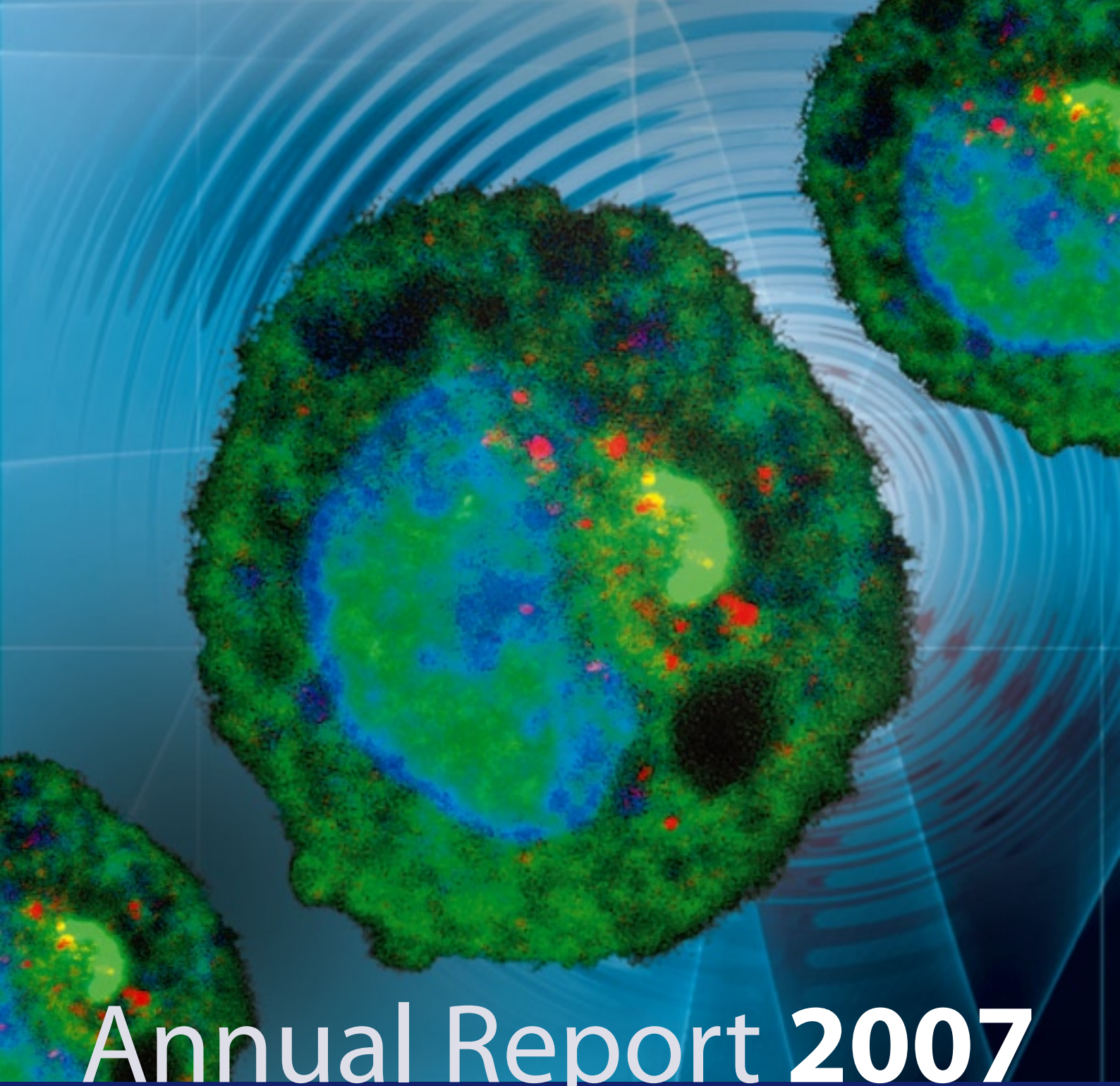

Annual Report **2007**

INTERNATIONAL INSTITUTE OF MOLECULAR
AND CELL BIOLOGY IN WARSAW

Director
Jacek Kuznicki

Deputy Scientific Director
Michal Witt

Deputy Administrative Director
Jaroslaw Filinski

Financial Manager
Hanna Iwaniukowicz

Chairman of the International Advisory Board
Angelo Azzi

Deputy Chairman of the International Advisory Board
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This Report was edited by:
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Photos: Anna Urbanska

