Annual Report 2006

INTERNATIONAL INSTITUTE OF MOLECULAR
AND CELL BIOLOGY IN WARSAW

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This Report was edited by:

Agnieszka Wagner-Ziemka and Michal Witt

Cover illustration:

DNase II is essential for degradation of DNA from apoptotic cells and for mammalian development. The Bujnicki group has discovered that DNase II belongs to the phospholipase D (PLD) superfamily and exhibits pseudosymmetric structure with the active site located at the interface between two domains (illustrated by the model). Research described in Cymerman et al., Bioinformatics. 2005; 21:3959-62, and Schafer et al., Protein Sci. 2007; 16:82-91.

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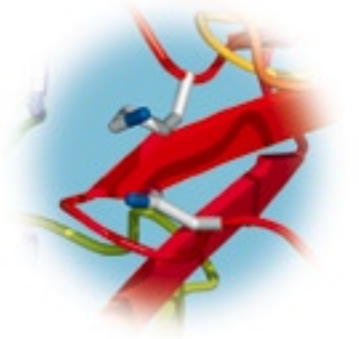


Map of the Ochota Campus

1 **International Institute of Molecular and Cell Biology in Warsaw**

Polish Academy of Sciences

2 Nencki Institute of Experimental Biology
3 Medical Research Center
4 Institute of Biochemistry and Biophysics
5 Institute of Biocybernetics and Biomedical Engineering
6 Institute of Fundamental Technological Research (in organisation)

Medical University of Warsaw

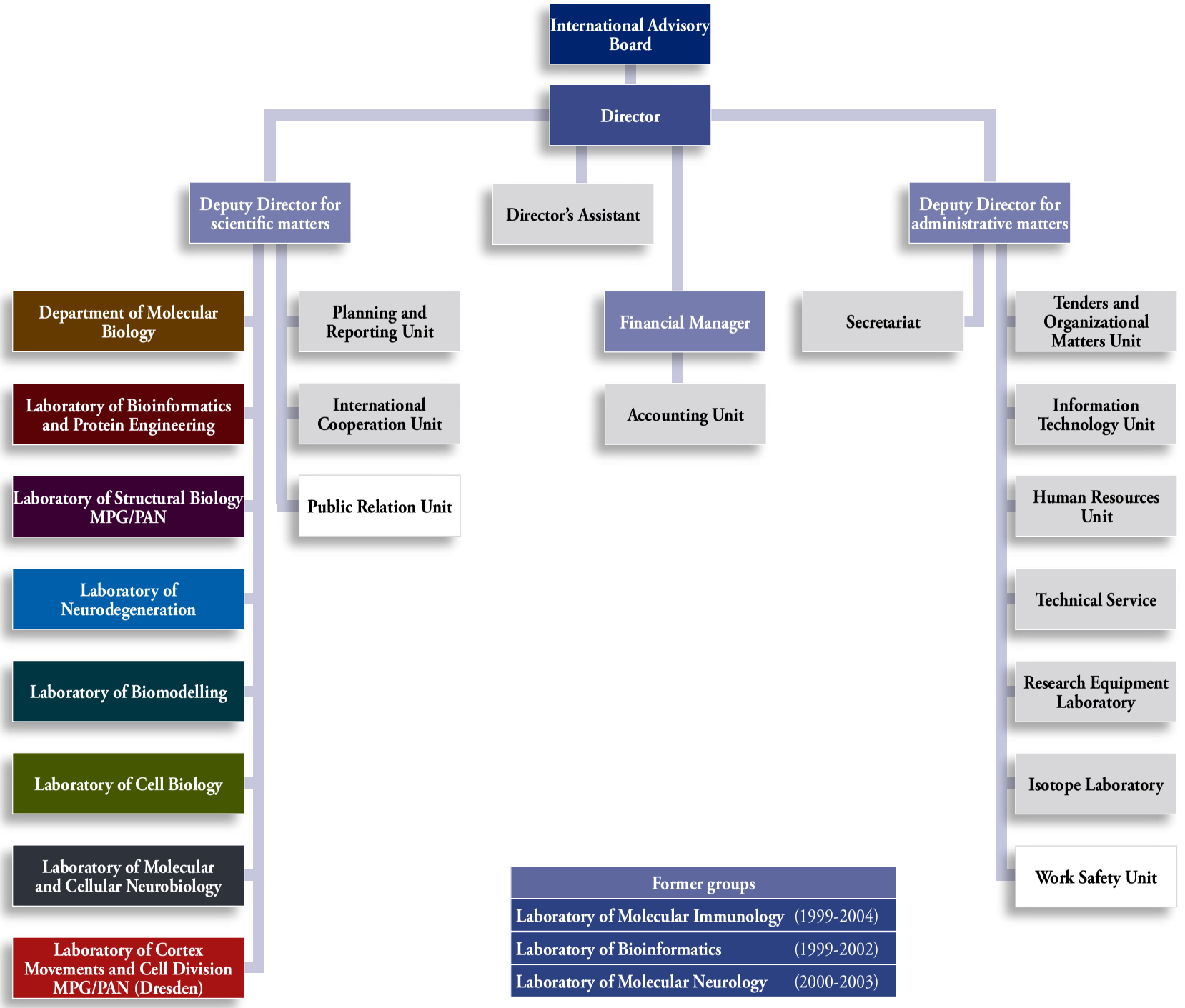
7 Faculty of Pharmacy
8 Hospital
9 Rector's office & Teaching Centre

Warsaw University

10 Faculty of Chemistry
11 Faculty of Biology
12 Heavy Ion Laboratory - cyclotron
13 Faculty of Geophysics
14 Faculty of Geology
15 Faculty of Matematics, Informatics and Mechanics
16 Interdisciplinary Centre for Mathematical and Computational Modeling

17 Oncology Hospital
18 Pulmonology Hospital
SD Student Dormitories
SC Sports Center

Structure of the International Institute of Molecular and Cell Biology in Warsaw



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Deputy Director for scientific matters

Maria Kleska
Deputy Director for administrative matters

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Financial Manager

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Monika Kacprzak
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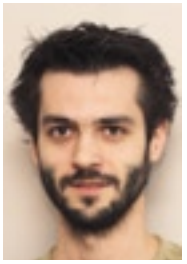
Rafal Flis
IT Manager

Dorota Libiszowska
Foreign Grants Manager

Sylwia Adamiec
International Cooperation Specialist



Przemyslaw Slusarczyk
IT Specialist

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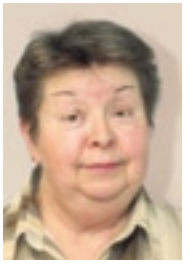

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2006-2010 term

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J. Gregor Sutcliffe

Professor, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA, USA

Anna Tramontano

Professor of Biochemistry, I Medical Faculty, University of Rome "La Sapienza", Rome, Italy

Participants of the meeting of the International Advisory Board, May 2006

From left (first row): A. Tramontano, A. Azzi, K. Hahlbrock, M. J. Nalecz, J. Kuznicki, M. Z. Ratajczak; (second row) L. Kaczmarek, J. G. Sutcliffe, A. A. Bogdanov, K. Arai, M. Witt; (third row) O. A. Krishtal, J. Mallet, R. P. Ericsson, M. Zylicz, R. Przewlocki, I. Baines.

Former International Advisory Board (term 2002-2006):

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Robert P. Erickson, Tucson, USA
Frank Gannon, Heidelberg, Germany
Willem H. Gispen, Utrecht, The Netherlands
Robert Huber, Martinsried, Germany
Wieland Huttner, Dresden, Germany
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Ryszard Przewlocki, Cracow, Poland
Mariusz Z. Ratajczak, Louisville, USA
Wojciech Stec, Lodz, Poland
J. Gregor Sutcliffe, La Jolla, USA

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
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. Kuznicki appointed as Acting Director
July 1999	Dr. J. Dastych is appointed as Leader of the Laboratory of Molecular Immunology
Oct. 1999	Prof. M. Zylicz 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
Nov. 2000	Dr. M. Bochtler is appointed as Leader of the Laboratory of Structural Biology (Joint MPG-PAN Junior Research Group), and Dr. M. Hetman as Leader of the Laboratory of Molecular Neurology
Dec. 2000	Dr. J. Rychlewski is appointed as Leader of the Laboratory of Bioinformatics
Jan. 2001	The MPG-PAN Junior Research Group commences its activities
June 2001	Prof. J. Kuznicki 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
Mar. 2002	Dr. J.M. Bujnicki is nominated as Acting Leader of the Laboratory of Bioinformatics and in June being appointed as Leader of the Laboratory of Bioinformatics
June 2002	Dr. S. Filipek is appointed as Leader of the Laboratory of Biomodelling
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 2003	Evaluation of first two research groups
June 2005	Professor J. Kuznicki re-elected as Director of the Institute (term 2006-2010). Dr. J. Jaworski accepted as a new lab leader
May 2006	New members of the International Advisory Board nominated for 2006-2010 term and Dr. M. Nowotny selected as a new lab leader

Directors' note

The Institute remains on a growth curve: its size still allows us to search for new lab leaders and to plan new research groups to settle in, however, it is slowly but steady filling up. Already, we were obliged to start thinking about the final shape of IIMCB, its internal topography and communication, access to core facilities, use of remaining space, the final number of new groups to be accommodated, the growing demand of equipment and technical infrastructure, etc. It is not that we didn't think about it to date, but currently, we are at the point where we have to face these issues definitively and conclusively. Thinking along these lines, we are preparing lab space for a new faculty member – the latest nominee for a lab leader position, Dr. Marcin Nowotny, who is supposed to launch his laboratory at IIMCB in October 2007 while a new competition is under way. Dr. Nowotny is already a recipient of a prestigious EMBO Installation Grant which places him in the EMBO Young Investigator network. There was also a set of Polish Prime Minister's Awards for doctoral theses (Magda Banach-Orlowska, Renata Filipek, Malgorzata Rzychon) and for a habilitation thesis (Janusz M. Bujnicki). The long debated system of parametric evaluation of Polish research and academic institutions, which has been introduced in Poland a while ago, finally resulted in a ranking of these institutions. IIMCB was evaluated in a section of biological sciences and it had been placed second among all 38 institutes, departments, etc. in this field countrywide. While this should probably satisfy us, a translation of this success in the amount of Ministerial funds is quite far from what a rational distribution of budgetary assets should mean.

What greatly improves our financial situation are grants within the 6th Framework Program (eight have already been funded for over € 2 millions). We work on applications to the EU within the 7th FP, as well as other international sources. Not to be regarded only as a "taker" but also to become a constructive "giver," we opened our activities towards Far East inviting potential collaborators and doctoral students from Vietnam.

Description of the Institute's Activities

The Organization of Research at IIMCB

Eight research groups comprise the structure of IIMCB: Department of Molecular Biology (Zylicz), Laboratory of Bioinformatics and Protein Engineering (Bujnicki), Laboratory of Structural Biology MPG/PAN (Bochtler), Laboratory of Neurodegeneration (Kuznicki), Laboratory of Biomodelling (Filipek), Laboratory of Cell Biology (Miaczynska), Laboratory of Molecular and Cellular Neurobiology (Jaworski), Laboratory of Cortex Movements and Cell Division MPG/PAN in Dresden (Paluch).

The scope of research carried out at IIMCB is mainly focused on fundamental biomedical problems. Among the major research topics are:

1. The role of molecular chaperones in cell transformation, analysis of interactions between human p53 - stress kinases and molecular chaperones, the characterisation of novel human testes specific protein kinase and regulation of its activity, factors of adverse prognosis in non-small lung cancer (Zylicz's group)
2. Theoretical and experimental studies on enzymes acting on nucleic acids (protein structure prediction, evolutionary analyses, functional characterization, protein engineering), and development of computer software for structural bioinformatics of proteins and nucleic acids (Bujnicki's group)
3. The crystallographic structure determination of proteins (Bochtler's group)
4. The studies on neurodegenerative disease (identification of mutations in Alzheimer disease-related genes of Polish patients and functional analysis of these mutations, search for bio-markers and potential therapeutic targets of Alzheimer disease), as well as studies of proteins implicated in the mechanisms of learning and memory and pathogenesis of Alzheimer's disease (cyclin-dependent kinase 5, Ca^{2+}-sensors belonging to calmyrin family, β- and γ-catenins, CHORD containing protein-1) (Kuznicki's group).
5. The structural modelling of membrane proteins and their complexes involving rhodopsin and other G protein-coupled receptors – structures and processes; the molecular role of mutations of presenilins in neurodegenerative diseases (Filipek's group)
6. Interdependence between intracellular endocytic transport and nuclear signal transduction (Miaczynska's group)
7. Molecular processes underlying neuronal development, plasticity, and "physiological" programmed cell death, including gene transcription, kinase-dependent cell signaling and intracellular trafficking (Jaworski's group)
8. Mechanics of the actomyosin cortex; study of cortical flows and of their contribution to the establishment of the mitotic division ring (Paluch's group).

Awards, Honors and Titles

- Habilitation degree to Dr. Andrzej Lewandowicz
- Award from Prime Minister for the habilitation thesis to Dr. Janusz M. Bujnicki
- Award from Prime Minister for the doctoral thesis to Dr. Magdalena Banach-Orlowska
- Award from Prime Minister for the doctoral thesis to Dr. Renata Filipek
- Award from Prime Minister and Polish Biochemical Society for the doctoral thesis to Dr. Malgorzata Rzychon

Education

IIMCB continues its doctoral program in partnership with other research and educational institutes of the Ochota Campus (23 students). The international PhD program run in collaboration with Utrecht University has entered the last phase: currently, four students are enrolled in this program. Additionally, the doctoral program of the Postgraduate School of Molecular Medicine (three students) and of the Foundation for Polish Science (six students) continues; (see section „Educational Activities" p. 64).

Media Visibility and Popularization of Science

In 2006, IIMCB faculty and staff members actively popularized science in media, etc. Results of their research were

presented on the *Academic Internet Television Network* and other TV channels, and within numerous press interviews. The articles about IIMCB and research conducted here were published by leading Polish newspapers and journals: *Wprost, Forum Akademickie, Gazeta Wyborcza* and *Nauka*. IIMCB researchers were present in various radio broadcasts and Internet portals (www.onet.pl, www.esculap.pl, www.naukawpolsce.pl, www.cordiseuropa.eu). They also presented several topics during the **Warsaw Science Festival**, the most popular and successful event bringing science to society in Poland. An IIMCB foreign grant specialist visited the **Polish Science Contact Agency of PAN in Bruxelles**, promoting the Institute's research activities and plans for the future. Among the events extensively covered by media was a lecture on scientific dishonesty given by **Prof. Nils Axelsen** from Copenhagen and a symposium on Alzheimer's disease organized within the APOPIS project of 6FP.

Twenty German and Polish high-school students visited the IIMCB program **"Einstein meets Curie-Sklodowska"**. In cooperation with the **Polish Children`s Fund**, as in previous years, the IIMCB organized a special event for talented high school students. Prof. Maciej Zylicz gave two lectures: one about the success and failure in biology, the second on the discovery of molecular chaperones. Moreover, four students carried out experiments in the selected laboratories for a few days. A South-African entrepreneur, **Mark Shuttleworth**, was hosted by IIMCB due to his involvement in open source computer software availability worldwide. He is also famous for becoming the first African national in space (though as a tourist). A group of **British journalists** (CORDIS News, Financial Times, Times of London, New Europe) **and PR specialists** dealing with the promotion of Polish science in old EU countries visited IIMCB. **Dr. Marta Miaczynska** was nominated to an competition for the title "Polish Woman 2006" by the Polish daily *Gazeta Wyborcza*.

Popularization activities for teachers and students at IIMCB have been performed mostly through the **Science Festival School (SFN)**. SFN, together with the Institute of Biochemistry and Biophysics PAN and the Nencki Institute of Experimental Biology PAN, runs two open laboratories: at IIMCB and the Warsaw Agricultural University. A total number of 1360 young participants visited laboratory workshops in 2006; 116 biology teachers and about 1000 students of various levels attended lectures organized by SFN (see section „Science Festival School – Popularization of Science" p. 66).

Publishing NEWSKO

Since 2000 e-bulletin NEWSKO provides the Ochota Campus community with current information on seminars, symposia, conferences, job opportunities and other essential events. NEWSKO, which has been published at the Institute every Thursday for the last seven years, integrates scientists, students and medical doctors at the Ochota Campus and plays a significant role as the communication platform for all Centres of Excellence at the Ochota Campus.

Computer Network

The IIMCB computer network, is implemented over a structured network of a copper fifth category cable. Active elements of the network are: two optic fiber transceivers, seven 3Com/HP 24-port Ethernet 10/100 Mb/s switches, one HP 48-port Ethernet 10/100 Mb/s and three HP 8-port Ethernet, 10/100/1000 Mb/s switches. The Network is connected directly to several different Research Institutes in the Ochota Campus through fiber optics. There are more than 100 workstations, notebooks, tablets and pads in the network protected by a local firewall operating under Windows W2K/XP/CE, Linux, BSD, Solaris and Mac OS. Fourteen Institute servers are used for e-mail, Intranet, Internet, DNS, DHCP, applications files, remote access, proxy, firewall, terminal services, multimedia and streaming video. These servers operate under Windows 2003, BSD and Linux. Users can access the local network remotely through VPN from home or elsewhere. There is a connection to the Ochota Campus through the Gigabit Ethernet. In the next years, we will continue upgrading the connections on the local network to the Gigabit Ethernet bone. A plan for future purchases includes mass storage, backup servers and multimedia for common usage of the Institute labs.

Mark Shuttleworth (a philanthropist, South African entrepreneur and founder of Shuttleworth Foundation and Canonical Ltd) giving a presentation on free software Ubuntu, a Linux distribution developed by Canonical Ltd. and describing his adventure - becoming the first African national in space as a tourist.

Activities of the Centre of Excellence in Molecular Bio-Medicine

Activities of the Centre of Excellence in Molecular Bio-Medicine focused on four objectives (i) improvement of research quality in biomedical sciences, (ii) extension of the range and scope of education and training in the field, (iii) promotion and popularisation of molecular medicine and human genetics as innovative and modern branches of basic and applicable research, and (iv) strengthening the international position of IIMCB as a centre, where basic and applied research, as well as education and training, were carried out at the highest level. These objectives were taken into consideration while implementing the Centre's activities depicted in the workplan throughout all workpackages.

Workpackage 1

Fourth International Annual Symposium took place in the 42nd month of the project. It consisted of an open research symposium and a closed meeting of the International Advisory Board of the Institute. The research symposium included lectures given by candidates for the new lab leader position at IIMCB: **Dr. Gennady Ermak**, USA; **Dr. Shermali Gunawardena**, USA; **Dr. Sebastien Holbert**, France; **Dr. Anna Moszczynska Mirecki**, Canada; **Dr. Marcin Nowotny,** USA; **Dr. Matthias Soller**, USA.

Workpackage 2

Within this workpackage, twinning activities were undertaken with three different European institutes. **Prof. Michal Witt** visited University Medical Center in Utrecht, the Netherlands; **Marta Brewinska, PhD student,** paid three visits to the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany; **Agnieszka Wagner-Ziemka MSc and Agnieszka Karbowska MSc** were hosted by the Italian National Research Centre on Aging in Ancona, Italy; and **Sylwia Adamiec MSc** went to the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany.

Workpackage 3

Within this workpackage 13 scientists from various European laboratories visited the Centre: **Dr. Manuela Bartolini** (Italy), **Dr. Pawel Smialowski** (Germany), Prof. **Nils Holger Axelsen** (Denmark)**, Dr. Seth Jon Davis** (Germany), **Prof. Pierre Formstecher** (France), **Prof. Luc Buee** (France), **Prof. Jean-Pierre Kerckaert** (France), **Prof. Fred van Leuven** (Belgium), **Dr. Casper Hoogenraad** (the Netherlands), **Prof. Volker Gerke** (Germany), **Prof. Jochen Herms** (Germany), **Dr. Daumantas Matulis** and **Dr. Jurgita Matulina** (Lithuania). Guests presented seminars for all scientists at the Centre and the Ochota Campus and led extensive scientific discussions with the researchers at the Centre. A number of scientific co-operations were initiated or further developed. They are expected to result in common research projects and publications. These visits were particularly important for young scientists at the Centre who gained an opportunity to discuss their projects with experts in the field and to develop new ideas. Moreover, 11 IIMCB fellows visited their European collaborators: **Anna Modzelewska** (**PhD student)** visited the University of Technology, Dresden, Germany; **Jakub Urbanski** (**PhD student)** went to the Utrecht University, the Netherlands, **Magdalena Drobek-Kaus** and **Monika Sokolowska** (**PhD students)** were hosted by the Institute of Biotechnology, Vilnius, Lithuania; **Prof. Jacek Kuznicki** visited Polish Science Contact Agency "PolSCA", Brussels, Belgium; **Dr. Jacek Jaworski** participated in the 5th Forum of European Neuroscience, Vienna, Austria; The University of Technology, Dresden, Germany hosted **Dr. Slawomir Filipek** (twice); **Katarzyna Voelkel** (**PhD student)** visited the University Hospital in Freiburg, Germany; **Lukasz Bojarski** (**PhD student)** paid a visit to the APOPIS 6th FP, München, Germany; and **Dr. Janusz Bujnicki** together with his **PhD student, Agnieszka Obarska,** went to the University of Edinburgh, United Kingdom.

Workpackage 4

According to its objectives, this workpackage was dedicated to the intense exchange of young researchers. In 2006, seven PhD students from IIMCB visited and worked in various European laboratories:

- **Agnieszka Chmiel** at the CEA/VALRHO-Marcoule, DSV - DIEP - SBTN, Service de Biochimie post-génomique et Toxicologie Nucléaire BP, Montpellier, France;

- **Magdalena Kaus-Drobek** at the Laboratory of Protein-DNA interaction, IBT Vilnius, Lithuania;
- **Monika Sokolowska** at the Laboratory of protein-DNA interactions, Institute of Biotechnology, Vilnius, Lithuania;
- **Bartosz Wawrzynow** at the CRUK Interferon and Cell Signaling Group, the Cell Signaling Unit, Cancer Research Centre and the University of Edinburgh, United Kingdom;
- **Malgorzata Dawidowska** at the Department of Immunology, Erasmus Medical Center, Rotterdam, the Netherlands;
- **Lukasz Swiech** at the Department of Neuroscience, Erasmus Medical Center, Rotterdam, the Netherlands;
- **Marta Kubala** at the Laboratory of Protein-DNA Interactions, Institute of Biotechnology, Vilnius, Lithuania.

Workpackage 5

The conference **"Molecular Mechanisms of Neurodegeneration and Neuroprotection",** a joint event organized by Prof. Jacek Kuznicki from the Centre and Prof. Bozena Kaminska from the Nencki Institute of Experimental Biology, took place in 2003.

Workpackage 6

According to the plan, all workshops and lecture series were carried out within the first three years of project duration and were described in the three IIMCB annual reports. Highlights of this workpackage were: **Molecular Medicine Lecture Series: Pneumonology, Hematology and Psychiatry** (organized by Prof. Michal Witt - IIMCB), **Lecture Series: Enzyme structure and mechanism** (organized by Dr. Matthias Bochtler - IIMCB), **workshop: Molecular basis of Alzheimer's disease** (organized by Dr. Cezary Zekanowski - IIMCB and Prof. Barcikowska - IMDiK), and **EMBO-FEBS Workshop on Biology of Molecular Chaperones: Heat Shock Proteins in Molecular Medicine, Misfolding Diseases and Cancer** organized by Prof. Maciej Zylicz from IIMCB and Prof. Ulrich Hartl from MPG, Germany.

Workpackage 7

Three integrated courses of the Postgraduate School of Molecular Medicine (SMM), which were planned within this workpackage, were carried out within the first three years of the project duration. The first one was focused on oncology (organizer: Prof. J. Lubinski, Szczecin), the second was devoted to molecular therapy in clinical practice (organizer: Prof. A. Mackiewicz, Poznan), and third was focused on molecular endocrinology (organizer: Prof. B. Jarzab). Their detailed descriptions were presented in three previous IIMCB annual reports.

Workpackage 8

One of the main objectives of the Centre of Excellence project was the **promotion of the International Institute of Molecular and Cell Biology** as a leading research center in molecular biomedicine, both domestically and internationally, and popularization of science in society. To meet these objectives, a number of related activities were undertaken, mostly within the first three years of project duration: organization of **Public Relation Unit**, issuing of **monthly Centre's bulletin**, co-organization of **Warsaw Science Festival**, annual public national event, as well as supporting activities of **Science Festival School**. Additionally, Centre issued two **promotional publications**: Annual Report 2005 and the Booklet on 18 CoEs Achievements.

Moreover, within this workpackage **Dorota Libiszowska MSc**, went to Brussels with some promotional tasks. She visited Polish Science Contact Agency of the Polish Academy of Sciences "PolSCA", CEMBM Project Officer at the European Commission and journalists from CORDIS News.

Top candidates at the 2006 competition for a lab leader position. From left: Dr. Shermali Gunawardena, Dr. Marcin Nowotny, Dr. Matthias Soller.

