White JK, **Tuszynska I**, Cooper LP, **Bujnicki JM**, Trinick J, Dryden DTF. The structure of M.EcoKI Type I DNA methyltransferase with a DNA mimic antirestriction protein. Nucleic Acids Res, 2009; 37:762-770
- **Kaminska KH**, Kawai M, **Boniecki M**, Kobayashi I, **Bujnicki JM.** Type II restriction endonuclease R.Hpy188I belongs to the GIY-YIG nuclease superfamily, but exhibits an unusual active site. BMC Struct Biol, 2008; 8(1):48
- **Purta E**, O'Connor M, **Bujnicki JM.** Douthwaite S YccW is the m5C methyltransferase specific for 23S rRNA nucleotide 1962. J Mol Biol, 2008; 383:641-651
- **Pawlowski M, Gajda MJ, Matlak R, Bujnicki JM.** MetaMQAP: a meta-server for the quality assessment of protein models. BMC Bioinformatics, 2008; 9(1):403
- **Orlowski J, Bujnicki JM.** Structural and evolutionary classification of Type II restriction enzymes based on theoretical and experimental analyses Nucleic Acids Res, 2008; 36:3552-69
- **Feder M, Purta E, Koscinski L**, Cubrilo S, Vlahovicek G, **Bujnicki JM**. Virtual screening and experimental verification to identify potential inhibitors of the ErmC methyltransferase responsible for bacterial resistance against macrolide antibiotics. ChemMedChem, 2008; 3:316-322
- **Pietal M, Tuszynska I, Bujnicki JM.** PROTMAP2D: visualization, comparison, and analysis of 2D maps of protein structure. Bioinformatics, 2007; 23:1429-30
- **Tkaczuk KL, Dunin-Horkawicz S, Purta E, Bujnicki JM.** Structural and evolutionary bioinformatics of the SPOUT superfamily of methyltransferases. BMC Bioinformatics, 2007; 8:73
- **Orlowski, J, Boniecki M, Bujnicki JM.** I-Ssp6803I: the first homing endonuclease from the PD-(D/E)XK superfamily exhibits an unusual mode of DNA recognition. Bioinformatics, 2007; 23:527-30

- **Dunin-Horkawicz S, Feder M, Bujnicki JM.** Phylogenomic analysis of the GIY-YIG nuclease superfamily. BMC Genomics, 2006; 7(1):98
- **Tkaczuk KL, Obarska A, Bujnicki JM.** Molecular phylogenetics and comparative modeling of HEN1, a methyltransferase involved in plant microRNA biogenesis. BMC Evol Biol, 2006; 6(1):6

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. The laboratory comprises three sections:

1. A section devoted to the development of computer software for the analysis of biological macromolecules. The bioinformatics tools include a suite of programs for protein structure prediction and analysis available on the website https://iimcb.genesilico.pl/toolkit/ (MetaServer for primary, secondary, and tertiary structure prediction, methods for template-based and *de novo* modeling of three-dimensional protein structures, MQAPs for quality assessment of protein models, and FILTREST3D for discrimination of models according to their agreement with experimental data). We also develop methods for the prediction of order/disorder in protein structures (http://iimcb.genesilico.pl/metadisorder/) and databases of nucleic acid metabolism, including MODOMICS, a database for systems biology of RNA modification (http://modomics.genesilico.pl/) and the REPAIRTOIRE database for systems biology of DNA repair (http://repairtoire.genesilico.pl/).

2. A section devoted to the application of bioinformatics software to make biologically and biomedically relevant predictions. Recently published research includes analyses of various enzymes that act on nucleic acids, such as RNA methyltransferases involved in bacterial resistance against various antibiotics. Theoretical research in this section frequently involves collaborations with other laboratories interested in obtaining a structural model of their favorite proteins and experimentally testing our predictions. Recent analyses of protein structures (published in 2010) include, for example, a novel type of nuclease involved in the degradation of uracil-containing DNA in insects and the PluTI restriction enzyme.

3. A section devoted to experimental research on proteins and nucleic acids that uses methods of biochemistry, molecular biology, and cell biology. 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., restriction enzymes that recognize and cut new sequences).

The research in all three sections is tightly integrated, demonstrated by the publication of articles comprising a combination of theoretical and experimental analyses (e.g., prediction and characterization of new RNA methyltransferases or nucleases). In particular, protein engineering involves iterative protein structure model building, model-based experiment planning, a series of experimental analyses, and experiment-based improvement of the models and tools used for model building.

Recent highlights

Researchers from our laboratory, in collaboration with Joanna Krwawicz (IBB PAS, Warsaw), developed REPAIRtoire (http://repairtoire.genesilico.pl/), a database of processes and molecular entities involved in the repair of damaged DNA in cells. It contains information about the chemical structures of damaged nucleotides, biochemical pathways leading from a particular type of altered DNA to repaired DNA, genes and enzymes responsible for the biochemical reactions, and diseases caused by defects in DNA repair systems. REPAIRtoire connects DNA damage to potential causes of each lesion as well as to effects if they are not removed. It provides tools with which to study important aspects of DNA metabolism in the cell. The description of REPAIRtoire has been published in the *Nucleic Acids Research* annual database issue (2011).

Rankings of the 9th Community-Wide Experiment on the Critical Assessment of Techniques for Protein Structure Prediction (CASP9), organized by the Protein Structure Prediction Center (University of California, Davis), have shown high positions of protein structure prediction methods developed in this laboratory. In particular, the updated version of the GeneSilico metaserver for protein disorder prediction has again been ranked #1 in its category, similar to the previous edition, CASP8, in 2008. Among the model quality assessment tools, MQAPmulti was ranked #4 in the general ranking, and MQAPsingle was ranked #1 among non-clustering MQAPs. High scores have also been obtained by our new experimental methods for protein modeling by recombination of fragments (RecombineIt).

FILTREST3D (http://filtrest3d.genesilico.pl/) is another method recently added to the list of bioinformatics tools developed in our laboratory. Its purpose is to score and rank models of macromolecular structures according to consistency with user-defined restraints. Automatic methods for macromolecular structure prediction (fold recognition, *de novo* folding, and docking programs) typically produce large sets of alternative models. These large model sets often include many native-like structures that cannot be

discriminated from non-native structures according to purely computational criteria. However, such native-like models can be more easily identified based on data from experimental analyses used as structural restraints (e.g. identification of nearby residues by crosslinking, chemical modification, site-directed mutagenesis, deuterium exchange coupled with mass spectrometry, etc.). FILTREST3D allows the scoring of models based on a combination of distance restraints with other factors, such as local or global structure or molecule shape, and implements logical operators to enable sets of alternative restraints. It has been implemented as a standalone open-source Python program and a freely available web server. A publication describing FILTREST3D has appeared in *Bioinformatics,* 2010 Dec 1, 26(23):2986-2987.

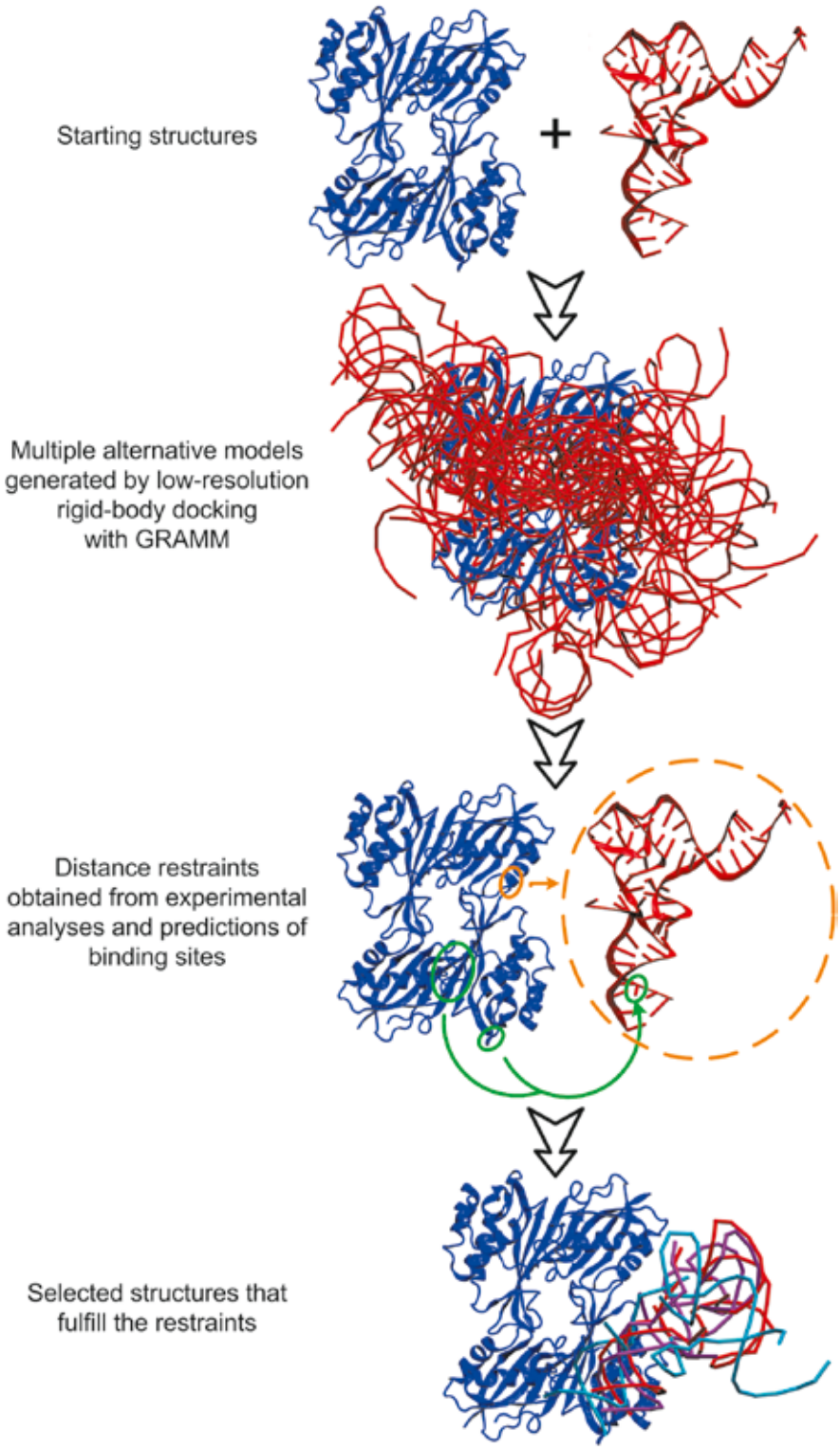
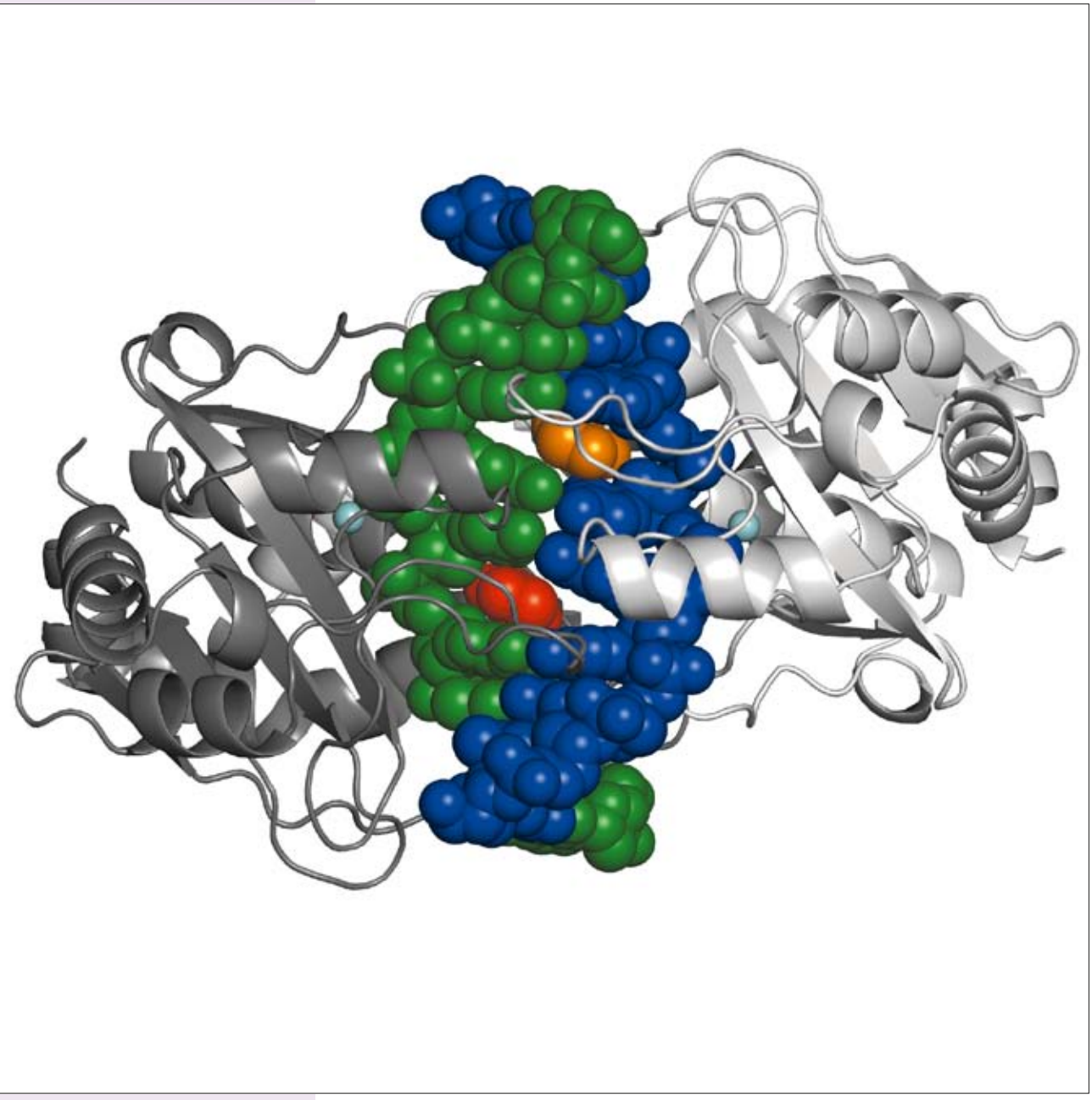


Fig. 1 A typical application of FILTREST 3D: Discrimination of native-like complexes among multiple decoys. Here, tRNA has been docked to a pseudouridine synthase TruA by GRAMM.



Crystal structure of the ThaI-DNA complex. DNA is shown in blue and green (in all atom representation), protein in grey (in ribbon representation), and the intercalating amino acids in red and orange (in all atom representations).

Laboratory of
Structural Biology MPG/PAN

Lab Leader:

Matthias Bochtler, PhD, Professor

Postdoctoral Fellows:

Honorata Czapińska, PhD

Monika Sokołowska, PhD

Roman Szczepanowski, PhD (part-time)

Junior Researchers:

Patrycja Haniewicz, MSc

Marek Wojciechowski, MSc

Researchers who left during 2010:

Grzegorz Chojnowski, PhD

Renata Filipek, PhD

Henryk Korza, PhD

Izabela Sabała, PhD

Jean-Philippe Borges, PhD

Technician:

Elżbieta Grzelak

MAX-PLANCK-GESELLSCHAFT

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY

The equipment and running costs for the lab, including personnel, are partly provided by the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (MPI-CBG).

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, MPI 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:

2010 - Present	Head, Structural Biology Laboratory, International Institute of Molecular and Cell Biology, Warsaw, Poland
2007 - Present	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, MPI of Biochemistry, Martinsried, Germany

HONORS, PRIZES, AND AWARDS:

1. 2005 Pieńkowski Award
2. 2004 EMBO/HHMI Young Investigator Award
3. 2000 Crystal Award, Germany
4. 1998 Crystal Award, Germany
5. 1990-1992 Scholarship from Deutsche Studienstiftung and Bavarian State

Recent publications

Protein-nucleic acid interactions

- **Sokolowska M, Czapinska H, Bochtler M**. Hpy188I-DNA pre- and post-cleavage complexes - snapshots of the GIY-YIG 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 ThaI-DNA 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 DNA-binding domain with a nonspecific nuclease. Proc Natl Acad Sci USA, 2005; 102:15797-802

Other (2010 only)

- **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-phox-deficient 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 Å 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

2010 has been a transition year for the group. The association of the laboratory with MPI-CBG in Dresden has come to an end, and several major grants have been completed. Four senior group members have left and taken membrane protein and method development projects with them. Continued funding has been available for the protein-nucleic acid interaction projects, and therefore the main results in 2010 are in this area. New funding for work on the structural biology of DNA methylation and hydroxymethylation and RNA-guided nucleases was requested in 2010 and has since been awarded. The work on these projects will commence in 2011 and focus on prokaryotic enzymes that could be useful as genetic tools and on key eukaryotic enzymes that interact with methylated and hydroxymethylated DNA. Two key results from 2010 are the following:

Hpy188I (TCN/GA): The Hpy188I enzyme was predicted to be a member of the GIY-YIG nuclease superfamily. GIY-YIG nucleases are present in all kingdoms of life and have very diverse roles. Well-characterized functions include transposon migration, flap cutting, and Holliday junction resolution in eukaryotes and nucleotide excision repair in prokaryotes. Despite the many roles of GIY-YIG nucleases in prokaryotes, no GIY-YIG domain has been crystallized in complex with target DNA. Therefore, many questions about the catalytic mechanism of the GIY-YIG module still remain. We managed to crystallize Hpy188I in a ternary substrate and product complexes with DNA and metal ions in the active site (Fig. 1). Our structures suggest that GIY-YIG nucleases catalyze DNA hydrolysis by a single substitution reaction. They are consistent with a previous proposal that a tyrosine residue (which we expect to occur in its phenolate form) acts as a general base for the attacking water molecule. In contrast to the earlier proposal, our data identify the general base with the GIY and not YIG tyrosine. A conserved glutamate residue (Glu149 provided in trans in Hpy188I) anchors a single metal cation in the active site. This metal ion contacts the phosphate proS oxygen atom and leaving group 3′-oxygen atom, presumably to facilitate its departure. Altogether, our data reveal striking analogy in the absence of homology between GIY-YIG and ββα-Me nucleases.

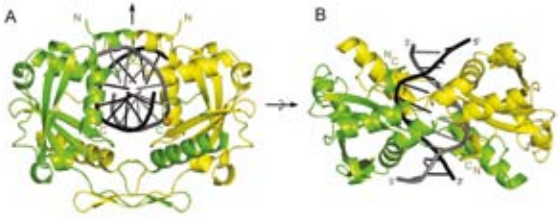


Fig. 1. Two perpendicular views of the GIY-YIG restriction endonuclease Hpy188I in complex with target DNA. Figure taken from Sokolowska et al., 2010.

endonuclease ThaI cuts the target sequence CG/CG with blunt ends. We solved the 1.3 Å resolution structure of the enzyme in complex with substrate DNA, with a sodium or calcium ion replacing a catalytic magnesium ion. The structure identifies Glu54, Asp82, and Lys93 as the active site residues, which is consistent with bioinformatics predictions. The interesting aspect of the otherwise canonical PD-(D/E)XK structure is the mode of DNA recognition. Two Met47 residues of the ThaI dimer symmetrically intercalate into the CG steps of the target sequence. They approach the DNA from the minor groove side and penetrate the base stack entirely. The DNA accommodates the intercalating residues without nucleotide flipping by a doubling of the CG step rise to twice its usual value, which is accompanied by drastic unwinding. Displacement of the Met47 side chains from the base pair midlines toward the downstream CG steps leads to large and compensating tilts of the first and second CG steps. Thus, DNA intercalation by ThaI is unlike intercalation by HincII, HinP1I, or proteins that bend or repair DNA and expands the "vocabulary" of protein-DNA interaction motifs.