Cover illustration:

N-terminal domain is dispensable for granular targeting of TNF. Confocal microscopy analysis of LAD2 cells transfected with p46–234hTNF-EYFP and 24 h posttransfection stained for tryptase. In overlay image, EYFP is represented by false color green, tryptase stain by false color red, and nucleus is visualized by TO-PRO3 stain (blue channel). Colocalization of EYFP and tryptase is represented by yellow color. Representative confocal section is shown. Bar 5 μm. Micrograph by M. Olszewski; J Immunol. 2007; 178:5701-9.

Contents

About IIMCB

The International Institute of Molecular and Cell Biology in Warsaw (IIMCB) is one of the most modern country´s research institutes in its field. The IIMCB´s research topics cover the wide area of cancer biology, neurobiology, protein structural biology, intracell comunication, dendritic tree formation, bioinformatics/computer modelling, etc. The International Advisory Board, the rules of recruitment of all faculty members through international competition and lack of tenure employment make it unique among Polish research institutions.

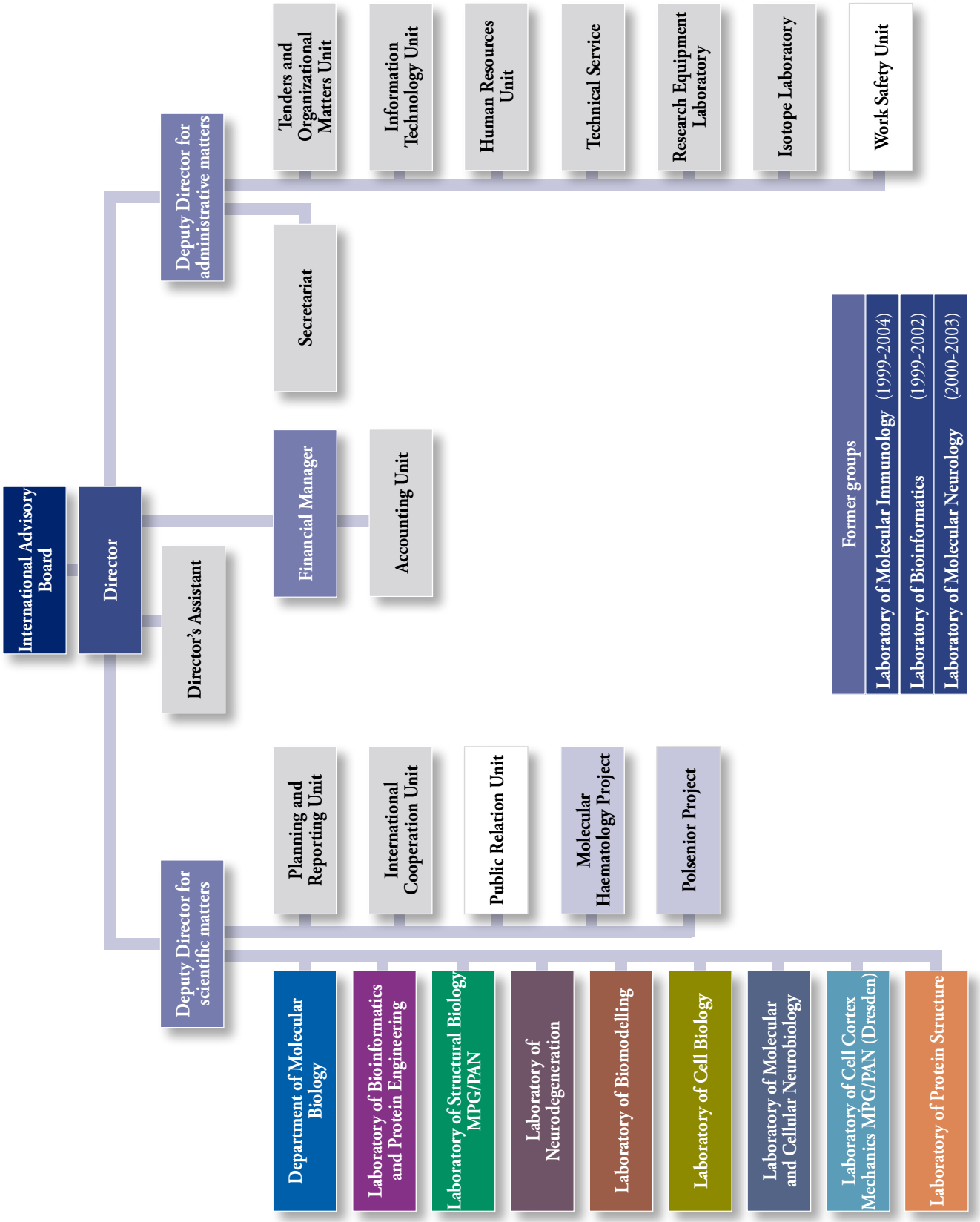
Created with the support of UNESCO and the Polish Academy of Sciences (PAN), the Institute started its activity on January 1, 1999, and is located at the building loaned to IIMCB by PAN. The principles of organisation of the Institute are distinct and differ from other research institutes in the country: an important body of the Institute is the International Advisory Board, acting as the Scientific Council; the leader of each research team of the Institute is selected in an international competition; group leader's initial employment is limited to a five-year contract; three years after beginning the research work, progress of research is assessed by the International Advisory Board. The professor's contract may be either terminated or extended.