Scientific Meetings and Lectures

- "Scientific Communication" - practical course for graduate students for whom English is a second language given by Prof. Edward Potworowski (Armand-Frappier Institute, Montreal, Canada), 8-12.05.2006, Warsaw, Poland, organized by IIMCB
- International Annual Symposium 26.05.2006, Warsaw, Poland, IIMCB within the „Centre of Excellence in Molecular Bio-Medicine" project
- IIMCB Annual Report Session, 2.06.2006, Lansk, Poland
- The MPI-CBG and IIMCB Trilateral Workshop for Young Scientists from Germany, Czech Republic and Poland, focused on „Cell Biology of Intracellular Transport Processes", supported by the Max Planck Society and IIMCB, co-organised by Dr. Marta Miaczynska (Laboratory of Cell Biology, IIMCB) and Dr. David Stanek (Charles University, Prague), 15-18.11.2006, Warsaw, Poland

Lectures within Centre of Excellence in Molecular Bio-Medicine project

Gennady Ermak (Ethel Percy Andrus Gerontology Center and Division of Molecular and Computational Biology, University of Southern California, Los Angeles, USA) RCAN1 Functions in Neuronal Development and Degeneration. 26.05.2006

Shermali Gunawardena (Department of Cellular and Molecular Medicine, University of California, San Diego, USA) Deadly pile-ups on neuronal highways: Transport problems in neurodegenerative disease. 26.05.2006

Sebastien Holbert (INRA, Tours, France) Large screen of neurodevelopmental diseases candidate genes. 26.05.2006

Anna Moszczynska Mirecki (Molecular Neurobiology Laboratory, Department of Neuroscience Research, Centre for Addiction and Mental Health, Toronto, Canada) Parkin-dopamine transporter protein-protein interaction: Implications for Parkinson's disease. 26.05.2006

Marcin Nowotny (Wei Yang Laboratory, National Institutes of Health, NIDDK/LMB, Bethesda, MA, USA) Structural studies of RNase H from substrate binding to two-metal catalysis. 26.05.2006

Matthias Soller (Department of Biology, Brandeis University, Waltham, USA) ELAV regulated pre-mRNA processing in synaptic growth. 26.05.2006

Manuela Bartolini (University of Bologna, Italy) Combined in vitro methodologies for drug discovery in Alzheimer's disease. 16.03.2006

Pawel Smialowski (Technische Universität München, Germany) Attempts to predict structure determination success from sequence. 3.03.2006

Nils Holger Axelsen (Department of Clinical Biochemistry, Statens Serum Institut, Copenhagen, Denmark) Scientific dishonesty and good scientific practice. 4.04.2006

Seth Jon Davis (Department of Plant Developmental Biology, Max Planck Institute for Plant Breeding Research, Cologne, Germany) Molecular-genetic perception of dawn and dusk: input regulation of the circadian clock. 7.04.2006

Fred Van Leuven (Experimental Genetics Group – LEGT EGG, Dept. of Human Genetics, Katholieke Universiteit Leuven, Belgium) Different transgenic mouse models for different aspects of Alzheimer's disease. 23.05.2006

Casper Hoogenraad (Department of Neuroscience, Erasmus Medical Center, Rotterdam, the Netherlands) Receptor trafficking regulates dendrite morphology. 2.10.2006

Volker Gerke (Institute of Medical Biochemistry ZMBE-Center for Molecular Biology of Inflammation, Muenster, Germany) Mechanisms controlling distinct steps in leukocyte transendothelial migration. 10.11.2006

Jochen Herms (Zentrum fur Neuropathologie und Prionforschung Ludwig Maximilians Universitat, Munich, Germany) Application of the in vivo two-photon microscopy in neurodegenerative diseases. 9.11.2006

Daumantas Matulis (Laboratory of Biothermodynamics and Drug Design, Institute of Biotechnology, Vilnius, Lithuania) Radicicol binding to Hsp90 by thermal shift assay. 15.12.2006

Seminars of invited speakers

Dorota Religa (Karolinska Institute, Stockholm & Medical Research Center, Warsaw) Polish Brain Power - development of Biobank. 5.01. 2006

Lukasz Jaroszynski (Institute of Human Genetics, Georg-August-University, Göttingen, Germany) Expression and functional analysis of Tex18 and Stra8 genes in male germ cells. 12.01.2006

Malgorzata Borowiak (Max Delbruck Center for Molecular Medicine, Berlin, Germany) Met as a part of general, defensive response to tissue injury. 9.02.2005

Hansjürgen Volkmer (NMI Natural and Medical Sciences Institute at the University of Tübingen, Germany) Function and regulation of cell adhesion molecules for the establishment of neuronal connectivity. 10.02.2006

Jakub Golab (Department of Immunology, Center of Biostructure Research, Medical University of Warsaw, Poland) Potential antitumor effects of statins. 16.02.2006

Kristian Rother (Institut für Biochemie CCM, Charite - Universitätsmedizin Berlin, Germany) Columba - integrating annotation on protein structures. 12.04.2006

Magda Kosmopoulou (The National Hellenic Research Foundation, Athens, Greece) Glycogen phosphorylase as molecular target for the development of new potent drugs for type 2 diabetes therapy. 25.04.2006

Lyudmila L. Sidorik (Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kiev, Ukraine) The role of molecular chaperons in dilated cardiomyopathy progression. 27.04.2006

Björn Wallner (Stockholm University, AlbaNova University Center, Stockholm Bioinformatics Center, Sweden) Prediction of protein model quality. 8.05.2006

Jaroslaw Marszalek (Intercollegiate Faculty of Biotechnology UG-AMG Department of Molecular and Cellular Biology, Gdansk) The evolution of mitochondrial chaperones utilized in Fe-S cluster biogenesis. 23.06.2006

Anthone W. Dunah (Mass General Institute for Neurodegenerative Disease, Harvard Medical School and Massachusetts General Hospital, Charlestown, USA) Functions and mechanisms of LAR receptor protein tyrosine phosphatases in the development and maintenance of excitatory synapses in brain. 13.07.2006

John Moses (The London School of Pharmacy University of London, Great Britain) Biomimetic synthesis of complex natural products. 28.07.2006

Richard J. Roberts - (New England Biolabs, Inc, Ipswich, USA) The genomics of restriction and modification. 15.09.2006

Marek Cieplak (Institute of Physics PAN, Warsaw, Poland) Stretching to understand proteins. 28.11.2006

Ashok S. Bhagwat (Department of Chemistry, Wayne State University, Detroit, USA) Antibody maturation caused by human AID: roles of transcription and DNA repair. 16.05.2006

IIMCB researchers seminars

Urszula Wojda (Laboratory of Neurodegeneration, IIMCB) Ca^{2+} - an universal regulator in the machinery of life: concepts in Ca^{2+} signaling. 19.01.2006

Slawomir Filipek (Laboratory of Biomodelling, IIMCB) Basics of MM & MD (Molecular Mechanics, Molecular Dynamics) methods for experimentalists. 2.02.2006

Krystiana Krzysko, (Laboratory of Biomodelling, IIMCB) True story of chocolate. 23.02.2006

Jacek Jaworski (Laboratory of Molecular and Cellular Neurobiology, IIMCB) Molecular basis of dendritic arbor development. 9.03.2006

Michal Witt (IIMCB) Ciliary disfunction as a basis for various human disorders. 23.03.2006

Malgorzata Gutkowska (Department of Molecular Biology, IIMCB) Hsp90 chaperones evolution - old and new ideas. 30.03.2006

Malgorzata Mossakowska (Laboratory of Neurodegeneration, IIMCB) Inflammatory bowel disease: principles of diagnosis and treatment. 13.04.2006

Marta Brewinska (Laboratory of Cell Biology, IIMCB) Fruity microscopy: imaging in cell biology. 20.04.2006

Anna Zarebska (Laboratory of Cell Biology, IIMCB) Cells contra g-force. 18.05.2006

Jakub Urbanski (Department of Molecular Biology, IIMCB) Froth on the Daydream? On exosomes and extracellular Hsp90. 25.05.2006

Maciej Olszewski (Department of Molecular Biology, IIMCB) Heads or tails: molecular determinants of TNF-alpha trafficking in mast cells. 8.06.2006

Magdalena Blazejczyk (Laboratory of Neurodegeneration, IIMCB) Quantum dots in live cell imaging. 12.10.2006

Marek Wojciechowski (Laboratory of Structural Biology, IIMCB) The mosaic pool: bacteriophage genomics. 19.10.2006

Agnieszka Choluj (Science Festival School) Scientist - an endagered species? 23.10.2006

Iwona Pilecka (Laboratory of Cell Biology, IIMCB) RNA interference in theory and practice. 26.10.2006

Agnieszka Chmiel (Laboratory of Bioinformatics and Protein Engineering, IIMCB) MSMS – mass spectrometry made simple. 29.11.2006

Magdalena Kaus-Drobek (Laboratory of Structural Biology, IIMCB) DNA mimicry by proteins. 14.12.2006

Malgorzata Perycz (Laboratory of Molecular and Cell Neurobiology, IIMCB) Local translation in neurons. 21.12.2006

Grants

International

6th Framework Programme

- "Structural studies of membrane proteases" (MTKD-CT-2006-042486); 626,800 EUR; 2006-2010; M. Bochtler
- "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. Miaczynska
- "A multidisciplinary approach to the study of DNA enzymes down to the single molecule level" (MRTN-CT-2005-019566); 254,452 EUR, matching funds 606,181 PLN; 2005-2009; J.M. Bujnicki
- "From cell-cell recognition to memory formation. New strategies for the treatment of dysfunctional plasticity, learning and memory" (LSHM-CT-2005-512012); 478,000 EUR, matching funds 1,203,600 PLN; 2005-2009; J. Kuznicki
- "Co-ordinated internet-linked networks for promoting innovation, exchanging knowledge and encouraging good practice to enhance bioscience education in European schools" (Sub-contract No1 to EC Contract 511180; SAS6); 38,534 EUR, matching funds 77,520 PLN; 2005-2008; J. Lilpop (SFN)
- "Genetic testing in Europe - Network for test development harmonization, validation and standardization of services" (LSHB-CT-2004-512148); 30,000 EUR, matching funds 70,591 PLN; 2005-2009; M. Witt
- "Abnormal proteins in the pathogenesis of neurodegenerative disorders" (LSHM-CT-2003-503330); 161,200 EUR, matching funds 457,000 PLN; 2004-2006; J. Kuznicki
- "Mechanisms of transgene integration and expression in crop plant plastids: underpinning a technology for improving human health" (LSHG-CT-2003-503238); 164,160 EUR, matching funds 477,000 PLN; 2004-2007; J.M. Bujnicki

5th Framework Programme

- "Centre of Excellence in Molecular Bio-Medicine" (QLK6-CT-2002-90363); 350,000 EUR and supplementary grant from KBN 996,000 PLN, matching funds 30 000 PLN 2003-2006; J. Kuznicki
- "Novel non-antibiotic treatment of staphylococcal diseases" (QLK2-CT-2002- 01250); 238,382 EUR, matching funds 776,000 PLN; 2002-2006; M. Bochtler

Other International Funds

- International Research Scholars: "Signaling from endosomes to the nucleus: The role of APPL proteins and their interacting partners"; Howard Hughes Medical Institute; 500,000 USD; 2006-2010; M. Miaczynska
- "Communication between intracellular organelles in trafficking and signalling: The role of APPL proteins", International Senior Research Fellowship of the Wellcome Trust (UK); 4,315,706 PLN; 2006-2010; M. Miaczynska
- The MPI-CBG/IIMCB Partner Group at the IIMCB; 60,000 Euro; 2006-2008; M. Miaczynska
- Grant NIH CA097899-01 Innovations in Biomedical Information Science and Technology: „Discovering new human DNA repair genes by bioinformatics"; 160,000 USD subcontract within a collaborative grant coordinated by Dr. A.S. Bhagwat (Wayne State University, Detroit, MI); 2005-2007; J.M. Bujnicki
- Grant NIH "Kinetoplastid SL RNA biogenesis"; 100,000 USD; 2004-2009; J.M. Bujnicki
- Grant NIH: Fogarty International Research Collaboration Award (FIRCA) "Low-resolution Structural Genomics of Nucleases" 53,365 USD; 2004-2006; J.M. Bujnicki
- Utrecht University fellowships for five PhD students (M. Witt's lab, IIMCB and Institute of Human Genetics PAN, Poznan; M. Zylicz's lab, IIMCB; A. Lipkowski's lab, Center for Experimental and Clinical Medicine, PAN, Warsaw; L. Kaczmarek's lab, Nencki Institute PAN, Warsaw; 10,000 EUR annually from 2004 to 2007

- EMBO & HHMI Young Investigator Programme Award; 78,000 USD and 150,000 PLN; 2004-2006; M. Bochtler
- The Max Planck Society (MPG) – the Polish Academy of Sciences (PAN) MPI-CBG Junior Research Group Program - Laboratory of Structural Biology MPG/PAN; 1,500,000 EUR, 2001-2008; M. Bochtler

Polish

Ministerial Research Grants

- 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.Kuznicki
- Research Developmental Grant „New tools for analysis and manipulations of nucleic acids: restriction enzymes acting on RNA and DNA-RNA hybrids (R12 002 02)"; 1,000,000 PLN; 2007-2010; J.M. Bujnicki
- Polish-Spanish Special Grant "Computer prediction and simulation of RNA tertiary structure formation" (HISZPANIA/152/2006); 553,600 PLN; 2007-2010; J.M. Bujnicki
- Polish-German Special Grant "The role of cell cortex contractility in the establishment and positioning of the cleavage furrow", (JRGP/37/2005) The Max Planck Society (MPG) – the Polish Academy of Sciences (PAN) MPI-CBG Junior Research Program - Laboratory of Cortex Movement and Cell Division MGP/PAN; 3,024,200 PLN; 2006-2008; E. Paluch
- "Role of mTOR-regulated proteins in development of dendritic tree of hippocampal and cortical neurons" (0966/P01/2006/30); 220,800 PLN; 2006-2009; J. Jaworski
- "Biochemical and microscopical characterization of APPL-positive endosomes" (0394/P04/2005/28); 390,040 PLN; 2005-2008; M. Miaczynska
- "Identification of natural substrates for enzymes from the HINT family of phosphoroamidases and identification of enzymes that synthesize these substrates" (0453/P04/2005/29); 352,000 PLN; 2005-2008; P. Bieganowski
- "Investigation of structure of presenilin protein and significance of its mutations in Alzheimer's disease development" (0695/P05/2005/29); 220,000 PLN; 2005-2007; K. Jozwiak
- "STRUF- the novel software for classification and prediction of proteins functions" (1581/T11/2005/29); 105,000 PLN; 2005-2007; J. Sasin
- "Differences in action of stress-induced and constitutively synthesized Hsp70" (KBN-0408/P04/2004/27); 550,200 PLN; 2004-2007; M. Zylicz
- "Combating bacterial resistance against MLSb antibiotics by design of a novel type of inhibitors against Erm methyltransferases" (KBN-0611/P05/2004/27); 93,000 PLN; 2004-2006; M. Feder
- „Receptors and drugs. Modeling of G Protein-Coupled Receptor and their interactions with drugs in case of opioid receptors"; (KBN-3/P05F/026/25); 120,000 PLN; 2003-2006; S. Filipek
- "Identification of specificity determinants of restriction endonucleases by bioinformatics and mutagenesis" (KBN-0344/P04/2003/24); 300,000 PLN; 2003-2006; J.M. Bujnicki

Ministerial Habilitation Grants

- „Variation of restriction enzymes sequence specificities by combination of different methods of bioinformatics and protein engineering" (3043/P01/2006/31); 278,000 PLN; 2006-2009; K. Skowronek

Ministerial Doctoral Grants

- „Sequence-structure-function relationships in apoptotic nuclease DNase II" (2409/P01/2006/31); 30,080 PLN; 2006-2007; J.M. Bujnicki
- „Modelling of the structure and the process of formation of the complex of oligomeric rhodopsin and trimeric G protein" (3154/P01/2006/31); 33,200 PLN; 2006-2007; S. Filipek
- "Investigation of the structure of arrestin-rhodopsin complex by theoretical methods" (0121/P01/2006/30); 33,000 PLN; 2006-2007; S. Filipek

Ministerial Commissioned Grants

- „Mechanism of biosynthesis of unusual protein-glycosaminoglycan linkage that plays key role in inflammatory processes" POL-POSTDOC II grant; 106,560 PLN; 2007-2008; A. Kaczmarczyk
- „Structural studies of restriction endonucleases generating unusual cleavage patterns" POL-POSTDOC II grant; 160,000 PLN; 2007-2008; H. Czapinska
- "Advanced molecular methods in haematology. Development and implementation of standardized research procedures for minimal residual disease, posttransplantation chimerism and marker translocations" (PBZ-KBN-120/P05/2004); 3,027,500 PLN; 2005-2008; 13 groups in Poland Director: M. Witt
- "New bioinformatics tools for proteomics and structural genomics" (KBN-K089/P04/2004); 1,850,000 PLN; 2004-2007; 5 groups in Poland, including 2 in IIMCB (Bujnicki and Bochtler); Director: J.M. Bujnicki
- Bilateral Polish-German Ordered Research Grant (KBNK064/P05/2003) "The transduction of neuronal Ca^{2+}-signals via EF-hand calcium-binding proteins caldendrin and calmyrin in Alzheimer's disease and psychotic

disorders"; 955,400 PLN; 2003-2006; Director: U. Wojda with cooperation with Dr. M.R. Kreutz, Department of Neurochemistry, Molecular Biology, Leibniz Institute for Neurobiology, Magdeburg

Ministerial Commissioned Grants coordinated by other institutions

- Three tasks within an ordered grant (PBZ-MNiI-2/1/2005) „Application of contemporary functional genomics and bioinformatics to characterize and develop models of biological processes of medical and agricultural interest": 1) Modeling of protein structures and their complexes, 2) A database of systems for DNA repair and degradation, 3) Experimental analyses of DNA repair proteins; 340,000PLN; 2006-2009; J.M. Bujnicki
- „From patterns of gene expression and regulatory motifs towards prediction and modeling of global gene expression in brain physiology and pathology", Director: B. Kaminska-Kaczmarek from Nencki Institute; within the ordered grant: „Application of functional genomics and bioinformatics for characterization and modeling of biological processes of critical importance for medicine and agriculture"; 375,000 PLN; 2006-2009; J. Jaworski
- "Search for diagnostic methods of Alzheimer's disease and identification of pathogenic mechanisms as potential targets of therapies based on proteomic research in human lymphocytes" (K129/P05/2005/UMED6); 400,000 PLN; 2005-2008; U. Wojda (within ordered grant directed by Medical University of Lodz)
- "Role of Hsp90 in regulation of gene expression involved in tumorgenic transformation", (PBZ–KBN–107/P04/2004); 985,000 PLN; 2004-2007; M. Zylicz (within ordered grant directed by Jagiellonian University)

Other Research Grants

- Professorial Grant from Foundation for Polish Science (SP10/04) „Beta-catenin metabolism in health and disease"; 240,000 PLN; 2004-2006; J. Kuznicki
- Scientific Network organized by Institute of Pharmacology of Polish Academy of Science - „Looking for systemic targets of potential neurotropic drugs" (26/E-40/SN-023/2006); 52,500 PLN; M. Wisniewska

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

- Dawidowska M, Derwich K, Szczepanski T, Jolkowska J, van der Velden VHJ, van Dongen JJM, Wachowiak J. Witt M, Characterisation of immunoglobulin and T-cell receptor (Ig/TCR) gene rearrangements in Polish pediatric acute lymphoblastic leukemia patients implications for RQ-PCR-based assessment of minimal residual disease. Leukemia Res, 2006, 30:1119-25
- Wojda A, Zietkiewicz E, Mossakowska M, Pawlowski W, Skrzypczak K, Witt M. Correlation between the level of cytogenetic aberrations in cultured human lymphocytes and the age and gender of donors. J Gerontol A Biol Sci Med Sci, 2006; 61(8):763-772
- Geremek M, Zietkiewicz E, Diehl SR, Alizadeh BZ, Wijmenga C, Witt M. Linkage analysis localizes a Kartagener syndrome gene to a 3.5 cM region on chromosome 15q24-25. J Med Genet, 2006; 43: e1. doi: 10.1136/jmg.2005.031526
- Dawidowska M, Wachowiak J, Witt M. Molecular diagnostic methods and assessment of effectivenes of therapy in modern pediatric hematooncology. Postepy Biochemii, 2006; 52:408-416.

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 based on the "rolling tenure" mechanism; 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 prolonged. There are no permanent positions at the Institute.

A history of these competitions dates back to 1998 when the first one was resolved. The table below shows details of each of the competitions completed to date.

Competition	Year	Number of candidates	**Winners employed at IIMCB**
I	1998	6	**Jaroslaw Dastych**
II	1999	3	**Maciej Zylicz**
III	2000	6	**Michal Hetman**
IV[1)]	2000	7	**Matthias Bochtler, Leszek Rychlewski**
V	2002	9	**Janusz M. Bujnicki, Slawomir Filipek**
VI[2)]	2002	9	-
VII	2003	18	**Marta Miaczynska**
VIII[3)]	2004	26	-
IX	2005	26	**Jacek Jaworski**
X[1)]	2005	17	**Ewa Paluch**
XI	2006	25	**Marcin Nowotny**

[1)]these competitions fulfilled the MPG/PAN agreement

[2)]no result

[3)]the winner did not accept the offer

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 Nature and/or Science and other highly internationally visible sources, including electronic 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 participate 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. This results in a job offer given to the winner(-s) of the competition.

The last competition, resolved in 2006, attracted to IIMCB 25 candidates, among them: 19 foreigners, five Polish nationals from abroad and one Polish citizen applying within the country. Dr. Marcin Nowotny, who accepted an offered position, should start his research activities at IIMCB in November of 2007. He already received the EMBO Installation Grant.

International Cooperation

With the Max Planck Society

The **Laboratory of Cortex Movements and Cell Division**, a twin lab of Matthias Bochtler's MPG/PAN laboratory operating at IIMCB since 2001, started its activities on the 1st of February 2006 and is headed by Dr. Ewa Paluch. The equipment and running costs for the lab, including personnel, are provided by the Polish site. The Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (MPI-CBG), being a host for this laboratory, covers local operational costs, maintenance, and provides administrative support. Dr. Ewa Paluch's group focuses on biochemical and physical mechanisms of cell shape and deformations. The research is concentrated on movements of the actomyosin cortex, the involvement of spontaneous cortical ruptures and flows in cell division in particular. The project-oriented cooperation between IIMCB and MPI-CBG is significantly intensified by the activity of the **Partner Group of the MPI of Molecular Cell Biology and Genetics** called the MPI-CBG/IIMCB Partner Group at the International Institute of Molecular and Cell Biology. Dr. Marta Miaczynska, head of the Laboratory of Cell Biology, leads the Partner Group and Prof. Marino Zerial, is the cooperating partner and scientific mentor at MPI-CBG. The Partner Group, set up for 3 years with the possibility of a 2-year extension, commenced its work in the beginning of 2006. Within the Partner Group programme, in November 2006 IIMCB hosted Trilateral Workshop for Young Scientists from Germany, Czech Republic and Poland, focused on „Cell Biology of Intracellular Transport Processes" which included also the MPI Partner Group of Dr. David Stanek at the Charles University in Prague.

With Utrecht University

A research collaboration program with Utrecht University was initiated by Prof. Willem Gispen, Rector of Utrecht University. The main goal of this program was to foster Polish – Dutch exchange of scientific information and to strengthen the research cooperation through bilateral visits of staff members and their students. Furthermore, eight Polish doctoral students received four-year fellowships to work in Poland on their doctoral thesis; to date, two of them defended their thesis in Utrecht.

With the Italian National Research Centre on Ageing (INRCA), Ancona

After a few years of successful cooperation, existing collaborations of scientists between the Italian National Research Centre on Ageing in Ancona and IIMCB were formalized. Prof. Claudio Franceschi representing INRCA and Prof. Jacek Kuznicki representing IIMCB have signed an agreement on the creation of an exchange program in the area of biomedical sciences. In 2006, IIMCB administration staff members visited Ancona in order to share experiences in particular issues of research organisation and management.

With the Palladin Institute of Biochemistry of NAS of Ukraine

Director of IIMCB, J. Kuznicki and the Director of the Palladin Institute of Biochemistry of NAS of Ukraine, S. V. Komissarenko, have signed an official agreement on scientific cooperation.

Foreign Visits to IIMCB:

1) 7.02.2006: visit of delegation from Federal Ministry for Education and Research, Bonn, Germany:
- **Dr. Herbert Diehl**, Director–General, European and International Affairs;
- **Dr. Erika Rost,** Head of Division, Cooperation with Central and Eastern European Countries;
- **Dr. Stephanie Splett–Rudolph,** German Aerospace Center.

2) 27.04.2006: working visit of delegation from Institut National de la Sante et de la Recherche Medicale (INSERM), Inserm – Universite Lille 2, Lille, France
- **Prof. Pierre Formstecher,** Head of Jean–Pierre Aubert Research Center;
- **Prof. Renata Polakowska,** Inserm Research Director;
- **Dr. Luc Buee,** Departament of Neuroscience, Jean–Pierre Aubert Research Center;
- **Dr. Jean–Pierre Kerckaert,** Inserm Research Director, Coordinator of the Project: Human Genome Exploration by High Resolution CGH–Array;

– **Prof. John Wood,** Chief Executive, Council for the Central Laboratory of the Research Councils (CCLRC), Chilton Didcot, UK.

3) 19.05.2006:
– **Dr. Salem Chouaib,** Research Director INSERM;
– **Prof. Pierre Formstecher,** Head of the Jean–Pierre Aubert Research Center, Inserm – Universite Lille 2;
– **Dr. Anne Bisagni,** Head of the Department for Regional and European Policy;
– **Dr. Philippe Arhets,** Deputy Director of the Department for Regional and European Policy, Coordinator of the French National Contact Point for the Life Sciences and Health of the 6th FP;
– **Mr. Pierre Michael,** Scientific Attache, the French Embassy in Poland;
– **Ms. Sandra Bravard,** Deputy Scientific Attache, the French Embassy in Poland

4) 13. 09.2006: **Prof. Abraham Tamir,** Professor of Chemical Engineering, founder the Museum of Art and Science, Ben–Gurion University of Negev, Beer–Sheva, Israel.

5) 14–15.09.2006: visit of the Nobel Laureate – **Dr. Richard J. Roberts**

6) 20.09.2006: Prof. Giorgi Kvesitadze, Director of the Durmishidze Institute of Biochemistry and Biotechnology, Tbilisi, Georgia.

7) 3.11.2006: visit of delegation from Vietnam:
– **Prof. Nguyen Quang,** Vice Director, Central Commission for Science and Education (CCSE), Department for Natural Science, Technology and Environment, Hanoi, Vietnam;
– **Dr. Trinh Van Tu,** General Director, Department for Local Science and Education, The Commission for Science and Education (CSE), Hanoi, Vietnam;
– **Bui Si Tieu,** Vice Chairman of CCSE, Hanoi, Vietnam;
– **Nguyen Quang Thuan,** First Secretary, Embassy of S.R. of Vietnam.

8) 24–31.05.2006: working visit of **Prof. Klaus Hahlbrock,** Laureate of Alexander von Humboldt Honorary Research Fellowship of Foundation for Polish Science; Professor Emeritus; Max-Planck Institute for Plant Breeding Research, Köln, Germany.

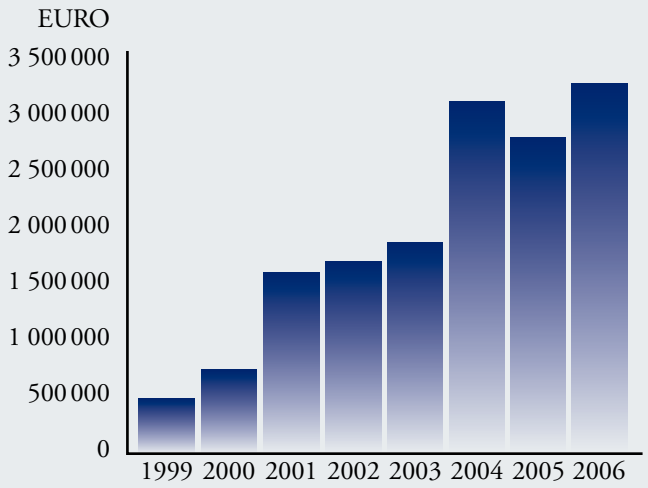
Mid- and long-term research visits (1999-2007)

- **Frank King,** MSc (USA) – PhD student in the Department of Molecular Biology, 1999-2001; graduated in October, 2001
- **Sanne Mikkelsen,** MSc (Denmark) - was involved in Polish Centenarians Program PolStu99, then worked in the Laboratory of Neurodegeneration, 1999-2001
- **Sophie Chiron** (France) – chief of 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, since Nov. 2006
- **Kristian Rother,** PhD (Germany/Finland) – Post-doctoral fellow in the Laboratory of Bioinformatics and Protein Engineering, since Oct. 2006
- **Neli Kachamakova,** PhD (Bulgaria) – Post-doctoral fellow in the Laboratory of Neurodegeneration, since Sept. 2006
- **Tran Cat Dong,** PhD (Vietnam) – Post-doctoral fellow in the Laboratory of Neurodegeneration, since Feb. 2007

Richard J. Roberts of New England Biolabs, Ipswich, MA, USA, Nobel Laureate in 1993 in medicine, visiting Bujnicki's lab.

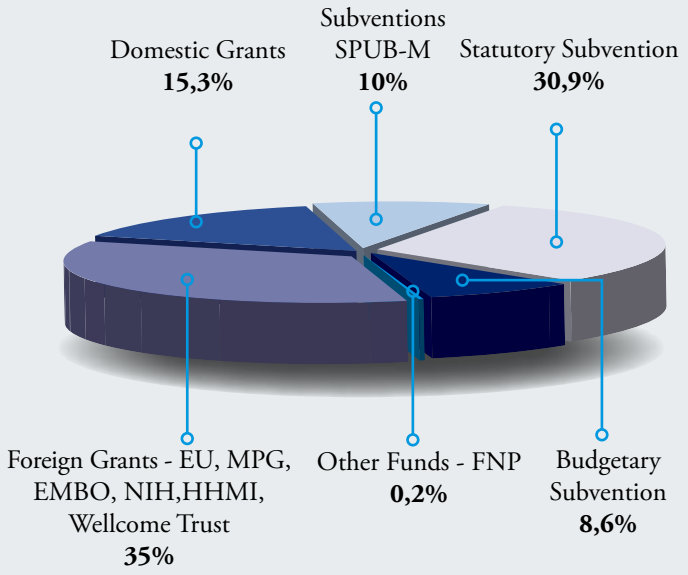
Diversity of Funding IIMCB '2006

Annual budget



Sources of Funding	amounts in PLN	amounts in EURO*
Budgetary Subvention	1 090 000	284 506
Statutory Subvention	3 899 000	1 017 697
Subventions SPUB-M	1 248 487	325 874
Domestic Grants	1 933 120	504 573
Foreign Grants - EU, MPG, EMBO, NIH, HHMI, Wellcome Trust	4 423 466	1 154 590
Other Funds - FNP	28 400	7 413
Total	**12 622 473**	**3 294 653**

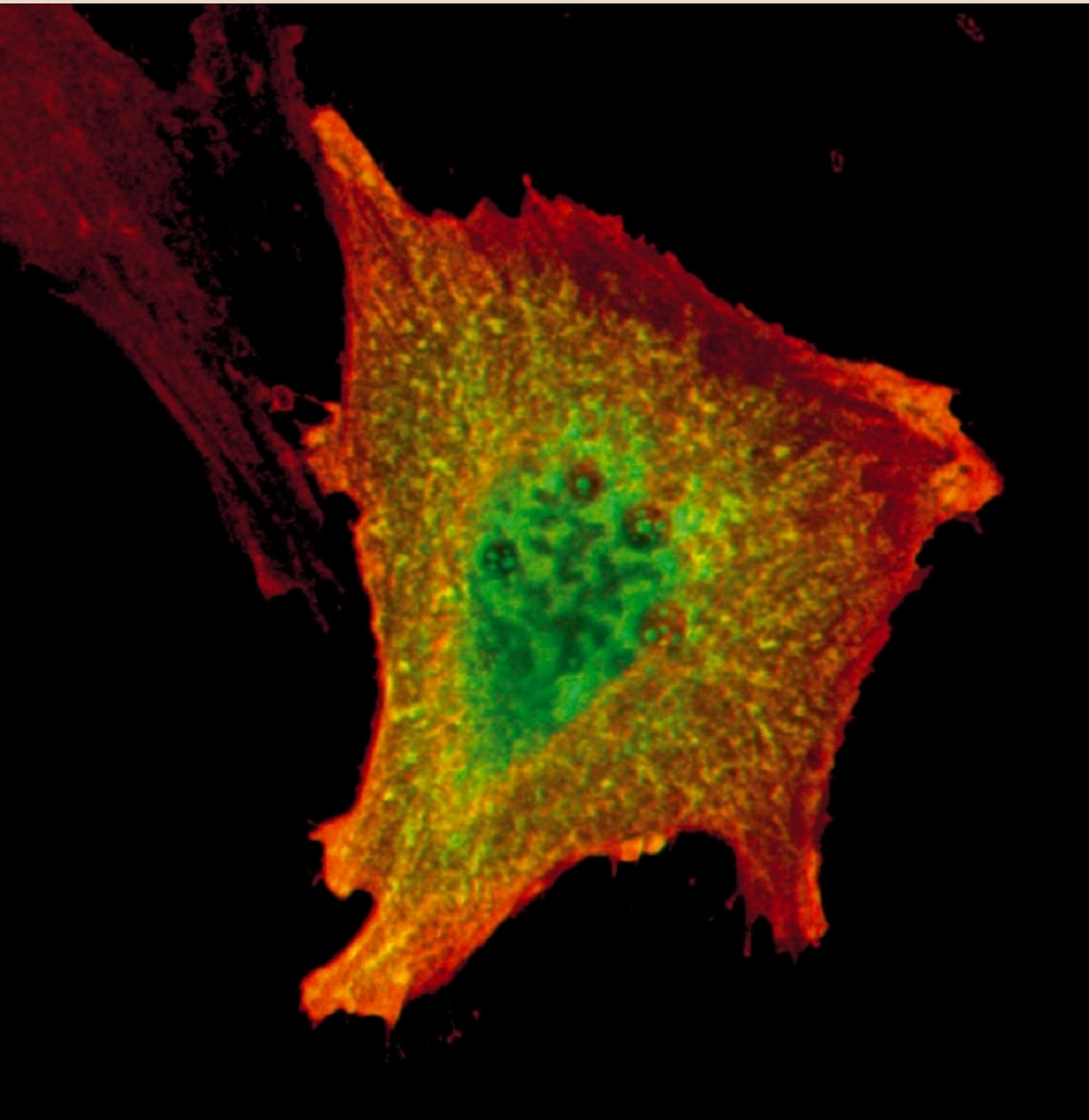
** 1 EURO = 3,8312 PLN @31st Dec `2006*



Profit & loss statement

	amounts in PLN
A. NET REVENUE ON SALES & EQUIVALENTS (Grants Completed)	11 425 737
B. OPERATIONAL ACTIVITY COSTS, in this:	12 382 766
Depreciation	1 396 565
Materials and energy	3 531 296
Services	950 027
Fees and taxes	488 630
Salaries and wages	3 553 020
Social and health insurance	816 602
Other operational expenses, in this:	1 646 626
- business trips	694 315
- property insurance	31 484
- grant indirect costs	591 942
- fellowships	328 885
C. OTHER OPERATIONAL INCOME (subventions)	959 373
D. OTHER OPERATIONAL EXPENSES	2 261
E. FINANCIAL INCOME, in this:	71 949
Interests	70 026
Other	1 923
F. FINANCIAL EXPENSES, in this:	47 764
Interests	218
Other	47 546
PROFIT / LOSS ON BUSINESS ACTIVITY (A-B+C-D+E-F)	**24 268**

Department of Molecular Biology



Lab Leader

Maciej Zylicz, PhD, Professor

Vice Head:
Alicja Zylicz, PhD, Professor

Research Associate:
Pawel Bieganowski, PhD
Marcin Klejman, PhD

Research Assistant:
Maciej Olszewski, MSc (since January 2006)

PhD students:
Malgorzata Gutkowska, MSc
Leszek Lipinski, MSc
Jakub Urbanski, MSc
Dawid Walerych, MSc
Bartosz Wawrzynow, MSc (until December 2006)
Anna Zurawska, MSc (since September 2006)

Secretary:
Grazyna Orleanska, MSc

Technician:
Wanda Gocal

Picture on the left:
SAOS-2 cells transfected with p53V143A temperature-sensitive mutant (green) and Hsp70 (red), cultured for 24hrs at 37°C.