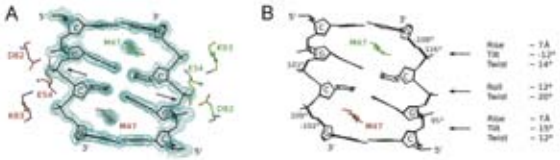
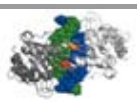
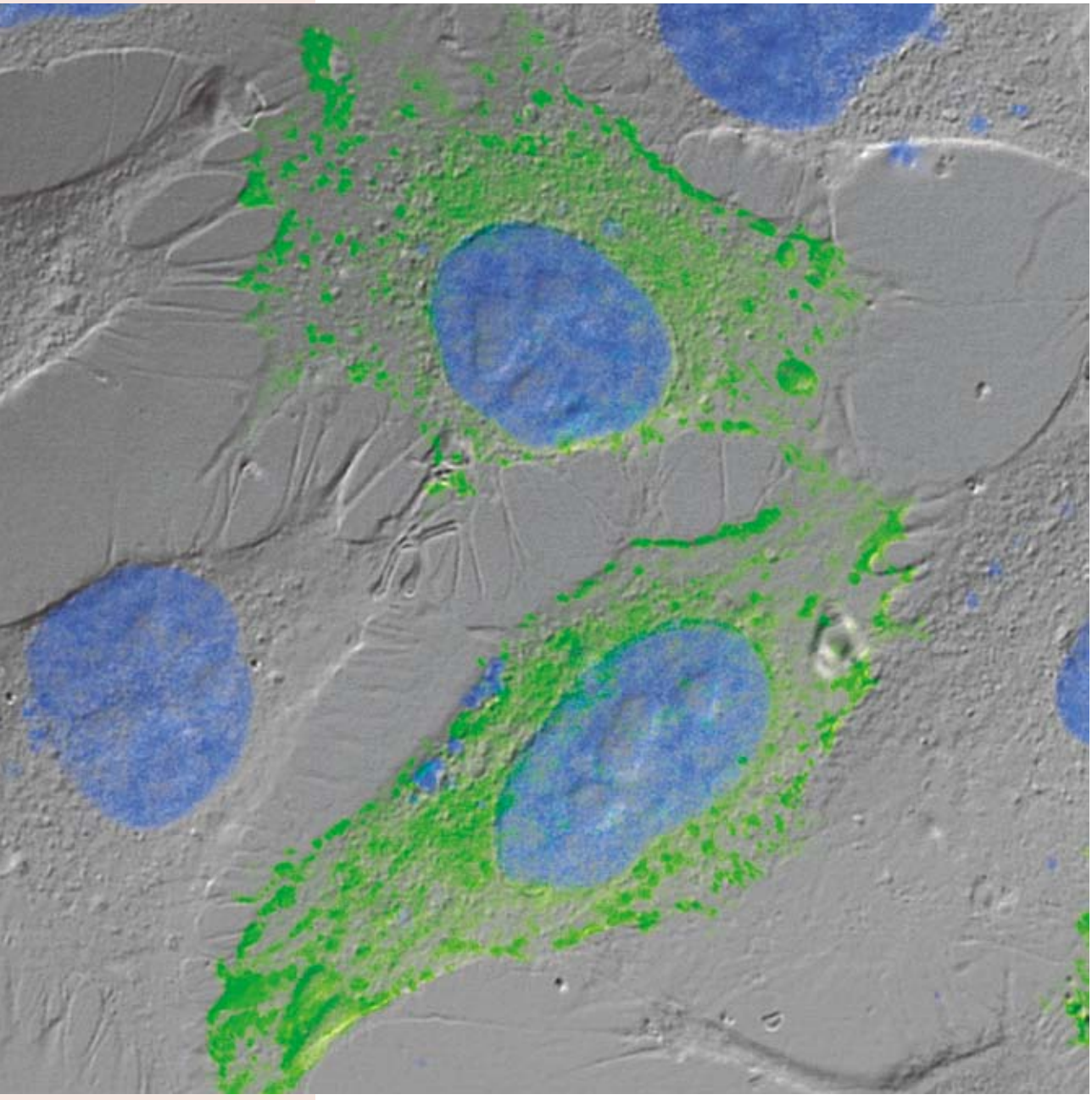


Fig. 2. Protein-DNA interactions in the ThaI-DNA complex. (A) Electron density for the DNA and intercalating methionines. (B) The most drastic DNA distortions are highlighted. Figure taken from Firczuk et al., 2010.





YFP-tagged STIM1 protein (green) in HeLa cells following depletion of calcium stores with thapsigargin (author T. Węgierski)

Laboratory of **Neurodegeneration**

Lab Leader:

Jacek Kuźnicki, PhD, Professor

Associate Professor, Vice Head:

Urszula Wojda, PhD, DSc. Habil.

Post-doctoral Fellows:

Joanna Gruszczyńska-Biegała, PhD

Tomasz Węgierski, PhD

Marta Wiśniewska, PhD

Junior Researchers:

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Katarzyna Dębowska, MSc

Anna Jaworska, MSc

Katarzyna Misztal, MSc

Andrzej Nagalski, MSc

Aleksandra Szybińska, MSc

MSc Students:

Nicola Brożko

Danuta Korona

Current affiliations of former PhD and MSc students:

Mateusz Ambrożkiewicz, MSc/PhD Program in Neurosciences, Georg-August University and European Neurosciences Institute in Göttingen/International Max Planck Research School

Magdalena Błażejczyk, a postdoctoral research fellow, Laboratory of Molecular and Cellular Neurobiology, IIMCB

Łukasz Bojarski, a research group leader, New Therapies of Neurological Diseases, Celon Pharma, www.celonresearch.com

Bożena Kuźniewska, PhD student at 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 MA

Adam Sobczak, a postdoctoral research fellow, Institute of Genetics and Biotechnology, Warsaw University, and Technology Transfer Unit of BioCentrum Ochota.

Jacek Kuźnicki, PhD, Professor

DEGREES:

- Professor, 1993
- 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, Science Policy Committee, Ministry of Science and Higher Education
1 Jul 2010-31 Dec 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 FP 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 - Present	Member, Polish Society for the Advancement of Science and Arts
1996-1999	and 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:

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

- **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, accepted April 2011
- **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 Jan 6 Epub ahead of print
- **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
- **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, 2010 Jun 10 Epub ahead of print
- **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**. Presenilin-dependent 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:

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. Biomarkers and potential therapeutic targets in Alzheimer's disease, based on:
   - 2.1. Cell cycle analyses
   - 2.2. Mutated p53

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, Aleksandra Szybińska, Tomasz Węgierski)*

The interaction between Ca^{2+} sensors STIM1 and STIM2 and Ca^{2+} 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. To gain insights into the neuronal function of STIM proteins, we focused on identifying their interacting partners in the brain by undertaking both a proteomic approach, using mass spectrometry, and a yeast genetics approach, using a split-ubiquitin screening system. The identified hits are currently being analyzed ***(Danuta Korona, Joanna Gruszczyńska-Biegała, Tomasz Węgierski)***.

In parallel, we tested the hypothesis that the mechanism of SOCE in neuronal cells is based on STIM proteins and that alterations of SOCE may lead to pathology, such as Alzheimer's disease. Our data indeed indicate that STIM1 and STIM2 proteins are involved in calcium homeostasis in neurons. Real-time PCR from cortical neurons proved that these cells contain significant amounts of *Stim*1 and *Stim*2 mRNA. In cultured cortical neurons that overexpress YFP-STIM1, YFP-STIM2, and ORAI1, we found that calcium depletion from the endoplasmic reticulum (ER) increased the number of STIM1/ORAI1 puncta much more than STIM2/ORAI1 puncta. In contrast, a reduction of extracellular calcium levels triggered puncta formation to a greater number for YFP-STIM2/ORAI1 than for YFP-STIM1/ORAI1. Our preliminary data indicate that the number of endogenous STIM2/ORAI1 complexes visualized by the Proximity Ligation Assay (Fig. 1) was enhanced by EGTA treatment. Thapsigargin (TG) treatment increased the amount of both endogenous STIM proteins in neuronal membrane fractions. Our results indicate that STIM1

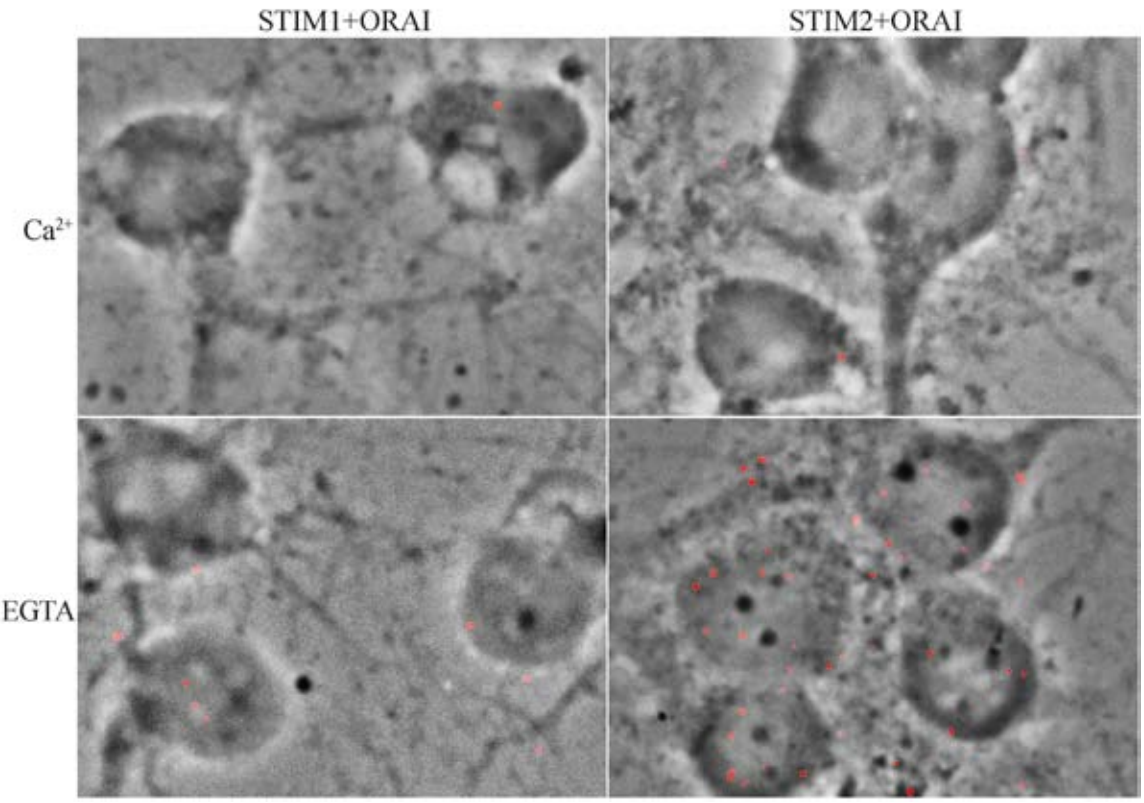


Fig. 1. Analysis of the complexes formed from endogenous STIMs and ORAI1 using the Proximity Ligation Assay. Cortical neurons treated with 2 mM Ca^{2+} or 2 mM EGTA were incubated with mouse anti-STIM1 and rabbit anti-ORAI1 or goat anti-STIM2 and rabbit anti-ORAI1. The PLA signal, visualized as a fluorescent red dot, suggests the close proximity of STIM and ORAI1 antigens. Sample cell images with an overlay of fluorescence signal over phase contrast show that endogenous STIM and ORAI1 complexes are localized in the cells.

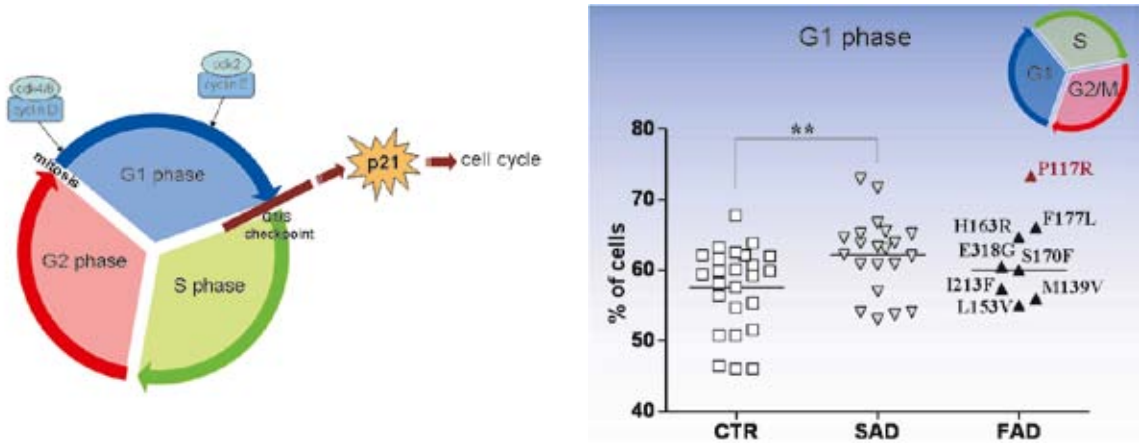


Fig. 2. Cell cycle aberrations in lymphocytes from patients with Alzheimer's disease
(Left) Schematic illustration of aberrant G1 phase cell cycle regulators identified in SAD lymphoblasts. The most significant change was detected in p21 protein levels. (Right) Increased percentage of cells in G1 phase in lymphoblasts from SAD patients detected by flow cytometry upon labeling with propidium iodide.

is the main activator of TG-induced SOCE in neurons, whereas STIM2 regulates resting Ca^{2+} levels and activates constitutive calcium entry (*Gruszczynska-Biegala et al.,* PLOS one, 2011).

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

Multiple neuronal functions rely on calcium signaling mediated by a group of Ca^{2+}-binding proteins, such as calmodulin and calcineurin. 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 and localization of CaMy1 in the brain and found that CaMy1 is involved in Alzheimer's disease *(Calcium Binding Proteins 2008; BBA-Mol Mech Diseases 2006; Neuropathol Appl Neurobiol 2005; Acta Biochim Pol 2005).* Additionally, we identified the SCG10 protein (stathmin2) as a novel CaMy1 ligand in developing human brain. SCG10 is a microtubule-destabilizing factor that plays a role in neuronal growth during brain development. We found increased mRNA and protein levels of CaMy1 during neuronal development, which paralleled the changes in SCG10 levels. In developing primary rat hippocampal neurons in culture, CaMy1 and SCG10 colocalized in cell soma, neurites, and growth cones. Pull-down, coimmunoprecipitation, and proximity ligation assays demonstrated that the interaction between CaMy1 and SCG10 is direct and Ca^{2+}-dependent in vivo. CaMy1 interfered with SCG10 activity in a microtubule polymerization assay, and CaMy1 overexpression inhibited SCG10-mediated neurite outgrowth in nerve growth factor-stimulated PC12 cells. Altogether, these data suggest that CaMy1, via SCG10, couples Ca^{2+} signals with the dynamics of microtubules during neuronal outgrowth in the developing brain *(Sobczak et al., BBA-Mol Cell Res 2011 Jan 5. [Epub ahead of print]).* We further investigate the physiological significance of CaMy1/SCG10 interactions in developing neurons.

Moreover, we pursued studies on CaMy2. CaMy2 transcript and protein were detected mainly in the hippocampus and cortex in the rat brain. Studies of rat primary hippocampal neurons showed that CaMy2 levels are controlled by NMDAR and Ca^{2+} and suggest a role for CaMy2 in the Ca^{2+} signaling that underlies NMDAR activation *(Arch Biochem Biophys 2009).* We cloned CaMy2 from the rat brain and demonstrated that CaMy2 binds Ca^{2+} and exhibits features of the Ca^{2+}-sensor protein. We also identified new potential targets of CaMy2 in the rat brain by affinity chromatography followed by mass spectrometry and confirmed these interactions by several methods in vitro. The physiological significance of these interactions in neurons is currently under investigation.

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)*

Many studies showed that disturbed cellular calcium homeostasis is one of the features of Alzheimer's disease (AD). Calcium dyshomeostasis is an early event in AD pathogenesis that precedes other disease symptoms and can affect many cellular processes. Thus, finding a drug that is able to restore normal calcium signaling is important. In collaboration with Dr. Jochen Herms, we started a novel approach using high-throughput screening of chemical compounds based on intracellular calcium level measurements. The screen was conducted on several stably transfected HEK 293 cell lines that bear human mutated or wildtype PS1 and FRET-based fluorescent calcium sensor Yellow Cameleon 3.60. The created cell lines were subjected to treatment with a set of compounds. Changes in their fluorescence levels, reflecting the calcium response, were measured using an OPERA system. Preliminary data were presented at the Society for Neuroscience meeting in San Diego in 2010.

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

Some molecular changes in AD 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 AD. Additionally, human lymphocytes have potential diagnostic value. In our studies, we use B-lymphocytes from AD patients immortalized with EB-virus.

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

Mounting evidence indicates that the aberrant expression of cell cycle molecules in the brain contributes to the development of AD and causes neuronal death. We analyzed whether cell cycle alterations occur in lymphocytes from patients with sporadic and familial forms of AD with distinct PS1 mutations (SAD and FAD). The results of our experiments using real-time PCR arrays, immunoblotting, and flow cytometry demonstrated differences in the regulation of G1/S phases between SAD lymphocytes and cells from non-demented subjects, as well as between SAD and FAD cells. Compared with FAD lymphocytes, SAD lymphocytes showed differences in the expression profiles of 90 cell cycle genes, such as the genes encoding cyclin D and cyclin E, and a marked increase in the level of the p21 protein, which promotes G1-arrest. Accordingly, SAD but not FAD cells had a prolonged G1-phase. These data showed that SAD involves a prolongation of the G1 phase driven by the p21 pathway, which is not activated in FAD cells. Thus, the mechanism of SAD differs from FAD *(Bialopiotrowicz et al., Neurobiol Aging 2010)*. We are continuing studies of the molecular and cellular aspects of cell cycle regulation in lymphocytes obtained from AD patients and investigate whether these cells may have diagnostic potential.