The International Institute of Molecular and Cell Biology is directly subordinate to the President of the Polish Academy of Sciences, who supervises the organisation and activities of the Institute. The President of PAN nominates members of IAB and the Institute's Directors. An important link between the Institute and the President of PAN is the 2nd Department of Biological Sciences of PAN, to which the Institute belongs together with sixteen institutes of PAN. The Institute is financed in part from the national budget (statutory subvention; budgetary subvention via PAN) and in part from other sources (Ministry of Science and Higher Education, Foundation for Polish Science, UNESCO, Framework Programs of EU, Max Planck Society, Howard Hughes Medical Institute, European Molecular Biology Organisation, National Institutes of Health Wellcome Trust, etc.). About 60% of funds arrive as competitive grant awards received by the group leaders.

The building offers 16,820 m^3 of cubic space, with 4,032 m^2 of internal surface. It is divided into seven floors and a basement. The administrative sector is located on the ground floor and partly on 1st floor (Directors' offices, the lecture hall for 60 people, the Institute's library/meeting room, other offices and social rooms). Floors from the 1st to the 5th are arranged as a typical laboratory space, a "Faraday – cage" lab for sensitive electronic measurements (e.g. electrophysiology), cold-rooms, a dark room, offices and common space for heavy laboratory equipment (see: http://www.iimcb.gov.pl/equipment.php). On the 5th floor, a part of the laboratory space is prepared to accommodate a cell and molecular biology school – SFN (training laboratory for 18 students).

The growing number of foreign researchers working here additionally justifies the name of this institute. The research equipment of the Institute together with shared resources of neighbouring institutes of the whole Ochota campus set the high technical standard of research conducted here. Welcome to the IIMCB!