Maciej Zylicz, PhD, Professor

Degrees

Professor, 1992

DSc.Habil. in molecular biology, Institute of Biochemistry and Biophysics PAN, Warsaw, Poland, 1986

PhD in biochemistry, Medical University of Gdansk, Poland, 1979

MSc in physics and biology, University of Gdansk, Poland, 1977 (student of physics and biology)

Post-doctoral Training

1982-1984 University of Utah, Department of Cellular, Viral and Molecular Biology, Salt Lake City, UT, USA

1979-1981 University of Gdansk, Department of Biochemistry, Gdansk

Professional Employment

since 2005 President, Executive Director of the Foundation For Polish Science (FNP)

since 1999 Head of the Department of Molecular Biology, IIMCB

1994-1999 Head of the Department of Molecular and Cellular Biology, Faculty of Biotechnology, University of Gdansk

1991-1994 Head of the Department of Molecular Biology, University of Gdansk

1993-1994 Visiting Professor, University of Utah, Medical Center, Institute of Oncology, Salt Lake City, UT, USA

1990-1993 Vice President, University of Gdansk

1988-1991 Associate Professor, Department of Molecular Biology, University of Gdansk

1981-1988 Assistant Professor, Department of Biochemistry, University of Gdansk

Other Professional Activities

2000-2004 Chair of Biology, Earth Sciences and Environmental Protection Commission of the State Committee for Scientific Research

Membership in Scientific Societies, Organizations and Panels

- Member of Polish Academy of Sciences
- Member of Polish Academy of Arts and Sciences
- Member of Academia Europaea
- Member of American Society of Biochemistry and Molecular Biology
- Member of EMBO
- Member of Advisory Editorial Board of EMBO Journal, EMBO Reports and IUBMB Life
- Member of EMBO Council (2004-2009)
- Member of the Selection Committee for EMBO YIP (2001-2003)
- Polish delegate to EMBC (2001-2004)
- Member of the State Committee for Scientific Research (1997-2004)
- Polish delegate to Life Science Committee of ESF (2003-2005)
- Member of the Selection Committee for the special DFG programs (2001-2005)

Honors, Prizes, Awards

1. Prime Minister Award for Scientific Achievements, 2002
2. "L. Marchlewski" Award from the Biochemistry and Biophysics Committee PAN, 2001
3. Award from the Foundation for Polish Science (FNP) in biological/medical sciences, 1999
4. Award from the Polish Biochemical Society for the best biochemistry work performed in Polish laboratories, 1996
5. Award from the Ministry of Education, 1994
6. "Heweliusz" Prize for the Scientific Achievements, awarded by the President of Gdansk, 1993
7. Award from the Polish Academy of Sciences, 1990
8. Individual Award from the Polish Academy of Sciences for Scientific Achievements, 1986

Doctorates

Liberek K, Skowyra D, Osipiuk J, Banecki B, Wojtkowiak D, Jakobkiewicz J, Puzewicz J, Barski P, King F, Bucko-Justyna M, Kudla G.

DSc Habil. Performed in the Department

Liberek K, Werel W, Marszalek J, Konieczny I, Wawrzynow A, Banecki B.

Professor Titles Received:

Liberek K, Marszalek J, Konieczny I, Wawrzynow A.

Publications

81 publications in primary scientific journals including: two papers published in Cell, six in EMBO J., six in PNAS and 25 in J. Biol. Chem. These papers were cited more than 4,500 times with an average citation of 60 per paper.

Selected publications since 2001

- Bieganowski P, Seidle HF, Wojcik M, Brenner C. Synthetic lethal and biochemical analyses of NAD and NADH kinases in Saccharomyces cerevisiae establish separation of cellular functions. J Biol Chem, 2006; 281(32):22439-45
- Wojcik M, Seidle HF, Bieganowski P, Brenner C. Glutamine-dependent NAD^+ synthetase. How a two-domain, three-substrate enzyme avoids waste. J Biol Chem, 2006; 281(44):33395-402
- Galazka G, Stasiolek M, Walczak A, Jurewicz A, Zylicz A, Brosnan CF, Raine CS, Selmaj KW. Brain-derived heat shock protein 70-peptide complexes induce NK cell-dependent tolerance to experimental autoimmune encephalomyelitis. J Immunol, 2006; 176(3):1588-99
- Kudla G, Lipinski L, Caffin F, Helwak A, Zylicz M. High guanine and cytosine content increases mRNA levels in mammalian cells. PLoS Biol, 2006; 4(6):0933-0942
- *Ilyushik E, Pryce DW, Walerych D, Riddell T, Wakeman JA, McInerny CJ, McFarlane RJ. Psc3 cohesin of Schizosaccharomyces pombe: cell cycle analysis and identification of three distinct isoforms. Biol Chem, 2005; 386:613-21
- *Klejman MP, Zhao X, van Schaik FMA, Herr W, Timmers HTM. Mutational analysis of BTAF1-TBP interaction: BTAF1 rescues DNA-binding defective TBP mutants. Nucl Acids Res, 2005; 33:5426-36
- Bucko-Justyna M, Lipinski L, Burgering BMT, Trzeciak L. Characterization of testis specific serine-threonine kinase 3 and its activation by phosphoinositide-dependent kinase-1-dependent signalling. FEBS J, 2005; 272:6310-23

*Papers marked with an asterisk have no IIMCB affiliation

- Mycko PM, Cwiklinska H, Szymanski J, Szymanska B, Kudla G, Kilianek L, Odyniec A, Brosnan CF, Selmaj KW. Inducible heat shock protein 70 promotes myelin autoantigen presentation by the HLA Class II. J Immunol, 2004; 172:202-213
- Kudla G, Helwak A, Lipinski L. Gene conversion and GC-content evolution in mammalian Hsp70. Mol Biol Evol, 2004; 21:1438-44
- Jassem J, Jassem E, Jakobkiewicz-Banecka J, Rzyman W, Badzio A, Dziadziuszko R, Kobierska-Gulinda G, Szymanowska A, Skrzypski M, Zylicz M. p53 and K-ras mutations are frequent events in microscopically negative surgical margins from patients with non-small cell lung carcinoma. Cancer, 2004; 100:1951-60
- 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
- Muller L, Schaupp A, Walerych D, Wegele H, Buchner J. Hsp90 regulates the activity of wild type p53 under physiological and elevated temperatures. J Biol Chem, 2004; 279:48846-54
- 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
- Jassem E, Niklinski J, Rosell R, Niklinska W, Jakobkiewicz J, Monzo M, Chyczewski L, Kobierska G, Skokowski J, Zylicz M, Jassem J. Types and localisation of p53 gene mutations. A report on 332 non-small cell lung cancer patients. Lung Cancer, 2001; 34:47-51
- Zylicz M, Wawrzynow A. Insights into the function of Hsp70 chaperones. IUBMB Life, 2001; 51:283-287
- King FW, Wawrzynow A, Hohfeld J, Zylicz M. Co-chaperones Bag-1, Hop and Hsp40 regulate Hsc70 and Hsp90 interactions with wild-type or mutant p53. EMBO J, 2001; 20:6297-6305
- Zylicz M, King FW, Wawrzynow A. Hsp70 interactions with the p53 tumour suppressor protein. EMBO J, 2001; 20:4634-4638
- Genevaux P, Wawrzynow A, Zylicz M, Georgopoulos C, Kelley WL. DjlA is a third DnaK co-chaperone of Escherichia coli, and Dj1A-mediated induction of colanic acid capsule requires Dj1A-DnaK interaction. J Biol Chem, 2001; 276:7906-12
- Banecki B, Wawrzynow A, Puzewicz J, Georgopoulos C, Zylicz M. Structure–function analysis of the zinc binding region of the ClpX molecular chaperone. J Biol Chem, 2001; 276:18843-18848
- Kaczanowski R, Trzeciak L, Kucharczyk K. Multitemperature single-stranded conformation polymorphism. Electrophoresis, 2001; 22:3539-45

Current Research

The research conducted in our department is predominantly focused on the role of molecular chaperones in mammalian cells including cell transformation (review Zylicz et al., 2001). Previously, using highly purified recombinant human proteins, we have identified intermediate reactions that lead to the assembly of molecular chaperone complexes with wild type or mutant p53 tumour suppressor protein (King et al., 2001). We have discovered that Hsp90 possesses higher affinity towards wild type p53 than to the conformational mutant p53. Lately we have demonstrated that Hsp90 molecular chaperone is required for binding of wt p53 to the promoter sequences under physiological temperature of 37°C in an ATP-dependent reaction (Walerych et al., 2004; Muller et al., 2004). These results obtained in vitro were supported by the observation that the treatment of human fibroblasts with geldanamycin or radicicol (Hsp90 specific inhibitors) resulted in dramatic decrease of the p21 mRNA and, in consequence, the p21 protein level, while the p53 mRNA and Ser-15P-p53 protein levels were mostly unaffected. Additionally, using ChIP technology and real-time PCR, we showed that Hsp90 inhibitors decreased the amount of chromatin-bound p53 located near the *p21/waf1* promoter sequence (Walerych et al., 2004). Moreover, using in vivo FRET analysis, we showed that p53 forms a transient complex with Hsp90 and using DNA chip technology, we showed that transcription from other p53-dependent promoters is also affected by Hsp90 inhibitors. Surprisingly, MDM2 E3 ligase, in the absence of E2 and E1 ubiquitination system, can substitute for Hsp90 molecular chaperone in promoting ATP-dependent binding of p53 to the *p21/waf1* promoter - derived sequence. We have shown that the ATP-binding mutant MDM2 protein (K454A) lacks the chaperone activity both in vivo and in vitro. The *mdm2* cotransfected with wild-type *p53* stimulates efficient p53 protein folding in vivo and this effect is abrogated when the ATP-binding defective form of MDM2 is used. This is the first demonstration that MDM2, which overexpression is a new independent factor of adverse prognosis in non-small cell lung cancer (Dworakowska et al., 2004), possesses an intrinsic molecular chaperone activity and indicates that the ATP-binding function of MDM2 can mediate its chaperone function towards p53 tumour suppressor. It was reported previously that MDM2 interacts with, but does not ubiquitinate, several transcription factors, which could affect cell transformation. Our findings that MDM2 is a novel molecular chaperone could help to explain the p53-independent oncogenic activity of MDM2.

Extensive analysis of human genes, which code for members of Hsp70 family, showed that heat shock inducible HSPA-1 contains 92% of G or C in the silent, third positions of codons (GC3=92%), while for constitutively expressed HSPA-8 GC3 is only 46%. This finding supports the biased gene conversion hypothesis of GC-content evolution (Kudla et al., 2004) but, more importantly, leads to a more general discovery that high GC3 content increases the mRNA level in mammalian cells (Kudla et al., 2006). We performed transient and stable transfections of mammalian cells with GC-rich and GC-poor versions of Hsp70, green fluorescent protein and IL2 genes cloned under the same promoters and found that GC-rich genes were expressed 7-fold up to over 100-fold more efficiently than their GC-poor counterparts. This effect was due to the increase in mRNA level, but not to different translation or degradation rates of GC-rich and GC poor mRNA. We conclude that silent-site GC content correlates with gene expression efficiently in mammalian cells and that this finding could be applied in biotechnology (patent number P370282).

Projects conducted in the laboratory:

1. Human p53 oligomerization observed by the FRET method
2. Rescue of human p53 activity by molecular chaperones
3. Yeast functional assay for testing the interaction between human Hsp90 and p53
4. Hsp70 family proteins involvement in wild-type and mutant p53 structure and function maintenance under normal and stress conditions
5. Physical and enzymatic properties of human Hsp90 alpha and beta isoforms. Identification of isoform specific Hsp90 interacting proteins by systematic approach
6. Hsp90 involvement in cancer cell invasion and Matrix Metalloproteinase 2 (MMP2) activation
7. The molecular process of ΔN p63 α and γ isoforms activation by MDM2 molecular chaperone
8. Family of testes-specific protein kinases-genetic and biochemical characterization.

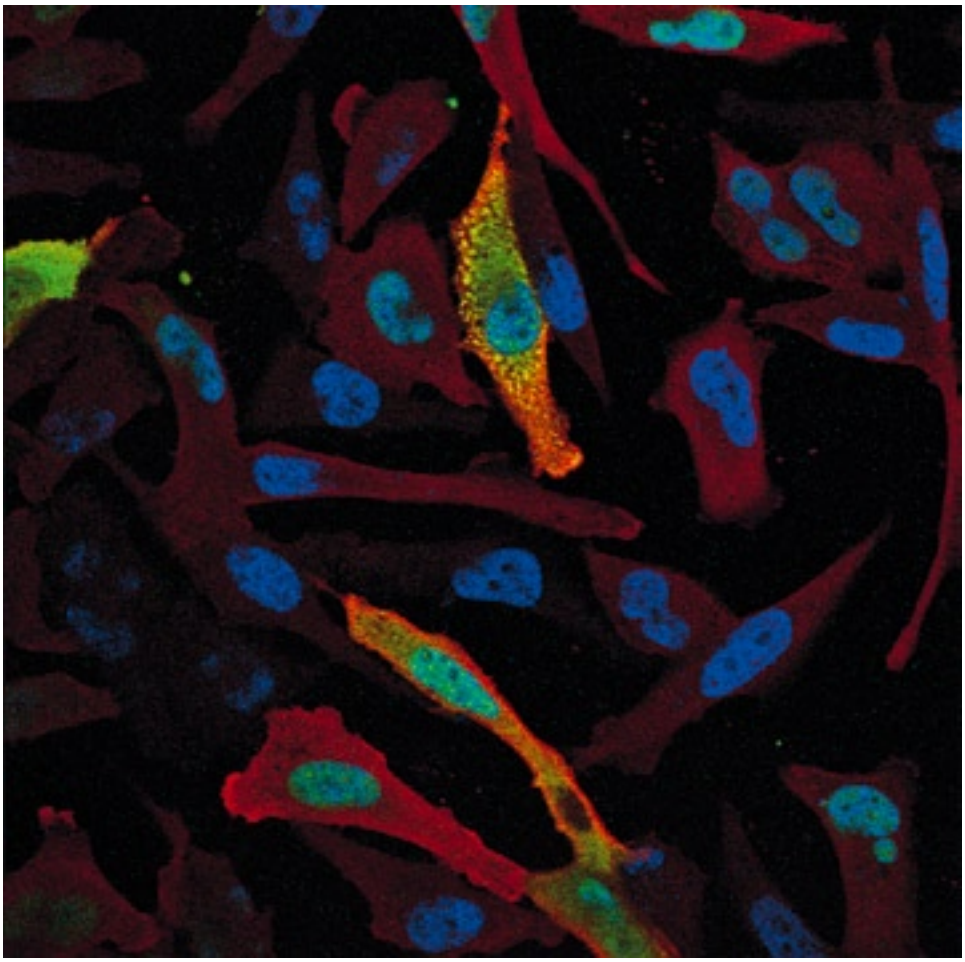


Fig. 1 HeLa cells transfected with p53V143A temperature-sensitive mutant (green) and Hsp70 (red), cultured for 24hrs

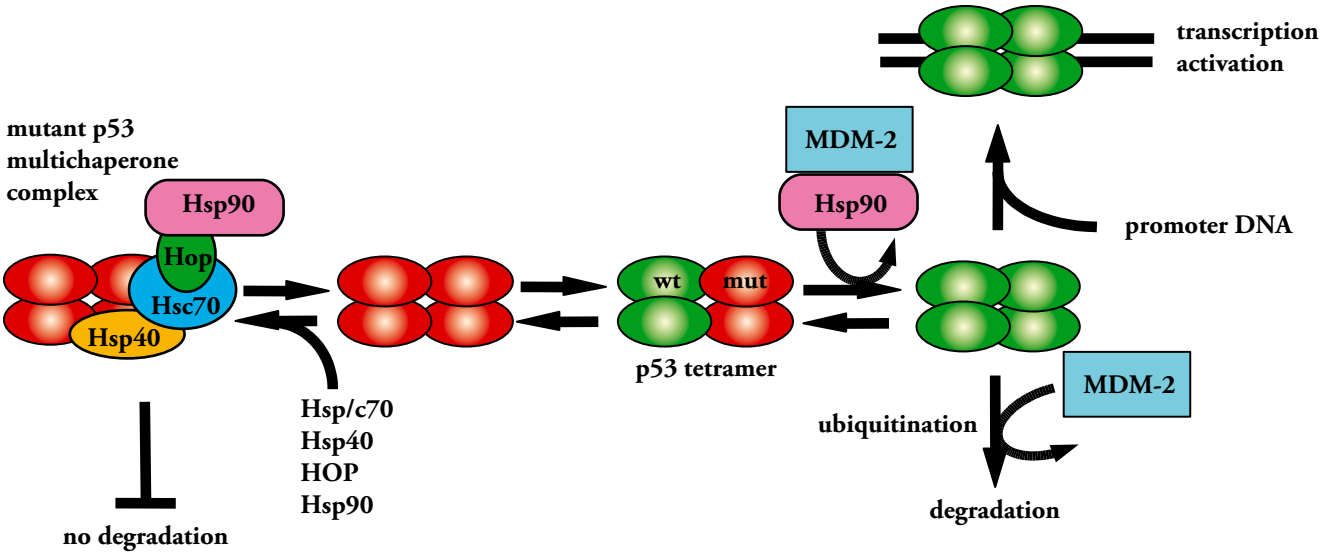


Fig. 2 The proposed model for a role of molecular chaperones in maintaining of p53 in cells at 37^{0}C.
The wild-type structure of p53 is represented by green ovals while the mutant conformation by red ones. p53 amino-acid changes, oncoproteins and other factors may shift the equilibrium between wild-type, mutant conformation and aggregation. Among those factors are molecular chaperones. With mutant p53 the stable multichaperone complex is formed while wild-type p53 interacts more transiently with Hsp90.

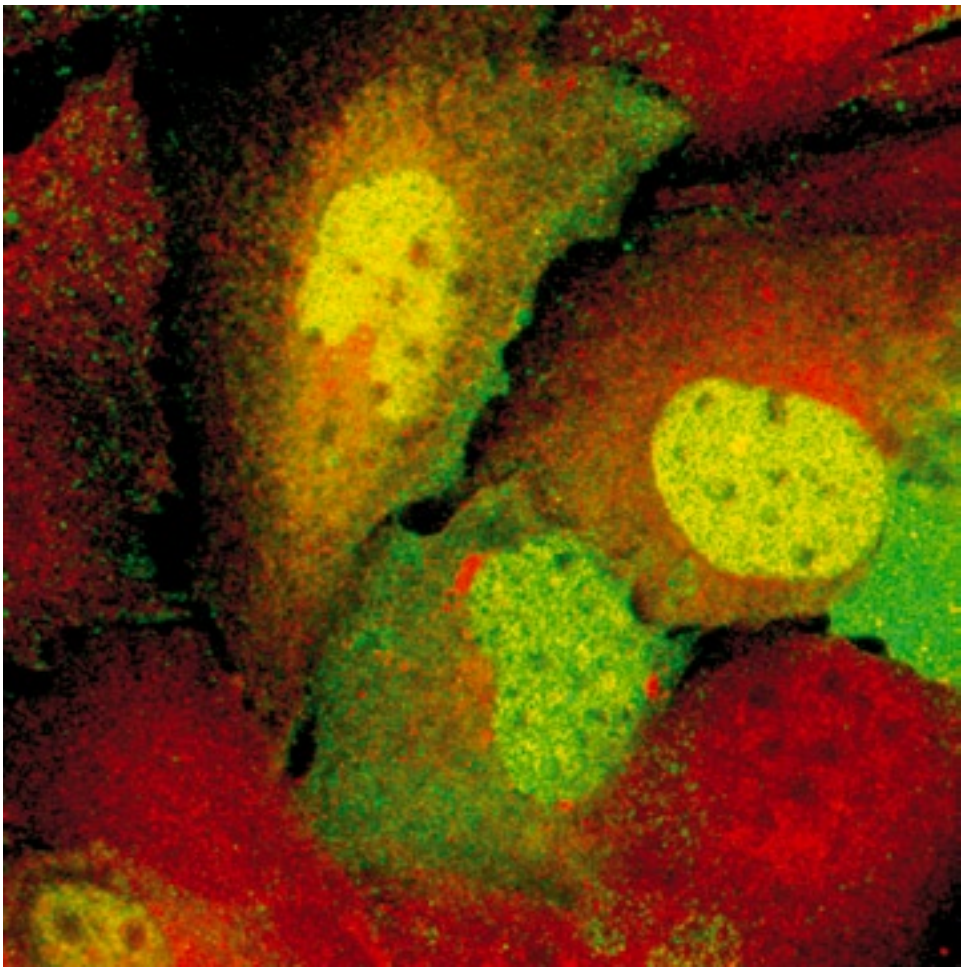
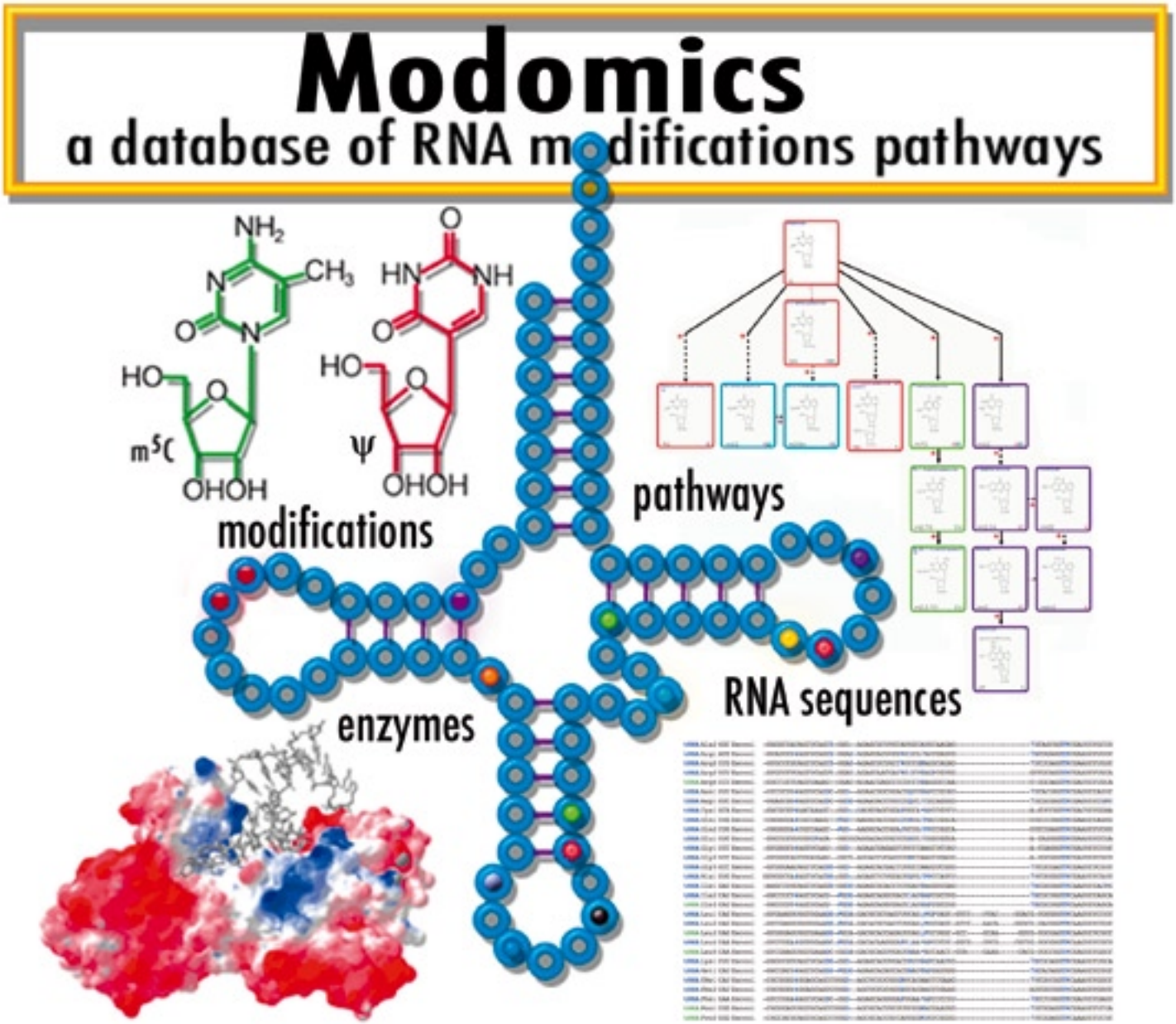
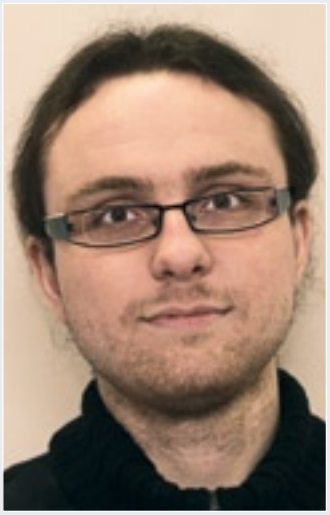


Fig. 3 SAOS-2 cells transfected with p53V143A temperature-sensitive mutant (green) and following 24hrs at 37^{0}C stained for Hsp70 (red).

Laboratory of Bioinformatics and Protein Engineering





Lab Leader

Janusz M. Bujnicki, PhD, DSc.Habil.