2.2. Mutated p53 *(Aleksandra Szybińska; cooperation: Maurizio Memo and Daniela Uberti, University of Brescia)*

Our collaborative studies revealed an increased level of conformationally altered p53 protein in immortalized B lymphocytes from patients with sporadic and familial AD compared with lymphocytes from healthy controls. Thus, the conformational p53 mutant may be used as a marker to discriminate between AD and non-AD individuals *(C. Lanni et al., Mol Psych 2008)*. Because the p53 conformational tertiary structure is influenced by the redox status of cells, an evaluation of the oxidative profile of these patients was performed. We found that among the markers of oxidative stress, hydroxytransnonenal-modified proteins were significantly increased in FAD patients. Furthermore, in addition to increased levels of oxidative markers, the antioxidant defense mechanisms were compromised in these patients because of decreased enzyme levels, such as superoxide dismutase. We also measured p53-regulated CD44 gene expression levels in lymphocytes from AD patients and healthy controls. Alzheimer's disease lymphocytes showed significantly higher CD44 levels compared with controls, with increased misfolded p53 levels. This finding may suggest that interactions between these two proteins can influence the peripheral immunological response during disease development *(Uberti et al., Neurodeg Dis 2010)*. These results support the evidence of an association between peripheral unfolded p53 and AD pathology and indicate that immortalized peripheral cells from AD patients are suitable models for the identification of disease markers.

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

β-catenin participates in two distinct functions in a cell, serving as (i) a component of cadherin-based adherens junctions, and (ii) a gene expression regulator in canonical Wnt signaling as a cofactor of LEF1/TCF transcription factors. In the developing brain, nuclear β-catenin activates genes involved in the proliferation and differentiation of neuronal precursor cells. Interestingly, aberrant regulation of the Wnt pathway in the adult brain has been associated with neurodegenerative diseases and mood disorders. However, the issue of the physiological role and regulation of the Wnt/β-catenin pathway in mature neurons is far from resolved.

We recently demonstrated that, specifically in thalamic neurons of the adult brain, β-catenin is constitutively nuclear. By exploring the mechanism of this phenomena, we found that neither disruption of the thalamic environment nor inhibition of Wnt/Dishevelled signal transduction affects nuclear levels of β-catenin in these cells, suggesting the existence of a mechanism that operates downstream of the WNT receptor. We reported that the β-catenin degradation rate is lower in thalamic neurons than in cortical neurons, which appears to be a consequence of low level of the β-catenin degradation complex (APC/AXIN1/GSK3β). Thus, the nuclear localization of β-catenin in thalamic neurons appears to be a cell-autonomous and cell-intrinsic feature *(Misztal, Wisniewska, Ambrozkiewicz, Kuznicki, under revision)*. We also showed that β-catenin, together with LEF1 transcription factor, regulates the Cacna1g gene, encoding the Cav3.1 T-type calcium channel subunit that contributes to electrical signal propagation in thalamic neurons and is involved in epilepsy *(Wisniewska et al., J Neurosci 2010)*.

Our main goal is to further identify new β-catenin-LEF1/TCF target genes, specific for neurons, which may help understand the role of nuclear β-catenin in the adult brain. We perform in silico analyses (e.g., screening of conserved noncoding sequences for transcription factor motifs) and analyses of spatially correlated gene expression in the brain. These investigations are performed in collaboration with Dr. Michal Dąbrowski, Nencki Institute, Warsaw. We also use custom-designed PCR arrays to profile gene expression in the brain and adenovirus-transduced primary neurons (Fig. 3). To experimentally confirm the actual targets, we perform luciferase assays, footprinting, and chromatin immunoprecipitation.

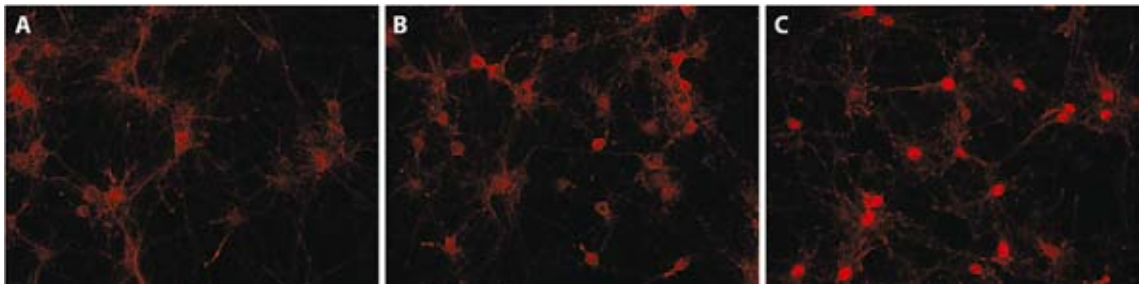
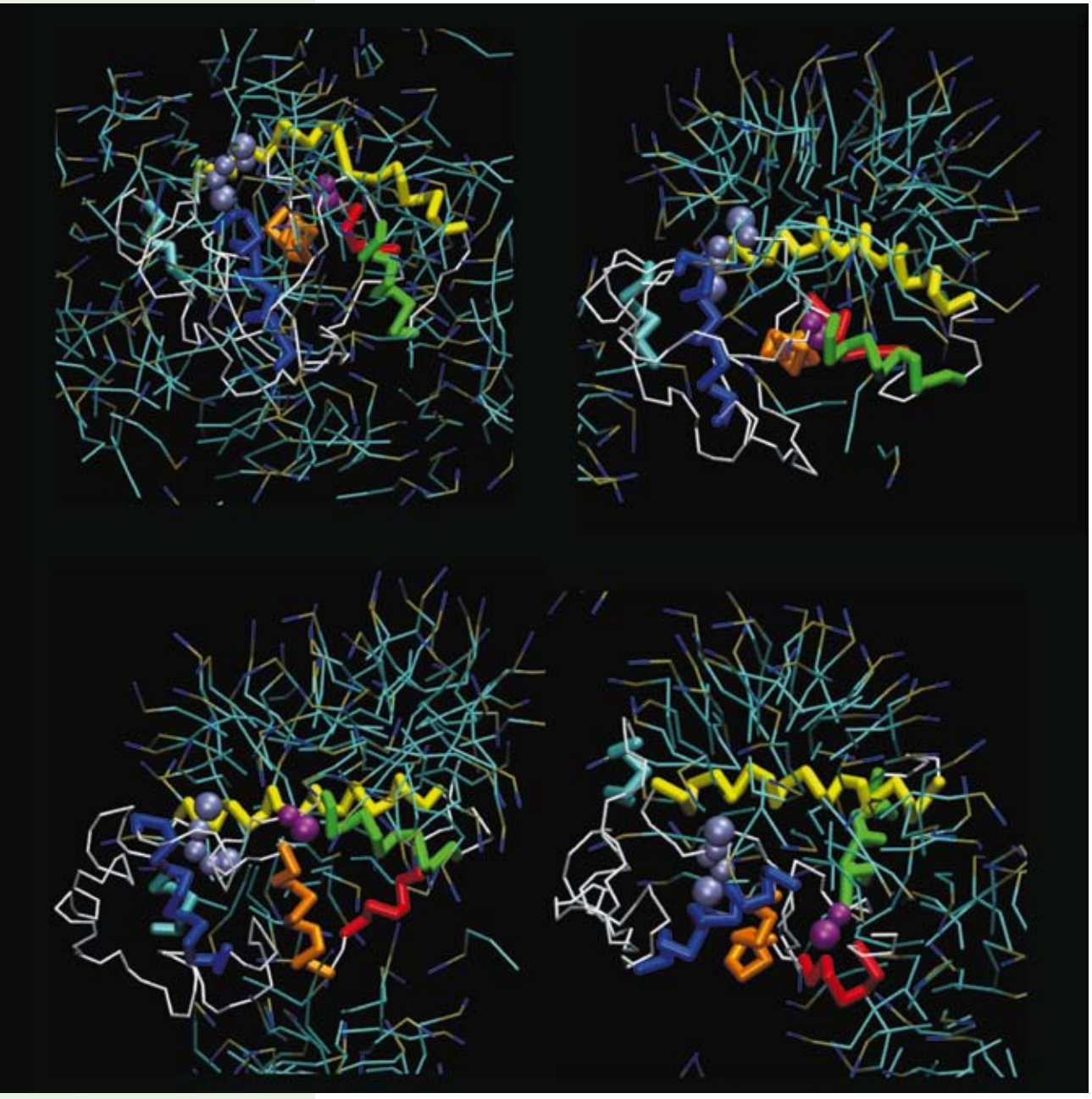
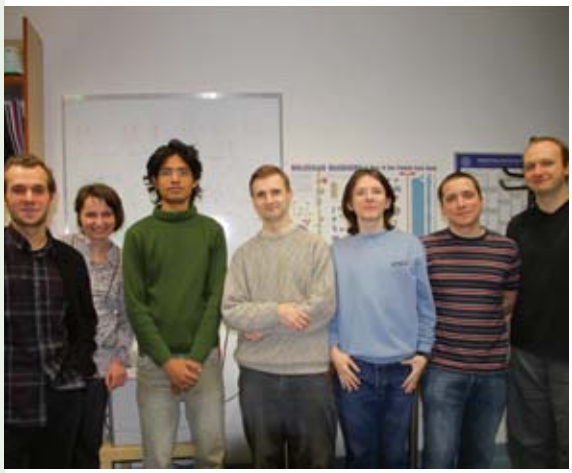


Fig. 3. Localization of β-catenin in cortical neurons treated with LiCl and overexpressing LEF1/TCF7L2.
Cortical primary cultures were labeled with β-catenin-specific mouse monoclonal antibody. (A) Control neurons. (B) Neurons treated with 10 mM LiCl. (C) Neurons transduced with LEF1 and TCF7L2 expressing adenoviruses and treated with 10 mM LiCl.



The structure of CTF presenilin during 3 µs coarse-grained molecular dynamics simulation in DPC micelles. The starting structure of CTF was taken from NMR measurements while detergent molecules were randomized (left-top panel). The catalytic residue D385 is shown as purple spheres (two spheres in coarse-grained representation), and the PAL motif is shown as blue spheres (Umesh Ghoshdastider).

Laboratory of **Biomodelling**



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- *The Open Structural Biology Journal*

PUBLICATIONS:

- Over 70 publications in primary scientific journals
- Over 2000 citations

Selected publications

- **Latek D, Kolinski M, Ghoshdastider U, Debinski A**, Bombolewski R, Plazinska A, Jozwiak K, **Filipek S**. Modeling of ligand binding to G protein coupled receptors: cannabinoid CB1, CB2 and adrenergic β_2AR. J Mol Model, 2011, accepted.
- Sobhanifar S, Schneider B, Löhr F, Gottstein D, Ikeya T, **Mlynarczyk K, Pulawski W, Ghoshdastider U, Kolinski M, Filipek S**, Güntert P, Bernhard F, Dötsch V. Structural investigation of the C-terminal catalytic fragment of presenilin-1. Proc Natl Acad Sci US, 2010; 107:9644-9
- **Kolinski M, Filipek S**. Structurally similar pair of agonist and antagonist of kappa opioid receptor studied by molecular dynamics simulations. J. Mol. Model. (2010) 16, 1567-1576.
- **Jozwiak K, Krzysko KA, Bojarski L**, Gacia M, **Filipek S**. Molecular models of the interface between anterior pharynx-defective protein 1 (APH-1) and presenilin involving GxxxG motifs. ChemMedChem, 2008; 3:627-634
- **Jozwiak K**, Zekanowski C, **Filipek S**. Linear patterns of Alzheimer's disease mutations along alpha-helices of presenilins as a tool for PS-1 model construction. J Neurochem, 2006; 98:1560-72
- **Modzelewska A, Filipek S**, Palczewski K, Park PS. Arrestin interaction with rhodopsin: conceptual models. Cell Biochem Biophys, 2006; 46:1-15

Publications in 2010-2011

- **Latek D**, Kolinski M, **Ghoshdastider U, Debinski A**, Bombolewski R, Plazinska A, **Jozwiak K, Filipek S**. Modeling of ligand binding to G protein coupled receptors: cannabinoid CB1, CB2 and adrenergic β_2AR. J Mol Model, 2011, accepted
- Koch R, Lipton AS, **Filipek S,** Renugopalakrishnan V. Arginine interactions with anatase TiO2 (100) surface and the perturbation of 49Ti NMR chemical shifts – A DFT investigation: Relevance to Renu-Seeram Bio Solar Cell. J Mol Model, 2011, accepted
- *Kilanczyk E, **Filipek S**, Filipek A. ERK1/2 is dephosphorylated by a novel phosphatase - CacyBP/SIP. Biochem Biophys Res Commun, 2011; 404:179-183
- Sobhanifar S, Schneider B, Löhr F, Gottstein D, Ikeya T, **Mlynarczyk K, Pulawski W, Ghoshdastider U, Kolinski M, Filipek S**, Güntert P, Bernhard F, Dötsch V. Structural investigation of the C-terminal catalytic fragment of presenilin-1. Proc Natl Acad Sci USA, 2010; 107:9644-9
- **Kolinski M, Filipek S**. Structurally similar pair of agonist and antagonist of kappa opioid receptor studied by molecular dynamics simulations. J Mol Model, 2010; 16:1567-76
- **Zurawska A, Urbanski J**, Matuliene 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.

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Current Research

1. Studies of activation mechanisms of G-protein-coupled receptors

Recent crystal structures of class A (rhodopsin-like) G-protein-coupled receptors (GPCRs), namely β_1- and β_2-adrenergic receptors and adenosine A_{2A} receptors, showed nearly identical transmembrane domain structures but differences in the states of molecular switches compared with rhodopsin, which was the first GPCR crystallized. Based on experimental data, agonist binding and receptor activation were proposed to occur through a series of conformational intermediates. The transition between these intermediate states involves disruption, creation, or reorganization of intramolecular interactions that stabilize the basal state of a receptor. These changes are elicited by the action of molecular switches (also called microswitches). The major switches proposed so far for different GPCRs, reflecting shared activation mechanisms, include the "rotamer toggle switch" that involves the CWxPx(F/H) sequence on transmembrane helix TM6, the switch based on the $NPxxY(x)_{(5,6)}F$ sequence that links helices TM7 and H8, and the "ionic lock" that links transmembrane helices TM3 and TM6 and employs the (E/D)RY motif on TM3. There are also switches not assigned to any particular sequence motifs, such as the "3-7 lock" that involves the interaction between TM3 and TM7 and is present only in selected receptor types.

In our earlier papers, we investigated early activation steps that occur simultaneously to ligand binding in the MOR (mu), DOR (delta), and KOR (kappa) opioid receptors. The first switch that was broken by agonist binding was the "3-7 lock," a hydrogen bond D3.32-Y7.43 that links transmembrane helices TM3 and TM7. It was the first activation event observed. We also detected the action of a second switch: a rotamer toggle switch that involves a simultaneous change of side chain conformations of W6.48 and adjacent residues and was therefore called the extended toggle switch. In the case of opioid receptors, the other residue in this extended switch was H6.52. This residue also participated in the agonist-antagonist sensor, determined by the propensity for creating a hydrogen bond with Y3.33 for antagonists and H6.52 for agonists. All studied ligands, analogs of morphine with a common tyramine structural scaffold, created a salt bridge with D3.32 with their protonated nitrogen atom of the tyramine group. This sensor was studied by us for MOR and later DOR and KOR. The proposed mechanism of its action was later confirmed by molecular dynamics simulations of a closely related agonist-antagonist pair of KOR ligands: 5'-GNTI and 6'-GNTI.

Cannabinoid receptors, similarly to opioid receptors, belong to class A (similar to rhodopsin) GPCRs. The docking of agonists and antagonists to cannabinoid CB_1 and CB_2 receptors revealed the importance of a centrally located rotamer toggle switch and its possible participation in the mechanism of agonist/antagonist recognition. The switch is composed of two residues, F3.36 and W6.48, located on opposite transmembrane helices TM3 and TM6 (which is dissimilar to other GPCRs) in the central part of the membranous domain of cannabinoid receptors. The CB_1 and CB_2 receptor models were constructed based on the adenosine A_{2A} receptor template. The two best-scored conformations of each receptor were used for the docking procedure. In all poses (ligand-receptor conformations) characterized by the lowest ligand-receptor intermolecular energy and free energy of binding, the ligand type matched the state of the rotamer toggle switch; antagonists maintained an inactive state of the switch, whereas agonists changed it (Fig. 1).

2. Structure of presenilin, a component of γ-secretase

The integral membrane protein ensemble γ-secretase is responsible for the proteolytic processing of various type I transmembrane domains, among them the amyloid precursor protein, whose final cleavage results in the release of the amyloid β peptide which is the major component of senile plaques found in Alzheimer's disease. The catalytic activity of γ-secretase requires the endoproteolytic cleavage of its presenilin subunit during the maturation of the complex. The cleavage results in

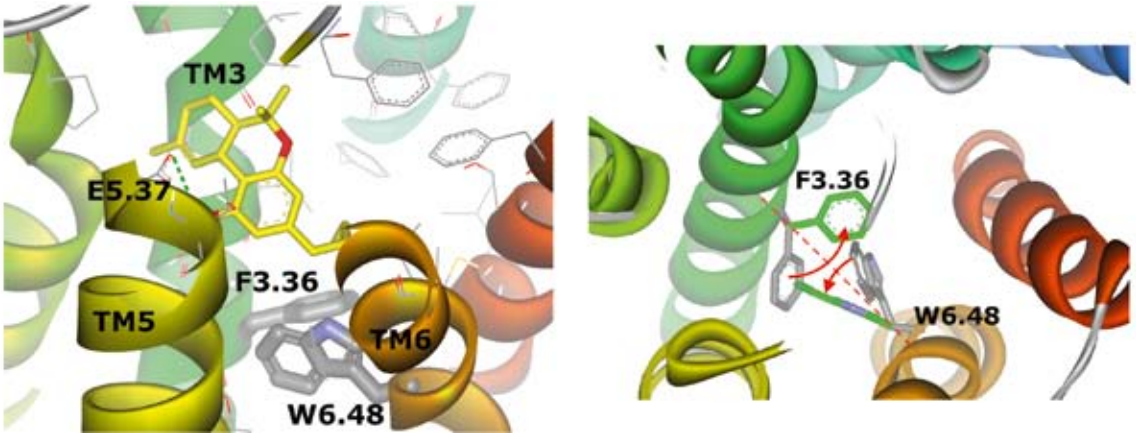


Fig. 1. The rotamer toggle switch in cannabinoid receptors comprises two residues, F3.36 and W6.48, which are located on transmembrane helices TM3 and TM6. The docking of agonists and antagonists to cannabinoid CB_1 and CB_2 receptors revealed the importance of this centrally located switch and its possible participation in the mechanism of agonist/antagonist sensing. The best-scored poses (ligand-receptor conformations) were obtained for the ligands that match the switch state—antagonists maintained the state of the rotamer toggle switch, whereas agonists changed it (Dorota Latek).

the generation of natural *N*- and *C*-terminal presenilin fragments, each of which harbors one of the two active-site aspartates that contribute to the formation of a hydrophilic cavity where catalysis is believed to occur. The *N*-terminal fragment of presenilin-1 is believed to have a classical transmembrane topology, consistent with all published models. Biochemical studies and topology predictions performed on the *C*-terminal fragment (CTF), however, have thus far yielded a plethora of ambiguous and contradictory results, which may be difficult to reconcile in the absence of structural information. By employing NMR measurements on cell-free expressed protein in SDS micelles, determining the first structural model of the *C*-terminal fragment of human presenilin-1 was possible. The structure revealed a topology by which the membrane would likely be traversed three times, consistent with the more generally accepted model, but contain unique structural features that may be adapted to accommodate the unusual intramembrane catalytic process and could account for the lack of consensus observed in most studies.