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



Directors and Administration

Jacek Kuznicki
Director

Michal Witt
Deputy Scientific Director

Maria Kleska
Deputy Administrative Director
(until Dec. 2007)

Jaroslaw Filinski
Deputy Administrative
Director (since Jan. 2008)

Hanna Iwaniukowicz
Financial Manager

Beata Tkacz
Director's Assistant

Monika Kacprzak
Secretary

Ewa Blazewicz
Secretarial Assistant

Agnieszka Ziemka
Planning and Reporting
Specialist

Urszula Bialek-Wyrzykowska
International Cooperation Manager

Dorota Libiszowska
Foreign Grants Specialist

Agnieszka Karbowska
Tenders Specialist

Sylwia Adamiec
International Cooperation Specialist

Krystyna Domanska
Human Resources Specialist

Rafal Flis
IT Manager

Przemyslaw Slusarczyk
IT Specialist

Marcin Biedacha
IT Specialist

Renata Knyziak
Accounting Specialist

Monika Nowicka
Payroll Specialist

Robert Banasiak
Maintenance Specialist

International Advisory Board of the International Institute of Molecular and Cell Biology in Warsaw

2006-2010 term

Chairman:

Angelo Azzi

Deputy Chairman:

Leszek Kaczmarek

Members:

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Professor, Vascular Biology Laboratory, Tufts University, Boston, MA, USA

Francisco E. Baralle

Director-General of International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

Alexey A. Bogdanov

Head of Department of Chemistry and Biochemistry of Nucleoproteins, Department of Chemistry, Moscow State University, Moscow, Russia

Nicolaus Blin

Professor of Molecular Genetics, Institute of Human Genetics, University of Tuebingen, Tuebingen, Germany; Foreign member of Polish Academy of Sciences

Ineke Braakman

Professor, Department of Cellular Protein Chemistry, Utrecht University, Utrecht, The Netherlands

Ivan Dikic

Professor of Biochemistry, Institute of Biochemistry II, Goethe University Medical School, Frankfurt am Main, Germany

Jerzy Duszynski

Director, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Robert P. Erickson

Professor, Department of Pediatrics, Section of Medical and Molecular Genetics, The University of Arizona Health Sciences Center, Tucson, AZ, USA

Klaus Hahlbrock

Professor Emeritus, Max-Planck Institute for Plant Breeding Research, Köln, Germany; Laureate of Alexander von Humboldt Honorary Research Fellowship of Foundation for Polish Science

Robert Huber

Head, Department of Structure Research, Max-Planck Institute of Biochemistry, Martinsried Germany

Wieland Huttner

Scientific Member and Director, Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Leszek Kaczmarek

Professor, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Oleg A. Krishtal

Deputy Director of the Bogomoletz Institute of Physiology, Head of the Department of Cellular Membranology, Bogomoletz Institute of Physiology, Kiev, Ukraine

Jacques Mallet

Professor, Laboratoire de Genetique Moleculaire de la Neurotransmission et des Processus Neurodegeneratifs, CNRS UMR 9923, Hopital de la Pitie-Salpetriere, Paris, France

Maciej J. Nalecz

Director, Division of Basic and Engineering Sciences, UNESCO, Paris, France

Ryszard Przewlocki

Professor, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

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, June 2007

From left (first row): O. A. Krishtal, R. Przewlocki, K. Hahlbrock, N. Blin, J. Hasler-Scott, J. Kuznicki, A. Tramontano; (second row) R. P. Ericsson, J. Duszynski, L. Kaczmarek, J. G. Sutcliffe, A. Azzi, I. Dikic, (third row) W. Huttner, I Braakman, F. E. Baralle, M. Zylicz, M. Witt.