Post-doctoral fellows:
Krzysztof J. Skowronek, PhD
Kristian Rother, PhD
Michal Boniecki, PhD

PhD students & Research Assistants:
Agnieszka Chmiel, MSc
Iwona A. Cymerman, MSc
Malgorzata Durawa, MSc
Marcin Feder, MSc
Michal J. Gajda, MSc
Andrzej Kaminski, MSc
Jan Kosinski, MSc
Michal A. Kurowski, MD
Grzegorz Papaj, MSc
Sebastian Pawlak, MSc
Marcin Pawlowski, MSc
Michal J. Pietal, MSc
Elzbieta Purta, MSc
Joanna M. Sasin, MSc
Karolina L. Tkaczuk, MSc
Irina Tuszynska, MSc
Agnieszka Obarska-Kosinska, MSc

Undergraduate students:
Stanislaw Dunin-Horkawicz, BSc, Katarzyna Filip, BSc, Paweł Sztromwasser, BSc, Tomasz Jarzynka, Laura Lopez-Munoz

Office Manager:
Michal Wrzesinski, MSc

Computer administrator:
Jan Kogut

Picture on the left:
Home page of MODOMICS. A schematic representation of cytosolic Phe-tRNA secondary structure from Saccharomyces cerevisiae with modified nucleosides depicted by color dots. Satellite images illustrate the types of data stored in MODOMICS. The figure was prepared by Dr. Adriana Magalska from the Nencki Institute (Warsaw).

Janusz Bujnicki, PhD

Degrees

2005 DSc.Habil, Institute of Biochemistry and Biophysics, PAN, Warsaw

2001 PhD in bioinformatics; University of Warsaw, Faculty of Biology

1998 MSc in microbiology; University of Warsaw, Faculty of Biology

Professional Experience

since 2002 Head of the Laboratory of Bioinformatics and Protein Engineering IIMCB

since 2006 Visiting Associate Professor, Bioinformatics Laboratory at the Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland

2004-2006 Assistant Professor, Bioinformatics Laboratory at the 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, NLM, NIH, Bethesda, MD, 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, MI, USA (with Dr. L.C. Lutter)

Awards

2006 Award from the Prime Minister for the habilitation thesis

2006 Young Researcher Award in Structural and Evolutionary Biology of the Visegrad Group Academies of Sciences

2005 Group award from the Ministry of Health for co-authorship of series of publications regarding the biological function of protein K (head of the team: Prof. J. Ostrowski)

2003 Fellowship for Young Scientists from the Foundation for Polish Science

2002 EMBO/Howard Hughes Medical Institute Young Investigator Program award

2002 Award from the Polish Society of Genetics (the best Polish genetics-related publication in the year 2001: Trends Biochem Sci. 2001 Jan; 26(1): 9-11)

2001 Award from the Polish Biochemical Society (the best Polish publication on nucleic acid biochemistry in the year 2000: FASEB J. 2000 Nov; 14(14): 2365-2368)

Publications in 2006

- Gros L, Renodon-Corniere A, de Saint Vincent BR, Feder M, Bujnicki JM, Jacquemin-Sablon A. Characterization of PRMT7alpha and beta isozymes from Chinese hamster cells sensitive and resistant to topoisomerase II inhibitors. Biochim Biophys Acta, 2006; 1760(11):1646-56
- Purta E, van Vliet F, Tkaczuk KL, Dunin-Horkawicz S, Mori H, Droogmans L, Bujnicki JM. The *yfhQ* gene of *Escherichia coli* encodes a tRNA:Cm32/Um32 methyltransferase. BMC Mol Biol, 2006; 7:23
- Carpenter M, Divvela P, Pingoud V, Bujnicki JM, Bhagwat AS. Sequence-dependent enhancement of hydrolytic deamination of cytosines in DNA by the restriction enzyme PspGI. Nucleic Acids Res, 2006; 34(13):3770-8
- Cymerman IA, Obarska A, Skowronek KJ, Lubys A, Bujnicki JM. Identification of a new subfamily of HNH nucleases and experimental characterization of a representative member, HphI restriction endonuclease. Proteins, 2006; 65(4):867-76
- Han R, Lukomska E, Caswell CC, Keene DR, Pawlowski M, Bujnicki JM, Lukomski S. Binding of the low density lipoprotein by streptococcal collagen-like protein Scl1 of *Streptococcus pyogenes*. Mol Microbiol, 2006; 61(2):351-67
- Dunin-Horkawicz S, Feder M, Bujnicki JM. Phylogenomic analysis of the GIY-YIG nuclease superfamily. BMC Genomics, 2006; 7(1):98
- Zamudio JR, Mittra B, Zeiner GM, Feder M, Bujnicki JM, Sturm NR, Campbell DA. Complete cap 4 formation is not required for viability in *Trypanosoma brucei*. Eukaryotic Cell, 2006; 5(6):905-15
- Gabant G, Auxilien S, Tuszynska I, Locard M, Gajda MJ, Chaussinand G, Fernandez B, Dedieu1 A, Grosjean H, Golinelli-Pimpaneau B, Bujnicki JM, Armengaud J. THUMP from archaeal tRNA:m^2_2G10 methyltransferase, a genuine autonomously folding domain. Nucleic Acids Res, 2006; 34(9):2483-94
- Obarska A, Blundell A, Feder M, Patel J, Vejsadova S, Sisakova E, Weiserova M, Bujnicki JM, Firman K. Structural model for the multisubunit Type IC restriction-modification DNA methyltransferase M.EcoR124I in complex with DNA. Nucleic Acids Res, 2006; 34(7):1992-2005
- Zegers I, Gigot D, van Vliet F, Tricot C, Aymerich S, Bujnicki JM, Kosinski J, Droogmans L. Crystal structure of *Bacillus subtilis* TrmB, the tRNA (m7G46) methyltransferase. Nucleic Acids Res, 2006; 34(6):1925-34
- Sikora E, Bielak-Zmijewska A, Magalska A, Piwocka K, Mosieniak G, Kalinowska M, Widlak P, Cymerman IA, Bujnicki JM. Curcumin induces caspase-3 dependent apoptotic pathway but inhibits DFF40/CAD endonuclease in human Jurkat cells. Mol Cancer Ther, 2006; 5(4):927-34
- 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
- Bheemanaik S, Bujnicki JM, Nagaraja V, Rao DN. Functional analysis of amino acid residues in the dimerisation interface of KpnI DNA methyltransferase. Biol Chem, 2006; 387(5):515-23
- Samaranayake M, Bujnicki JM, Carpenter M, Bhagwat AS. Evaluation of molecular models for the affinity maturation of antibodies: roles of cytosine deamination by AID and DNA repair. Chem Rev, 2006; 106(2):700-19
- Chiang PK, Bujnicki JM, Su XZ, Lanar DE. Malaria: therapy, genes and vaccines. Current Mol Med, 2006; 6(3):309-26
- Skowronek KJ, Kosinski J, Bujnicki JM. A theoretical model of restriction endonuclease HpaI in complex with DNA, predicted by fold-recognition and validated by site-directed mutagenesis. Proteins, 2006; 63(4):1059-68
- Mikula M, Karczmarski J, Dzwonek A, Rubel T, Hennig E, Dadlez M, Bujnicki JM, Bomsztyk K, Ostrowski J. Casein kinases phosphorylate multiple residues spanning the entire hnRNP K length. Biochim Biophys Acta, 2006; 1764(2):299-306
- Klimek-Tomczak K, Mikula M, Dzwonek A, Paziewska A, Karczmarski J, Hennig E, Bujnicki JM, Bragoszewski P, Denisenko O, Bomsztyk K, Ostrowski J. Editing of hnRNP K protein mRNA in colorectal adenocarcinoma and surrounding mucosa. Br J Cancer, 2006; 94(4):586-92
- Metz J, Wachter A, Schmidt B, Bujnicki JM, Schwappach B. The yeast Arr4p ATPase binds the chloride transporter Gef1p when copper is available in the cytosol. J Biol Chem, 2006; 281(1):410-7
- Dunin-Horkawicz S, Czerwoniec A, Gajda MJ, Feder M, Grosjean H, Bujnicki JM. MODOMICS: a database of RNA modification pathways. Nucleic Acids Res, 2006; 34(Database issue):D145-9

- Bujnicki JM. Protein structure prediction by recombination of fragments. ChemBioChem, 2006; 7(1):19-27
- Godlewska R, Dzwonek A, Mikula M, Ostrowski J, Pawlowski M, Bujnicki JM, Jagusztyn-Krynicka EK. *Helicobacter pylori* protein oxidation influences the colonization process. Inter J Med Microbiol, 2006 Mar 15.

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 in macromolecular complexes. The laboratory comprises three sections:

- A section devoted to the development of computer software for analysis of biological macromolecules. The bioinformatics tools include a suite of programs for protein structure prediction and analysis available via the website https://genesilico.pl/toolkit/ (MetaServer for primary, secondary, and tertiary structure prediction, methods for template-based and de novo modeling of three-dimensional protein structures, MetaMQAP for quality assessment of protein models, FILTREST3D for discrimination of models according to their agreement with experimental data, and COLORADO3D for mapping of sequence features onto the protein structure), a standalone program PROTMAP2D for analysis of contact and distance maps in protein structures (http://genesilico.pl/protmap2d.htm), and the MODOMICS database for systems biology of RNA modification (see the research highlight below).

- A section devoted to the application of bioinformatics software to make biologically and biomedically interesting predictions. Recently published research includes phylogenomic analyses of various nuclease (e.g. PD-(D/E)XK, GIY-YIG, HNH) and methyltransferase (SPOUT and RFM) superfamilies and detailed structure prediction and modeling of individual proteins that are of wide interest (e.g. HEN1, a methyltransferase involved in plant microRNA biogenesis). Theoretical research of this section frequently involves collaboration with other laboratories, interested in obtaining a structural model for their favorite proteins and experimental testing of our predictions. Recent modeling analyses (published in 2006) include various nucleases involved in DNA degradation or repair, various tRNA methyltransferase, yeast Arr4p ATPase, hnRNP K, Streptococcal collagen-like protein Scl1, cytosine deaminase AID, etc.

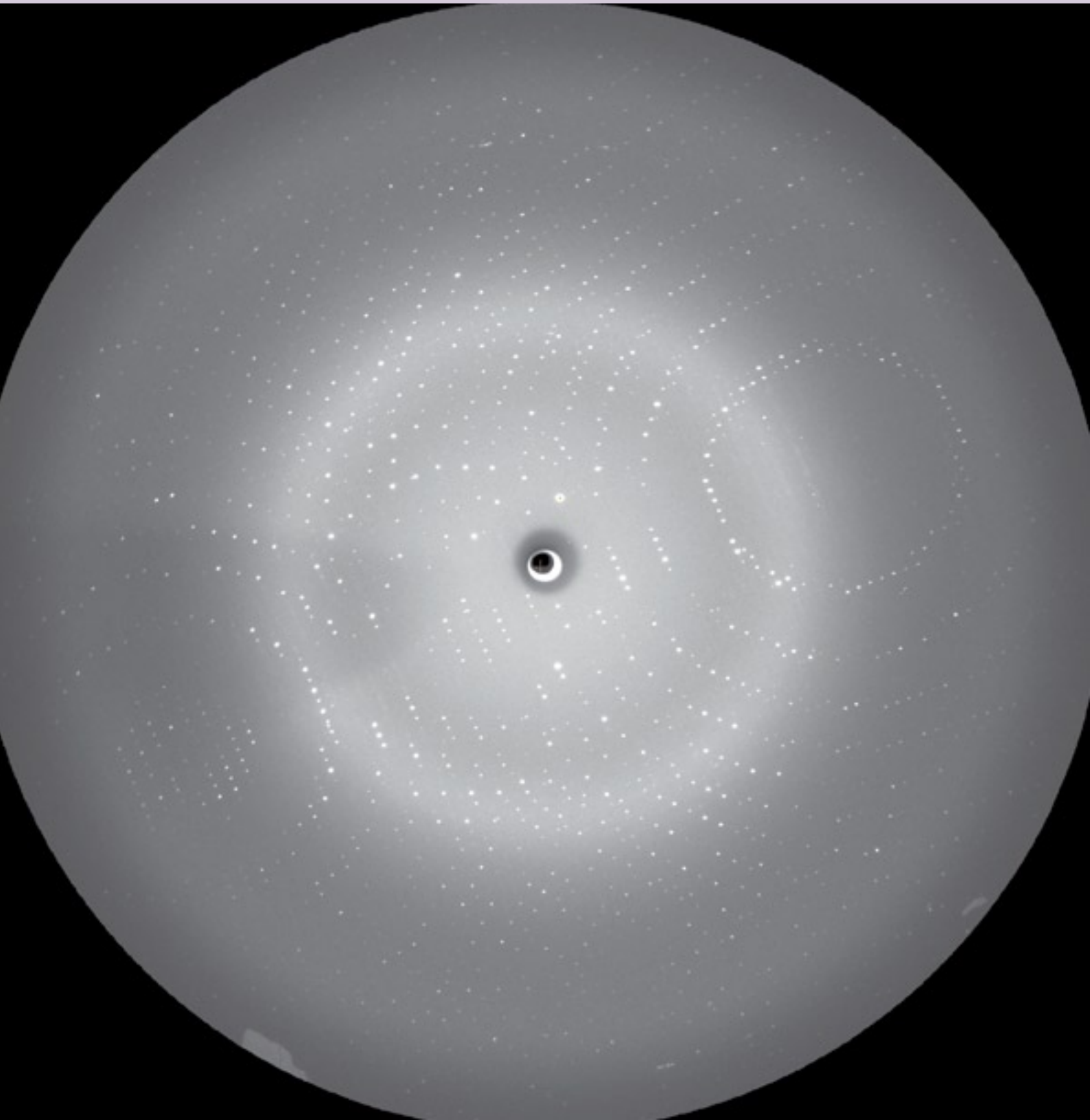
- A section devoted to experimental research on proteins and nucleic acids using methods of biochemistry, molecular biology, and cell biology. There are three principal types of analyses carried out by researchers from our „wet lab": 1) Experimental testing of functional predictions made by the theoretical section by gene cloning, protein expression, purification, development of in vitro and in vivo functional assays and biochemical and cellular characterization. 2) experimental testing of structural predictions by application of low-resolution structure probing methods such as mutagenesis, chemical modification, cross-linking, mass spectrometry, circular dichroism, limited proteolysis etc. 3) Protein engineering to obtain enzymes with new, useful features, in particular altered substrate specificity (e.g. restriction enzymes that recognize and cut new sequences). Other protein engineering projects include attempts to design and obtain proteins with altered tertiary and quaternary structure.

The research in all three sections is tightly integrated, as demonstrated by publications of articles comprising the combination of theoretical and experimental analyses, e.g. „Identification of a new subfamily of HNH nucleases and experimental characterization of a representative member, HphI restriction endonuclease" and „Theoretical model of restriction endonuclease HpaI in complex with DNA, predicted by fold recognition and validated by site-directed mutagenesis." In particular, protein engineering involves iterative protein structure model building, model-based experiment planning, series of experimental analyses, and experiment-based improvement of the models and the tools used for model building.

Recent highlight: MODOMICS

MODOMICS is the first comprehensive database resource for systems biology of RNA modification. It integrates information about the chemical structure of modified nucleosides, their localization in RNA sequences, pathways of their biosynthesis and enzymes that carry out the respective reactions. MODOMICS also provides literature information, and links to other databases, including the available protein sequence and structure data. MODOMICS can be queried by the type of nucleoside (e.g. A, G, C, U, I, m1A, nm5s2U, etc.), type of RNA, position of a particular nucleoside, type of reaction (e.g. methylation, thiolation, deamination, etc.), enzyme name or sequence (to conduct a BLAST search against sequences deposited in the database). Options for data presentation include graphs of pathways involving the query nucleoside, multiple sequence alignments of RNA sequences and tabular forms with enzyme and literature data. The contents of MODOMICS can be accessed through the World Wide Web at http://genesilico.pl/modomics/.

Laboratory of Structural Biology MPG/PAN



Lab Leader

Matthias Bochtler, PhD, DSc.Habil.

Post-doctoral fellows:
Honorata Czapinska, PhD
Renata Filipek, PhD
Aneta Kaczmarczyk, PhD
Izabela Sabala, PhD

PhD students:
Grzegorz Chojnowski, MSc
Magdalena Kaus-Drobek, MSc
Henryk Korza, MSc
Magdalena Lipka, MSc
Malgorzata Firczuk, MSc
Monika Sokolowska, MSc
Roman Szczepanowski, MSc
Marek Wojciechowski, MSc

MAX-PLANCK-GESELLSCHAFT

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY

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

Picture on the left:
X-ray diffraction pattern of the protein crystal.

Matthias Bochtler, PhD, DSc.Habil.

Degrees

DSc. Habil, Institute of Bioorganic Chemistry PAN, Poznan, Poland, 2006

PhD in biochemistry, Technische Universitaet München, Germany, 1999

MSc in experimental physics, Ludwig Maximilians-Universitaet München, Germany, 1995

Research Training

1999-2000 Max Planck Institut für Biochemie, Martinsried, Germany

1996-1999 Research Assistant, MPI für Biochemie, Martinsried, Germany

1995-1996 internship, the Department of Medical Microbiology, University of Regensburg, Germany

1992-1993 guest student, Cambridge University, United Kingdom

1990-1992 studies in physics, Munich University, Germany

Professional Employment

since 2001 Head of a joint MPG-PAN Junior Group at the International Institute of Molecular and Cell Biology in Warsaw

2000 Patent training (Weickmann & Weickmann)

1999-2000 Post-doctoral fellow at the Max Planck Institute for Biochemistry in Martinsried near Munich

Honors, Prizes, Awards

Pienkowski award, 2005

EMBO/HHMI Young Investigator award, 2004

Crystal award, Germany, 2000

Crystal award, Germany, 1998

Scholarship from Deutsche Studienstiftung and the Bavarian State, 1990-1992

Selected publications

- 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, in press
- 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, Epub ahead of print
- Bochtler M, Chojnowski G. The highest reflection intensity in a resolution shell. Acta Crystallogr A, 2007; 63(Pt 2):146-55
- Firczuk M, Bochtler M. Mutational analysis of peptidoglycan amidase MepA. Biochemistry, 2007; 46(1):120-8
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- Odintsov SG, Sabala I, Bourenkov G, Rybin V, Bochtler M. Substrate access to the active sites in aminopeptidase T, a representative of a new metallopeptidase clan. J Mol Biol. 2005; 354(2):403-12
- Korza HJ, Bochtler M. Pseudomonas aeruginosa LD-carboxypeptidase, a serine peptidase with a Ser-His-Glu triad and a nucleophilic elbow. J Biol Chem, 2005; 280(49):40802-12
- Odintsov SG, Sabala I, Bourenkov G, Rybin V, Bochtler M. Staphylococcus aureus aminopeptidase S is a founding member of a new peptidase clan. J Biol Chem, 2005; 280(30):27792-9
- Azim MK, Goehring W, Song HK, Ramachandran R, Bochtler M, Goettig P. Characterization of the HslU chaperone affinity for HslV protease. Protein Sci, 2005; 14(5):1357-62
- Szczepanowski RH, Filipek R, Bochtler M. Crystal structure of a fragment of mouse ubiquitin-activating enzyme. J Biol Chem, 2005; 280(23):22006-11
- Groll M, Bochtler M, Brandstetter H, Clausen T, Huber R. Molecular machines for protein degradation. Chembiochem, 2005; 6(2):222-56
- Filipek R, Potempa J, Bochtler M. A comparison of staphostatin B with standard mechanism serine protease inhibitors. J Biol Chem, 2005; 280(15):14669-74.

Current Research

The Laboratory of Structural Biology MPG/PAN is mostly focused on the determination of atomic resolution structures of proteins. In 2006, we have worked on (a) peptidases, (b) proteases and proteins that are involved in protein degradation, (c) restriction endonucleases and on (d) methods development.

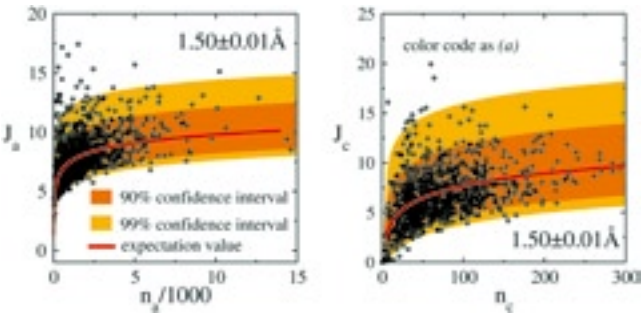
(a) Peptidases: We have concentrated on peptidases that are specific for peptidoglycan, the main building block of bacterial cell walls. In recent years, it has become clear that there is at least one peptidase activity for every amide linkage. The spectrum of peptidases that can cleave peptidoglycan ranges from serine and cysteine peptidases to metallopeptidases. Aspartic peptidases that can cleave peptidoglycan have not (yet) been identified. At least the serine and metallopeptidase groups of peptidoglycan amidases can be further subdivided into different fold groups that share no overall similarity. Peptidases with different folds often have different specificities, but there is no strict correlation. A detailed survey of the literature (Firczuk and Bochtler, to be published) shows that in some cases peptidases of vastly different folds have identical specificities whereas in other cases, peptidases in one fold group can have very different specificities. For our experimental work, we have focused on peptidoglycan amidases without detectable sequence similarity to peptidases of known structure. Our expectation to find new folds was fulfilled in the cases of the serine peptidase LD-carboxypeptidase (Korza and Bochtler, JBC, 2005, 280, 40802-12) and the metallopeptidase LytM (Firczuk et al., JMB, 2005, 354, 578-90). However, in both cases, we found very familiar active sites in the context of the new folds. In the case of LD-carboxypeptidase, the active site serine is anchored in a kink at the N-terminus of a helix, which starts out as a 3/10-helix and continues as an α-helix. This arrangement is well known from the so-called "αβ-hydrolases", and is thought to provide several advantages: it exploits the helix dipole moment for catalysis, maximally exposes the active site nucleophile, and elegantly solves the problem of dangling hydrogen bonds at the end of the helix. In the case of the metallopeptidase LytM, the similarity to VanX-type peptidases is not limited to the active site, but also includes a core β-sheet of identical topology. Using MepA as a model system for enzymes in this group, we have attempted

to define the role of conserved catalytic residues in more detail. This work has recently been published (Firczuk and Bochtler, Biochemistry, 2007, 46, 120-8).

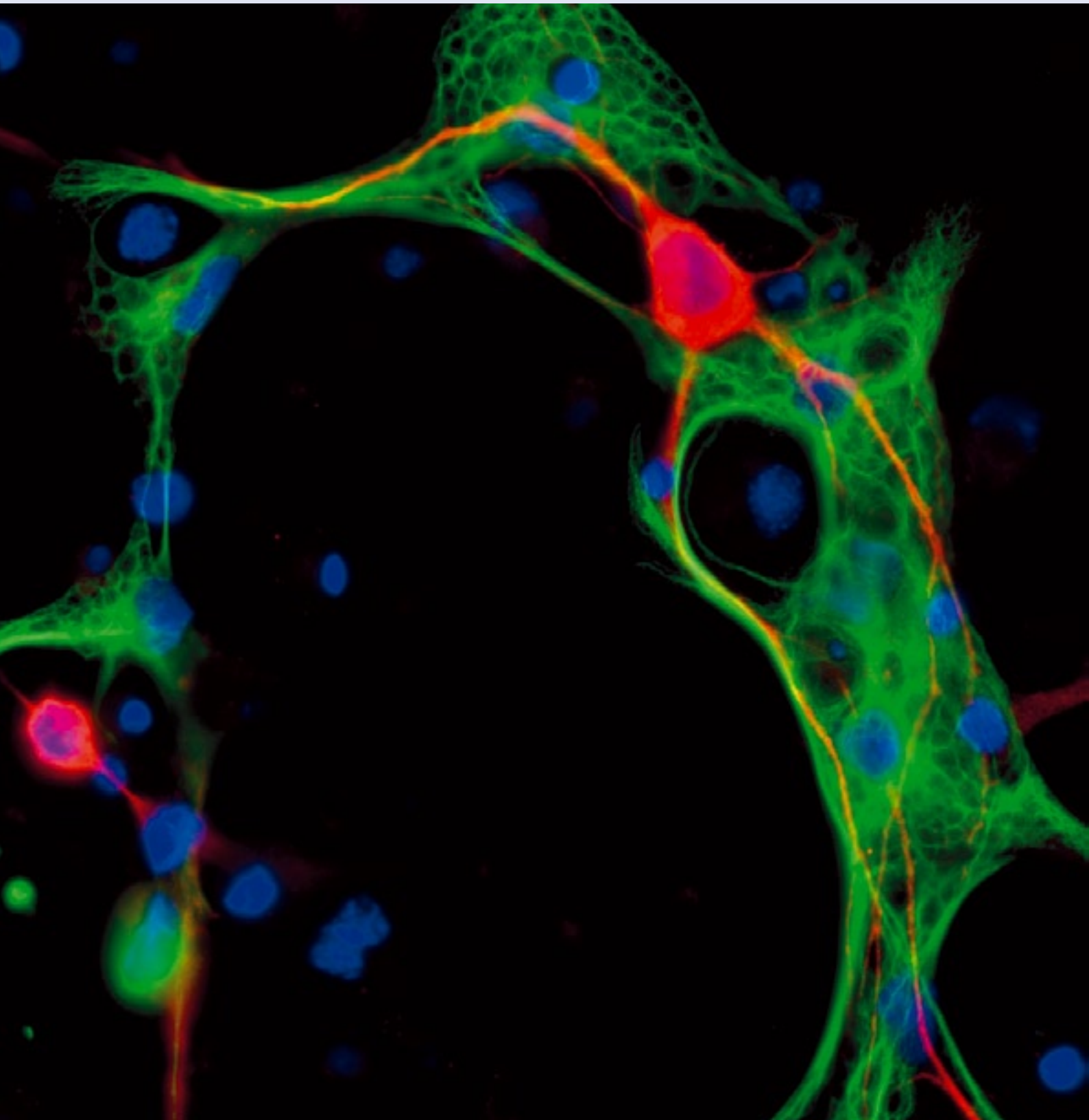
(b) In the area of protein degradation, a long-standing interest of the group is the structure of ubiquitin-activating enzyme, which is the most upstream component of the ubiquitin-proteasome pathway. The enzyme catalyzes the conversion of ubiquitin to the adenylate, and the subsequent conversion to an enzyme bound thioester. The adenylation domain of the enzyme can be modeled with confidence based on related structures. We have focused on the two domains that mediate the conversion to the thioester, which we have termed the first and second catalytic cysteine half-domains. We have previously solved the structure of the second catalytic half-domain by X-ray crystallography. In 2006, we have collaborated with Dr. Zhukov of the Institute of Biochemistry and Biophysics on the structure of the first catalytic half domain. The assignment has been published (Jaremko L, J. Biomolecular NMR, epub ahead of print) and the manuscript on the structure is in preparation.

(c) In the restriction endonuclease field, we have focused on restriction enzymes that are exotic, because their ''footprints", the overhangs of the DNA cleavage products, have unusual lengths. The abundances of such enzymes vary widely, but there are at least some enzymes for every possible footprint with a 5'-overhang length between minus and plus 5. We have focused on restriction endonucleases that generate 5 nucleotide 5'-overhangs or 1 nucleotide 5'-overhangs. In the former case, our structure of the restriction endonuclease Ecl18kI has shown that the enzyme achieves its unusual specificity by flipping nucleotides in its target sequence (Bochtler et al., EMBO J., 2006, 5, 2219-2229). In the latter case, we focused on the related restriction endonucleases MvaI (CC/WGG, "/" denotes the cleavage site) and BcnI (CC/SGG). For both enzymes, we could show biochemically that they act as monomers and crystallographically that they recognize their target DNAs asymmetrically (Kaus-Drobek et al., Nucl. Acids Research, in press, Sokołowska et al., JMB, manuscript accepted). Moreover, the structures confirmed the predicted similarity of MvaI and BcnI to MutH, a component of the DNA repair machinery. Interestingly, MutH is a nickase that introduces single strand breaks in hemi-methylated DNA. In contrast, BcnI and MvaI are endonucleases that cleave both strands of DNA. Apparently, the distinction between nickases and some endonucleases is only gradual: those enzymes that can bind target sequences of approximate twofold symmetry in both possible orientations (such as MvaI and BcnI) act as endonucleases, those that prefer one binding mode over the alternative (such as MutH) are nickases. Our findings suggest mutagenesis strategies to convert some endonucleases to nickases and vice versa.

(d) In the method development area, we have focused on a specific aspect of the so-called crystallographic "phase problem". This problem arises because X-ray crystallography is in some sense equivalent to conventional imaging, rather than to holography, and therefore looses information, the so-called "phase information" during the recording. There are multiple solutions to this problem. It is possible to determine the missing phase information experimentally, by a comparison of the diffraction pattern of the crystal before and after a minor modification by the addition of one or several heavy atoms per unit cell (MIR). In recent years, it has become more common to collect all diffraction data from one crystal, but to alter the diffraction properties of a selected atom (typically selenomethionine) by varying the X-ray wavelength near an atomic resonance of a selected scatterer (typically selenium) (MAD). Experimental phase information is often not needed at all, if the unknown molecule in the crystal is sufficiently similar to a molecule of known structure. In this case, approximate phase information can be derived if the known molecule can be placed in the crystal. So why not use canonical secondary structure elements, such as helices and strands, as the model? The conventional answer is that these are too small to be located with confidence. But are helices really so difficult to locate? We have specifically looked at the 1.5 Å peak from helices in the diffraction pattern of 3D-crystals and asked whether this peak was strong enough to distinguish it from random background fluctuations (Bochtler and Chojnowski, Acta Cryst. A, 2007, 63, 146-155, Chojnowski and Bochtler, revisions required). We find that the signature of very long helices can indeed be spotted in the diffraction pattern, but so far we can only spot helix orientation, but not location. Moreover, we cannot (yet) distinguish the N- and C-terminal ends of helices.