To further evaluate the structure of CTF, we performed molecular dynamics (MD) simulations both in micelles and in a lipid bilayer. To achieve the sufficient simulation time needed for micelle formation and extensive sampling of the conformational space, we chose a coarse-grain approach using the MARTINI method, which was primarily developed to study the behavior of large biological membrane systems. Simulations were performed with 200 coarse-grain dodecylphosphocholine (DPC) molecules, which formed a micelle consisting of approximately 80 molecules around CTF during the first 50 ns, during which time the structure of CTF was frozen. In the ensuing 3 γs simulation, the coarse-grain structure of CTF was allowed to change. The resulting structure, compared with the NMR input structure, is displayed in Fig. 2. The comparison with the initial NMR structure revealed that the interhelical angle between helices 7 and 8 remained relatively stable (140° in the NMR structure and 120° after the 3 γs simulation), and helices 9a and 9b became more antiparallel (105° in the NMR structure and 130° after the simulation).

We also performed coarse-grained simulations in DLPC (dilauroylphosphatidylcholine) and DPPC (dipalmitoylphosphatidylcholine) bilayers using the MARTINI method (Fig. 3) and MD simulations of all-atom representation of CTF by employing continuous environments with the Implicit Membrane Model (IMM1) method in the CHARMM program. As expected, the resulting structures showed larger differences both with respect to the NMR input structure and to each other. Although the angle between helices 7 and 8 remained relatively stable, the angle between helices 9a and 9b changed depending on the thickness of the membrane. Additionally, the position of the catalytic helix also shifted from the center of the membrane toward its border, again depending on the membrane width. Notably, however, in the present case, the bilayer is not necessarily a better suited environment than a micelle because several elements of CTF are believed to reside in close proximity or even contribute to the formation of the water-filled cavity that exists within the γ-secretase complex. In such a case, the more accommodating micelle may indeed provide a better hydrophobic environment for CTF in the absence of the other γ-secretase components.

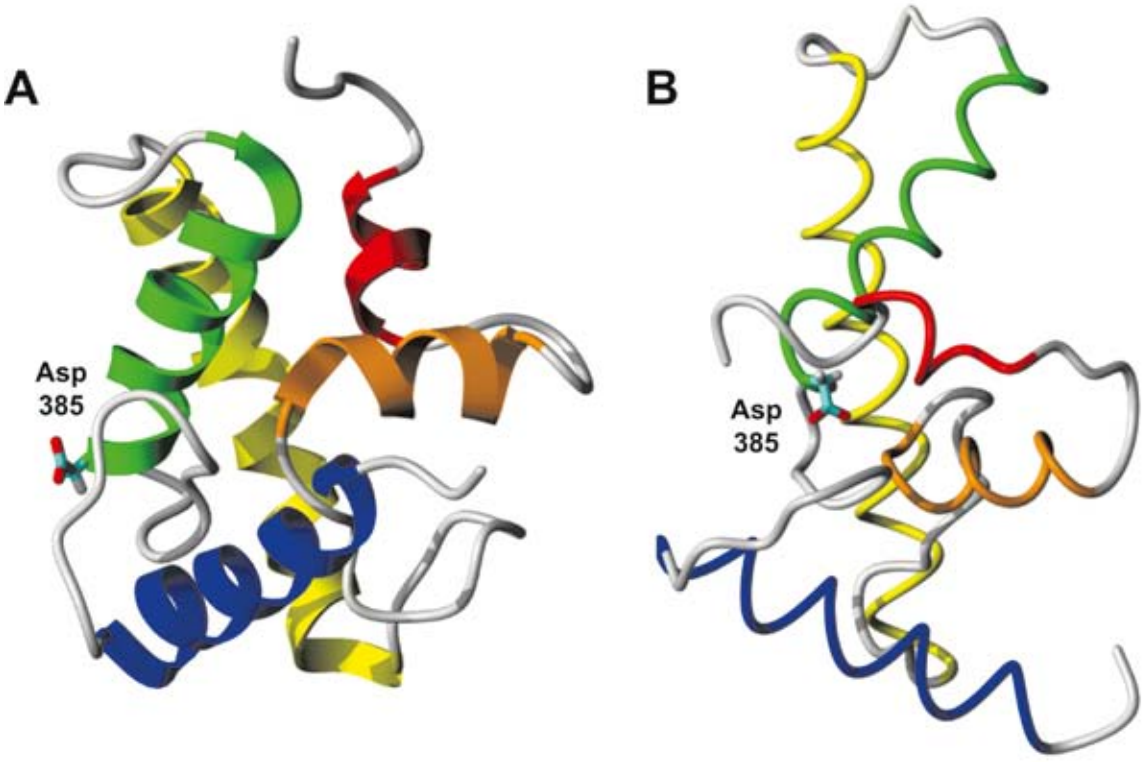


Fig. 2. Comparison of CTF NMR and MD simulated structures. (A) Ribbon diagram of the lowest energy conformer from NMR investigation. (B) C^{α}-trace after 3 μs of coarse-grained MD simulation in a DPC detergent/water mixture. Color scheme: helix β in blue, helix 7 in green, helix 8 in yellow, helix 9a in orange, and helix 9b in red. Structures are shown without the long *N*-terminal loop for simplicity (Sławomir Filipek).

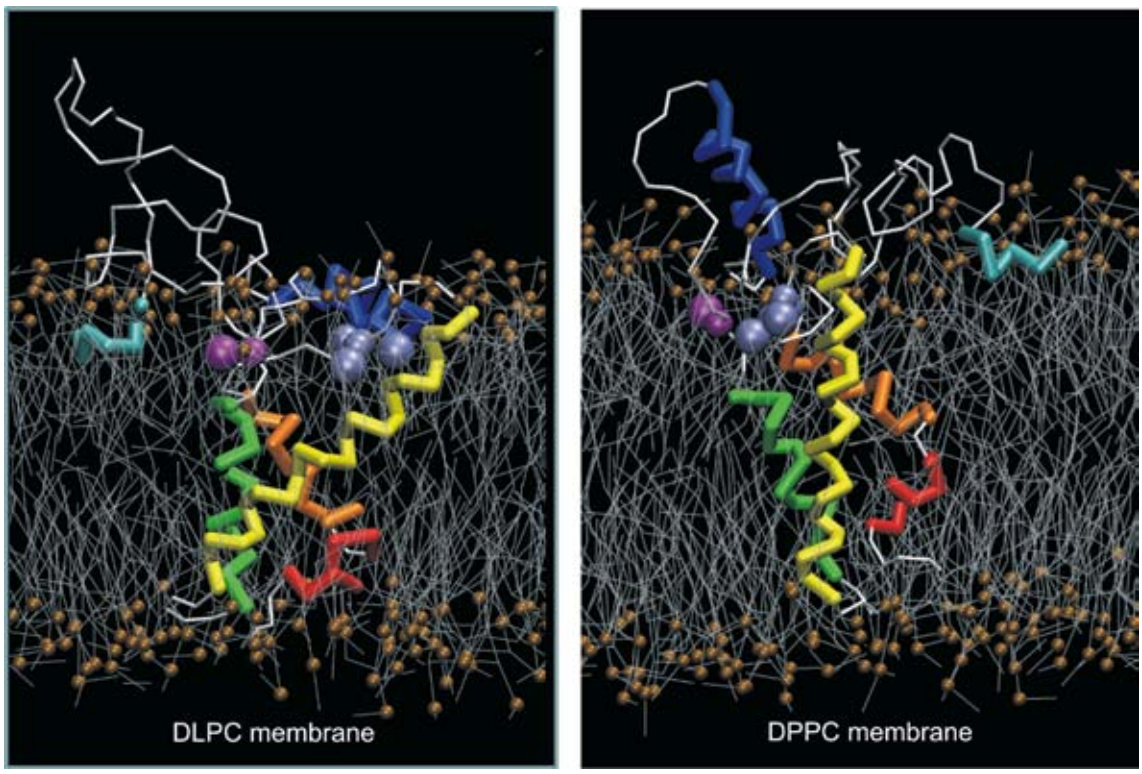
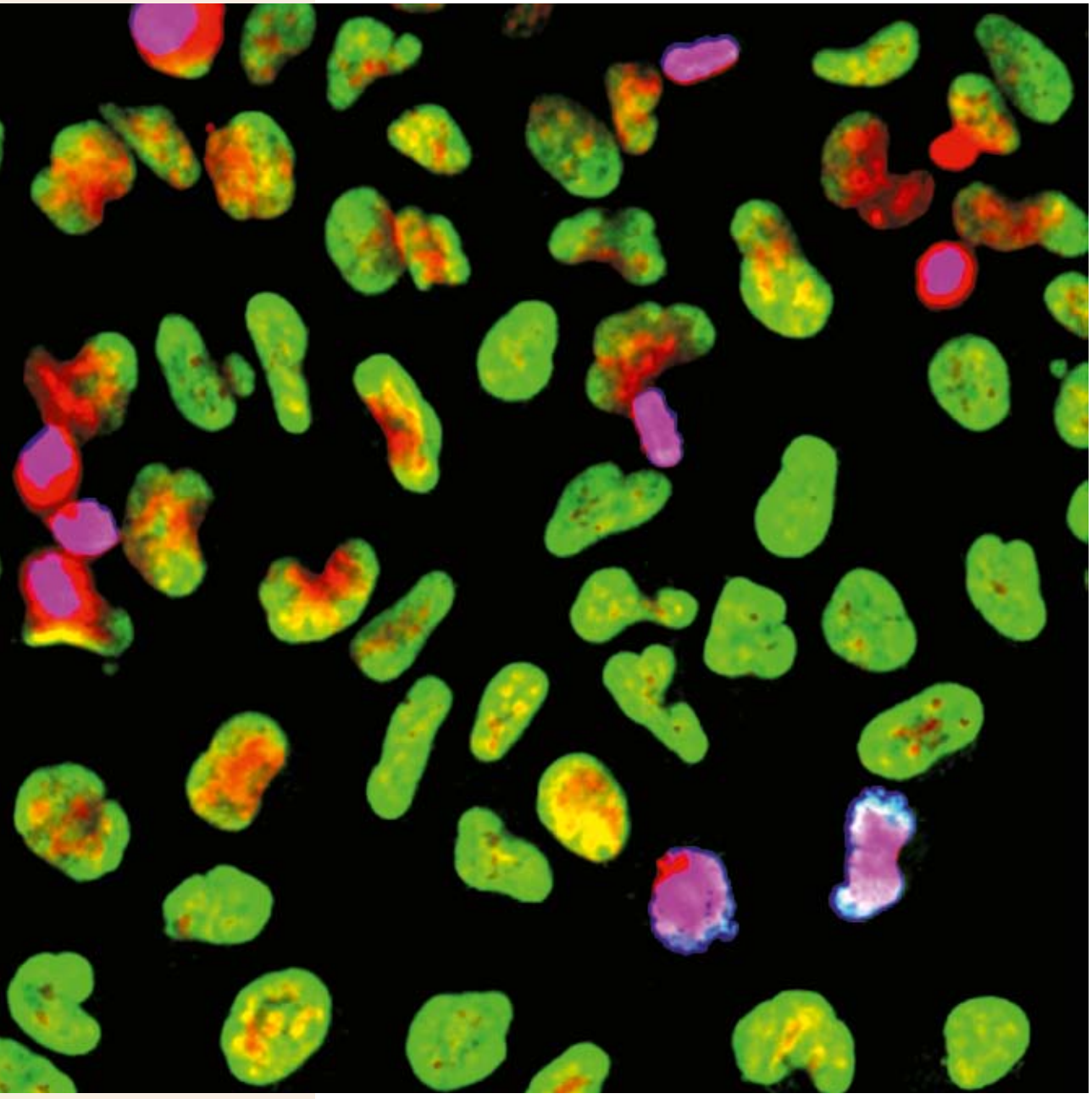


Fig. 3. Representative structure of CTF presenilin-1 in a DLPC (dilauroyl-) and DPPC (dipalmitoyl-phosphatidylcholine) membrane after 1 μs of coarse-grained simulation following a 1 μs simulation that started from a random distribution of lipids. The catalytic D385 is shown as two purple spheres (coarse-grained representation), and the PAL motif is represented as blue spheres (Krzysztof Młynarczyk).



Nuclei of HEK293 cells stained with DAPI (red) and antibodies against bromodeoxyuridine (green) and phosphorylated histone H3 (blue) (author: Daniela Chmiest).

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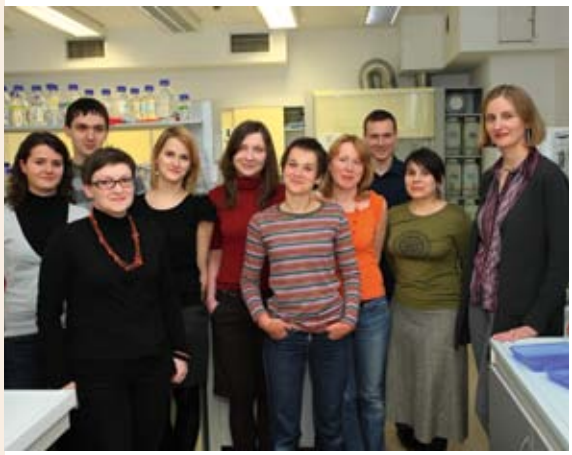

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DEGREES:

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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

- **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(8):1093-107
- **Miaczynska M**, Bar-Sagi D; Signaling endosomes: seeing is believing. 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
- **Olchowik M**, **Miaczynska M**. Effectors of GTPase Rab5 in endocytosis and signal transduction. Postepy Biochem, 2009; 55:171-180
- *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
- **Pilecka I**, **Miaczynska M**. Clathrin-dependent endocytosis – mechanisms and significance. (in Polish), chapter of a book „At the frontiers of chemistry and biology", edited by J. Barciszewski, the Publishing House of Mickiewicz University in Poznan, 2006
- *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
- ***Miaczynska M**, Zerial M. Mosaic organisation of the endocytic pathway (Review). Exp Cell Res, 2002; 272:8-14
- *Lippe R, **Miaczynska M**, Rybin V, Runge A, Zerial M. Functional synergy between Rab5 effector Rabaptin-5 and exchange factor Rabex-5 when physically associated in a complex. Mol Biol Cell, 2001; 12:2219-28
- *Nielsen E, Christoforidis S, Uttenweiler-Joseph S, **Miaczynska M**, Dewitte F, Wilm M, Hoflack B, Zerial M.

Rabenosyn-5, a novel Rab5 effector is complexed with hVPS45, and is recruited to endosomes through a FYVE finger domain. J Cell Biol, 2000; 151:601-612

- *Rubino M, **Miaczynska M**, Lippe R, Zerial M. Selective membrane recruitment of EEA1 suggests a role in directional transport of clathrin-coated vesicles to early endosomes. J Biol Chem, 2000; 275:3745-48
- *Christoforidis S, **Miaczynska M**, Ashman K, Wilm M, Zhao L, Yip SC, Waterfield MD, Backer JM, Zerial M. Phosphatidylinositol-3-OH kinases are Rab5 effectors. Nat Cell Biol, 1999; 1:249-252

* 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 (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).

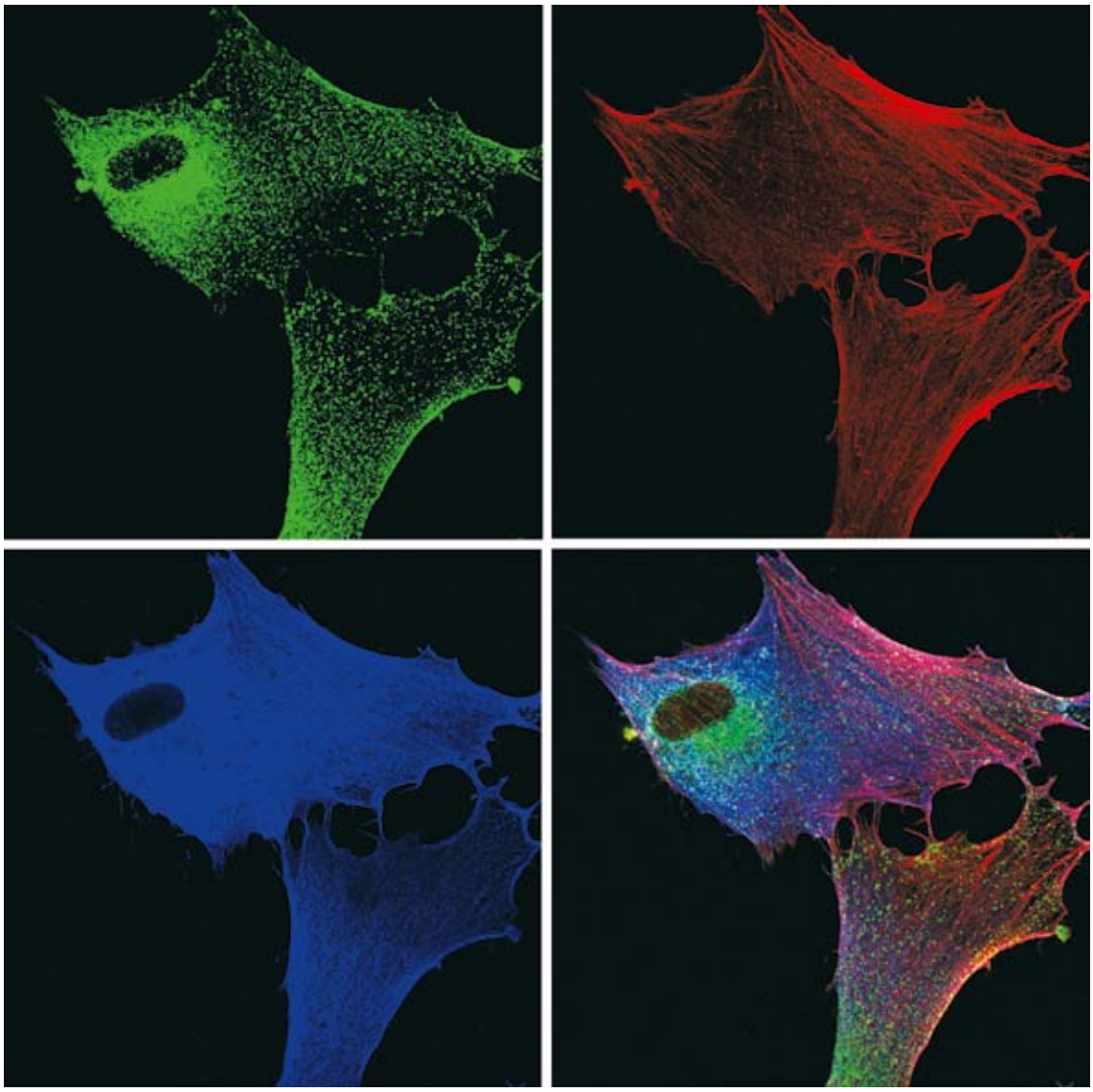
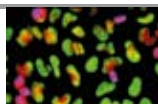


Fig. 1. APPL endosomes in CCD-1070SK human fibroblasts, visualized with anti-APPL2 antibodies (green). Actin (red) was visualized with phalloidin and Annexin A2 (blue) with specific antibodies (author: Łukasz Sadowski).