Important Dates in the Institute's History

Sept. 1991	The proposal to create the Institute was published in the UNESCO Bulletin of MCBN
June 1994	State Committee for Scientific Research (KBN) accepts the activities aimed at establishing the Institute
Oct. 1994	Presidium of Polish Academy of Sciences (PAN) votes to support the Institute
May 1995	An agreement between Poland and UNESCO to establish the Institute
June 1996	The Molecular and Cell Biology Department is created by PAN with Prof. M.J. Nalecz as the Head
June 1997	Polish Parliament passes a bill to found the Institute
May 1998	Prof. A. Azzi is nominated as the Director of IIMCB
Jan. 1999	The Institute commences its independent activities; Prof. J. 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
Dec. 2007	Dr. M. Nowotny is appointed as Leader of the Laboratory of Protein Structure

Directors' note

Exciting times for the Polish academic community seem to be on the doorsteps. First, Poland's access to the European Union has resulted in new funding opportunities that could significantly supplement Polish universities and research institutes. Secondly, this imposes requirements on the modification of the structure of Polish science that is one of the priorities for the new Polish government. The reformatory movements are being planned not only in the government, but also at the headquarters of the Polish Academy of Sciences (PAN). When the President of PAN presented plans for profound changes in the structure of institutes belonging to PAN it became clear that our Institute is already well advanced in this matter, paving the way for potential followers: regular assessments of research group progress, external reviewers, terminal employment, competitive recruitment of leaders' and institute directors' positions, etc. All this was already implemented at IIMCB from the very beginning and has been well rehearsed and exercised until now with quite satisfying results. In fact, in PAN's quarterly journal "NAUKA", we presented this as a model for the reforms of public academic institutions. Thus, we have the feeling that the Academy's initial decision which made the organization of IIMCB possible and further support of PAN turned out to be a good investment for future global changes.

These trends also shape activities at IIMCB. In this context, eight running grants of the 6th Framework Programme of the EU and ten from other international sources like Howard Hughes Medical Institute, Wellcome Trust, National Institutes of Health, and European Molecular Biology Organization should be regarded as a good score. Our recently fully reactivated Grant Office, after a series of successful deliveries, is back in a full swing; in fact it's overwhelmed with new applications, doing its best in an effort to meet all the closely dated deadlines within the first quarter of this year. The applications are being submitted in reply to calls of the 7th Framework Program, structural funds via governmental channels, and others.

The new Polish government stresses its desire to restructure Polish science on one hand, and a willingness to push research performed in Poland on a track leading to innovative, science based technologies on the other. In essence, this would perfectly create an extremely positive climate for institutions like IIMCB. However, what the whole scientific community really suffers from, apart from a miniscule budget, is a constant feeling of uncertainty about further developments, the lack of clear-cut strategic plans on evolving the organization of the Polish research sector, the fussiness of assessment criteria, constant changes in rules and regulations, etc. In this reformatory fervor, rules are being changed while the ball is in play, which does not increase the number of supporters of changes. In these circumstances it was figured out at IIMCB, encouraged by formal ministerial directives, that a diversification of funding sources, coming as a supplement of governmental funds, should not only bring resources, but also a feeling of some financial security. Currently we are testing this hypothesis.

Description of the Institute's Activities

The Organization of Research at IIMCB

Nine 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 Cell Cortex Mechanics MPG/PAN in Dresden (Paluch) and Laboratory of Protein Structure (Nowotny).

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, which includes analysis of interactions between human p53 and molecular chaperones and oncogenic activity of MDM2, 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 molecular basis of 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), and studies of proteins implicated in the mechanisms of learning and memory and pathogenesis of Alzheimer disease (cyclin-dependent kinase 5, Ca^{2+}-sensors belonging to calmyrin family, beta and delta-catenins, CHORD containing protein-1) (Kuznicki's group).
5. The molecular modelling of structures and processes of membrane proteins, their oligomerization and complexes, focusing on rhodopsin and other G protein-coupled receptors; 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 and plasticity, as well as central nervous system pathologies (spinal cord injury, epilepsy, neurodegenerative disorders, including gene transcription, kinase-dependent cell signaling, cytoskeleton dynamics 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).
9. Structural and biochemical studies of nucleic acid enzymes (Nowotny's group).