Laboratory of Neurodegeneration



Lab Leader

Jacek Kuznicki, PhD, Professor

Associate Professors:
Urszula Wojda, PhD, DSc.Habil.

Post-doctoral fellows:
Neli Kachamakova, PhD
Monika Klejman, PhD
Andrzej Lewandowicz, MD, DSc.Habil. (until December 2006)
Marta Wisniewska, PhD

PhD students:
Magdalena Blazejczyk, MSc
Lukasz Bojarski, MSc
Wojciech Michowski, MSc
Katarzyna Misztal (since September 2006)
Adam Sobczak, MSc
Aleksandra Szybinska, MSc

MSc students:
Katarzyna Debowska, Mirosław Drab, Kamila Skieterska, Bozena Zebrowska

Projects' manager:
Malgorzata Mossakowska, PhD

Picture on the left:
Immunofluorescent staining of rat brain primary cells: red - neurons (anti-Map2), green - astrocytes (anti-GFAP), blue - nuclei (DAPI).

Jacek Kuznicki, PhD

Degrees

Professor, 1993

DSc.Habil., Nencki Institute of Experimental Biology PAN, Warsaw, Poland, 1987

PhD in biochemistry, Nencki Institute of Experimental Biology PAN, Warsaw, 1980

MSc in biochemistry, Warsaw University, 1976

Post-doctoral Training

1981-1984 Visiting Fellow, Laboratory of Cell Biology headed by E.D. Korn, National Institutes of Health, Bethesda, MD, 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 located at the Nencki Institute of Experimental Biology PAN, Warsaw

1999-2001 Acting Director, IIMCB; Organizer and Director of Centenarian Program

1996-2002 Head of Laboratory of Calcium Binding Proteins, the Nencki Institute of Experimental Biology PAN, Warsaw

1992-1995 Visiting Professor at the National Institute of Mental Health, Laboratory of Clinical Science, Bethesda, MD, USA

1991-1992 Deputy Director (Scientific Director), Nencki Institute of Experimental Biology PAN, Warsaw

1986-1992 Associate Professor and Head of Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology PAN, Warsaw

1984-1985 Research Associate, Nencki Institute of Experimental Biology PAN, Warsaw

1981-1984 Visiting Fellow, NIH, Laboratory of Cell Biology, Bethesda, MD, USA

1980-1981 Post-doctoral Fellow, Nencki Institute of Experimental Biology PAN, Warsaw

1976-1980 PhD Student, Nencki Institute of Experimental Biology PAN, Warsaw

Membership in Scientific Societies, Organizations and Panels

- Member of the 7th FP European Commission - Health Research Advisory Group, since 2006
- Member of the Polish Academy of Science (PAN), since December 2004
- Member of American Society for Biochemistry and Molecular Biology, since 2003
- Head of Advisory Board of the Science School Festival, since 2002
- Member of the Biochemical Society (England), since 1995
- Member of the Polish Neuroscience Society, since 1991
- Member of the Polish Society for the Advancement of Science and Arts, since 1991
- Vice-president of the Polish Biotechnology Committee, 1996-1999 and 2000-2002
- Member of the Polish Biotechnology Committee, 1990-2002
- Co-Editor of Advances in Biochemistry (published in Polish), 1989-1992
- Member of the Polish Biochemical Society, since 1977, General Secretary, 1989-1991

Honors, Prizes, Awards

- Professorship Award from Foundation for Polish Research (FNP), 2004-2007
- Prime Minister Award for the scientific achievements, 2003
- Award from Division of Biological Sciences PAN for the work on calcium binding proteins, 2001
- Polish Anatomical Society Award for the article on calcium binding proteins published in "Advances in Cell Biology", 1987
- Skarzynski Award from Polish Biochemical Society for the best review article in Advances in Biochemistry, 1986
- Parnas Award from Polish Biochemical Society for the publishing of the best paper in biochemical research, 1977
- Mozolowski Award, Polish Biochemical Society for outstanding Polish young biochemists, 1977
- Magna cum laude, University of Warsaw, 1976

Publications in 2006

- Blazejczyk M, Wojda U, Sobczak A, Spilker C, Bernstein HG, Gundelfinger ED, Kreutz MR, Kuznicki J. Ca^{2+}-independent binding and cellular expression profiles question a significant role of calmyrin in transduction of Ca^{2+}-signals to Alzheimer's disease-related presenilin 2 in forebrain. Biochim Biophys Acta, 2006; 1762(1):66-72
- Zekanowski C, Golan MP, Krzysko KA, Lipczynska-Lojkowska W, Filipek S, Kowalska A, Rossa G, Peplonska B, Styczynska M, Maruszak A, Religa D, Wender M, Kulczycki J, Barcikowska M, Kuznicki J. Two novel presenilin 1 gene mutations connected with frontotemporal dementia-like clinical phenotype: genetic and bioinformatic assessment. Exp Neurol, 2006; 200(1):82-88
- Spiechowicz M, Bernstein HG, Dobrowolny H, Lesniak W, Mawrin C, Bogerts B, Kuznicki J, Filipek A. Density of Sgt1-immunopositive neurons is decreased in the cerebral cortex of Alzheimer's disease brain. Neurochem Int, 2006; 49(5):487-493
- Lesniak W, Kuznicki J. Binding and functional characteristics of two E-box motifs within the S100A6 (calcyclin) gene promoter. J Cell Biochem, 2006; 97(5):1017-1024
- Groves P, Palczewska M, Kuznicki J. Calretinin, an EF-hand calcium-binding proteins, binds zinc and Cooper. Calcium Binding Proteins, 2006; 1:3, 156-159
- Baranowska B, Bik W, Baranowska-Bik A, Wolinska-Witort E, Szybinska A, Martynska L, Chmielowska M. Neurohormonal control of food intake and metabolic homeostasis during ontogenesis. J Phys Pharmacol, 2006; 57, Supp 6, 55-61
- Bik W, Baranowska-Bik A, Wolinska-Witort E, Martynska L, Chmielowska M, Szybinska A, Broczek K, Baranowska B. The relationship between adiponectin levels and metabolic status in centenarian, early elderly, young and obese women. Neuro Endocrinol Lett, 2006, 27(4):493-500
- Pietruszka B, Kollajtis-Dolowy A, Szulc K, Kaluza J, Broczek K, Pawlinska-Chmara R, Mossakowska M. Characteristics of nutrition among centenarians living in Warsaw. Pol J Food Nutr Sci, 2006; 15/56:111-115
- Wieczorowska-Tobis K, Niemir ZI, Podkowka R, Korybalska K, Mossakowska M, Breborowicz A. Can an increased level of circulating IL-8 be a predictor of human longevity? Med Sci Monit, 2006; 12(3):CR118-121

- Pawlinska-Chmara R, Mossakowska M. Evaluation of teeth and dentures state of Polish centenarians (in Polish). Ann Univ M. Curie-Sklodowska, 2006; Supp16; 564: 452-455
- Wollmer MA, Sleegers K, Ingelsson M, Zekanowski C, Brouwers N, Maruszak A, Brunner F, Huynh K-D, Kilander L, Brundin R-M, Hedlund M, Giedraitis V, Glaser A, Engelborghs S, De Deyn PP, Kapaki E, Tsolaki M, Daniilidou M, Molyva D, Paraskevas GP, Thal DR, Barcikowska M, Kuznicki J, Lannfelt L, Van Broeckhoven C, Nitsch RM, Hock C, Papassotiropoulos A. Association study of cholesterol-related genes in Alzheimer's disease. Neurogenetics, accepted.

Current Projects

We are interested in molecular mechanisms involved in learning and memory, as well as in neurodegeneration, and we employ genetic, biochemical and bioinformatics methods to study these processes at the genomic, proteomic, and cellular levels.

Our major projects are:

1. Identification of mutations in Alzheimer disease-related genes of Polish patients and functional analysis of these mutations
2. Search for bio-markers and potential therapeutic targets of Alzheimer's disease
3. Studies on the cyclin-dependent kinase 5 involvement in pathogenesis of Alzheimer's disease
4. Studies on protein Ca^{2+}-sensors in normal and pathological processes in neurons
5. Role of β- and γ-catenins in signaling connected with neuronal cell adhesion molecules
6. Characterization of biological functions of CHORD containing proteins in the nervous system.

1. Identification of mutations in Alzheimer disease-related genes of Polish patients and functional analysis of these mutations

(Aleksandra Szybinska, Cezary Zekanowski in collaboration with the group of Maria Barcikowska at the Medical Research Center, PAN, and with Krzysztof Jozwiak and Slawek Filipek from Laboratory of Biomodelling at IIMCB)

More than 100 mutations linked to early-onset familiar Alzheimer's disease (FAD) have been identified in presenilin proteins. Presenilin 1 and presenilin 2 are the catalytic components of the γ-secretase enzymatic complex, which also comprises of nicastrin, Aph-1 and Pen-1 proteins. γ-secretase is responsible for intramembranous cleavage of amyloid precursor protein (APP) and some other cellular substrates. Most FAD mutations in presenilins are located in their transmembrane domains, indicating that the intramembrane interactions play a crucial role in the stabilization and proper functioning of the enzyme. Presenilins interact with β-catenin, calmyrin, and several other proteins. Despite extensive efforts, the structure and mechanism of presenilin activity remains unclear. To determine the spectrum of mutations in a group of Polish patients with clinically diagnosed early-onset Alzheimer's disease, frontotemporal dementia and related dementias, we performed a screening for mutations in presenilin 1 (PSEN1), presenilin 2 (PSEN2), amyloid precursor protein (APP), tau protein (MAPT), and prion protein (PRNP) genes. The total frequency of mutations in a group of familial AD patients was 21%. Screens in a group of 65 Polish patients with early onset AD identified three previously known mutations in PSEN1 gene: L153V, S170F, and L224H. Clinical outcome of our living patient bearing S170F mutation strongly resembles the outcome of previously diagnosed patient with S170F mutation with atypical AD and Lewy bodies. We also identified novel mutations in PSEN1 (F226L, I213F, P117R) and in PSEN2 (Q228L). Lymphocytes of patients with identified mutations in presenilins have been immortalized and collected in the cell bank consisting of about 200 lymphoblasts lines including those obtained during Polish Centenarian Project. We examined previously recognized pathogenic mutation in PSEN1 gene and novel, identified by us mutations, on β-amyloid production. Stable clones of human embryonic kidney HEK 293 Flp-In-239 cells with Swedish APP mutation KM670/671NL (obtained from Dr. Jessie Theuns, University of Antwerp), were stably transfected with constructs bearing the above-mentioned PSEN mutations or empty vector. β-amyloid 1-40 and 1-42 levels in serum-free culture media were estimated by ELISA. Cells with mutated presenilins produced much more β-amyloid than control cells and β-amyloid 42/β-amyloid 40 ratio was significantly increased comparing to controls, indicating that novel mutations identified in Polish patients are likely responsible for FAD.

To visualize possible effects of mutations on PS1 structure, homology modeling based on bacteriorhodopsin structures and molecular dynamics were performed. This bioinformatics analysis shows how the mutations of one amino acid residue affect some other residues. (Zekanowski C, et al., Exp Neurol 2006; 200(1):82-88)

2. Search for functional bio-markers and potential therapeutic targets of Alzheimer's disease

(Lukasz Bojarski, Mirosław Drab, Neli Kachamakova, Andrzej Lewandowicz, Aleksandra Szybinska, Urszula Wojda, Bozena Zebrowska in collaboration with other laboratories)

In this respect, several projects are carried out:

2.1 To explain the nature of different γ-secretase complexes and their activities, we aimed at identification of biochemical properties and proteins differentiating membranous complexes of PS1 from complexes of PS2. We demonstrated that despite similar isoelectric points, endogenous PS1 and PS2 have different elution profiles from ion exchange resin

MonoQ, allowing separation of both proteins. This observation can be explained either on the basis of different posttranslational modifications of both presenilins, or on the basis of different compositions of their protein complexes. The latter was supported by the affinity chromatography results showing that endogenous PS2 from lymphocytes, but not PS1, binds calmyrin 1, an EF-hand calcium binding protein known also as Calcium and Integrin Binding protein (CIB1) or Kinase Interacting Protein (KIP1). Using co-immunoprecipitation and immunoblotting methods, we analysed whether CaMy1 is present in γ-secretase complexes containing PS2 (Bojarski L. et al., 2006,submitted).

2.2. In collaboration with Dr. Axel Wollmer, we participated in an association studies to determine, whether cholesterol metabolism genes are risk factors for AD in a group of 250 controls, and 223 late onset AD patients. He screened 115 sporadic AD cases and 191 healthy control subjects and showed, on average, three tagging single nucleotide polymorphisms (SNPs) in 28 cholesterol-related genes associated with AD risk. HMGCS2, FDPS, RAFTLIN, ACAD8, NPC2, and ABCG1 were associated with AD at a significance level of $P<0.05$. Replication trials in five independent European samples, including the Polish groups, confirmed association of HMGCS2, FDPS, NPC2, and ABCG1 were found to be in at least one sample ($P=0.05$ to $P=0.001$); (Wollmer A. et al., 2006, accepted).

2.3. In cooperation with Dr. Daniela Uberti (University of Brescia) the conformational mutant p53 as a new putative marker to discriminate AD from non-AD patients was analysed. Conformation of p53 protein was studied in cell lysates from our immortalised B lymphocytes from 13 sporadic AD (SAD) and 9 familial AD (FAD) patients and 12 control subjects by immunoprecipitation experiments. Cells from SAD and FAD patients specifically expressed an increased amount of conformationally altered p53 that makes them distinguishable from cells of age-matched non-AD subjects. This suggests a role for a dearrangement of protein controlling the cell cycle in AD pathogenesis (C. Lanni, et al., submitted).

2.4. In collaboration with Dr. Kathrine Tissot (University of Zurich), analysis of plasma samples of Polish centenarians for the presence of anti-β amyloid antibodies was performed. Almost 80 plasma samples from centenarians with an MMST assay score from 0 to 30 were checked against β-amyloid 1-42 immunoreactivity. No correlation between immunoreactivity and the MMSTE score was observed. Interestingly, two samples showed strong immunoreactivity in TAPIR (Tissue Amyloid Plaque Immunoreactivity) assay. Culture media of immortalised B cells from these centenarians' cell did contain anti-Aβ1-42 antibodies.

2.5. In collaboration with Dr. Jochen Herms (Ludwig Maximilians University) we analyse lymphocytes from patients with presenilin 1 mutations, showing very similar alterations in the calcium homeostasis to neurons from transgenic animal models of familial AD. We will perform cell imaging screens for new potential therapeutic targets for AD and also analyse features of calcium-related mechanisms of synapse formation and spine morphology in hippocampal neurons from wild type and PS1 mutant transgenic mice.

3. Studies on cyclin-dependent kinase 5 involvement in pathogenesis of Alzheimer disease *(Aleksandra Szybinska, Cezary Zekanowski in collaboration with Aleksandra Wyslouch-Cieszynska from laboratory of Mass Spectroscopy, Institute of Biochemistry and Biophysics PAN)*

Cyclin-dependent kinase 5 in complex with p35 protein has brain-specific activity and is known to play an important role in a variety of neuronal processes in both developing and adult brains. In an adult brain, cdk5 via its interactions with different synaptic, cytoskeletal and cellular adhesion proteins as well as NMDA receptors and calcium channels, is involved in synaptic plasticity, memory and learning processes impaired in Alzheimer's disease. It was shown recently that in AD patients, the brains expression and activation of cdk5 is upregulated. That upregulation results in MAP tau overphosphorylation together with that caused by GSK beta. Other consequences of cdk5 activity impairment regarding AD are poorly understood. In our studies, we compare by proteomics methods protein expression and modifications in synaptosomes of transgenic mice, AD models bearing human mutated presenilin 1 and APP genes and p25 overexpressing animals, which are cdk5 hyperactivation models versus wild type animals to find mechanisms of neurodegeneration processes in AD connected with cdk5 upregulation. The first step of our studies is an optimisation of synaptosomal samples preparations from mouse brains for LC/MS analysis, as well as conditions for 2D electrophoresis of the samples.

4. Analysis of protein Ca^{2+}-sensors in norm and in pathological processes in neurons *(Magdalena Blazejczyk, Katarzyna Dębowska, Bozena Zebrowska, Adam Sobczak, under the supervision of Urszula Wojda and in collaboration with other laboratories)*

Ca^{2+}-binding neuronal calcium sensors (NCS) participate in Ca^{2+}-control of neuronal development, plasticity, and neurodegeneration and draw much attention due to implications in multiple brain pathologies including Alzheimer's disease. Genomic databases indicate the existence of a novel family of Ca^{2+}-binding proteins. Structurally, they most are similar to the NCS, called calmyrin (CaMy, known also as KIP or CIB). This family consists of 4 genes in humans (CaMy1-CaMy4) and several CaMy-like sequences in other species. The only characterized member of this family is CaMy1. We have previously demonstrated that CaMy1 is implicated in Alzheimer's disease and that it interacts specifically with Alzheimer's disease associated presenilin 2 in vitro and in vivo (Bernstein et

al, Neuropathol Appl Neurobiol. 2005, 31(3):314-24; Blazejczyk et al, Biochim Biophys Acta. 2006;1762(1):66-72). Recently, we analysed another member of this family, CaMy2. We cloned rat recombinant CaMy2 protein and obtained polyclonal anti-CaMy2 antibodies. CaMy2 transcript and protein were detected mainly in the hippocampus and cortex of rat brains. In order to gain insights in the role and the molecular mechanisms by which CaMy2 exerts its function, we analysed structural and biochemical features of recombinant CaMy2 underlying the transduction of Ca^{2+} signals by homology modeling, $^{45}Ca^{2+}$ gel overlay, spectral characteristics, trypsin susceptibility assay and gel filtration. We also searched for protein ligands of CaMy2 in rat brain. Obtained data showed that CaMy2, like CaMy1, is able to bind Ca^{2+} with physiologically relevant affinities which results in changes of conformation. Our work revealed differences between CaMy2 and CaMy1 in their structure, protein interactions, and cellular and subcellular localization, suggesting that they can function as Ca^{2+} sensors in different signaling pathways at their specific locations in the brain (M. Blazejczyk, et al., submitted).

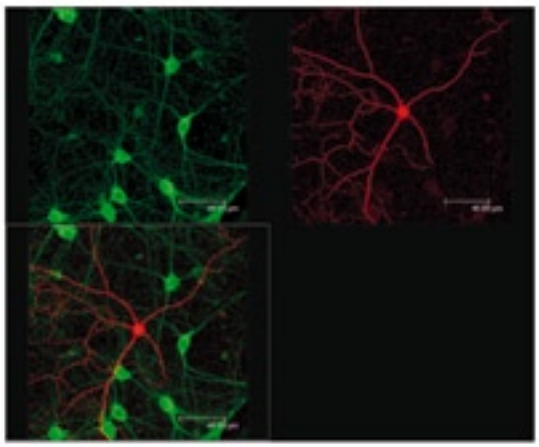
5. Role of β- and δ-catenins in signaling connected with neuronal cell adhesion molecules *(Monika Klejman, Katarzyna Misztal, Marta Wisniewska in collaboration with partners from PROMEMORIA 6th FP of EU)*

β-catenin plays a crucial role in cell proliferation and development, and is a component of the adherens junctions. In addition to the membrane localized protein there is also a cytosolic pool of β-catenin, which is controlled by phosphorylation and subsequent ubiquitination and degradation. After wnt signaling activation, β-catenin phosphorylation is inhibited, the protein translocates to the nucleus and activates gene transcription as a cofactor of Lef1/Tcf4 transcription factor. We are interested in the function of β-catenin in the adult brain, since new data suggests it might be involved in learning and memory formation. We analyse β-catenin expression in the forebrain of adult mice and rats using immunocytochemistry and immunofluorescent methods, as well as biochemical analysis of the brain protein extracts. We also analyse the level and distribution of proteins potentially involved in β-catenin degradation in the brain, namely Siah 1, Sgt1 and CacyBP/SIP. The influence of Ca^{2+}-signaling on β-catenin ubiquitination is being studied in mouse embryonic fibroblasts and HEK293 cell lines.

γ-catenin is a component of cell adhesion complex and is exclusively expressed in the nervous system. It was shown to be important for the processes of learning and memory. However, the mechanism of γ-catenins activity is not fully understood. The aim of our work is to identify all interacting partners for γ-catenins in the brain using proteomic analyses of brain samples and affinity chromatography with recombinant γ-catenin expressed in the baculovirus system. Baculoviruses carrying γ-catenin gene were constructed and protein expression was achieved.

6. Characterization of biological function of CHORD containing proteins in the nervous system *(Wojciech Michowski, Kamila Skieterska in collaboration with Guido Tarone from University of Turin)*

Two genes for CHORD containing proteins are present in the mammalian genome, melusin and chp-1. Melusin is a protein expressed in heart and skeletal muscles. It specifically senses mechanical stress induced by chronic aortic hypertension, mediates development of adaptive cardiac hypertrophy and protects cardiac muscle from consequences of pressure overload. We identified melusin as a novel protein target of the S100 Ca^{2+}-signal sensing proteins. Chp-1 is a ubiquitously expressed protein which functions under stress conditions. It exhibits chaperoning activity and its mutants show mitotic aberrations. Since high level of chp-1 is observed in neuronal tissue we decided to explore its function in neurons. Using imunofluorescence technique, we monitored the level and changes in the distribution of chp-1 in primary rat hippocampal neurons under temperature and oxidative stresses. We applied siRNA against chp-1 to determine the effect of chp-1 loss in neuronal cells. The treatment decreased the chp-1 level, but seemed to have no effect on the dendtritic tree (Fig. 1). For efficient delivery of the siRNA to primary neurons, we developed an adenoviral system. By means of immunohistochemistry, we studied the distribution of chp-1 in the rat brain. In parallel, we looked for molecular pathways in which chp-1 takes part by searching for its new protein targets using biochemical methods.



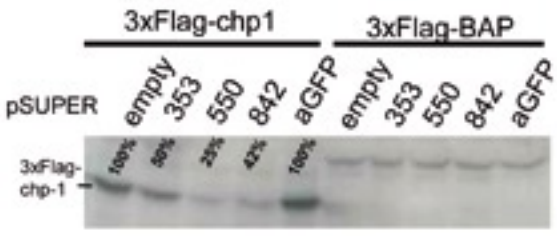
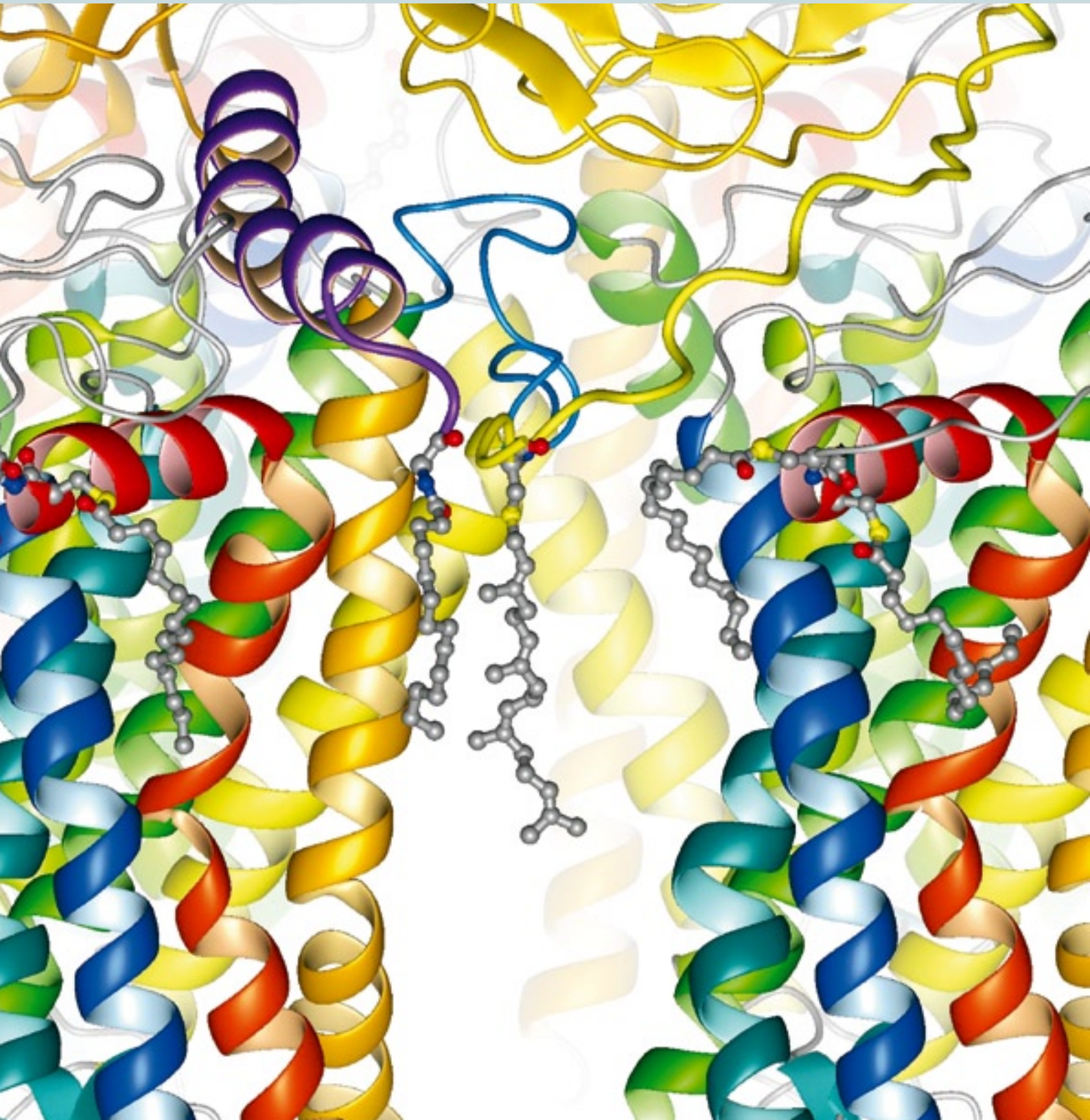
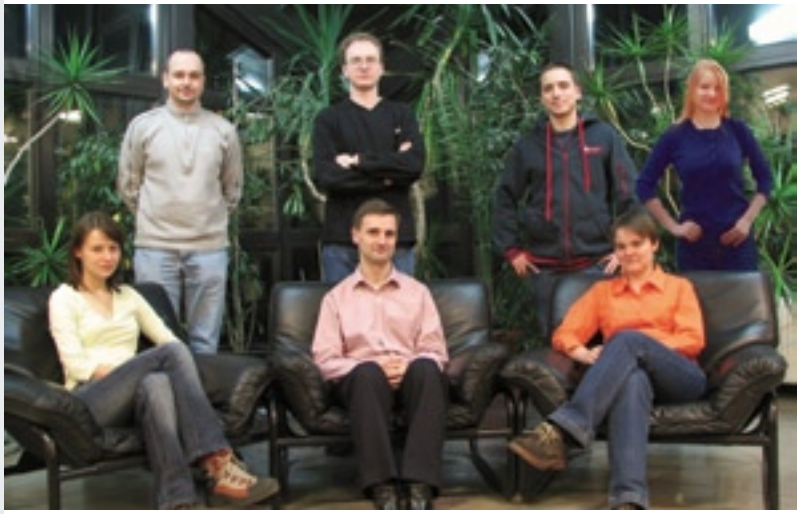


Fig. 1
A. Hippocampal neurons in vitro. Green-anti chp-1 monoclonal antibody. Red-neuron containing pSUPER 550 with siRNA against chp-1

B. Western Blot with anti flag antibody. HEK293 cells were cotransfected with vectors coding for flag-chp-1 (3xFlag-chp-1) shRNAs (pSUPER) or with flag-BAP and pSUPER as a control. 353, 550, 842 are siRNA against chp-1 and aGFP is a control siRNA. siRNA 550 proved to be the most effective in silencing of flag-chp-1.

Laboratory of Biomodelling





Lab Leader

Slawomir Filipek, PhD, DSc.Habil.

Post-doctoral fellow:
Krzysztof Jozwiak, PhD

PhD students:
Anna Modzelewska, MSc (until Jan. 2007)
Krystiana Krzysko MSc (until Jan. 2007)
Michal Kolinski, MSc

Undergraduate students:
Aleksander Debinski
Anna Zwolinska

Picture on the left:
The location of hydrophobic modifications of rhodopsin and G protein in the complex of rhodopsin tetramer (double dimer) and G-protein trimer (Gtαβγ). Myristoyl (Gtα N-terminus) and farnesyl (Gtγ C-terminus) are centrally located while rhodopsin's double palmitoyl chains are located on the left and right.

Slawomir Filipek, PhD, DSc.Habil.