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 research lines 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-positive endosomes and studies of endocytic trafficking of platelet-derived growth factor (PDGF). Early endosomes are one of the main stations that sort endocytic cargo after internalization. Their typical markers are the small GTPase Rab5, its effector protein EEA1, and a phospholipid species, phosphatidylinositol 3-phosphate (PI[3]P). However, recent reports demonstrated the existence of Rab5-positive, PI(3)P-negative early endosomes that harbor one or both homologous adaptor proteins APPL1 and APPL2. APPL-positive membrane structures, now termed APPL endosomes, show only limited colocalization with EEA1 and are presently considered a distinct subpopulation of early endosomes (Fig. 1). Nonetheless, no specific markers of this compartment, other than APPL proteins, have been described. We sought to characterize APPL endosomes both biochemically and microscopically. To this end, various cell fractionation and gradient purification techniques were established to separate different populations of endosomes. We analyzed the distribution of APPL endosomes during density gradient ultracentrifugation compared with canonical EEA1-positive early endosomes. Although APPL endosomes appear to consist of heterogeneous membrane structures of various densities, they can be partially separated from the 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 (Urbanska et al., submitted). In parallel, we set up quantitative microscopy methods to study the transport pathways that lead cargo via APPL endosomes compared with canonical EEA1-harboring early endosomes. We demonstrated that APPL endosomes are involved in the early trafficking of cargo molecules internalized via clathrin-mediated endocytosis and destined for recycling (transferrin) or degradation (epidermal growth factor, EGF).

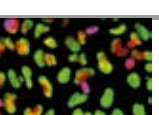
Intriguingly, the inhibition of proteasomes by drugs, such as MG132, ALLN, and bortezomib, leads to the solubilization of APPL1 protein from APPL endosomes and its clustering in the perinuclear region. Such treatment specifically affects APPL endosomes but not canonical early endosomes marked by EEA1.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

In another project, we focused on investigating the endocytic routes of internalized platelet-derived growth factor (PDGF). The ultimate goal of these studies, performed in collaboration with Prof. Carl-Henrik Heldin and Dr. Carina Hellberg (Ludwig Institute for Cancer Research, Uppsala, Sweden), is to evaluate the impact of endocytosis on PDGF-dependent signaling events. We established methods to label PDGF molecules for microscopy 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 can be altered. We showed that such changes affect the activation of certain signaling molecules, arguing that PDGF endocytosis directly impacts intracellular signal transduction downstream of this growth factor.

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

In the projects dealing with this general topic, we uncovered novel roles and interactions of the APPL protein with nuclear factors, and we have undertaken RNA-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, as well as with 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.

APPL1 was previously shown to interact with the NuRD (nucleosome remodeling and deacetylase) complex (Miaczynska et al., Cell, 2004), but the biochemical basis or functional relevance of this interaction remained unknown. We characterized the binding between APPL1 and NuRD in more detail, identifying HDAC2 as the key NuRD subunit responsible for this association. However, the extent of APPL1-NuRD interactions is regulated by the cellular levels of HDAC1, concomitantly affecting the nucleocytoplasmic distribution of APPL1. Increased binding of APPL1 to NuRD upon silencing of HDAC1 promotes the nuclear localization of APPL1, whereas HDAC1 overexpression exerts an opposite effect. Moreover, we uncovered a NuRD-independent interaction of APPL1 with HDAC1. APPL1 overexpression affects the composition of the HDAC1-containing NuRD complex and 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.

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 are currently performing systematic RNAi-based screens for the involvement of candidate proteins in transcriptional regulation mediated by several transcription factors.

With respect to the methodology used in our laboratory, our main experimental system are cultured mammalian cells, but we have also initiated collaborative studies performed in primary neurons (with the Jacek Jaworski group at IIMCB) and in mice to broaden the impact of our cell-based observations in the context of cell-cell communication or the whole organism. In our research, we use a variety of 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.

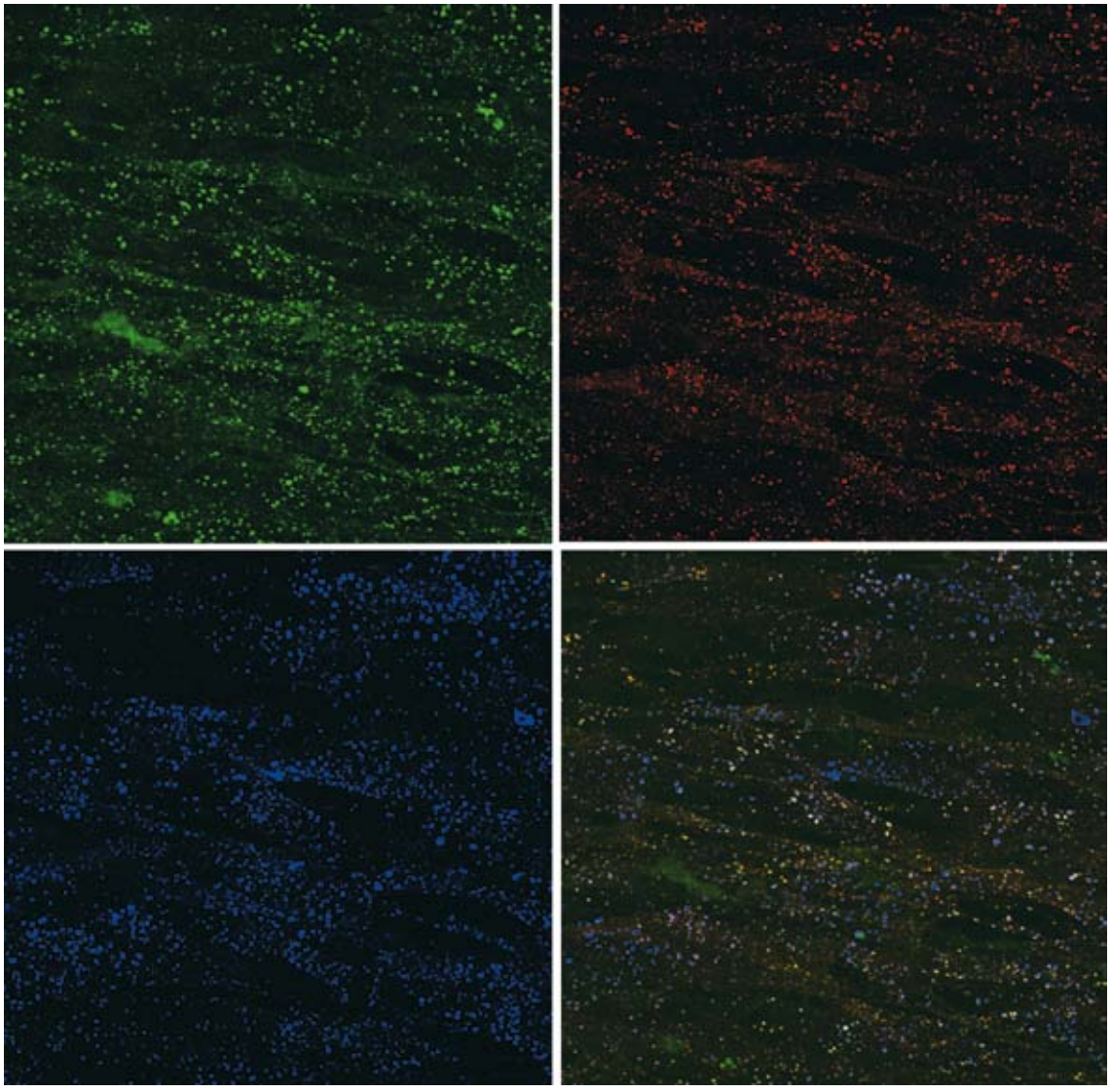
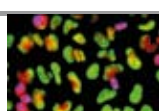
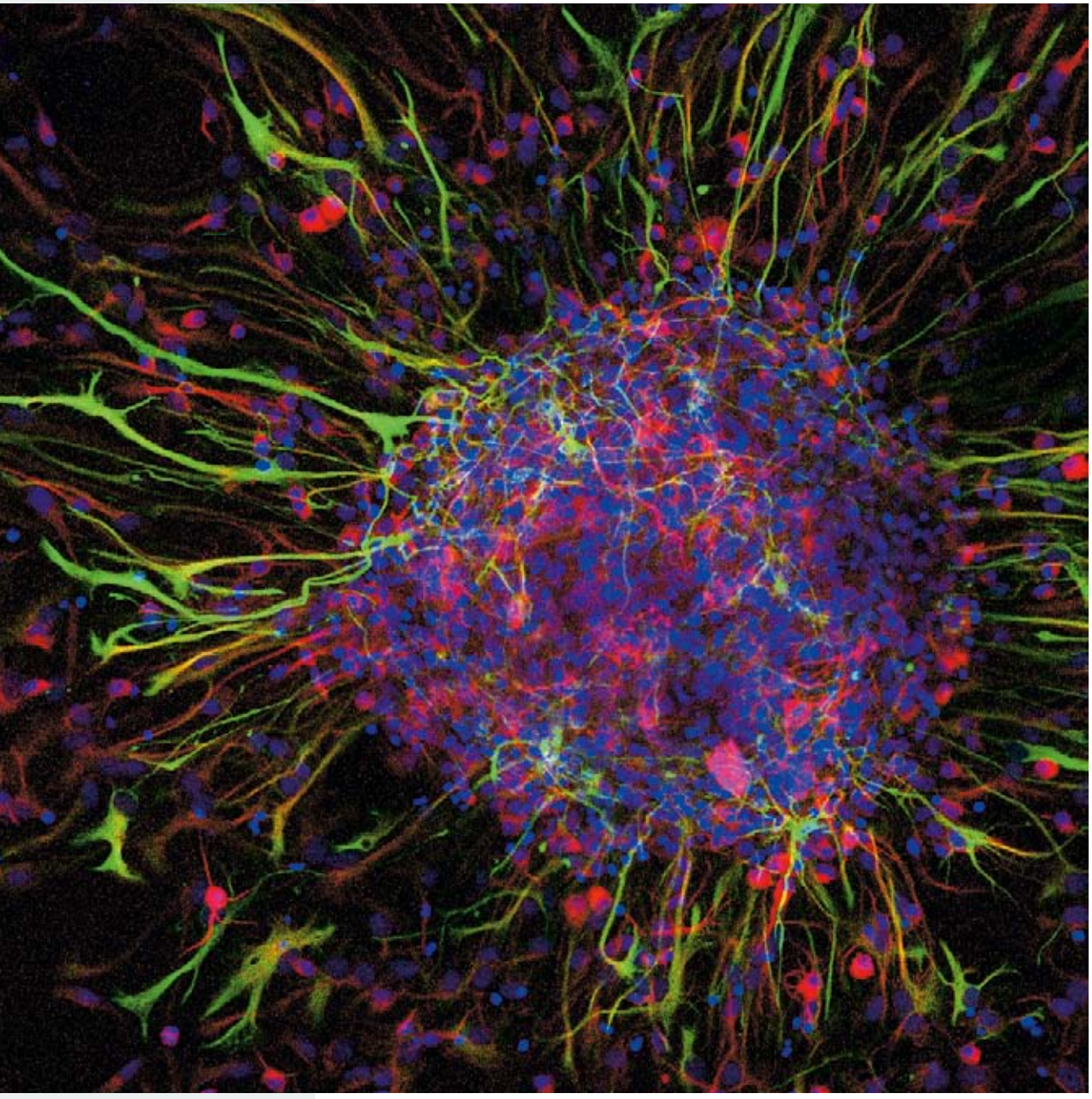


Fig. 2. Internalized PDGF (green) visualized in CCD-1070SK human fibroblasts, together with immunostaining for its receptor (red). The marker of early endosomes (EEA1 protein) is stained in blue (author: Łukasz Sadowski).





Representative image of neurospheres differentiating *in vitro* for 4 days (blue – cell nuclei, green – glial cells; red – neurons). Neurospheres are used in our lab to compare development of embryonic- and adult-born neurons (author: Agnieszka Skałecka)

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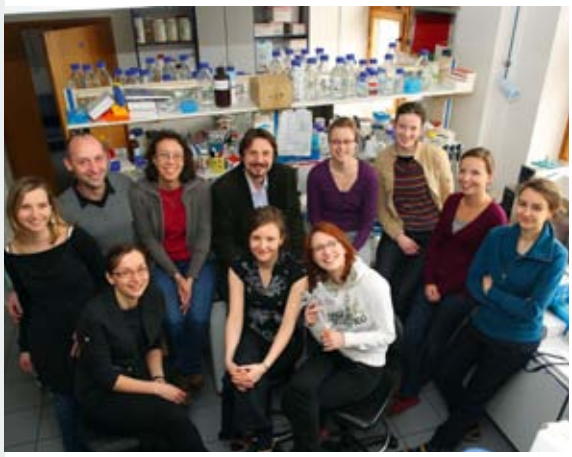

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Iwona Cymerman, PhD

Agata Góźdź, PhD

Matylda Macias, PhD

Łukasz Świech, MSc (PhD thesis-Feb. 2011)

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Agnieszka Skałecka, MSc

Anna Urbańska, MSc

Małgorzata Urbańska, MSc

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Małgorzata Zarębska (thesis defended March 2010)

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Marcelina Pieprzyk, MSc

Jacek Jaworski,
PhD, DSc Habil

RESEARCH TRAINING:

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 CNRS, 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

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

FELLOWSHIPS AND AWARDS:

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., JCB, 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	Bourse de Stage du Gouvernement Francaise (French Government Scholarship)

Selected publications

Publications in 2010-2011

- **Swiech L, Blazejczyk M, Urbanska M, Pietruszka P**, Dortland B, **Malik A**, Wulf P, Hoogenraad CC, **Jaworski J**. CLIP-170 and IQGAP1 cooperatively regulate dendrite morphology. J Neurosci, 2011; 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, 2011; 31(14):5271-8
- 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
- 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, doi: 10.1093/nar/gkr038
- 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 maintenance of plasticity of neuronal connectivity are at the center of interest of molecular neurobiology. Dendrites are the main site of information input onto 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, among other factors, on 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 been also 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, 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 three 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.
3. Characterizing both mTOR-regulated cellular processes and the role of local protein synthesis in pathologies of the central nervous system.

Progress in areas 1 and 2 is discussed in more detail in the following sections.

Identification of mTOR partners and mTOR regulated proteins involved in the process of dendritic branching

Our major effort toward identifying mTOR-regulated proteins involved in dendritic arborization has been to design a shRNA library against mRNA that encode those proteins and perform a screen in neurons cultured *in vitro*. We selected 150 proteins potentially regulated by the mTOR-Raptor complex based on a bioinformatic approach and designed a library of shRNAs against all selected candidates. We then performed a screen and identified 30 genes crucial for dendritic arbor development and stability using this library. Several of them appeared to be involved in endocytosis regulation. We currently focus on investigating the potential relationship between mTOR and β-adaptin and ESCRT proteins, which regulate the early and late steps of endocytosis, respectively. Indeed, our preliminary results

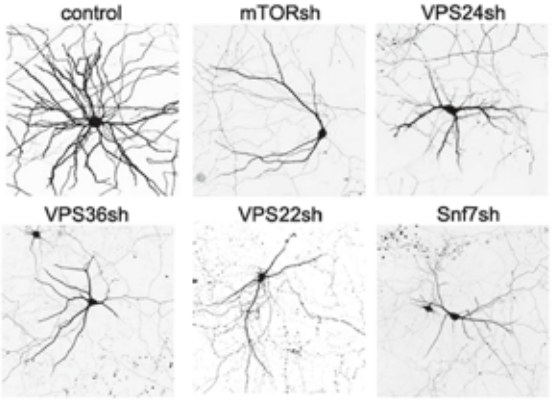

Fig. 1. ESCRT proteins are needed for dendritogenesis. DIV7 neurons in culture were transfected with control vector, shRNAs directed against different ESCRT proteins or shRNA against mTOR (positive control) for 3 days. GFP was cotransfected for visualization of morphology of transfected cells. Knockdown of ESCRT proteins similarly to knockdown of mTOR substantially reduced number of dendrites. *Photo: Marcelina Pieprzyk*

suggest that several ESCRT proteins, in addition to those identified in our screen, can regulate the dendritic arborization of hippocampal neurons (Fig. 1).