Awards, Honors and Titles

- The title of Doctor Honoris Causa of the University of Wroclaw to Prof. Maciej Zylicz.
- Full Membership of the Polish Academy of Sciences honored to Prof. Maciej Zylicz.
- START fellowships for the most talented young researchers in Poland, Foundation for Polish Science honoured four of our graduate students: Iwona Cymerman, Malgorzata Firczuk, Jan Kosinski and Leszek Lipinski.
- L'Oreal National Habilitation Fellowship for women researchers in biomedical sciences to Dr. Marta Miaczynska.
- Polish Genetic Society award to School of Science Festival (SFN) for their activities in 2004-2006.

Education

IIMCB continues its doctoral program in partnership with other research and educational institutes of the Ochota Campus (35 students). The international PhD program run in collaboration with Utrecht University has entered the last

phase: currently, four students are still 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. 69).

Media Visibility and Popularization of Science

In 2007, IIMCB faculty and staff members actively popularized science in media, etc. Results of their research were presented on radio and TV channels, and within press interviews. **Maciej Zylicz** expressed his views on a condition of Polish science and developments at the Foundation for Polish Science which he presides in interviews for *Forum Akademickie* and for *Polish Voice – Developing Ideas*, the supplement for *Warsaw Voice*. The latter also featured a description of **Ewa Paluch's** activity in Dresden. **Michal Witt** presented a progress in the multicenter commissioned project on molecular methods in hematology which he coordinates at IIMCB, in *Puls Medycyny*. The longevity studies conducted at IIMCB gained interest again of *Warsaw Voice* in a supplement *The Polish Science Voice* where it was announced on a cover. The conclusions from Polish Centenarians Program were published in a monography *Skazani na dlugowiecznosc (Destined for longevity: In Search of Determinants of Succesful Aging)*, edited by **Malgorzata Mossakowska, Katarzyna Broczek** and **Michal Witt** and published by Osrodek Wydawnictw Naukowych, Poznan. Marta Miaczynska's success of getting L'Oreal National Habilitation Fellowship was widely publicized. An ongoing discussion on a necessity of reforms of Polish research sector was continued by **Jacek Kuznicki** and **Marta Miaczynska** in a polemic letter to Nature. The photograph of a mast cell made by **Maciej Olszewski** got to a cover of the issue of *Journal of Immunology.* IIMCB was presented as a model for reforming Polish research institutes by **Michal Witt** and **Agnieszka Ziemka** in *Nauka*.

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 University of Life Sciences. A total number of over 1500 young participants visited laboratory workshops in 2007; 100 biology teachers and about 800 students of various levels attended lectures organized by SFN (see section „Science Festival School – Popularization of Science" p. 71). **National Children's Fund** brought four gifted youngsters to IIMCB to participate in several bench experiments.

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

In 2007 a huge investment, in the range of upgrading the whole network infrastructure, was made by IIMCB. In consequence, a new computer network was built. As a result of the conducted works, all active and passive elements constituting the whole implementation were replaced. There was a new structural cabling system employed, based on a fully shielded category 7 TrueNet CopperTen copper cable, capable of transmitting data of 10 Gbps. At present, the new network is operated by advanced switches working in Fast GigabitEthernet standard. Each level has its own 3Com 4200G network equipment, communicating with one another via autonomous fiber optic devices with the 3Com 5500G core switch. The communication speed in the interior network reaches 1 Gbps. The new implementation is fully redundant, scalable and easy to manage. Its output is realized through a fiber optic cable, connected directly to one of the Institutes at the Ochota Campus. Moreover, in 2007 IIMCB purchased new advanced antispam equipment, which allowed getting rid of bothersome spam problem. Additionally, the electronic mail server was replaced; further plans being to substitute all servers with professional equipment, mounted in 19" racks. At the end of 2007, a new server room was successfully arranged to function as a new, fully equipped server room. In 2008, we plan to set up a project aiming at covering the whole IIMCB building with a wireless range. At the moment, two new file servers and advanced backup equipment are being purchased. We also intend to start replacing workstations with the high quality equipment.