Degrees

DSc.Habil. in medicinal chemistry, Warsaw University, Faculty of Chemistry, 2004

PhD in theoretical chemistry, Warsaw University, Faculty of Chemistry, 1993

MSc in quantum chemistry, Warsaw University, Faculty of Chemistry, 1985

Post-doctoral Training

2001, 2002 Visiting scientist, Department of Ophthalmology, University of Washington, Seattle, WA, USA

Professional Employment

since 2002 Head of the Laboratory of Biomodelling, IIMCB

1993-2002 Post-doctoral fellow, Warsaw University, Faculty of Chemistry

1985-1993 Assistant, Warsaw University, Faculty of Chemistry

Honors, Prizes, Awards

2000-2002 Scientific awards-stipends of Rector of Warsaw University

Professional Memberships

Molecular Graphics and Modelling Society
Biophysical Society

Publications

over 50 publications in primary scientific journals
over 1000 citations
over 800 citations with IIMCB affiliation (years 2003-2006)

Selected publications

Cieplak M, Filipek S, Janovjak H, Krzysko KA. Pulling single bacteriorhodopsin out of a membrane: Comparison of simulation and experiment. Biochim Biophys Acta, 2006; 1758(4):537-44

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(5):1560-72

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Mirzadegan T, Benko G, Filipek S, Palczewski K. Sequence Analyses of G-Protein-Coupled Receptors: Similarities to Rhodopsin. Biochemistry-US, 2003; 42:2759-67

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Noorwez SM, Kuksa V, Imanishi Y, Zhu L, Filipek S, Palczewski K, Kaushal S (2003) Pharmacological Chaperone-mediated in Vivo Folding and Stabilization of the P23H-opsin Mutant Associated with Autosomal Dominant Retinitis Pigmentosa. J Biol Chem, 2003; 278:14442-50

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*Filipek S, Stenkamp RE, Teller DC, Palczewski K. Protein-Coupled Receptor Rhodopsin: A Prospectus. Annu Rev Physiol, 2003; 65:851-79.

*Paper marked with an asterisk have no IIMCB affiliation

Publications in 2006

- Cieplak M, Filipek S, Janovjak H, Krzysko KA. Pulling single bacteriorhodopsin out of a membrane: Comparison of simulation and experiment. Biochim Biophys Acta 2006; 1758(4):537-44
- 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(5):1560-72
- Modzelewska A, Filipek S, Palczewski K, Park PS. Arrestin interaction with rhodopsin: conceptual models. Cell Biochem Biophys. 2006; 46(1):1-15
- Sapra KT, Park PS, Filipek S, Engel A, Muller DJ, Palczewski K. Detecting molecular interactions that stabilize native bovine rhodopsin. J Mol Biol. 2006; 358(1):255-69
- Zekanowski C, Golan MP, Krzysko KA, Lipczynska-Lojkowska W, Filipek S, Kowalska A, Rossa G, Peplonska B,

Styczynska M, Maruszak A, Religa D, Wender M, Kulczycki J, Barcikowska M, Kuznicki J. Two novel presenilin 1 gene mutations connected with frontotemporal dementia-like clinical phenotype: Genetic and bioinformatic assessment. Exp Neurol. 2006; 200(1):82-88

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Description of Current Research

1. Unfolding studies of molecular interactions that stabilize membrane proteins

Atomic force microscopy (AFM) and single-molecule force spectroscopy (SMFS) allow high-resolution imaging of individual membrane proteins in their native environment, and detecting and locating the forces that stabilize these proteins. Such forces are a direct measure of interactions established within the membrane protein and depend on environmental changes such as temperature, pH, ion concentration, and oligomeric assembly. The molecular interactions within rhodopsin, embedded in a native rod outer-segment membrane, were established and mapped onto the protein structure resolving stable structural segments. The map showed the highly conserved residues among G protein-coupled receptors to be centered in the stable structural segments of rhodopsin. In the absence of the stabilizing Cys110-Cys187 bond the molecular interactions establishing structural segments changed their strengths and locations. Such changes may build a molecular mechanism of protein destabilization, misfolding and malfunction.

We participate in this project by performing simulation of pulling rhodopsin using the Steered Molecular Dynamics (SMD) method. Theoretical methods are the only way to see what is going on within protein structure beneath the stretch. Although the pulling speed in all-atom simulations is about six-seven orders of magnitude higher than in AFM experiment, we obtained nearly the same structurally stable segments as in experiment (Fig. 1).

Another technique, Go-like model approximation, was used for bacteriorhodopsin (Fig. 2) to overcome the problem of high pulling speed, but in the same time, simplifying the protein to Cα chain. Obtained pulling scenario (Fig. 3) was very similar to obtained AFM experiments, indicating that this method may be very useful for tracing the stretching protein (see snapshots of bacteriorhodopsin structure in Fig. 2). Unfortunately, because of lack of structural details, this method cannot be used for investigations of small molecules interacting with the protein. Both theoretical methods are complementary to investigate protein stability and misfolding.

2. Alzheimer's Disease mutation patterns as a tool for presenilin model construction

Presenilins (PS-1 and PS-2) are highly evolutionary conserved integral membrane proteins that – as a part of a large, multiprotein γ-secretase complex - cleave other transmembrane proteins, such as the Notch receptor or β-amyloid precursor protein. Presenilins contain ten identified hydrophobic regions (HRs) in their primary structure and nearly all of them, except HR-7, were suggested to form transmembrane helices leading to many topological models of these proteins. Proteolytic processing of APP by the sequential action of β-secretase and γ-secretase releases amyloid-β peptides (Aβ) – highly aggregative components of senile plaques. Presently, more than 140 Alzheimer's Disease (AD) mutations are in the PSEN1 gene. The lowest age of onset is for the L166P mutation located in HR-3. This mutation not only induces very high increases in $A\beta_{42}$ level, but also impairs APP and Notch intracellular domain production and signalling.

We performed the analysis of mutation patterns in all ten HRs using the most up-to-date information about AD mutations and we have built a conceptual model of PS-1 based on the distribution of these mutations. The membrane spanning HRs of PS-1 were predicted to fold into a helical secondary structure. Linear patterns of mutations along each α-helix were reported five years ago (Hardy and Crook 2001), and nearly all novel mutations fulfilled this pattern so far (Fig. 4). We tried to explain the reason why AD mutations fall into lines or more extended structures by building an appropriate model of PS-1 (Fig. 5a). It appears that the discordant amino acids in PS-1 vs. PS-2 proteins can also be clustered in unique spatial patterns while mapped on the α-helices of HRs (Fig. 5b). As a result, we estimated regions less important for the function of presenilins and they proved to be complementary to areas of AD mutations. The model properly distinguishes residues belonging to AD-affected sites and non-pathogenic areas, and may be used for classification purposes. It also complies with experimental results such as different accessibilities of the catalytic residues in uncleaved PS-1 and the binding of PEN-2 by the PS-1 HR-4 NF motif.

3. Rhodopsin in oligomeric state and its complexes

G protein-coupled receptors (GPCRs) form a superfamily of receptors essential for signalling across plasma membranes. In humans, over 800 genes encode GPCRs with half of them

being odour and taste receptors, and the rest being receptors of endogenous ligands and the light. Each GPCR responds to an extracellular stimulus by activating specific G protein. Then, the trimeric Gαβγ protein dissociates into Gα and Gβγ and one of them (depending on GPCR) modulates specific enzymes that produce second messenger small molecules giving rise to a highly amplified signalling cascade.

Rhodopsin (Rh) proved to be useful, not only as a template for homology modelling of other GPCRs, but also in studying dimerization of these receptors. Currently, it is believed that most GPCRs exist and act as dimers. It is even suggested that GPCRs spend their whole life cycle in a cell as dimers (both homo- and heterodimers), starting from the formation of dimers in endoplasmic reticulum with the help of dimer-probing cytosolic chaperons to the internalization of these receptors.

Kota et al. (PNAS 2006) and Guo et al. (PNAS 2005), using cysteine mutants for Rh and D2 receptors, respectively, confirmed our model of Rh oligomer that both helices TM4 and TM5 are involved in the formation of intradimeric interface. W175, located on the extracellular loop between TM4 and TM5, and Y206 located on TM5, were found to form the interface in the case of human Rh. The other residues shown on Fig. 6, proved to form the interface, were found for D2 receptor. Thirteen residues on TM4 were found to crosslink in the D2 receptor. They were divided into three classes depending on their location on TM4. The first set is coloured in orange in Fig. 6. These residues are on the face of TM4 predicted by using AFM geometric constraints (TM4-TM5 interface). The second set of residues coloured in red (three residues including W4.50 – the most conserved amino acid residue on TM4) belongs to the TM4-TM4 Rh interface, which is obtained by activation of the receptor. The third set (coloured in dark green) consists of nearly all residues starting from residue 4.56 to 4.62. These residues occupy a highly movable part of TM4 consisted of one turn of α-helix and tilted to the rest of TM4.

Using our model of rhodopsin-transducin complex (consisting of two Rh dimers and Gt trimer) in the three-component membrane, we investigated hydrophobic posttranslational modifications of these proteins. Without them, the rhodopsin-transducin complex is not formed. We studied the stabilizing role of these modifications (palmitoyl chains at rhodopsin and myristoyl and farnesyl at transducin) on the structure of the complex.

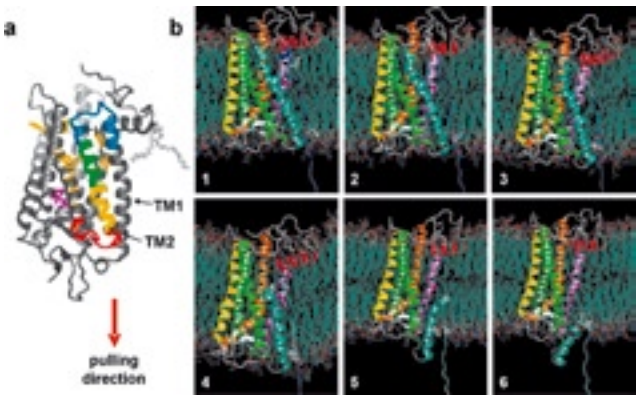


Fig. 1 (a) The location of structurally stable segments on transmembrane helices TM1 and TM2 of WT rhodopsin from AFM experiments (each segment is painted in a different color). The ends of structurally stable segments correspond to force peaks during pulling of N-terminus of rhodopsin. (b) Panels 1-6 show rhodopsin structures corresponding to force peaks during SMD simulations of rhodopsin pulling.

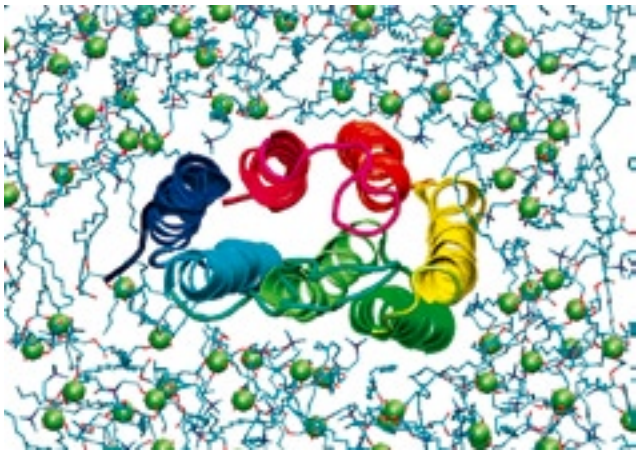


Fig. 2 The bacteriorhodopsin-membrane system at the end of the all-atom simulations. The helices of BR are coloured from blue (helix TM1) to red (helix TM7). The green spheres in the membrane indicate locations of the phosphorus atoms.

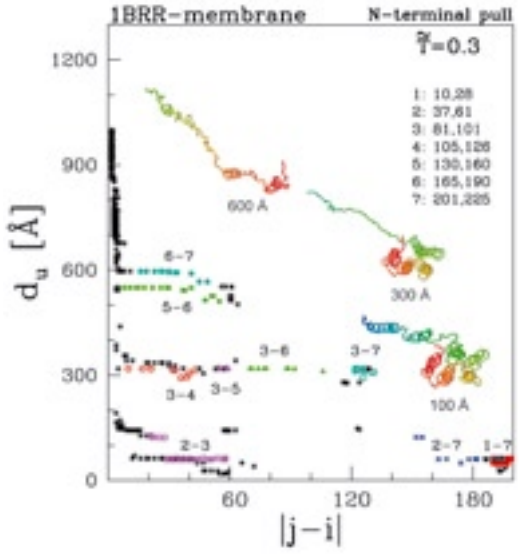


Fig. 3 The stretching of the BR scenario corresponding to pulling by the N-terminus. The Go-like model approximation was used and the protein was represented by their Cα atoms only. The scenario is defined in terms of last tip displacement, d_u, at which specific native contacts still hold. The contacts between amino acids i and j are identified by the sequential distance, |j-i|, between them. Labels, such as 1-7, specify contacts between specific helices.

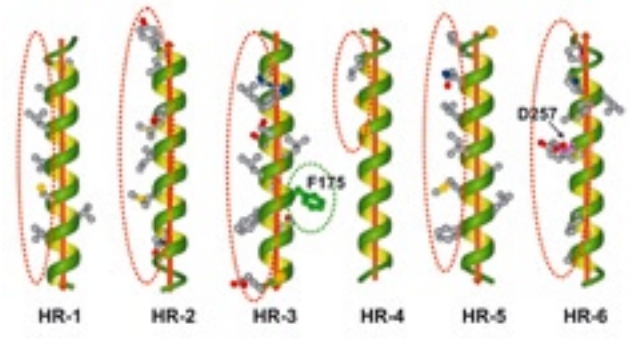


Fig. 4 Linear patterns of AD mutations on individual hydrophobic regions (HRs) of presenilin-1 (PS-1). Residues are coloured in atom types. AD residues – carbon atoms in grey; catalytic residues – carbon atoms in violet; neutral polymorphism – carbon atoms in green. Cα atoms of glycine residues mutated in AD are shown as gold spheres. Solid arrows inside the helices indicate the direction of a sequence. Helices are aligned antiparallelly. Dashed red ellipses denote unilateral location of AD mutation residues.

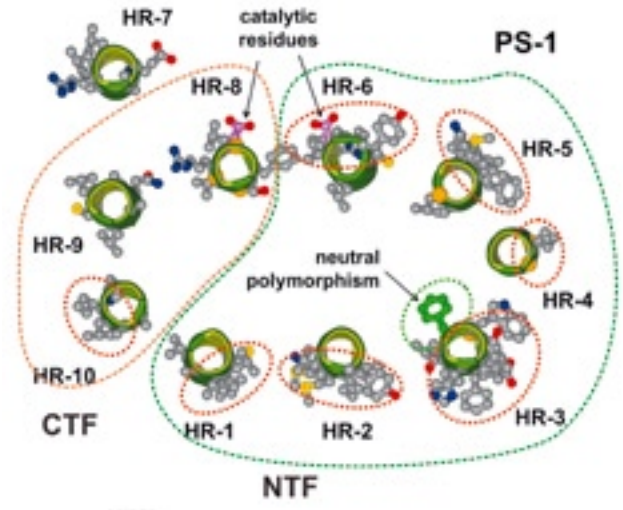


Fig. 5a The areas on PS-1 HRs containing AD mutation residues. N- and C-terminal fragments are marked by dashed lines. Residues are coloured in the same way as in Fig. 4. Dashed red ellipses indicate concentrations of AD mutations on particular faces of helices. The dashed green ellipse denotes the region containing neutral polymorphism.

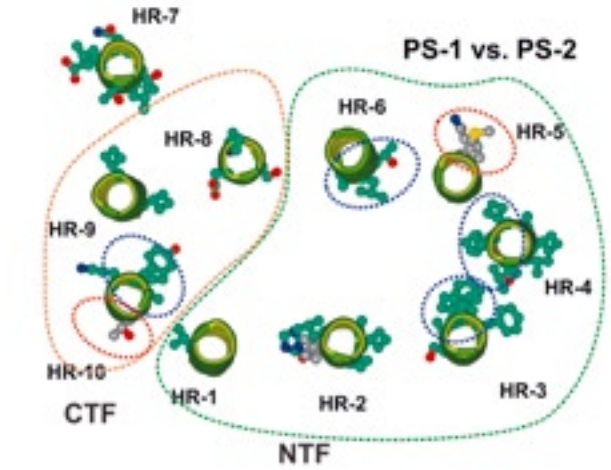


Fig. 5b PS-1 versus PS-2 amino acid discordances (carbon atoms in blue-green). AD mutation residues - carbon atoms in grey. Dashed blue ellipses indicate regions of concentrations of discordances.

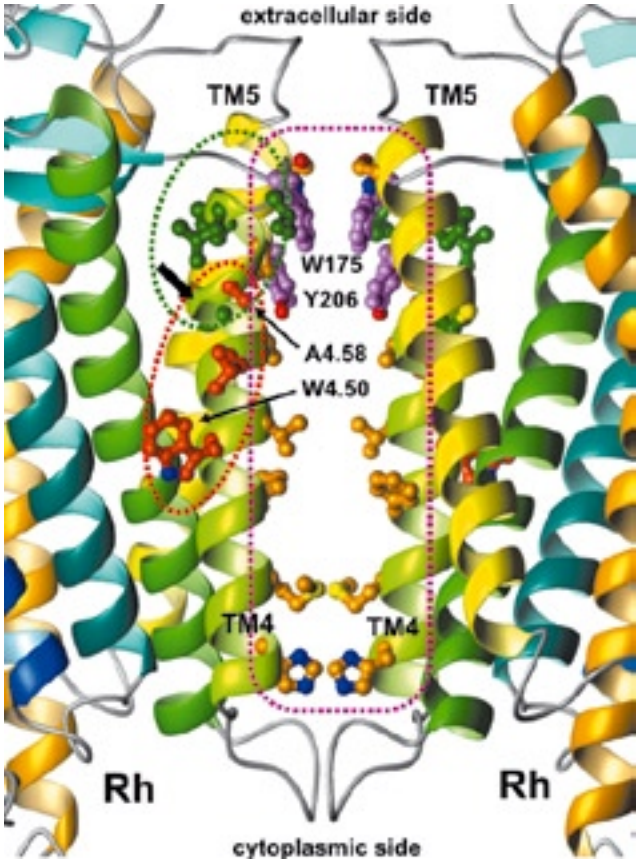


Fig. 6 TM4-TM5 rhodopsin intradimeric interface with experimentally proved crosslinking residues. Residues in violet were confirmed for rhodopsin, others for D2 dopamine receptor. Central encircled area involves residues belonging to TM4-TM5 interface. The dashed red ellipse indicates residues belonging to TM4-TM4 interface. The dashed green ellipse marks residues on the flexible part of TM4. The wide black arrow indicates the beginning of this flexible area.

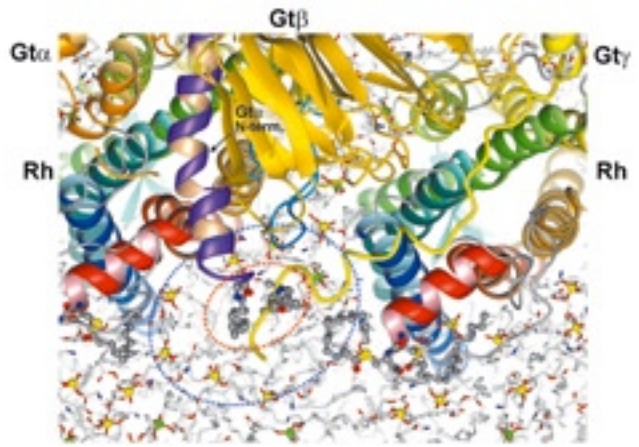
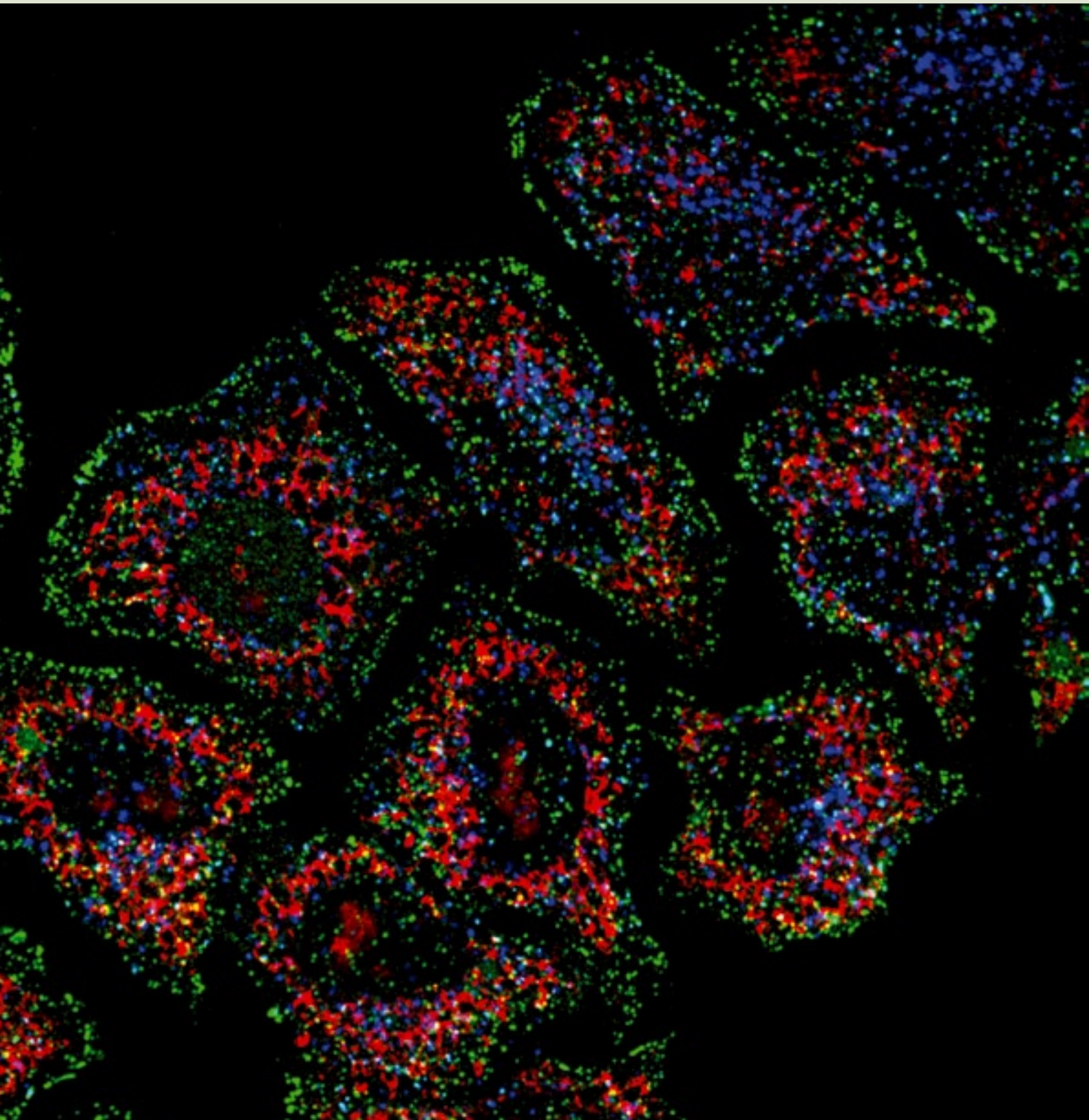


Fig. 7 Transducin membrane anchor area (marked by a dashed red ellipse) being in complex with rhodopsin oligomer. Gtα N-terminal helix is coloured in purple. The loop between TM5 and TM6 linking adjacent Rh dimers is coloured in blue. Hydrophobic modifications of Rh and Gt are shown in ball and stick representation. The view from cytoplasm. Phosphorus atoms of phospholipids are shown as spheres coloured in yellow (PEDS) and green (PSDS). Phospholipids involved in interaction with Gt membrane anchor area are marked by a dashed blue ellipse.

Laboratory of Cell Biology



Lab Leader

Marta Miaczynska, PhD

Post-doctoral fellows:
Iwona Pilecka, PhD (since July 2006)
Beata Pyrzynska, PhD (since November 2006)
Sajid Rashid, PhD (since November 2006)

Research assistants:
Beata Bielinska, PhD (half-time)
Magdalena Banach-Orlowska, PhD (since August 2006)

PhD students:
Marta Brewinska, MSc
Anna Zarebska, MSc
Anna Hupalowska, Msc
Lukasz Sadowski, MSc

Grant administrator:
Vanessa Formas, MA

Picture on the left:
Confocal image of mitochondria and endosomes in HeLa cells. Mitochondria were visualized with Mitotracker (red). Two populations of early endosomes were stained with antibodies against APPL1 (green) and EEA1 (blue).

Marta Miaczynska, PhD

Degrees

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 in the Max Planck Institute for Molecular Cell Biology and Genetics (MPI-CBG) in Dresden, Germany

1997-2000 postdoctoral training at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany

1993-1996 PhD studies in the Institute of Microbiology and Genetics, University of Vienna, Austria

1990-1991 exchange student at the University of Wolverhampton, Wolverhampton, UK

Fellowships and awards

2005 International Research Scholar of Howard Hughes Medical Institute, USA, (2006-2010)

2005 International Senior Research Fellowship of the Wellcome Trust, (UK), (2006-2011)

2005 Partner Group grant from the German Max Planck Society, (2006-2008)

2001-2004 Postdoctoral Fellowship of the Max Planck Society, Germany

1999-2000 Long Term Postdoctoral Fellowship of the Human Frontier Science Program Organization (HFSPO)

1998-1999 Erwin Schrödinger Postdoctoral Fellowship from the Austrian Science Fund (FWF)

1993-1996 Bertha von Suttner PhD Scholarship from the Austrian Ministry of Science

1990-1991 Studentship of the European Community Tempus Scheme

Selected publications

- 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; 116:445-456
- 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

Publications in 2006

- *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, accepted for publication at the Publishing House of Mickiewicz University in Poznan

*Paper marked with an asterisk have the IIMCB affiliation of the authors.

Description of Current Research

Research in the Laboratory of Cell Biology is focused on the relationship between the processes of intracellular membrane transport and signal transduction. We apply a variety of biochemical, microscopical and cell-based techniques to address a question of how intracellular signal transduction is modulated by the endosomal compartments and endocytic transport.

It is well established that signals initiated at the plasma membrane are transmitted to the nucleus through the cytoplasm via a series of protein-protein interactions. For many years, no other intracellular organelles were considered to be required for signal propagation. Similarly, endocytosis was viewed merely as a mechanism for signal termination by downregulation of surface receptors and their degradation. However, more recent studies strongly argue that endocytic organelles constitute intracellular platforms for active signal propagation. In particular, our previous studies characterising endosomal APPL proteins as signal transducers provided a striking example of the involvement of endosomes in signalling (Miaczynska et al., 2004). Two homologous proteins APPL1 and APPL2 are effectors of the small GTPase Rab5, a key regulator in the early steps of endocytosis. They are localized to a subpopulation of Rab5-positive endosomes that appear segregated from the well-characterized canonical early endosomes marked by another Rab5 effector EEA1. APPL-harbouring endosomes are accessible to certain endocytic cargo such as epidermal growth factor (EGF), thus raising a possibility that they may represent a specialized endosomal compartment devoted to signalling.

Interestingly, intracellular distribution of APPL1 appears to be dynamic and changes in response to extracellular stimuli such as EGF or oxidative stress. In our current model, EGF is internalized into APPL- and EEA1-positive endosomes and upon EGF endocytosis, GTP hydrolysis on Rab5 releases APPL1 from the membrane. Cytosolic APPL1 translocates into the nucleus where it interacts with the histone deacetylase and chromatin remodelling complex NuRD/MeCP1. Knockdown of APPL1/APPL2 proteins by RNAi demonstrated that each of them is required for efficient cell proliferation. By identifying an endocytosis regulator Rab5 and a nuclear chromatin remodelling complex NuRD/MeCP1 as interacting partners of both APPL proteins, these data pointed for the first time to a direct molecular link between the processes of endocytosis and chromatin remodelling. As histone deacetylase activities are essential for cell cycle progression, APPL binding to NuRD/MeCP1 may serve the purpose of subjecting this function to regulation by extracellular signalling. Moreover, APPL-harbouring endosomes appear as an intermediate in signalling between the plasma membrane and the nucleus.

Identification of a novel APPL-mediated signalling pathway that is essential for cell proliferation posed a number of novel questions. The research in our Laboratory currently focuses on the following projects:

- Biochemical and microscopical characterization of an endosomal compartment occupied by APPL proteins. We apply cell fractionation and gradient purification techniques to separate

various populations of endosomes in order to enrich APPL-harbouring compartments and determine their protein content. In a parallel approach, we use quantitative microscopy analyses to characterize transport pathways leading through APPL-containing endosomes. To this end, we determine colocalization of fluorescent cargo molecules in APPL endosomes at various time points post-internalization. The quantitative analyses of confocal images are performed in collaboration with Drs. Yannis Kalaidzidis and Marino Zerial (MPI Dresden). We expect that these studies will determine both the molecular identity and the function of APPL-containing endosomes in trafficking of various cargo molecules.

- The mechanisms responsible for APPL1 shuttling in the cell. We would like to understand the exact roles played by various intracellular pools of APPL1 (endosomal, cytoplasmic and nuclear). We search for compartment-specific determinants (interacting partners and/or post-translational modifications) localising APPL1 to various organelles. We would further like to understand whether these pools are interchangeable and which of them is related to APPL function in the regulation of cell proliferation.