In 2010, we also successfully finalized the first phase of our studies on the mTOR-dependent regulation of dendritic arborization that involves CLIP-170. CLIP-170 was identified as an TOR target primarily in yeast and then consequently in mammalian cells. CLIP-170 belongs to a group of microtubule

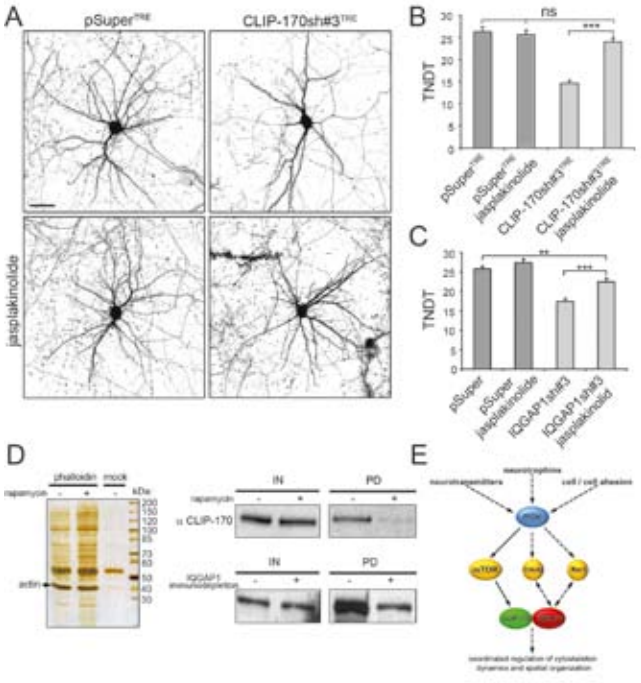

Fig. 2. Forced actin stabilization prevents effects of CLIP-170 or IQGAP1 depletion in developing hippocampal neurons. **(A)** Representative images of hippocampal neurons co-transfected at DIV7 with pSuper^{TRE} vector or CLIP-170sh#3^{TRE} treated with 1 μg/ml doxycycline and 10 nM jasplakinolide 24 h later and fixed 6 days after transfection. Cells were co-transfected with GFP encoding vector for morphology visualization. Scale bar = 20 μm. **(B)** Analysis of the total number of dendritic tips (TNDT) of transfected neurons. **(C)** TNDT of hippocampal neurons after IQGAP1 knockdown with sh#2 and 4 day treatment with 10 nM jasplakinolide. Error bars indicate SEM (***$p < 0.001$, **$p < 0.01$; Kruskal-Wallis test). **(D)** Pull-down of F-actin from vehicle or rapamycin-treated (100 nM) rat hippocampal homogenates. Silver stained gel confirming presence of actin bound with biotinylated phalloidin (left panel). Western blot analysis revealing rapamycin-sensitive and IQGAP1 dependent interaction of CLIP-170 with F-actin resin (right panel). **(E)** A proposed model of a novel mTOR-dependent mechanism of dendritic arbor development regulation. *Photo: Łukasz Świech*

plus-end tracking proteins (+TIPs) and is believed to regulate microtubule dynamics at their plus-ends by promoting their growth. However, CLIP-170 plays additional roles (e.g., bridging microtubules to a cortical actin). Our studies (Swiech et al., 2011, *J. Neurosci.*, in press) describe a novel mechanism of control of dendritic arbor morphology in mammalian neurons via +TIP family member CLIP-170. This mechanism likely involves an mTOR-dependent interaction between CLIP-170 and IQGAP1, an actin-regulating protein that coordinates cross-talk between dynamic microtubule plus-ends and the actin cytoskeleton. Interactions between CLIP-170 and IQGAP1 and subsequently F-actin are decreased by mTOR inhibition in neuronal cells (Fig. 2). Both CLIP-170 and IQGAP1, similar to mTOR, are indispensible for proper dendritic arbor morphology, and dendritic arbor deterioration caused by their knockdown can be rescued by F-actin stabilization (jasplakinolide treatment) (Fig. 2). Moreover, the presence of CLIP-170 and IQGAP1 and microtubule dynamics are needed to support mTOR-dependent, PI3K-induced increases in dendritic arbor complexity in rat hippocampal neurons. These observations suggest that in addition to previously described mTOR-dependent translational control of dendritogenesis, this kinase supports this process by enhancing interactions between microtubule and actin-binding proteins. This, in turn,

can lead to the anchoring and stabilization of microtubules or microtubule-induced changes in actin dynamics needed for dendritic growth or stabilization. Based on these results and a literature search, we speculate that mTOR activation by extracellular signals may allow the formation of the IQGAP1-CLIP-170 complex with PI3K-activated Cdc42 or Rac1 as well as with Lis1 to converge mTOR and small Rho GTPase signaling pathways downstream of PI3K to coordinate the regulation of microtubules and actin during dendritic growth (Fig. 2). Indeed, our preliminary studies suggest that Lis1 is indispensible for dendritic growth. However, in addition to interactions with IQGAP1 and the regulation of cytoskeleton component cross-talk, CLIP-170 is involved in the regulation of trafficking of microtubule minus-end-directed cargo via its interaction with $p150^{Glued}$. Thus, our current efforts are focused on exploring the potential role of this particular interaction for mTOR-regulated dendritic growth.

Establishing a link between local protein translation and physiological dendritic arbor development

Local protein synthesis is now a well established mechanism that underlies several important aspects of cell development and polarization. This is a phenomenon of regulated protein translation that occurs in cell subdomains, often distant from the major sites of protein production. For example, local translation that occurs in growth cones of navigating axons has been shown to be crucial for the proper connectivity of the neuronal network. Several lines of evidence suggest that mTOR kinase is one of the crucial regulators of local translation. The major difference between general and local translation is the spatial separation of these two processes, which requires the transportation of translationally dormant mRNA to the destination point where it gets derepressed and becomes translationally competent. Dendritically targeted mRNAs contain tagging sequences that are recognized and bound by protein complexes that provide both translational silencing and microtubule attachment. Zipcode binding protein 1 (ZBP1) is one of the proteins involved in this process and the formation of such ribonucleoprotein particles (RNPs) that transport mRNA in neurons. Our preliminary results suggest that ZBP1 can be phosphorylated by mTOR. Therefore, in the past 2 years, we studied ZBP1-dependent dendritic growth as a proof-of-concept that local mRNA targeting and translation are important for proper dendritic growth. Similar to CLIP-170 studies, in 2010 we successfully finalized the first stage of the ZBP1 project (Perycz et al., 2011, *J. Neurosci.*, in press). Our work accomplished until now describes the essential contribution of ZBP1 to the development of proper dendritic morphology in hippocampal neurons. It also shows that ZBP1 is not required for the stabilization of already developed dendritic arbors. This difference correlates with the lower abundance and more even dendritic distribution of ZBP1 in mature neurons than developing neurons. A detailed analysis of the contribution of ZBP1 to dendritic development showed that the ability of ZBP1 to bind, transport, and release its cargo was necessary to sustain a correct branching pattern of dendritic arbors. Moreover, the negative effects of ZBP1 knockdown were significantly, but not completely, alleviated by ZBP1-independent overexpression of β-actin, a well-known target of ZBP1 (Fig. 3). This effect was quite specific because other ZBP-1 targets or locally translated proteins did not reverse the deleterious effects of ZBP1 knockdown. The only exception was MAP2, which was quite unexpected and opened new avenues for further studies. Thus, we conclude that β-actin mRNA transport and translational control contribute to the role of ZBP1 in dendritogenesis. Our future studies will mostly focus on mTOR-dependent ZBP1 phosphorylation and the contribution of this posttranslational modification to ZBP1 function and neuronal development.

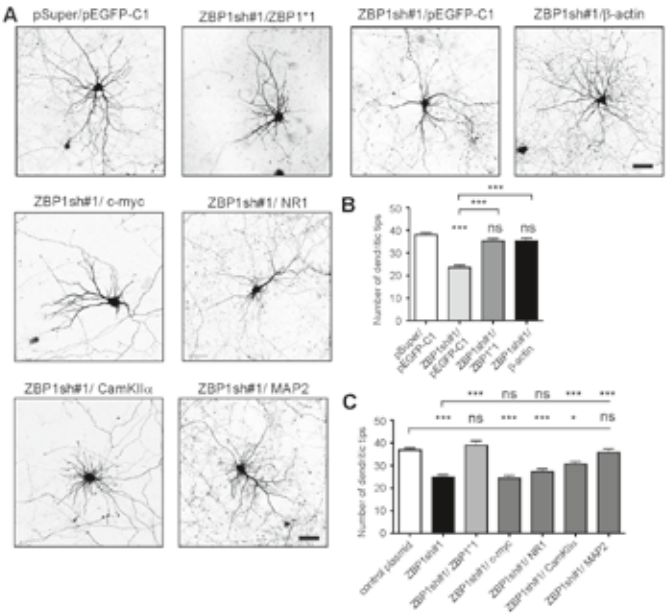

Fig. 3. β-actin overexpression in hippocampal neurons with ZBP1 knockdown is sufficient for partial phenotype rescue.
(A) Micrographs of hippocampal neurons transfected on DIV7 with pEGFP-C1 together with pSuper vector as a control, ZBP1sh#1, or ZBP1sh#1 together with EGFP-ZBP1*1, EGFP-β-actin, c-Myc, GW1-NR1, GFP-CamKIIα. Or EGFP-MAP2. Expression proceeded for 3 days. Neuronal morphology was visualized by staining for cotransfected β-gal. Scale bar = 50 µm **(B, C)** mean number of dendritic tips **(B)** ***p < 0.00033, **p < 0.0033 (Kruskal-Wallis test followed by Mann-Whitney post hoc test with Bonferroni adjustment; ns, not significant). **(C)** ***p < 0.00016, *p < 0.008 (Kruskal-Wallis test followed by Mann-Whitney post hoc test with Bonferroni adjustment; ns, not significant). *Photo: Małgorzata Perycz*

In addition to the aforementioned activities, our important goal is to understand how physiological processes regulated by mTOR are disturbed in nervous system pathology. In 2010, we continued research related to *(i)* the molecular role of mTOR in epileptogenesis (Polish-Norwegian Research Funds grant) and *(ii)* mTOR and GSK3 reciprocal communication in physiology and neurodegenerative disorders (FP7 "NeuroGSK" project). In the upcoming year, our plan is to test our basic findings and scientific questions in two new, clinically relevant models: *in vivo* development of adult-born neurons and the development of iPS cells reprogrammed to neurons. Both of these research directions are financed by Era-Net projects.

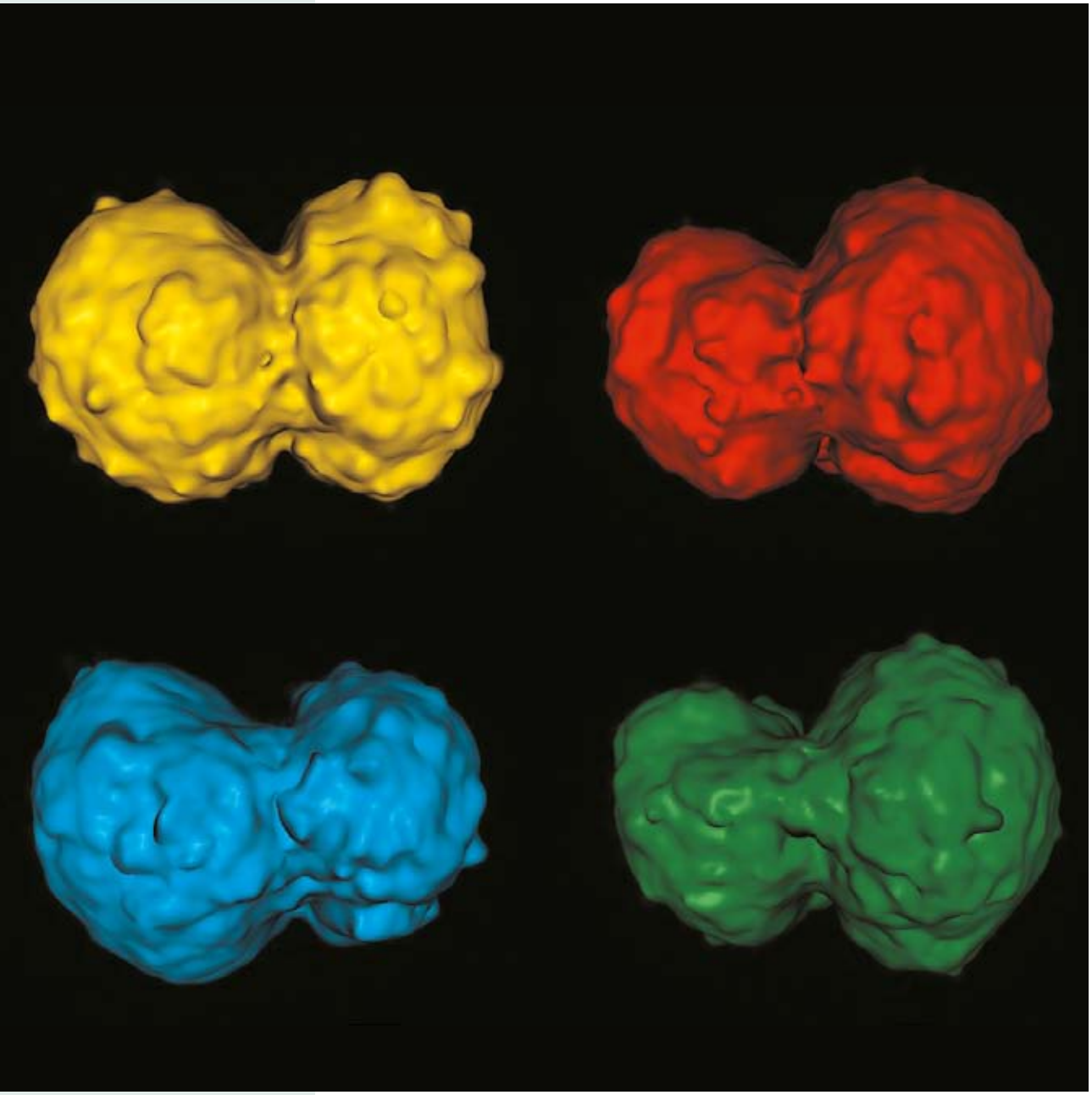

Image sequence of a cytokinetic cell displaying shape oscillation upon anillin depletion. (Autor: Jakub Sędziński)

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

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INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY

The equipment and running costs for the lab, including personnel, are provided by IIMCB (through research grants financed by the Polish Ministry of Science and Higher Education).

Ewa Paluch,
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DEGREES:

2005	PhD in Biophysics, University Paris 7, Paris, France
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2000	Agrégation of Physics
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1998	License in Physics, Ecole Normale Supérieure de Lyon, France

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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

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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); 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

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Selected publications

- **Diz-Muñoz 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
- Charras G, **Paluch E**. Blebs lead the way: how to migrate without lamellipodia (review). Nat Rev Mol Cell Biol, 2008; 9:730-736
- **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.

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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 goal of the group's research is to understand how the mechanical properties of the cell are regulated at the protein level to achieve controlled cellular deformations. To that aim, we study the cell cortex, a network of actin, myosin, and associated proteins that lies beneath the plasma membrane and determines the shape of the cell body. The cortex enables the cell to resist externally applied forces and exert mechanical work. As such, it plays a role in normal physiology during events that involve cell deformation, such as mitosis, cytokinesis, and cell locomotion, and in the pathophysiology of diseases, such as cancer, in which cortical contractility is often upregulated. Despite its importance, very little is known about how the cortex is assembled and regulated.

The biological function of the cortex relies on its ability to contract and exert forces. Therefore, the biological properties of the cortex cannot be understood in isolation from its 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. Moreover, *de novo* cortex assembly can be observed at the bleb membrane, and we use blebs as tools for the study of cortex nucleation and growth. The staff, composed of biologists and physicists, combines biophysical and molecular approaches. 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 the *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 membrane blebs can be studied in a semi-automated manner. Blebs are induced by laser ablation of the cell cortex (Tinevez et al., *Proc Natl Acad Sci USA*, 2009), and the recruitment of fluorescently labeled actin, actin-binding proteins, and myosin can then be quantitatively monitored (Fig. 1). Control experiments have shown that

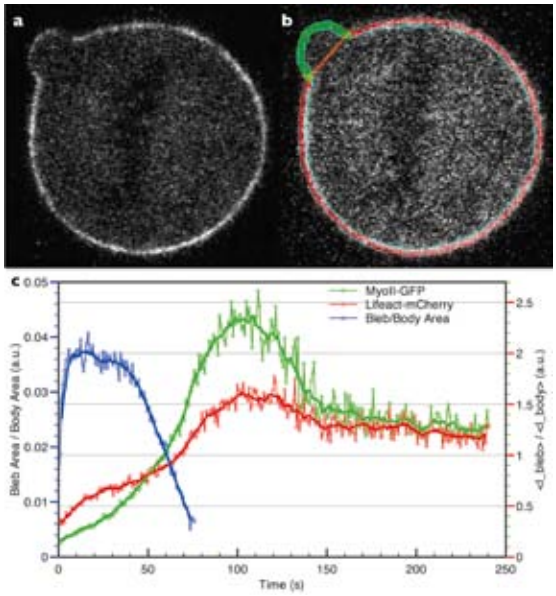
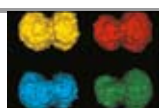


Figure 1: (A) HeLa fibroblast that stably expresses Actin-GFP following laser ablation, exhibiting a bleb in which the cortex has partly reassembled. (B) Representation of the segmentation of the cell in (A). The cortices in the bleb and cell body are monitored using custom code in Matlab. (C) Plot of the time evolution of bleb size (blue) and the mean fluorescence intensities of tagged myosin II (green) and F-actin (red) in the bleb cortex, normalized to the mean intensity of the cell body cortex. (Author: Maté Biro)

the dynamics of cortex assembly are tightly controlled and highly reproducible between cells. We are currently investigating the involvement of various actin-binding proteins in cortex nucleation at the bleb surface. This assay provides a precise, quantitative tool to study the regulation of cortex assembly. Moreover, to narrow the list of potential regulators, we are seeking to determine the composition of the cortices of isolated blebs. In collaboration with Dr. G. Charras (UCL, London) and Dr. P. Roux (IRIC, Montreal; partner groups in an HFSP Young Investigator Grant; see grants list above), we have developed a method of isolating blebs from mammalian cells. The group of P. Roux has analyzed the protein composition of the isolated blebs using mass spectrometry. The results provided a list of hits, which are currently being tested in our assay.



2. Mechanisms of bleb formation

The growth of blebs depends on myosin activity and is commonly believed to directly result from intracellular pressure. In a previous work, we directly tested this hypothesis and showed that bleb growth is driven by, and considerably reduces, intracellular pressure (Tinevez et al., *Proc Natl Acad Sci USA*, 2009). In combination with a physical model (collaboration with the group of Prof. J.F. Joanny, Institut Curie, Paris), these experiments allowed us to predict the mechanical factors that determine the size reached by a bleb. We are currently investigating the mechanics of bleb expansion. We aim to understand how the speed of bleb expansion, which is an important parameter during, for example, bleb-based migration, is controlled.

3. Mechanics of the dividing cell: cortex tension in cytokinesis

The formation and ingression of the cleavage furrow during cytokinesis relies on the controlled reorganization of the actin cortex. So far, most studies of cytokinetic mechanics have focused on force generation at the cell equator, where cortical actin and myosin accumulate in a contractile ring. However, a significant amount of actin and myosin is also present at the poles of a dividing cell. Mechanical resistance of this polar cortex has been shown to slow ingression dynamics. We investigated the contribution of contractile forces exerted at the poles to cytokinesis and showed that polar contractility makes the symmetric shape of the cytokinetic cell intrinsically unstable. Indeed, an

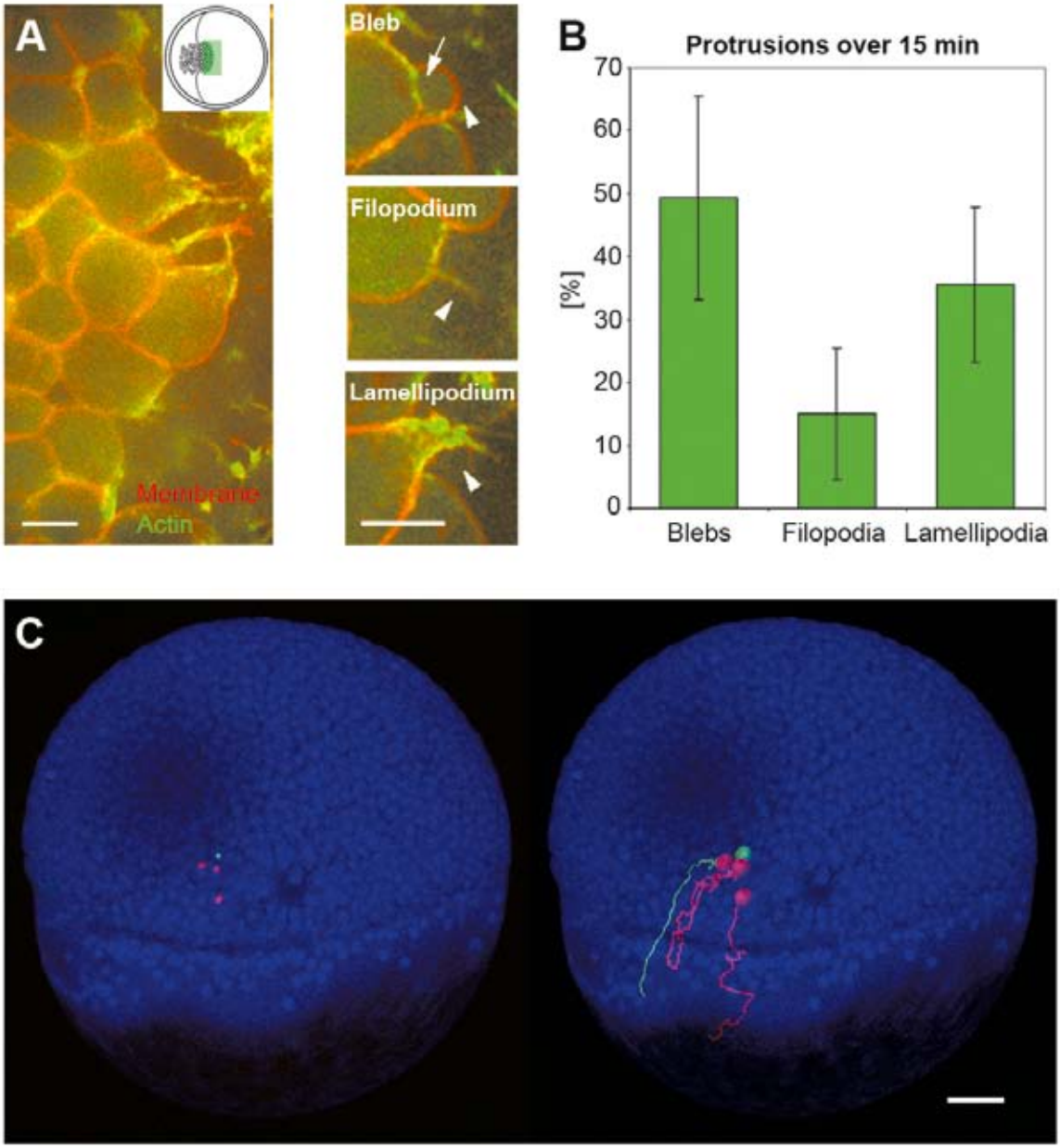


Figure 2: (A) Animal view of the leading edge of a wildtype zebrafish prechordal plate. The inset shows a schematic animal view of an embryo at 80% epiboly, with the green rectangle marking the imaged area. Examples of a bleb, filopodium, and lamellipodium in prechordal plate leading edge cells are shown. Arrowheads indicate protrusions. The arrow indicates the separation between the actin cortex and membrane in the bleb. Scale bars = 10 µm. (B) Percentage of blebs, filopodia, and lamellipodia in wildtype prechordal plate leading edge cells. (C) Lateral view of a MZoep mutant embryo (blue) with transplanted ERM-deficient mesendoderm cells (red). ERM-deficient cells form more blebs than control (green) cells and display a less straight migration path (compare the red and green tracks). Tracking time = 110 min. Scale bar = 50 µm. (Author: Alba Diz-Muñoz)

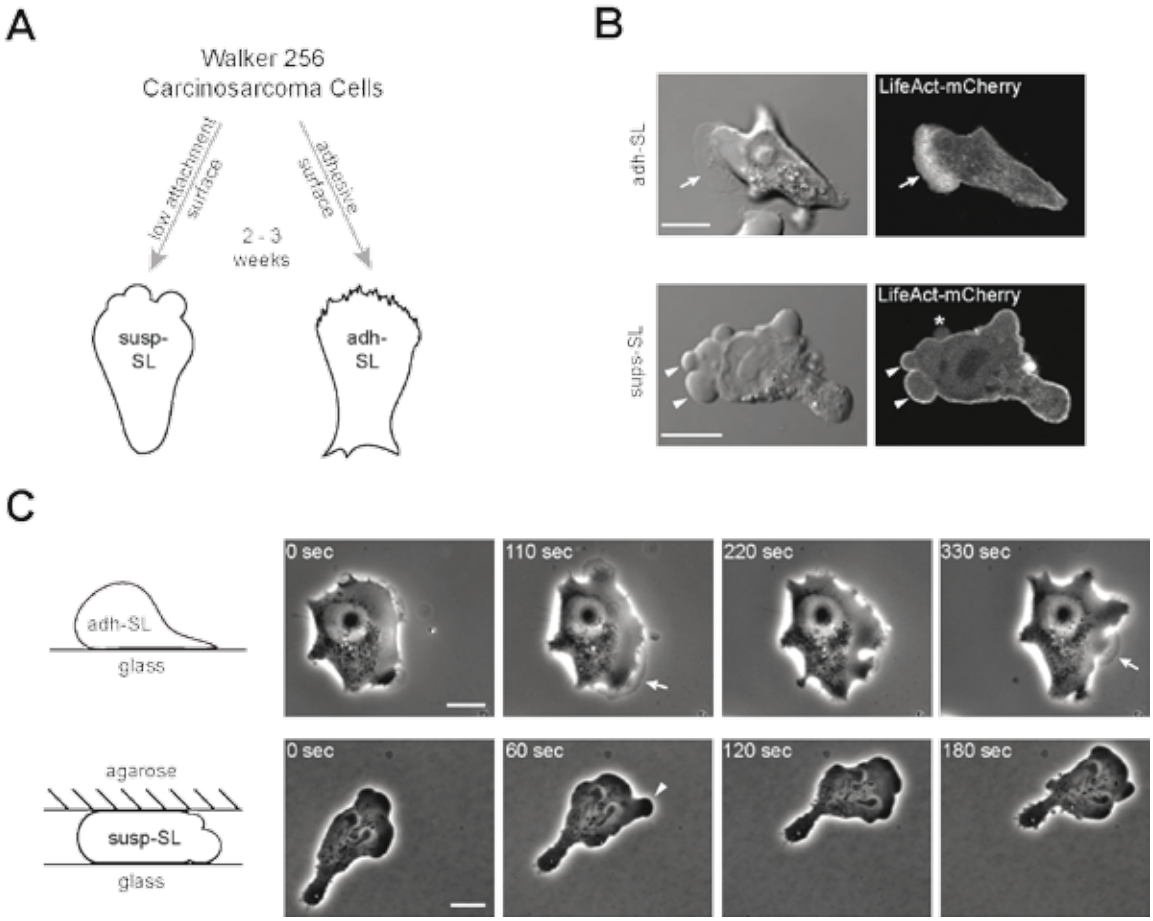


Figure 3: Two sublines of Walker 256 carcinosarcoma cells as a model system for protrusion formation. (A) Schematics of subline selection by culturing cells on different substrates. (B) Protrusions formed by the sublines. AdhSL cells form mainly thin, actin-filled lamellipodia (arrow). SuspSL cells show mostly polarized blebbing at their leading edge (arrowheads). Recently formed blebs do not contain F-actin (asterisk). Scale bar = 10 µm. (C) Time-lapses of migration of the sublines. AdhSL cells migrate on flat two-dimensional substrates, whereas SuspSL cells need confined environments. Arrows indicate lamellipodia. Arrowheads indicate blebs. Scale bar = 10 µm. (Author: Martin Bergert)

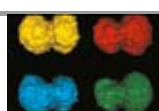
imbalance in contractile forces between the two poles can displace the cleavage furrow from its equatorial position, leading to shape instabilities that can result in division failure. We showed that such instabilities are present during control division and can be amplified by treatments that affect the actin cortex, leading to shape oscillations and division failure (cf. cover figure). We have further shown that blebs, which are commonly observed at the poles of dividing cells, can prevent the appearance of shape instabilities by constantly releasing polar contractility. We are currently working on a physical model of the shape instabilities.

4. Bleb and lamellipodia during cell migration in three-dimensional environments

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, *Nat Rev Mol Cell Biol*, 2008). What determines the type of protrusion formed by a migrating cell and how the various protrusion types contribute to cell migration are poorly understood. We investigate the formation and function of blebs and lamellipodia in two different systems:

– We study cell migration *in vivo* during *Danio rerio* (zebrafish) embryonic development (collaboration with the laboratory of Prof. C.P. Heisenberg, IST, Austria). We showed that mesendoderm progenitor cells in the zebrafish prechordal plate 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 showed that increasing bleb formation slows migration by reducing the directional persistence of the migrating cells (Fig. 2, Diz-Muñoz et al., *PLoS Biol*, 2010). We are currently investigating the effects of decreasing bleb or enhancing lamellipodia formation on mesendoderm progenitor migration. We are also characterizing the orientation of the various protrusion types with respect to the migration direction.

– In parallel, we are investigating the mechanisms of bleb and lamellipodia formation using an *in vitro* culture system. We use Walker carcinosarcoma cells, which can be induced to form either blebs or lamellipodia, depending on the culture conditions (Fig. 3). We are currently comparing the mechanical properties of the two sublines and the molecular pathways that lead to the formation of one or the other protrusion type.



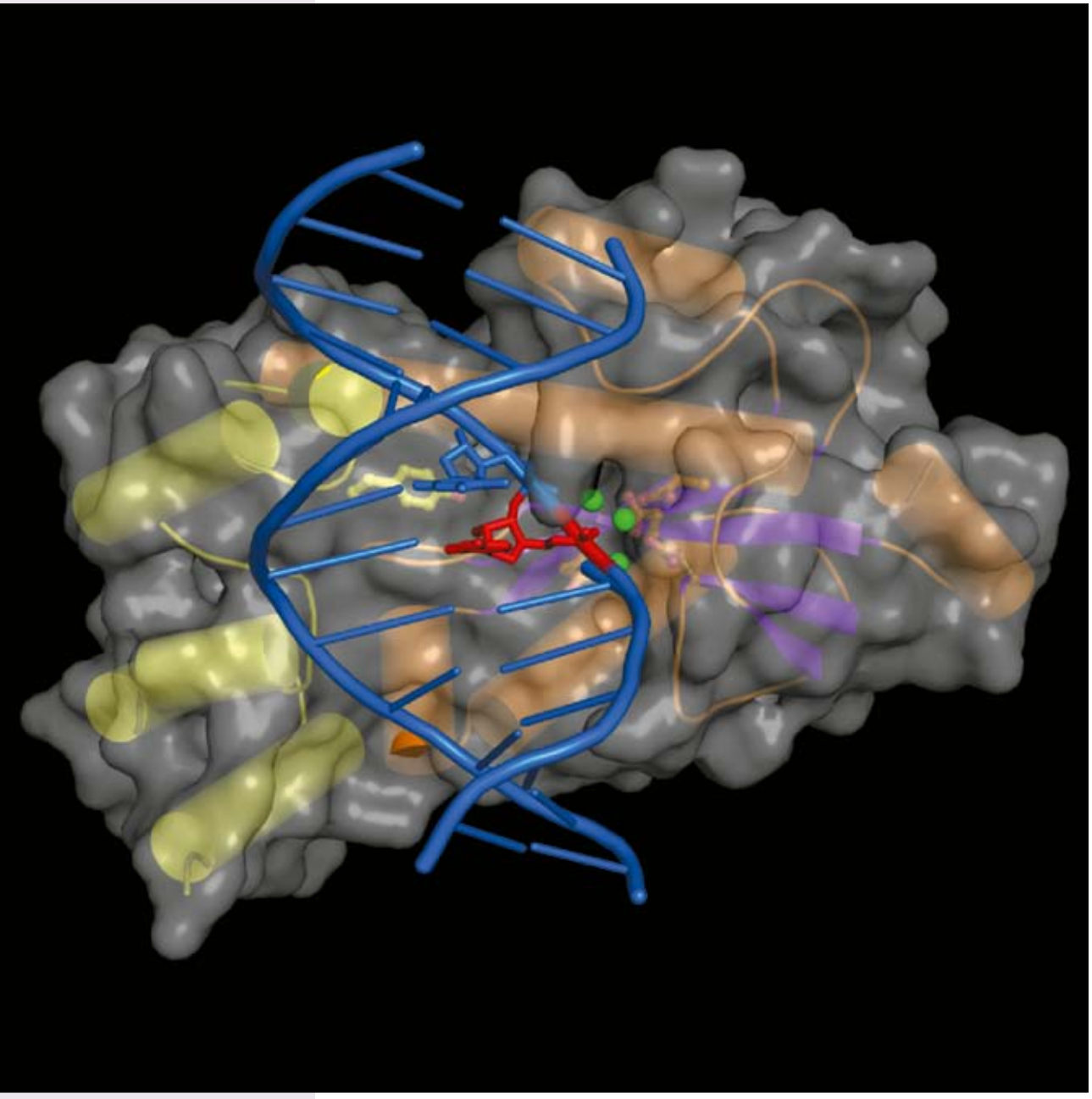


Fig. 1 The structure of T. *maritima* RNase H2 in complex with nucleic acid substrate solved at 2.0 Å resolution. The protein is shown in cartoon representation and colored by the domains (yellow and orange). The DNA is shown in ladder representation (blue for DNA and red for the single ribonucleotide located at the active site). The calcium ions are shown as green spheres.

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DEGREES

2002	PhD *magna cum laude* in Biochemistry, 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 Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, USA

PROFESSIONAL EMPLOYMENT

2008 - Present Head, Laboratory of Protein Structure, IIMCB

HONORS, PRIZES, AWARDS

2003	Prime Minister's Award for PhD thesis
2001, 2002	Annual Stipend for Young Scientists, Foundation for Polish Science
1999	Fellowship, Kronenberg Bank Foundation

Selected publications

- **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
- *Yang W, Lee JY, **Nowotny M**. Making and Breaking Nucleic Acids: Two-Mg^{2+}-ion Catalysis and Substrate Specificity, (review). Mol Cell, 2006; 22:5-13
- ***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. Ca^{2+}-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 in 2010 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, which differentiates 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 (Fig. 1). 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, which changes 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.

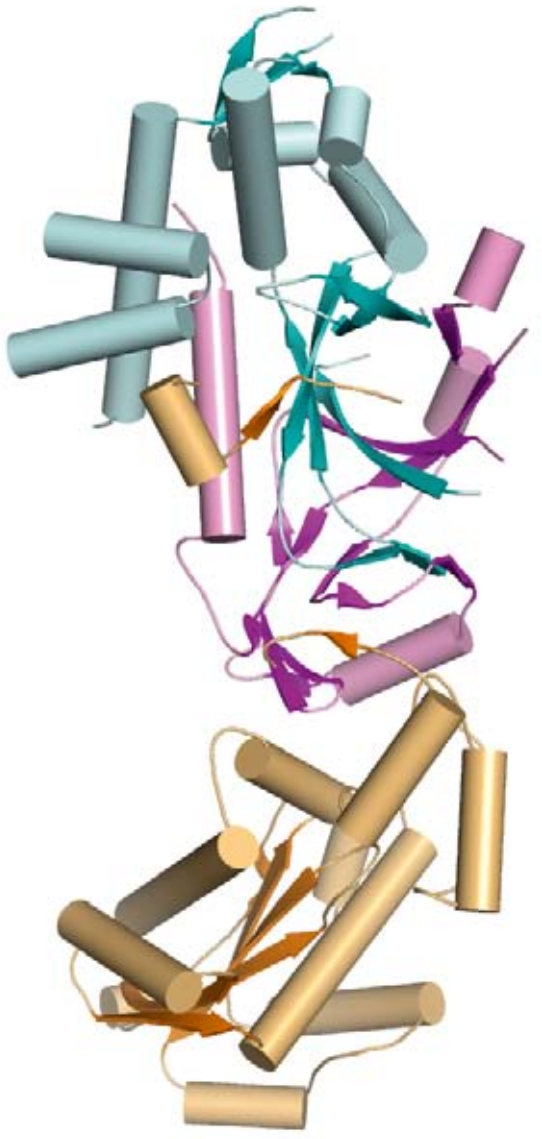
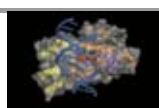


Fig. 2: Crystal structure of human RNase H2 solved at 3.1 Å resolution. The catalytic subunit is shown in orange, and the auxiliary subunits are shown in pink (subunit C) and cyan (subunit B).



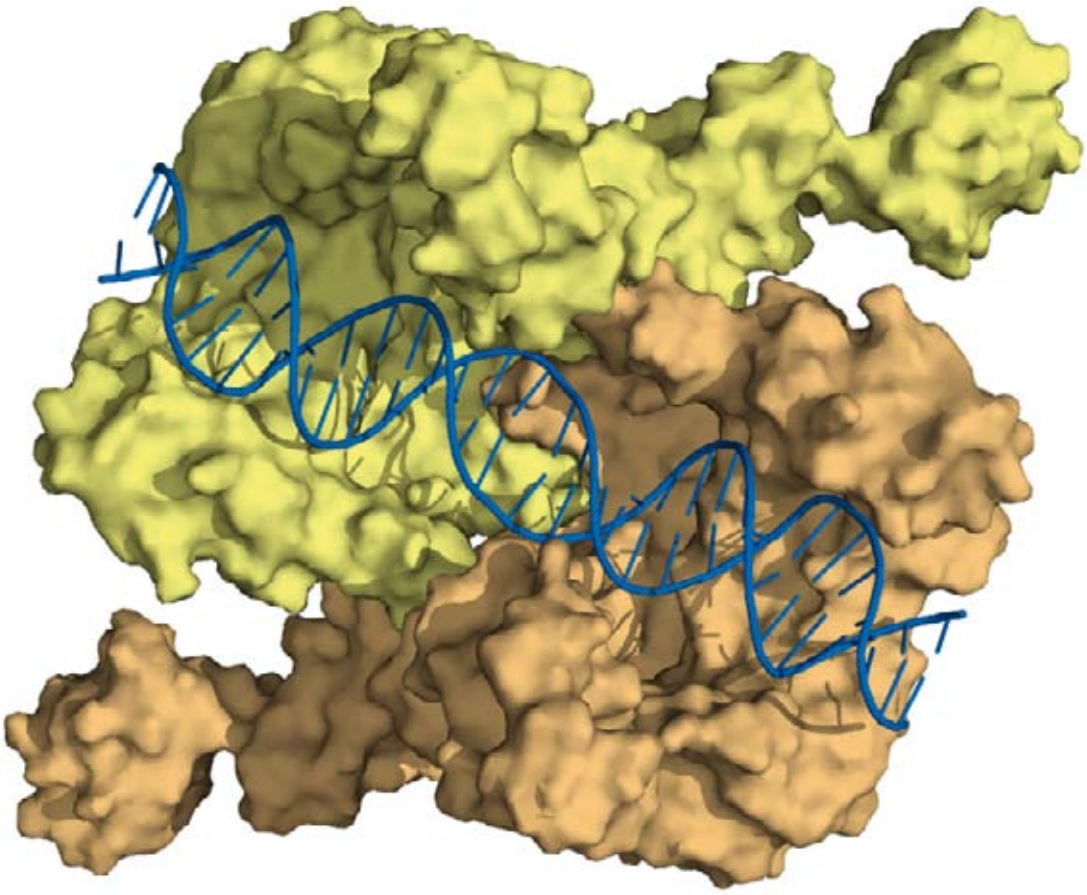


Fig. 3: Structure of *T. maritima* UvrA in complex with DNA solved at 2.9 Å resolution. The protein is shown in surface representation (the dimer subunits are colored in yellow and orange), and the DNA is shown in blue cartoon representation.