- The significance of signalling from endosomes to the nucleus via APPL proteins. We are characterizing the interactions between APPL proteins and their nuclear binding partners by biochemical methods (co-immunoprecipitation, GST pull-down). We also intend to clarify the intracellular topology of these interactions by analyzing the distribution and trafficking of APPL1 and its nuclear interacting partners by microscopy techniques. We expect that, in the long term, such studies will help to understand how intracellular compartmentalization affects the signalling processes and how molecular communication between endosomes and the nucleus is achieved.

- The importance of the APPL pathway in signalling downstream of other growth factors besides EGF. This task is undertaken in collaboration with other laboratories participating in a European Union Integrated Project entitled: Tracking the Endocytic Routes of Polypeptide Growth Factor Receptor Complexes and their Modulatory Role on Signalling (acronym EndoTrack).

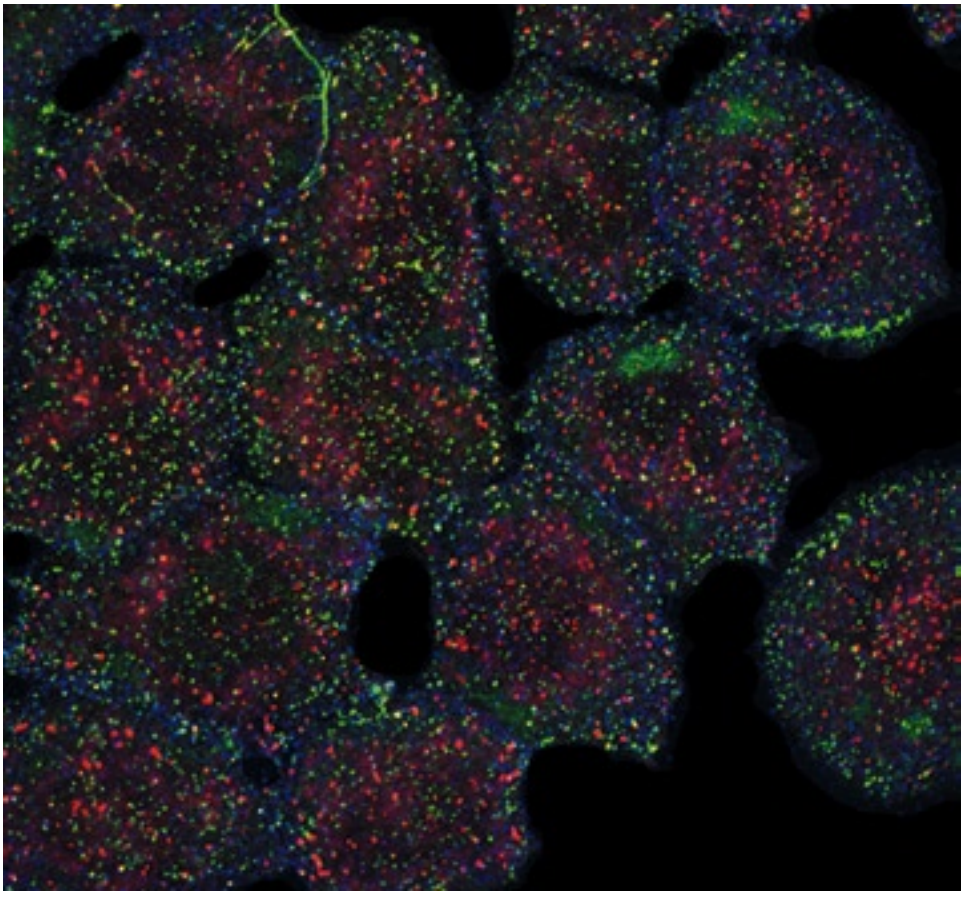
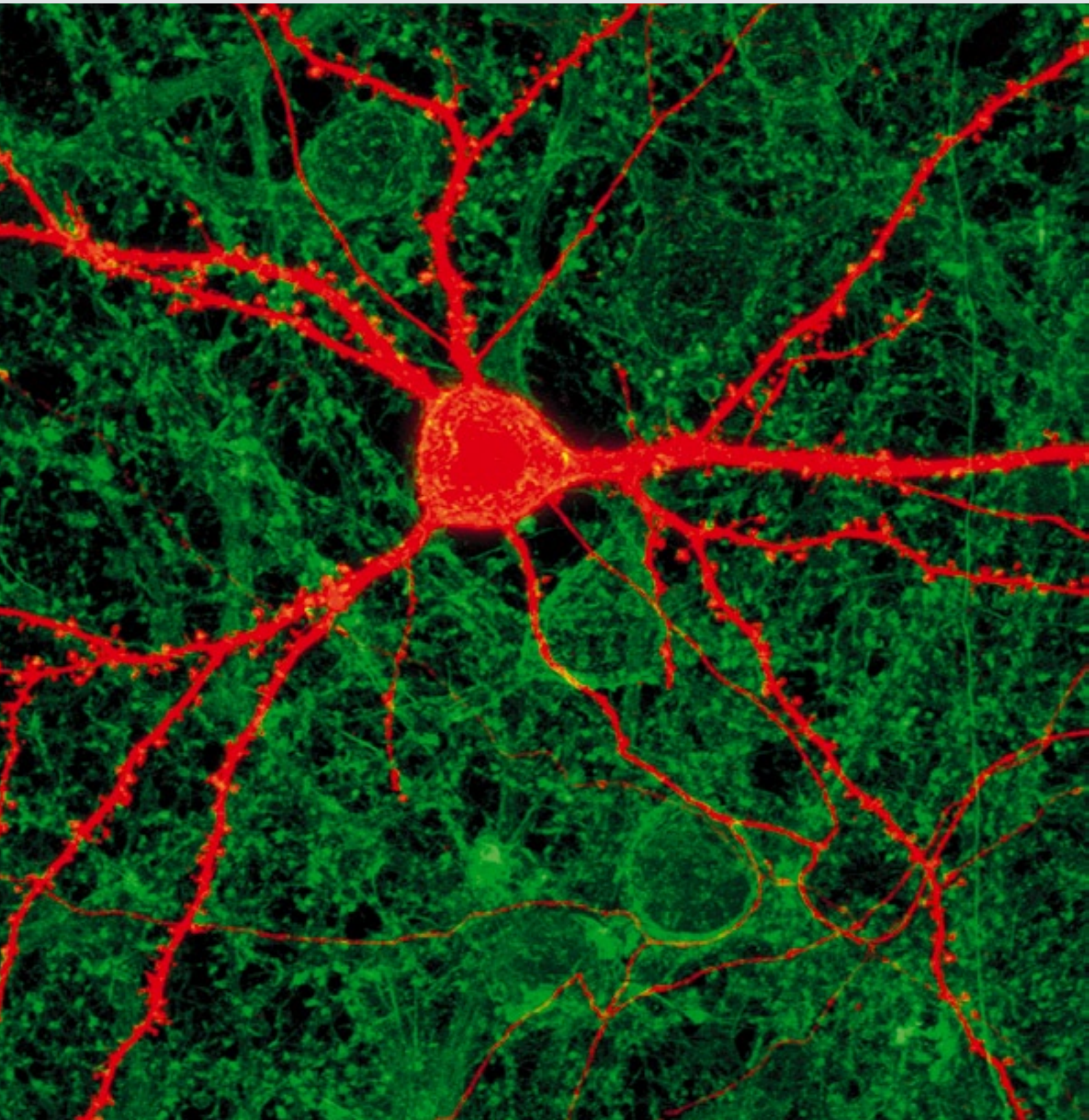
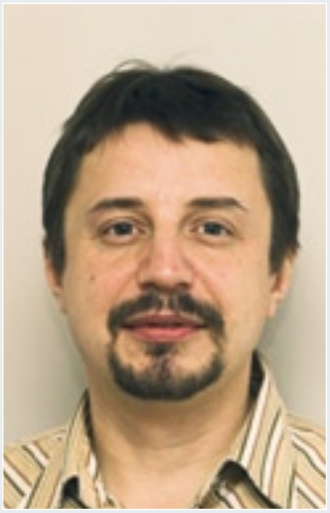


Fig. 1 HeLa cells contain two populations of early endosomes, marked by APPL1 and EEA1 proteins, respectively. Confocal image of HeLa cells, which were allowed to internalize Transferrin-Alexa 647 (blue) for 30 seconds and then fixed. APPL-containing endosomes were stained by APPL1 antibody (green), while red staining corresponds to EEA1.

Laboratory of Molecular and Cellular Neurobiology





Lab Leader

Jacek Jaworski, PhD

PhD students:
Malgorzata Perycz, MSc
Lukasz Swiech, MSc

Undergraduate students:
Malgorzata Urbanska
Patrycja Pietruszka
Kamil Parobczak

Technician:
Izabela Szamreta (until February 2007)

Picture on the left:
Staining of actin cytoskeleton with fluorescent phalloidin (green) of mature hippocampal neurons in culture in vitro. Morphology of the cell is visualized by staining with antibody against transfected β-galactosidase (red).

Jacek Jaworski, PhD

Degrees

2001 PhD in molecular neurobiology, Nencki Institute of Experimental Biology PAN, Warsaw, Poland

1996 MSc in Biology, Department of Genetics, Warsaw University, Poland

Research Training

2006 Erasmus Medical Center, Dr. C.C. Hoogenraad, Rotterdam, Holland, reseach visit, 1 month

2002-2005 Picower Center for Learning and Memory, Massachusetts Institute of Technology and Howard Hughes Medical Institute, Prof. Morgan Sheng, Cambridge, MA, USA; postdoctoral associate

2000 ARL Division of Neural Systems, Memory and Aging, University of Arizona, Dr. J. Guzowski, Tucson, USA (1 month), research training

1997-2001 Laboratoire de Genetique Moleculaire de la Neurotransmission et des Processus Neurodegeneratifs (L.G.N.), Prof. J. Mallet, UMR 9923 C.N.R.S., Paris, France (seven months in total), research training

1996-2002 Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Prof. Leszek Kaczmarek Warsaw, Poland; PhD student until 2001; postdoctoral associate until May 2002

1995-1996 Department of Genetics, Prof. P. Weglenski, Warsaw University, Poland, master degree

Fellowships and awards

2005 Konorski Award of Polish Neuroscience Society and Polish Academy of Science for the best publication of year 2004 in the field of neuroscience (for publication by Kowalczyk et al, 2004 JCB, 167:209-213)

2002 Polish Prime Minister Award for the PhD thesis

2001 Foundation for Polish Science National Scholarship for Young Investigators, 1 year scholarship

2000 EMBO Short Term Fellowship 2000

1999 Polish Network for Cell and Molecular Biology UNESCO/PAN Scholarship

1997 Bourse de stage du Gouvernement Francaise (French Government Scholarship)

Selected publications

- *Okulski P, Jay TM, Jaworski J, Duniec K, Dzwonek J, Konopacki FA, Wilczynski GM, Sanchez-Capelo A, Mallet J, Kaczmarek L TIMP-1 abolishes MMP-9-dependent long-lasting long-term potentiation in the prefrontal cortex. Biol Psychiatry, 2007; Jan 6; [Epub ahead of print]
- *Jaworski J, Sheng M. The growing role of mTOR in neuronal development and plasticity Mol. Neurobiol, 2006; 34: 205-219
- *Nolan EM, Ryu JW, Jaworski J, Feazell RP, Sheng M, Lippard SJ. Zinspy sensors with enhanced dynamic range for imaging neuronal cell zinc uptake and mobilization. J Am Chem Soc, 2006;128(48):15517-28
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- 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; 101:1129-1134
- Gozdz A, Habas A, Jaworski J, Zielinska M, Albrecht J, Chlystun M, Jalili A, Hetman M Role of N-methyl-D-aspartate receptors in the neuroprotective activation of Extracellular Signal Regulated Kinase1/2 by Cisplatin. J Biol Chem, 2003; 278:43663-71
- Mioduszewska B, Jaworski J, Kaczmarek L Inducible cAMP early repressor (ICER) in the nervous system - a transcriptional regulator of neuronal plasticity and programmed cell death. J Neurochem, 2003; 87:1313-1320
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fection of dentate gyrus neurons in vitro. J Neurosci Res, 2000; 60:754-760

- 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; 274:28106-28112

*Papers marked with an asterisk have the IIMCB affiliation of the author

Description of Current Research

The main scientific objective of the Laboratory of Molecular and Cellular Neurobiology is role of the mTOR protein kinase in physiological brain development as well as in the course of neurodevelopmental disorders. We are mostly focusing our research on two phenomena that are dependent on mTOR activity and are crucial for proper formation of the neuronal networks – dendritic arbor formation and synaptogenesis. In this context, we attempt to understand the role of phenomenon of local protein synthesis in dendrites of neurons – a process that was undoubtedly proven to relay on mTOR activity.

Dendrites are the main site of information input onto neurons, and different neurons have distinctive and characteristic dendrite branching patterns. Advances in electrophysiology and computational modeling have clearly shown that dendritic arbor shape is one of the crucial factors determining how signals coming from individual synapses are integrated. In fact several neurodevelopmental pathologies are characterized by abnormalities in the dendritic tree structure including a number of mental retardation (MR) syndromes (such as Down's, Rett's as well as Fragile X syndromes) and schizophrenia. Dendritic arbor development is a multi-step process that is controlled by both external signals and intrinsic genetic programs. Only in recent years have molecular mechanisms been elucidated for dendritic arbor development. Among the proteins that transduce extracellular or cell surface signals into changes in dendritic shape are several protein kinases. Our recent work demonstrated for the first time that PI3K and its downstream kinase, Akt, regulate the complexity of dendritic branching in neurons by protein kinase mTOR (mammalian target of rapamycin).

mTOR is a serine/threonine protein kinase that can be called a cellular sensor of metabolic state of the cell since several pathways sensing the presence of growth factors, the level of amino acids and the level of ATP convert on mTOR (Fig. 1). Not surprisingly, this kinase controls cell size in both non-neuronal and neuronal cells. In neurons, however, role of mTOR activity goes much beyond simple growth control. It has been implicated in neuronal differentiation, axon elongation and directional movements, synaptogenesis, long-term synaptic plasticity and finally in learning and memory. mTOR is thought to act primarily by phosphorylating eIF-4E binding protein (4E-BP) and p70 ribosomal S6 protein kinase (p70S6K), which are important regulators of protein translation. Moreover, recent findings have shown mTOR involvement to be important for local protein synthesis in neuronal dendrites. In the context of these findings our recent data describing mTOR-4EB-P1 and p70S6 kinase involvement in dendritic branching raises an interesting question whether local or general mTOR signaling is required for dendrite morphogenesis. It serves as a starting point for studying the more general question of the potential role of local protein synthesis in dendritic tree development.

However, "chemical genomics", performed on yeast as well as microarray studies with the use of Drosophila cells, identified hundreds of rapamycin-dependent mutants, the analysis of which suggest that mTOR might be involved in cellular functions other than translation such as transcription, ubiquitin-dependent proteolysis, autophagy and microtubule stability. Moreover, several recent research studies suggest the existence of non rapamycin dependent activities of mTOR due to its interaction with the protein called Rictor (Fig. 1). So far the list of functions regulated by mTOR-Rictor complex is very short and definetly not closed. Finally knowledge about mRNAs and proteins that levels of expression and activity depend on mTOR in mammalian cells in general and in neurons in particular is rather obscure. That raises a general question that should be answered first - what are the mTOR dependent proteins and cellular processes involved in dendritogenesis process?

In our quest to answer these questions our main goals are:

1) Identification of mTOR-regulated proteins in neurons by genomic and proteomic approaches.

2) Designing an siRNA library against mTOR-regulated proteins expressed in neurons and perform a systematical screen for these mTOR-dependent proteins that are involved in the process of dendritic branching.

3) Establishing a link between local protein translation and physiological dendritic arbor development. This task requires first establishing a strategy for specific inhibition of local protein synthesis. This unique technology will help us determine how local protein production in dendrites contributes to their development.

4) Characterization of both mTOR-regulated cellular processes and local protein synthesis role in dendritic arbor pathologies observed in MR and schizophrenia.

In the year 2006, our research mainly concentrated on addressing goals 2 and 3. Based on the bioinformatic approach, we analyzed all known proteins, which expression and/or activity depend on rapamycin sensitive actions of the mTOR-Raptor complex (Fig. 1). We identified over 400 mammalian orthologs of such proteins identified in lower organisms. Using stringent

criteria for sensitivity of these proteins to rapamycin and their levels of homology to mammalian proteins, we selected 100 proteins for further studies as targets for siRNA silencing. This initial list was additionally supplemented with known from literature mammalian targets for mTOR. Next, we designed a library of siRNAs against all selected candidates. Currently, we are in the course of preparing of an siRNA-pSUPER-plasmid based library. This library is going to be expanded further, based on the results obtained in the course of realization of our aim #1.

At the same time, we have proceeded with experiments aiming at establishing methods for visualization of local protein synthesis on one hand and selective silencing of local translation in dendrites. We successfully used genetic sensors of local translation (myr-YFP-CamKII3'UTR) to visualize its induction upon BDNF activation of neurons in cultures in vitro (Fig. 2). This important initial step was critical for further research on the link between local protein synthesis and dendritogenesis. On the other hand, our initial results suggest that, targeting with siRNA technology, major components of mRNA transport machinery is a very powerful tool for functional studies on the role of the local translation process for dendritic arbor formation and/or stability.

In addition to our main research activities described above, our group was involved in several collaborative projects in 2006. Most importantly, we started a research project with the several Polish groups (Commissioned Grant form Ministry of Science and Higher Education) with aim to define mTOR targets that are responsible for the progress of tuberous sclerosis – a multiorgan disease that severely affects the brain. One of the characteristic features of this illness is upregulation of mTOR activity due to mutations in its inhibitors – hamartin and tuberin (TSC1/2 complex, Fig. 1). We also closely collaborated with the group of Dr. Hoogenraad (Erasmus MC, Rotterdam, Netherlands) in order to study the role of microtubule dynamics in dendritic spine development. Finally, due to our group expertise in neuronal physiology and siRNA technology, we are involved in several collaborations at the IIMCB, including the groups of Prof. J. Kuznicki, Dr. Marta Miaczynska and Dr. Janusz Bujnicki.

Our research plans for 2007 are to proceed with started, already, topics (aims #2 and #3) toward more advanced experiments (siRNA library screen; analysis of correlations between the dendritogenesis process and activation of local protein synthesis) and kick off proteomic screens for mTOR interacting partners in neurons (aim #1).

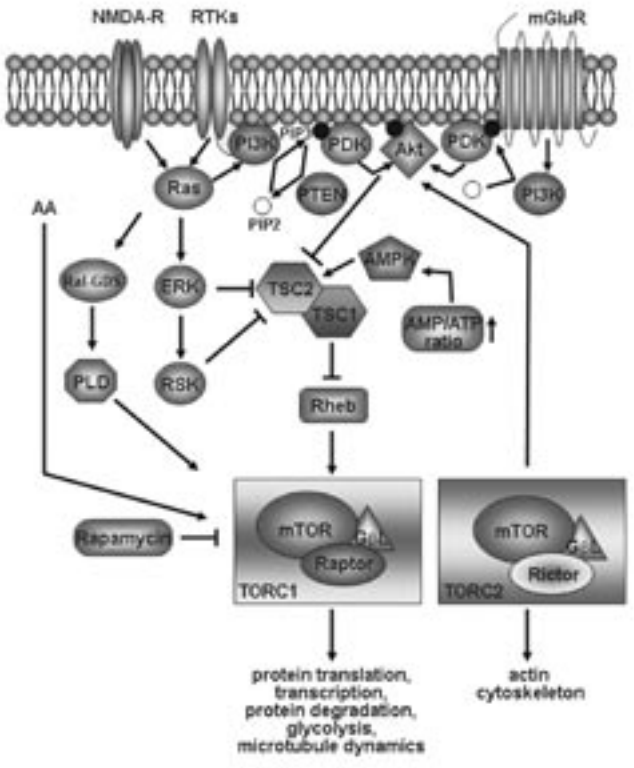


Fig. 1 Schematic diagram of signaling pathways leading to mTOR activation in mammalian cells. Stimulation of several receptors at the plasma membrane, such as receptor tyrosine kinases (RTKs; e.g., TrkB), ionotropic and metabotropic glutamate receptors (NMDA-R; mGluRI) through PI3K-dependent as well as PI3K-independent pathways leading to mTOR activation. PI3K is activated by Ras, leading to activation of PDK1 and Akt, which then inhibits the TSC1/2 complex. That allows GTP-bound Rheb protein to activate mTOR. Ras can also stimulate mTOR activation via ERK or phospholipase D (PLD) pathways, independently of PI3K. An increased level of amino acids (AA) induces mTOR activity in PI3K-independent manner. Increased AMP/ATP ratio, an indicator of energy deficits in the cell, activates TSC2 and inhibits mTOR. mTOR bound to Raptor protein forms the TORC1 complex that is responsible for the control of several cellular processes of which protein translation and transcription are the best described. mTOR can also form a TORC2 complex with Rictor, which regulates actin dynamics and can also phosphorylate Akt. Little is known about the regulation of TORC2.

Abbreviations: PI3K, phosphoinositide-3' kinase; Ral-GDS, Ral GDP dissociation stimulator; PDK, pyruvate dehydrogenase kinase; PIP2, phosphatidylinositol bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homolog deleted on chromosome ten; RSK, p90 ribosomal S6 kinase 1; TSC1, tuberous sclerosis complex protein 1, hamartin; TSC2, tuberous sclerosis complex protein 2, tuberin; Rheb, Ras homolog enriched in the brain; AMPK, AMP-dependent protein kinase.

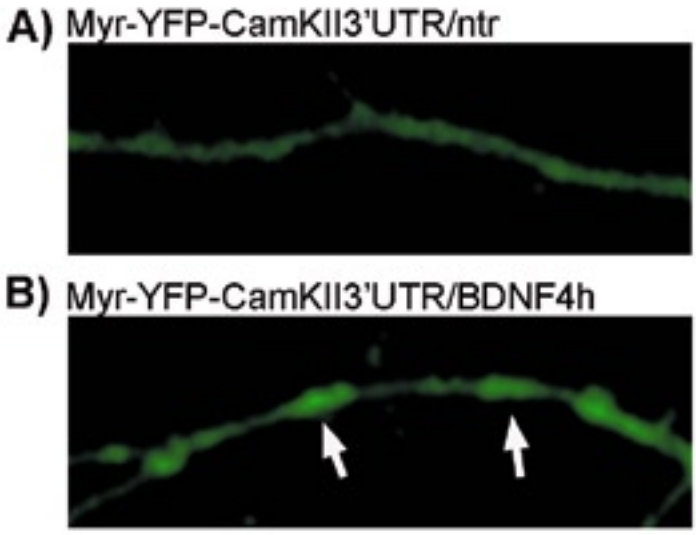


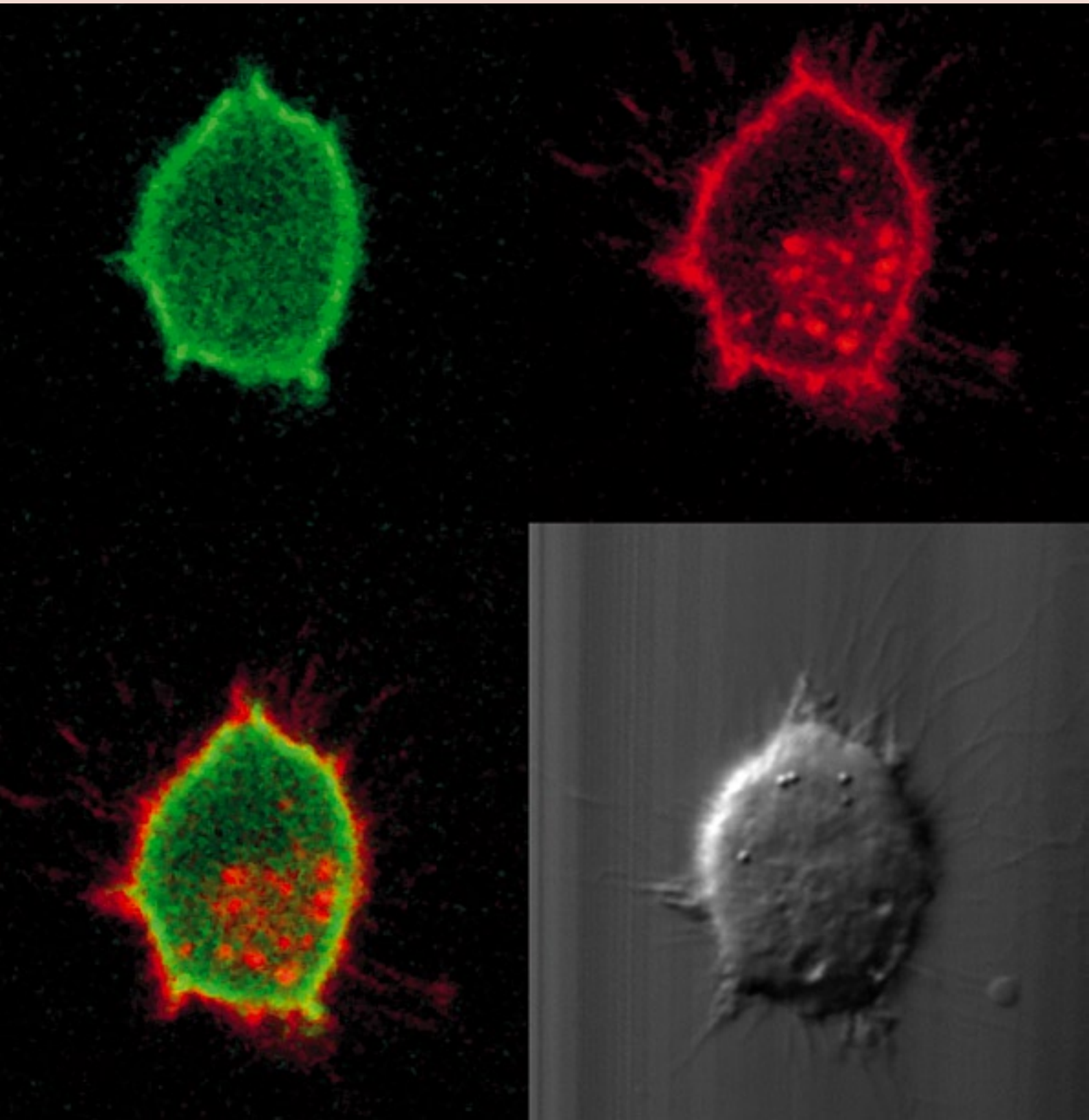
Fig. 2 Genetic sensors of translation can sense BDNF-induced local translation in dendrites.

A) Concfocal image of a section of a dendrite of cell transfected with Myr-YFP-CamKII 3'UTR (genetic sensor of translation) under control conditions.

B) Confocal image of a section of the dendrite cell transfected with the same sensor 4h after BDNF addition to the culture medium. BDNF is a known activator of mTOR dependent local translation. During the period of stimulation inhibitor of transcription, actinomycin D was present in the culture media to prevent potential increase of fluorescence due to an increase in sensor encoding mRNA transcription in response to BDNF treatment.

Laboratory of Cortex Movements and Cell Division MPG/PAN

(located at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, started its activity on the 1st of February 2006)



Lab Leader

Ewa Paluch, PhD

Post-doctoral fellow:
Jean-Yves Tinevez, PhD (since April 2006)

PhD students:
Jakub Sedzinski, MSc (since September 2006)

MSc student:
Ulrike Schulze, BSc (since January 2007)

Technician:
Julia Roensch, BSc (since June 2006)

MAX-PLANCK-GESELLSCHAFT

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY

The equipment and running costs for the lab, including personnel, are provided by IIMCB (MEiN special research project).

Picture on the left:
The actin cortex of a L929 mouse fibroblast. From left to right and from top to bottom: actin-GFP, cRider-RFP (membrane protein), merge, DIC.

Ewa Paluch, PhD

Degrees

2005 PhD in Biophysics, University Paris 7, Paris, France.

2001 DEA (Masters degree) "Interfaces Physique-Biologie", University Paris 7 (rank: 1st)

2000 Agrégation of Physics

1999 Maîtrise (equivalent BSc) in Physics at Ecole Normale Supérieure de Lyon, France

1998 Licence in Physics at Ecole Normale Supérieure de Lyon

Research Training

2001-2005 PhD studies at the Institut Curie, Paris, France

2000-2001 DEA (equivalent Masters) research project in Biophysics, Institut Curie, Paris, France

1999 Maîtrise (BSc) research project in particle Physics, CERN, Geneva, Switzerland

1998 Licence (part of BSc) research project in relativistic Astrophysics, Paris-Meudon Observatory, France

Professional Employment

since 2006 Joint MPI-CBG/PAN group leader at IIMCB, located at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden

Oct.-Dec. 2005 Scientist position at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden

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 from the Ligue Nationale contre le Cancer, France

2001-2004 PhD scholarship from CNRS, France

2000 Agrégation in Physics (French national competition, rank: 6th)

1998-2001 full salary from the 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

Selected publications

- *Paluch E, Van der Gucht J, Sykes C. Cracking up: symmetry breaking in cellular systems. J Cell Biol., 2006, 175(5):687-92
- Paluch E, van der Gucht J, Joanny J-F, Sykes C. Deformations in actin comets from rocketing beads. Biophys J, 2006, 91: 3113-22
- 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
- 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

*Papers marked with an asterisk have the IIMCB affiliation of the author

Publications (other than scientific articles)

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

Research

Concerted flows of the actomyosin cortex, associated proteins and cortical organelles are commonly observed in various modes of cell locomotion as well as at the onset of cytokinesis. They are essential for numerous cell biology processes, such as formation of the division ring, contraction of the cell body during cell migration, or segregation of polarity markers during asymmetric divisions. Moreover, these cortical flows are thought to reflect a basic physical mechanism where gradients of tension pull cortical components from regions of relaxation to regions of high tension. The group focuses on the biophysical and molecular study of cortex movements.