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 containing 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. 2). 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, which 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 could 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, which was 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 cleave regular hybrids efficiently. 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, which may lead 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.

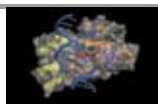
3. Structural and biochemical studies of UvrA DNA repair protein

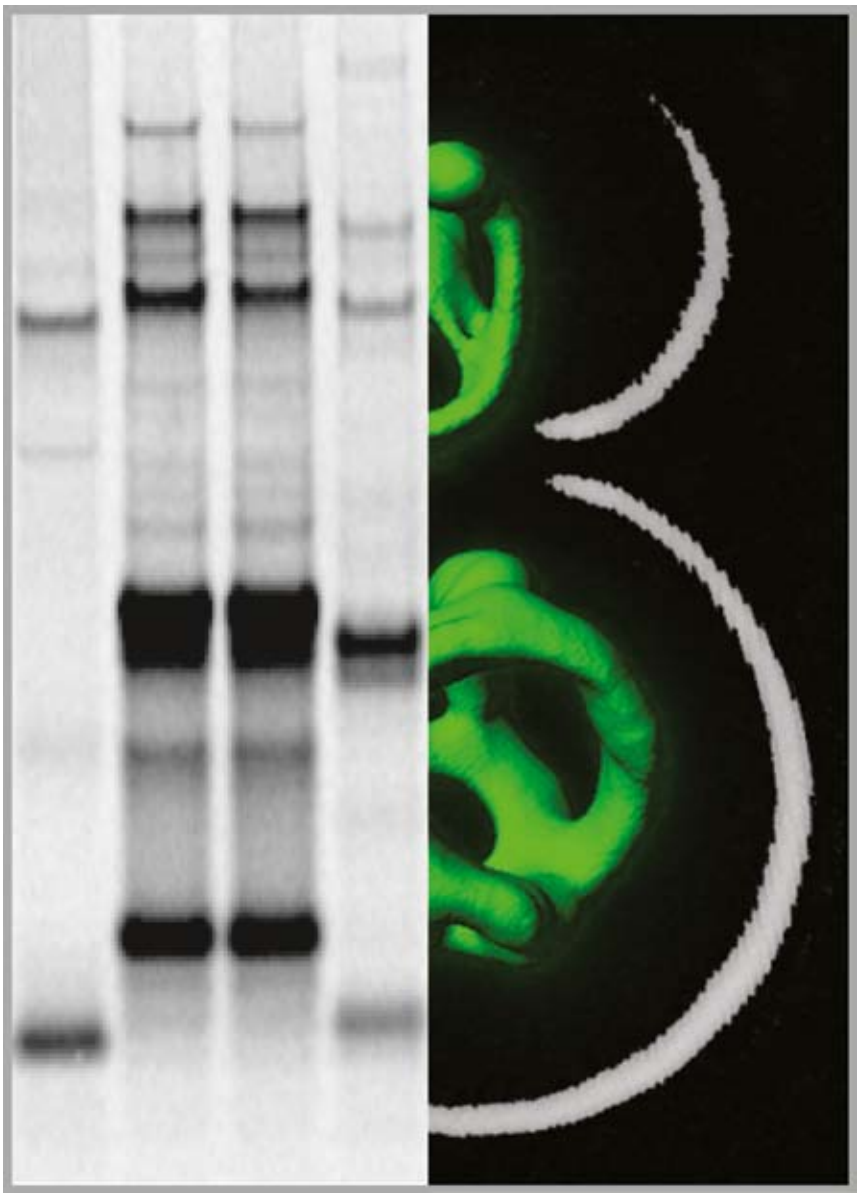
DNA constantly undergoes detrimental chemical modifications (also called DNA damage), which occur spontaneously or are caused by physical and chemical factors. To maintain the genetic stability of the cell and protect the organism, these modifications need to be corrected. One of the primary pathways 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 containing 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 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 was 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 consisting of palindromic oligonucleotides, which contained 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, which consists 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.





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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	Master Degree 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 Leader 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

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
- **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

All papers have no IIMCB affiliation

Description of Current Research

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, which are synthesized outside of the mitochondria in the cytosol. 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 our work with Prof. Nikolaus Pfanner 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 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.

Our current research, supported by a Welcome Programme Grant from the Foundation for Polish Science, an EMBO Installation Grant, and a grant from the Ministry of Science and Higher Education, will explore novel and exciting links between disulfide bond formation mechanisms and mitochondrial protein homeostasis. We postulate the presence of unique mechanisms involved in protein biogenesis that involve crosstalk between the cytosol and mitochondrial compartments. Our research addresses three major and related issues:

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

Our goal is to better understand the complex and dynamic processes involved in the formation of functional organelles, the maintenance of mitochondrial protein homeostasis, and their failure, which results in pathology.

Educational Activities

IIMCB continues its doctoral program in partnership with other research and educational institutes of the Ochota Campus. Currently 50 PhD students are on board within the doctoral programs of the Institute of Biochemistry and Biophysics, of the Nencki Institute, of the University of Warsaw, of the Postgraduate School of Molecular Medicine (SMM) and of the Foundation for Polish Science (FNP). The international PhD program run in collaboration with Utrecht University has entered the final phase.

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 (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 (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 – 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.

For details see page: www.biotechip.pl

Postgraduate School of Molecular Medicine (SMM) (www.iimcb.gov.pl/smm.php)

Medical Universities in Warsaw, Gdańsk, Wroclaw, Łódź and Lublin, as well as the International Institute of Molecular and Cell Biology, Nencki Institute of Experimental Biology, the Institute of Biochemistry and Biophysics, Mossakowski Medical Research Centre, the Cancer Centre and Institute of Oncology and the Foundation for Experimental and Clinical Oncology have jointly founded the Postgraduate School of Molecular Medicine (SMM). The main goal of the School is to offer a modern postgraduate PhD program in the field of molecular medicine, which is addressed to medical, biology and pharmacology postgraduate students in Poland. Since 2002, SMM has been admitting foreign students. SMM is formally affiliated with the Medical University of Warsaw, which is responsible for the administration of the School. According to its by-laws, the School is managed by a Director and Scientific Council elected by all the founding institutions. SMM enrolls students (up to twelve per year) for the four-year doctoral program. The candidates are requested to present a scientific program of their doctoral research, the scientific merit of which is carefully evaluated by the Recruitment Committee of SMM, as well as by independent reviewers from Poland and from abroad. Ten groups of students were accepted during the period of 1998-2011, including nine foreign individuals. Successful candidates accomplish their scientific programs, under the supervision of their tutors, in home laboratories throughout Poland. Members of the SMM Scientific Council evaluate students' progress annually. The tutorial program offered to students includes theoretical (lectures and seminars) and practical (laboratory sessions) courses on modern molecular biology and molecular aspects of medicine. Furthermore, SMM helps students to participate in short-term scientific training in leading Polish and foreign laboratories. Alongside funds generated by the founding institutions, SMM activities were supported so far by subsidies from the Polish Ministry of Health, the Ministry of Science and Higher Education, the Kronenberg Foundation, UNESCO-ROSTE, the European Commission and the National Centre for Scientific Research (CNRS), France. Additional financial support came from the French government supporting the costs of the participation of outstanding French scientists in the tutorial

and organizational activities of SMM, as well as in short-term scholarships for the training of SMM students in laboratories in France. SMM also offers a new international PhD program supported by the Polish Foundation of Science, providing interdisciplinary postgraduate training, focusing on the application of recent high throughput technologies and an integrated, interdisciplinary approach to molecular genetic and genomic processes in relation to cancer development. Projects are carried out in laboratories located: in Warsaw (Warsaw Medical University, the Cancer Centre and Institute of Oncology, the Interdisciplinary Centre for Mathematical and Computational Modelling, Nencki Institute of Experimental Biology), in Szczecin (the International Hereditary Cancer Centre, the Pomeranian Medical University), in Gliwice (the Cancer Centre and Institute of Oncology - Gliwice Branch). All teams have developed successful scientific cooperation: students will be working in collaborating laboratories for 6-12 months. In 2010, the following courses were organized:

- SMM Spring School lecture course "From gene to phenotype – advances in molecular biology and medicine", 12-15.04.2010, Warsaw. This annual course, obligatory for first-year students, was organized by Prof. Bożena Kamińska-Kaczmarek. The lectures were given by 17 outstanding scientists and academic teachers from the top clinical and research institutions in Poland and abroad
- Workshop - Scientific Communication, 26-30.04.2010, Warsaw, run by Prof. Edward Potworowski, organized by IIMCB
- Life science imaging – a workshop on the visualisation of molecules, interactions and biological processes 14-16.06.2010, Warsaw, organized by SMM and Nencki Institute
- SMM Summer School - High Throughput Genomics and Proteomics Technologies in Cancer Research, 12-14.10.2010, Gliwice, organized by SMM, Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology and the Silesian University of Technology
- XIII Annual Inaugural and Research Report SMM Session, 27-28.10.2010, Warsaw, organized by the SMM office and SMM students. The inaugural lecture was given by Prof. Daniel Olive from Université de la Méditerranée, Institut Paoli Calmettes, Institut de Cancérologie et d'Immunologie de Marseille.

Centre for Innovative Bioscience Education (CIBE)

(Formerly: Science Festival School)

The aim of the Center for Innovative Bioscience Education (CIBE) 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 four biological institutes: the International Institute of Molecular and Cell Biology (IIMCB), Nencki Institute of Experimental Biology PAN (IBD), the Institute of Biochemistry and Biophysics PAN (IBB), Warsaw University of Life Sciences (SGGW), the BioEducation Foundation and Warsaw Science Festival. IIMCB houses the CIBE laboratory, office and administration. CIBE also coordinates a second laboratory at Warsaw University of Life Sciences. In 2010, over 1,300 young participants attended laboratory workshops. At the same time, over 150 biology teachers attended laboratory workshops and courses and over 200 children attended hands-on practice experiments. In total, during its activity, CIBE dealt with 8800 school children.

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. Second edition. Participants become acquainted with the production, purification and use of different types of antibodies, including biotechnological methods allowing for large scale manufacturing. They also perform diagnostic tests routinely used in medical 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 2010, as part of teacher training, the following events were organized:

- Course for teachers "Volvox – let's teach how to experiment!" in Konin, February 12th - 13th
- Course for teachers "Volvox – let's teach how to experiment!" in Bytom, March 26th - 27th
- Course for teachers from Tadzhikistan in cooperation with the Partners Foundation in Warsaw, April 22nd

- Course for teachers "From independently performed, enzyme-focused experiments and observations towards ministry-approved pupils' biological knowledge" in collaboration with Warsaw Center for Educational and Public Innovations and Training, Warsaw, October 7th
- Lecture for teachers "Modern teaching of science - Inquiry Based Science Education" during the seminar "Interesting solutions for the methodology of natural sciences teaching" organized by Warsaw Center for Educational and Public Innovations and Training, Warsaw, October 12th
- Course for teachers "Experiments in biology lessons - developing creative and critical thinking" in collaboration with Warsaw Center for Educational and Public Innovations and Training, Warsaw, November 3rd
- Course for teachers "Self-reliant projects and experiments for pupils. A training course" in collaboration with Warsaw Center for Educational and Public Innovations and Training, Warsaw, November 17th - 18th
- 9th CIBE and Nencki Institute Symposium for teachers

14th Science Picnic (30 May 2009)

As in previous years, the BioEducation Foundation and CIBE organized an exhibition and science show during the 14th Science Picnic in Warsaw. The motto for 2010 was "The big micro - world". 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
- "How to see DNA" from DNA isolation to DNA visualization
- "Let's make DNA" cut-outs for youngsters

The XIV Science Festival (17-26 September 2010)

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 2010 CIBE organized open laboratory workshops for the public:

- "Explore your own DNA"
- "Do you know what you eat?" a workshop for students
- "Let's get to know Ochota – a science hub" a city game

Publication of the brochure "Change your perspective – become a scientist! What scientists can do in their lives"

The aim of the brochure was to show that scientific research can become either a life-time fascination or just one of many steps in a professional career. We presented examples to show that science is full of fascinating people who, although being crazy about their work, do find time to do extraordinary things outside their profession. We hope that this brochure will help to overcome the stereotype of a scientist as a laboratory-hidden geek.

The Center for Innovative Bioscience Education –a place for the discovery of gifted youngsters

The Polish Ministry of Education announced the 2010/2011 school year as the year of the discovery of talented youngsters. In effect, a webpage has been created with a nationwide map of extramural initiatives supporting the recognition of talented youngsters (www.roktalentow.men.gov.pl). The webpage promotes institutions and organizations which support skills development in youngsters. In principle, this initiative should enhance the experience exchange between selected organizations as well as help students, teachers and parents to get in touch with the institutions under the patronage of the Ministry. The Center for Innovative Bioscience Education has been selected by the Ministry of Education as one such place supporting the development of talented youngsters.

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 methods (procedures). The purpose of this project is for every junior high school pupil attending the program to take part in a school scientific club and lead a project using the skills acquired during such workshops. 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. CIBE, being an expert in biology education, 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 CIBE are: Agnieszka Chołuj, Joanna Lilpop, Marta Badurek, and Marcin Wiśniewski as a coordinator at Warsaw University of Life Sciences. Animators and co-workers: Krzysztof Brewczyński, Maja Cieplak, Kamil Koper, Marek Kulka, Maciej Kotliński, Paweł Krawczyk, Jakub Kruszewski, Aleksandra Kwiatkowska, Bartosz Zapisek, Monika Ostaszewska, Katarzyna Chomiela, Anna Fogtman, Andrzej Foik, Aleksandra Skrajna, Anna Rolicka, Piotr Gerlach, Aleksandra Kot-Horodyńska, Piotr Horodyński, Katarzyna Laskowska.

Staff at IIMCB (as of 31 March 2011)

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Michał Witt	Deputy Scientific Director	(1/2) IIMCB /Structural Funds
Hanna Iwaniukowicz	Financial Manager	IIMCB
Agnieszka Karbowska	Director's Representative for Administrative Matters	IIMCB/Structural Funds
Agnieszka Wagner-Ziemka	Director's Representative for Research Management	IIMCB/EU grant
Roman Szczepanowski	Director's Representative for Information Technology & Research Equipment	EU grant (1/2)
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Robert Banasiak	Maintenance Specialist	IIMCB

Department of Molecular Biology		
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Alicja Żylicz	Vice Head	IIMCB
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Marta Małuszek	Junior Researcher	IBB PhD School / Ministerial grant
Magdalena Pruszko	Junior Researcher	IBB PhD School / Structural Funds (MPD Project)
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Zuzanna Tracz	Junior Researcher	IBB PhD School / Ministerial grant
Milena Wiech	Junior Researcher	Nencki PhD School / Ministerial grant
Grażyna Orleańska	Secretary	IIMCB (1/2)

Laboratory of Structural Biology MPG/PAN		
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Martin Bergert	Junior Researcher	DFG Grant
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Steve Simmert	MSc Student	Volunteer
Julia Roensch	Technician	Ministerial grant

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Magdalena Mika	MSc Student	Volunteer
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Małgorzata Siemiątkowska	MSc Student	Volunteer
Paweł Tomaszewski	MSc Student	Volunteer
Piotr Wojciechowski	MSc Student	Volunteer
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Grzegorz Chojnowski	Post-doctoral Fellow / Programmer	Structural Funds
Jan Kogut	Programmer	Structural Funds
Tomasz Jarzynka	Programmer	Structural Funds
Łukasz Munio	Programmer	Structural Funds

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Marcin Jaciuk	PhD Student	IIMCB / IBB PhD School
Michał Miętus	PhD Student	Structural Funds (MPD Project) / IBB PhD School
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Marta Wiśniewska	Post-doctoral Fellow	EU grant
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Katarzyna Dębowska	PhD Student	IIMCB / Nencki PhD School
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Katarzyna Misztal	PhD Student	IIMCB / Nencki PhD School
Andrzej Nagalski	PhD Student	IIMCB /Nencki PhD School
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Danuta Korona	MSc Student	Volunteer

Laboratory of Molecular and Cell Neurobiology		
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Matylda Macias	Post-doctoral Fellow	EU grant
Łukasz Świech	Post-doctoral Fellow	IIMCB/ EU grant
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Małgorzata Zarębska	MSc Student	Volunteer

Laboratory of Cell Biology		
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Beata Pyrzyńska	Post-doctoral Fellow	HHMI
Ewelina Szymańska	Post-doctoral Fellow	EU grant
Maciej Lipko	Post-doctoral Fellow	Polish-Norwegian Research Fund
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Daniela Chmiest	Trainee	Polish-Norwegian Research Fund

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Monika Dudek	Technician	IIMCB
Jadwiga Dyttus	Technician	IIMCB
Elżbieta Grzelak	Technician	IIMCB

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Kamila Ornoch	MSc Student	Volunteer / Structural funds
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PolSenior Project		
Małgorzata Mossakowska	Coordinator	IIMCB
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Przemysław Ślusarczyk	IT Specialist	Ministerial grant
Sylwia Zakrzewska	Assistant	Ministerial grant
Magdalena Owczarz	Assistant	Ministerial grant

AriaDNA Project		
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Anna Łasińska	Microscopy Specialist	Ministerial grant

Ciliary Structure and Function Project		
Zuzanna Bukowy	Post-doctoral Fellow	EU grant
Małgorzata Szczepaniak	PhD Student	Structural Funds (MPD Project)/IBB PhD School

Biocentrum Ochota Project		
Szymon Niewieczerzał	Programmer	Structural Funds
Dorota Latek	Programmer	Structural Funds

SBMPs Project		
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Umesh Ghoshdastider	PhD student	EU grant
Shuguang Yuan	PhD student	EU grant
Wojciech Puławski	PhD student	EU grant

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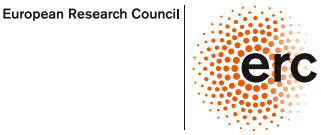
Maja Cieplak	Teacher	Volunteer
Katarzyna Chomiela	Teacher	Volunteer
Anna Fogtman	Teacher	Volunteer
Andrzej Foik	Teacher	Volunteer
Piotr Gerlach	Teacher	Volunteer
Piotr Horodyński	Teacher	Volunteer
Aleksandra Kot - Horodyńska	Teacher	Volunteer
Aleksandra Kwiatkowska	Teacher	Volunteer
Maciej Kotliński	Teacher	Volunteer
Paweł Krawczyk	Teacher	Volunteer
Jakub Kruszewski	Teacher	Volunteer
Marek Kulka	Teacher	Volunteer
Katarzyna Laskowska	Teacher	Volunteer
Monika Ostaszewska	Teacher	Volunteer
Anna Rolicka	Teacher	Volunteer
Aleksandra Skrajna	Teacher	Volunteer
Bartosz Zapisek	Teacher	Volunteer

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

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

Supported by the **EC FP7 grant „Proteins in Health and Disease", HEALTH-PROT, GA No 229676**

IIMCB ranked first among 36 institutions in the section of Biological Sciences, and classified into category 1, according to the latest parametric evaluation of Polish research institutions by Ministry of Science and Higher Education. The achievements of years 2005-2009 were taken into account.

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ISBN 978-83-917905-3-3