It has been shown that the cell cortex can spontaneously break. For example, when cortical contractility is enhanced by depolymerization of microtubules and in the absence of substrate adhesions, the cell cortex spontaneously ruptures and a bulge of bare membrane (a bleb) is expelled through the hole. Cortex flows triggered by the rupture then lead to the oscillation of a constriction ring across the cell. Based on these experiments, we proposed that bleb formation and cortical flows reveal an intrinsic behavior of the actomyosin cortex and that they are direct consequences of cortical contractility. By biochemically fine-tuning the level of cortex tension, the cell could use these spontaneous behaviors for polarization, locomotion and shape changes in general. The group focuses on the involvement of these spontaneous cortical ruptures and flows in cell division. The staff is composed of biologists and biophysicists. Combining biophysical and molecular approaches, we would like to understand the mechanism(s) that trigger cortical flows at the onset of cytokinesis and how the flows contribute to the establishment of the cleavage furrow. More generally, we investigate how the cortex mechanical properties control cell shape changes and how the cell biochemically regulates these mechanical properties.

Subprojects (future plans)

1. Characterization of the cortical flows (Jakub Sedzinski)

In spite of its importance for cell deformations, very little is known about the actual contents and structure of the cell cortex. We aim to identify the molecular players involved in cortical flows and characterize their roles in cortex movements. We are currently developing an assay where RNAi of candidate proteins in the oscillating cells described above will allow us to identify the role of these proteins in concerted cortical flows. A long term project will be to pin down the minimal ingredients necessary for the cortex activity, paving the way for a biomimetic system of the cell cortex.

2. Cortex contribution to accurate mitotic spindle positioning (Julia Roensch)

Recent data indicates that a tight control of myosin II activity is essential for accurate spindle positioning. We are investigating whether spontaneous movements of the cortex resulting from high myosin activity could lead to spindle mispositioning.

3. Cortex mechanics and cell shape (Jean-Yves Tinevez, Ulrike Schulze)

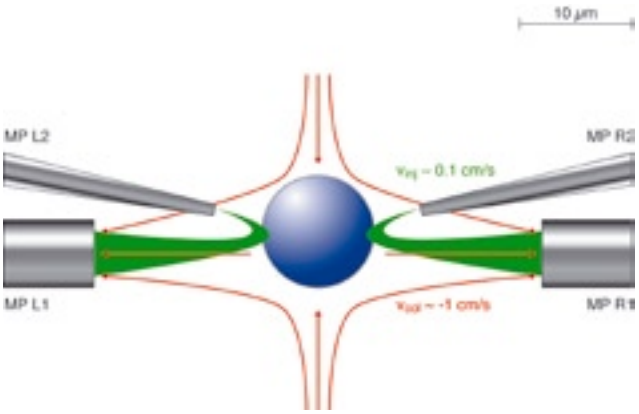
The aim of this project is to determine:

– how cortex mechanical properties influence cell shape

– which cortical proteins influence cortex mechanics.

We are planning to vary the cortex molecular composition (RNAi, microinjection of proteins) and observe corresponding cortical behaviors and deformations. We have built a micromanipulation experiment that allows us to microinject proteins inside living cells as well as to measure cortical tension. We will use it to assess how changes in the concentration of various cortical proteins affects cortex tension and cell shape.

The micropipette experiment also enables us to externally trigger cortex movements. Moreover, we can induce cortex flows by locally ablating the cell cortex with a UV pulsed laser.



Sketch of the double micropipette setup allowing for cortex flows induction, cell microinjection and tension measure.

We are using these setups to test the influence of cortex ruptures on various motility events. One question is whether synchronized flows of cortex from the poles towards the equatorial region are sufficient to induce the formation of a stable constriction ring.

4. Physical modeling of cortical flows

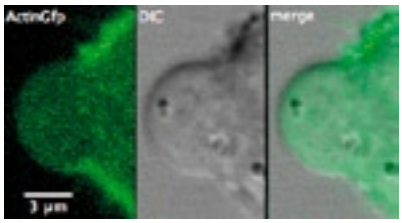
In parallel, we develop a collaboration with theoretical physicists (group of Frank Jülicher, MPI-PKS, Dresden) in order to model cortical mechanics that can lead to furrow formation. This physical analysis will help us determine which cortical behaviors may result from self-assembly, and which ones require further regulation.

Long term projects

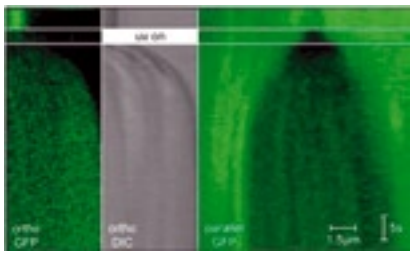
In the long term, and in collaboration with other groups at the MPI-CBG, Dresden and at the IIMCB, Warsaw, we would like to investigate:

– the role of the membrane and of lipid domains in cortical flows and cell shape changes

– how cortex spontaneous behaviors are involved in cell migration in tissues in developing embryos.



A bleb (spherical membrane protrusion) induced by photo-ablation of the cell cortex.



Kymograph of the cortex opening after laser ablation. Left and center: kymographs along a line orthogonal to the cortex. Right: kymograph along the cell cortex; the velocity of the hole opening allows to measure cortical tension.

Educational Activities

Utrecht University Doctoral Program

The Utrecht University international doctoral program is based on an agreement between the Polish Network for Cell and Molecular Biology UNESCO/PAN and the Utrecht University (The Netherlands). This is a part of the research collaboration program initiated by Prof. Willem Gispen to facilitate the exchange of scientific information and ideas among Polish and Dutch scientists and graduate students and to allow for short-term research visits of the staff members and their students from Poland to Utrecht and vice versa. The first turn of the doctoral program offered three four-year doctoral positions. The doctoral thesis were to be defended in front of the dissertation committee of the Utrecht Medical Center. As a result till now two students M. Bucko-Justyna (M. Zylicz's lab, IIMCB) and K. Starowicz (R. Przewlocki's lab, Institute of Pharmacology PAN, Cracow) defended their theses in due course. Because of the success of this part of the program, the next recruitment has been announced in 2003. Currently four students are enrolled in the program: M. Geremek (M. Witt's lab, IIMCB and Institute of Human Genetics PAN, Poznan: Genetic analysis of primary ciliary dyskinesia/Kartagener Syndrome [PCD/KS]), M. Lukowiak (A. Lipkowski's lab, Center for Experimental and Clinical Medicine PAN, Warsaw: Pharmacology of opioid peptides. The application of polymers as carriers of the opioid peptides), P. Michaluk (L. Kaczmarek's lab, Nencki Institute PAN, Warsaw: Role of MMP-9 in neuronal plasticity), Jakub Urbanski (M. Zylicz's lab, IIMCB: Molecular chaperones in tumor invasiveness). IIMCB coordinates the entire program on the Polish site.

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

Medical Universities in Warsaw, Poznan, Szczecin, Gdansk, Wroclaw, Lodz, as well as the International Institute of Molecular and Cell Biology, the Nencki Institute and the Foundation for Experimental and Clinical Oncology have jointly founded the Postgraduate School of Molecular Medicine. The main goal of the School is to offer a new post-graduate PhD program in the field of molecular medicine, which is addressed to medical, biology and pharmacology postgraduate students in Poland. Since the year 2002, SMM has been opened to 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 the Director and the Scientific Council elected by the founding institutions. At present, the Director's position is held by Prof. L. Konarska from the Faculty of Pharmacy Department of Biochemistry and Clinical Chemistry, Medical University of Warsaw. SMM admits 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 independent reviewers in Poland and from abroad. Nine groups of students were accepted during the period of 1998-2006, including six foreign individuals. Successful candidates accomplish their scientific program, under supervision of their tutors, in home laboratories throughout Poland. The members of SMM Scientific Council evaluate student progress annually. The tutorial program offered to the students includes theoretical (lectures, seminars) and practical courses (laboratory sessions) on selected topics of modern molecular biology and medicine. Each SMM student is awarded a stipend (full or supplemental). Furthermore, SMM helps students to participate in short-term scientific training in leading Polish and foreign laboratories. In parallel to funds generated by founding institutions, SMM activities are supported by subsidies from the Polish Ministry of Health, Ministry of Science and Higher Education, the Kronenberg Foundation, UNESCO-ROSTE, the European Commission within the 5th Framework Programme (Centre of Excellence in Molecular Bio-Medicine of IIMCB), CNRS (France). Additional financial support comes from the French government supporting the costs of participation of outstanding French scientists in tutorial and organizational activities of SMM as well as short-term scholarships for the training of SMM students in laboratories in France. In 2006, the following courses were organized:

- Practical course "Scientific communication", 8-12.05.2006, Warsaw, organized by SMM and IIMCB. This annual obligatory course for all first-year students was organized by Prof. Michal Witt. The course, run by Prof. Edward Potworowski of Armand-Frappier Institute, Montreal, Canada, was designed to heighten the students' awareness of what constitutes the clear and effective transmission of a scientific message, whether written or spoken.
- Practical course "Molecular methods applied in medicine", 26-30.06.2006, Poznan, outsourced at the Summer School „Progress in Molecular Biology".
- 8th Annual Inaugural and Research Report SMM Session, 23-24.10.2006, Warsaw, organized by prof. Liliana Konarska, Medical University of Warsaw and SMM students. Inaugural lecture "Adhesive and proteolytic phenotype of migrating endothelial cells – impications for angiogenesis" was given by Prof. Czeslaw Cierniewski, Medical University of Lodz and Medical Biology Centre Polish Academy of Sciences, Lodz. During the session 27 SMM students presented their research results obtained during the academic year 2005/2006. The presentations were divided into five subsessions: cancer cell biology and signaling, protein engineering and biomodelling, molecular diagnosis of cancer and human disorders, immune system in disease, neurobiology.



8th Inaugural and Annual Research Report Session of SMM, 23-24.10.2006, Warsaw.

Science Festival School (SFN) – Popularization of Science

Science Festival School – Nonscientists meet with Science

The Science Festival School (SFN) aims to reduce the gap between science and society in Poland by conducting educational activities popularizing biology: open lectures, workshops for students and all interested participants, as well as courses for biology teachers. All activities are focused on improving biology education and the awareness of biology in society. The co-founders of the Science Festival School are four biological institutes: International Institute of Molecular and Cell Biology (IIMCB), Nencki Institute of Experimental Biology PAN (IBD), Institute of Biochemistry and Biophysics PAN (IBB), Warsaw Agricultural University (WAU) and Warsaw Science Festival. IIMCB hosts the SFN laboratory, office and administration and allows for the School's activities at its premises. Science Festival School leads another laboratory at the Warsaw Agricultural University. In 2006, a total number of 1360 young participants took part in laboratory workshops together with 116 biology teachers and about 1000 lectures listeners.

In 2006 Science Festival School was awarded with Master of Science Popularization Prize; from left: J. Lilpop, M. Badurek, A. Choluj.

Master of Science Popularization Award

In the second contest, "Master of Science Popularization", organized by the Polish Press Agency and Ministry of Science and Higher Education, Science Festival School received the first prize in the category of "Journalist, Editorial Office or Non-Scientific Institution of Science". This award was given for the efforts of SFN to develop the interest of young students in biology and more over in science, and in encouraging teachers to incorporate a molecular biology curriculum into the biology courses at schools. In this way, the SFN changes biology education in Polish schools. The other ex-equo award in this category went to Mr. Wiktor Niedzicki, the leading Polish journalist specializing in topics related to science.

Researchers' Night

European Researchers' Night is a Europe – wide, annual event involving a wide range of scientific and research organizations – including museums, laboratories and academic institutions – that host a variety of entertaining and fun events planned to run late into the night of Friday. The aim is to give the public, young people in particular, the opportunity to meet researchers in a festive and fun atmosphere, and to highlight the appeal of pursuing a career in research. The 2006 Researchers' Night, held on 22 September was a huge success in Warsaw: thousands of people, from children to the elderly, took part in the myriad of events organized on that day. Science Festival School had organized two workshops:

- **"Genes Around Us"** dedicated to journalists, Members of Parliament and representatives of local authorities.
- **"Experiment at School? It is possible!!"** dedicated to biology teachers.

The Science Festival School was the only institution in Warsaw which scored the maximum points received from workshop participants and evaluators.

10th Science Picnic (3 June 2006)

BioEducation Foundation, Science Festival School and Mo-

lecular Biology Student's Association organized an exhibition and science show, with the motto "The world in ten years"

- The viruses of the future
- Let's see the bacteriophage!
- Let's make you own bacteriophage!
- Make an appointment with the physician of the future – check your DNA

10th Warsaw Science Festival (16-24 September 2006)

My carrier as a biologist. Open lecture – **Agnieszka Choluj**

Laboratory workshops: How to wring jellyfish out from bacteria? The miracles of biotechnology – **Sebastian Pawlak**

Laboratory workshops: Explore your own DNA – **Joanna Lilpop, Michal Mlacki**

Laboratory workshops: "Do you know what you eat?" – **Joanna Lilpop**

"Games with biology background": X Science Festival in Jablonna Palace – **Agnieszka Choluj**

"Let's see DNA!!", "The power of your saliva": experiments for small children, 10th Science Festival in Jablonna Palace – **Agnieszka Choluj**

Science Picnic

Laboratory workshops

The participants of workshops apply laboratory equipment, techniques and real life experiments. The practical experiments are supported by lectures presenting the theoretical basis of molecular biology, genetics and its techniques. The workshop lasts four hours over the course of one day and covers topics such as:

- examining DNA by PCR methods
- bacterial transformation
- gene cloning
- protein fingerprinting
- molecular diagnosis

Courses for biology teachers

Science Festival School in 2006 organized 3 courses and 3 workshops. Participating teachers had an opportunity to learn how to use modern equipment and molecular biology techniques, and how to make experiments that can be easily implemented in schools. During our workshops for teachers, we tried to build a connection between them and scientists so they can feel like part of science community. We also equipped them with lesson scenarios and affordable experimental kits that can be used at school laboratories. After each workshop, the participants received certificates, which in turn, help them to develop their own career. SFN helps all teachers, especially those from small towns and villages: their access to such forms of self-improvement are the most difficult.

The 5th conference for biology teachers was organized in cooperation with Nencki Institute of Experimental Biology. Over 50 teachers took part in 6 lectures given by scientists from the Institute, Warsaw University, and Faculty of Medical Microbiology.

Laboratory training for talented secondary school pupils

Two weeks laboratory training for seven gifted secondary school pupils was organized during 2006 summer holidays. Two laureates of the Polish Biology Olympiad joined the research groups at IIMCB: Laboratory of Neurodegeneration and Laboratory of Molecular and Cellular Neurobiology. SFN organized initial training in laboratory practice and covered the costs of accommodation in Warsaw for young researchers.

Educational projects

"VOLVOX " project under FP6, activities in 2006

In 2005, Science Festival School started the implementation of the Volvox Specific Support Action project funded by the European Commission within FP6, officially entitled: Coordinated internet-linked networks for promoting innovation, exchanging knowledge and encouraging good practice to enhance bioscience education in European schools. Volvox consists of nine partners from Denmark, Estonia, Germany, Italy, Luxembourg, Poland, Portugal, Sweden and the UK. New attractive resources should encourage more young people to develop positive attitudes towards studying science and to consider a scientific career.

During the second year of the operation of the Volvox project, Science Festival School participated in the following activities:

- SFN organized the fourth Consortium meeting in Warsaw. The group invited Christian Siatka from L'ecole de l'AND, which is a Francophone network of molecular biology education centers in France and Canada.
- New practical protocol about urease enzyme: "Urease or how our urine makes plants grow", was written by Anna Lorenc, co-worker of SFN. This protocol was tested in the

laboratory and practically with groups of students. Several materials offered by Volvox partners include: "The chocolate challenge" (by John Schollar, NCBE), and "More juice from apples" (by Dean Madden NCBE). Both were translated into Polish and adapted.

- A group of close cooperating science teachers was established by Science Festival School. The group has taken part in making decisions in choosing Volvox materials for translation into Polish and adaptation into Polish reality. They also has tested adapted materials in practice with students in the classrooms. The group has contributed towards preparing and testing new educational materials regarding woodlice, physical activity tests and fish dissection. The teachers decide which materials offered by other Volvox partners are the most suitable for Polish schools. SFN also sent invitations to a wider group of science teachers. Those who would like to participate, will receive completely prepared and adapted materials in Polish for practical tests.

Open lectures

Besides our offer directed toward schools and teachers, SFN, in 2006, presented theoretical issues of modern biology to the more general audience. Every two weeks, SFN organized open lectures on modern biology given by top Polish scientists. These lectures were accessible to everyone with a basic knowledge of biology. Among topics were: genomics, evolution, genetic diseases, genetically modified organisms, gene expression regulation, immunology and stem cells. Every year, one lecture is dedicated to the Nobel Prize in medicine and physiology awarded for this particular year. In 2006, it was a lecture given by Dr. Ewa Henning from the Medical Center of Postgraduate Education, "*Helicobacter pylori* – the old friend of Human". In 2006, the lectures attracted an audience of 1000.

Workshop for biology teachers.

Infrastructure and Working Environment

The building of the Institute offers 16820 m^3 of cubic space, with 4032 m^2 of internal surface. It is divided into seven floors and a basement. The administrative sector is located on the ground floor (Directors' offices, the lecture hall for 60 people, Institute's meeting room, other offices and social rooms). Five floors, from the 1st till the 5th, are arranged as a typical laboratory space, a "Faraday - cage" lab for sensitive electronic measurements (e.g. electrophysiology), a coldroom, a dark room, several offices, social rooms and common space for heavy laboratory equipment (see: http://www.iimcb.gov.pl/equipment.php). On the 4th floor there is a computer facility of the Institute (including servers) and on the 5th floor a part of the laboratory space is prepared to accommodate a cell and molecular biology school (training laboratory for 18 students – currently in use of SFN).

The infrastructure of the Institute is fully adapted to the safety and biosafety regulations for chemical and molecular biology laboratories. All laboratories have been equipped with modern apparatus in accordance with the highest international standards. In addition to the regular equipment in each wet laboratory, there are pieces of apparatus shared by all researchers. There are centrifuges and ultracentrifuges, sets of FPLC and HPLC systems, chromatography systems AKTA, a real-time thermocycler, fluorescence microscopes, phosphoimagers, incubators and shakers for bacterial cultures, electroporators for transfections and transformations, freezers (-70°C). Among new pieces of equipment are: surface plasmon resonance measuring system Biacore 3000, confocal microscope, flow cytometer, CD spectropolarimeter, microplate reader, two microcalorymeters. There are also seven cell culture labs fully equipped with incubators, laminar-flow hoods, and microscopes, three coldrooms, and two sets of water deionizing units. The isotope laboratory is equipped with a phosphofluoroimager and a scintillation counter compliant with the Polish and EU law regulations. The Laboratory of Structural Biology, financed by the Max Planck Society, Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, is furnished with the modern protein crystallography equipment including a high brilliance rotating anode generator (RU-H3RHB from MSC), Max-Flux confocal optical mirrors, a MAR345 low noise X-ray detector and a cryosystem. Due to its uniqueness, this equipment serves other members of the scientific community interested in protein crystallography analysis.

The building is equipped with ventilation, air conditioning, smoke alarms and fire escapes according to current regulations. Three lecture halls allow for intensive seminar programs, without any restrictions concerning time schedules. The practical courses are organized in a separate laboratory that is an important element for comfort and work safety. Being part of a large building complex, IIMCB has access to the following: six lecture halls (from 20 to 300 people), an exhibition hall, a hotel, a cafeteria, as well as other facilities belonging to the neighboring research institutes of the Ochota Campus. The IIMCB facilities, as well as the whole campus complex, are fully accessible for the disabled. Medical, social and legal services are accessible to the staff on-site. A security guard system operates on the entire campus around the clock.

Staff at IIMCB

(as of March 2007)

Name		Funding
Jacek Kuznicki	Director	IMCB
Michal Witt	Deputy Director for scientific matters	IIMCB(1/2)
Maria Kleska	Deputy Director For administrative matters	IIMCB
Hanna Iwaniukowicz	Financial Manager	IIMCB
Beata Tkacz	Director's Assistant	IIMCB
Monika Kacprzak	Secretary	IIMCB
Dorota Libiszowska	Foreign Grants Manager	IIMCB
Sylwia Adamiec	International Cooperation Specialist	IIMCB
Agnieszka Ziemka	Planning and Reporting Manager	IIMCB
Agnieszka Karbowska	Tender Specialist	IIMCB
Renata Knyziak	Accounting Specialist	IIMCB
Monika Nowicka	Payroll Specialist	IIMCB
Krystyna Domanska	Human Resources Specialist	IIMCB
Ewa Blazewicz	Secretarial Assistant	IIMCB
Rafal Flis	IT Manager	IIMCB
Przemyslaw Slusarczyk	IT Specjalist	IIMCB
Marcin Biedacha	IT Specjalist	IIMCB
Robert Banasiak	Maintenance Specialist	IIMCB

Department of Molecular Biology

Name		Funding
Maciej Zylicz	Head	IIMCB
Alicja Zylicz	Vice Head	IIMCB
Pawel Bieganowski	Research Associate	Ministerial grant(1/2)
Marcin Klejman	Research Associate	IIMCB
Maciej Olszewski	Research Assistant	Ministerial grant
Malgorzata Gutkowska	PhD Student	Nencki Fellowship
Leszek Lipinski	PhD Student	IBB
Jakub Urbanski	PhD Student	Utrecht Fellowship
Dawid Walerych	PhD Student	IIMCB
Anna Zurawska	PhD Student	IBB
Grazyna Orleanska	Secretary	IIMCB

Laboratory of Bioinformatics and Protein Engineering

Name		Funding
Janusz M. Bujnicki	Head	IIMCB
Krzysztof Skowronek	Research Coordinator	EU
Michal Boniecki	Postdoc	NIH
Kristian Rother	Postdoc	EU
Agnieszka Chmiel	PhD Student	IIMCB
Iwona Cymerman	PhD Student	SMM/Ministerial grant
Malgorzata Durawa	PhD Student	Ministerial grant(1/2)
Marcin Feder	PhD Student	SMM/NIH
Michal Gajda	PhD Student	EU
Andrzej Kaminski	PhD Student	NIH
Jan Kosinski	PhD Student	SMM/NIH
Grzegorz Papaj	PhD Student	Ministerial grant
Sebastian Pawlak	PhD Student	Ministerial grant
Marcin Pawlowski	PhD Student	IIMCB
Elżbieta Purta	PhD Student	Ministerial grant
Joanna Sasin	PhD Student	Ministerial grant
Karolina Tkaczuk	PhD Student	NIH, Ministerial grant
Irina Tuszynska	PhD Student	NIH
Agnieszka Obarska-Kosinska	PhD Student	NIH
Stanisław Dunin-Horkawicz	Msc Student	Volunteer
Katarzyna Filip	Msc Student	Volunteer
Pawel Sztromwasser	Msc Student	Volunteer
Tomasz Jarzynka	Msc Student	Volunteer
Jerzy Orlowski	Msc Student	Volunteer
Robert Orzechowski	Msc Student	Volunteer
Michal Wrzesinski	Office Manager	NIH
Olga Babicka	Office Manager	IIMCB
Krzysztof Kuchta	Programmer	NIH
Jan Kogut	Computer Administrator	EU

Laboratory of Structural Biology MPG/PAN

Name		Funding
Matthias Bochtler	Head	Max Planck
Honorata Czapinska	Post doctoral Fellow	Ministerial grant
Renata Filipek	Post doctoral Fellow	EU
Aneta Kaczmarczyk	Post doctoral Fellow	Ministerial grant
Izabela Sabala	Post doctoral Fellow	EU
Grzegorz Chojnowski	PhD Student	UW Fellowship
Magdalena Kaus-Drobek	PhD Student	IIMCB/Nencki Fellowship
Henryk Korza	PhD Student	EU
Magdalena Lipka	PhD Student	EU
Malgorzata Firczuk	PhD Student	IIMCB
Monika Sokolowska	PhD Student	Max Planck
Roman Szczepanowski	PhD Student	Max Planck
Marek Wojciechowski	PhD Student	IIMCB/Nencki Fellowship

Laboratory of Neurodegeneration

Name		Funding
Jacek Kuznicki	Head	IIMCB
Urszula Wojda	Associate Professor	IIMCB
Neli Kachamakova	Post-doctoral Fellow	Ministerial grant
Monika Klejman	Post-doctoral Fellow	IIMCB
Marta Wisniewska	Post-doctoral Fellow	EU
Magdalena Blazejczyk	PhD Student	Ministerial grant/Nencki Fellowship
Lukasz Bojarski	PhD Student	IIMCB/Nencki Fellowship
Wojciech Michowski	PhD Student	Nencki Fellowship
Katarzyna Misztal	PhD Student	FNP grant
Adam Sobczak	PhD Student	Ministerial grant/Nencki Fellowship
Aleksandra Szybinska	PhD Student	IIMCB
Malgorzata Mossakowska	Project - coordinator	IIMCB
Katarzyna Debowska	MSc Student	Volunteer
Miroslaw Drab	MSc Student	Volunteer
Kamila Skieterska	MSc Student	Volunteer
Bozena Zebrowska	MSc Student	Volunteer

Laboratory of Biomodelling

Name		Funding
Slawomir Filipek	Head	IIMCB
Krzysztof Jozwiak	Post-doctoral Fellow	Ministerial grant
Michal Kolinski	PhD Student	SMM
Aleksander Debinski	MSc Student	Volunteer
Anna Zwolinska	MSc Student	Volunteer

Laboratory of Cell Biology

Name		Funding
Marta Miaczynska	Head	Wellcome Trust
Iwona Pilecka	Post-doctoral Fellow	Wellcome Trust
Beata Pyrzynska	Post-doctoral Fellow	HHMI
Sajid Rashid	Post-doctoral Fellow	EU
Beata Bielinska	Research Assistant	HHMI(1/2)
Magdalena Banach-Orlowska	Research Assistant	Wellcome Trust
Marta Brewinska	PhD Student	HHMI/Nencki Fellowship
Anna Zarebska	PhD Student	IIMCB/Nencki Fellowship
Anna Hupalowska	PhD Student	EU
Lukasz Sadowski	PhD Student	EU
Vanessa Formas	Grant Administrator	HHMI/EU

Laboratory of Molecular and Cell Neurobiology

Name		Funding
Jacek Jaworski	Head	IIMCB
Malgorzata Perycz	PhD Student	Nencki Fellowship
Lukasz Swiech	PhD Student	Nencki Fellowship
Malgorzata Urbanska	MSc Student	Volunteer
Patrycja Pietruszka	MSc Student	Volunteer
Kamil Parobczak	MSc Student	Volunteer

Laboratory of Cortex Movements and Cell Division MPG/ PAN

Name		Funding
Ewa Paluch	Head	IIMCB
Jean-Yves Tinevez	Post-doctoral Fellow	Ministerial grant
Jakub Sedzinski	PhD Student	Ministerial grant
Ulrike Schulze	MSc Student	Volunteer
Julia Roensch	Technician	Ministerial grant

Research Equipment Laboratory

Name		Funding
Pawel Bieganowski	Fellow	IIMCB(1/2)
Beata Bielinska	Fellow	IIMCB(1/2)
Malgorzata Durawa	Fellow	IIMCB (1/2)
Wanda Gocal	Technician	IIMCB
Monika Dudek	Technician	IIMCB

School of the Science Festival

Name		Funding
Agnieszka Choluj	Head	IIMCB
Marta Badurek	Organizer	Volvox Project
Joanna Lilpop	Organizer	Volunteer
Anna Lorenc	Consultant	Volunteer
Jarosław Bryk	Consultant	Volunteer
Takao Ishikawa	Teacher, consultant	Volunteer
Aleksandra Kwiatkowska	Teacher	Volunteer
Agata Rogowska	Teacher	Volunteer
Maciej Kotlinski	Teacher	Volunteer
Sebastian Pawlak	Teacher, consultant	IIMCB
Grzegorz Papaj	Consultant	IIMCB
Anna Karnkowska	Teacher	Volunteer
Michal Mlacki	Teacher, technician	Volunteer
Roman Szczesny	Teacher	Volunteer
Monika Hejnowicz	Teacher	Volunteer
Justyna Rudzka	Teacher	Volunteer
Izabela Szczupakowska	Teacher	Volunteer
Damian Graczyk	Teacher	Volunteer
Monika Ostaszewska	Teacher	Volunteer
Justyna Wasil	Teacher	Volunteer

Notes