Skazani na dlugowiecznosc (Destined for longevity: In Search of Determinants of Succesful Aging), edited by Malgorzata Mossakowska, Katarzyna Broczek and Michal Witt and published by Osrodek Wydawnictw Naukowych, Poznan.

Grants

6th Framework Programme

- EURASNET "European alternative splicing network of excellence" (LSHG-CT-2005-518238); 120,000 EUR; 2006-2010; IIMCB participation 2008-2010; J.M. Bujnicki
- MemProt "Structural studies of membrane proteases" (MTKD-CT-2006-042486); 626,800 EUR; 2006-2010; M. Bochtler
- EndoTrack "Tracking the endocytic routes of polypeptide growth factor receptor complexes and their modulatory role on signalling" (LSHG-CT-2006-019050); 428,400 EUR, matching funds 1,011,709 PLN; 2006-2010; M. Miaczynska
- DNA Enzymes "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
- PROMEMORIA "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
- VOLVOX "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)
- EUROGENTEST "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
- PLASTOMICS "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

Other International Funds

- EMBO Installation Grant "Structural and biochemical studies of UvrA DNA repair protein" (1476); 250,000 EUR; 2007-2012; M. Nowotny
- Wellcome Trust International Senior Research Fellowship "Structural and functional studies of two members of integrase superfamily – type 2 RNase H and Ruvc resolvase – from substrate recognition to catalysis" (081760); 4,106,806 PLN, 2007-2012, M. Nowotny
- Howard Hughes Medical Institute, International Research Scholars: "Signaling from endosomes to the nucleus: The role of APPL proteins and their interacting partners"; 500,000 USD; 2006-2010; M. Miaczynska
- Wellcome Trust International Senior Research Fellowship "Communication between intracellular organelles in trafficking and signalling: The role of APPL proteins" (076469); 4,315,706 PLN; 2006-2010; M. Miaczynska
- NIH Grant "High-accuracy protein models derived from lower resolution data" subcontract (430-46-22 B) within a collaborative grant coordinated by A. Kloczkowski, Iowa State University, USA; 60,000 USD; 2007-2008; J.M. Bujnicki
- NIH Grant "Discovering new human DNA repair genes by bioinformatics" subcontract (WSU03043) within a collaborative grant coordinated by A.S. Bhagwat, Wayne State University, USA; 160,000 USD; 2005-2007; J.M. Bujnicki
- NIH Grant "Kinetoplastid SL RNA biogenesis", subcontract (2301 G EN541) within a collaborative grant coordinated by D.A. Campbell, University of California, USA; 100,000 USD; 2004-2009; J.M. Bujnicki
- NIH Grant: Fogarty International Research Collaboration Award (FIRCA) "Low-resolution structural genomics of nucleases" (952056); 53,365 USD; 2004-2006; J.M. Bujnicki
- The MPI-CBG/IIMCB Partner Group at the IIMCB; 60,000 Euro; 2006-2008; M. Miaczynska

- 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
- 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

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 & Development 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
- "Investigation of the mechanisms regulating expression of calmyrin2, a novel EF-hand Ca^{2+}-binding protein, and elucidation of its role in Ca^{2+}-signal transduction in physiology and in death of neurons" (N301 109 32/3854); 303,000 PLN; 2007-2010; U. Wojda
- "Role of dendrtic mRNA transport and local protein syntheis in development of dendritic arbor of neurons" (NN301314733); 300,000 PLN; 2007-2010; J. Jaworski
- "Investigations of activation of GPCRs by theoretical methods" (N/N301/2038/33); 205,000 PLN; 2007-2010; S. Filipek
- "S-nitrosylation and CDK5 dependent phosphorylation of proteins – proteomic analysis of synaptosomal fractions from transgenic mice Alzheimer disease models" (NN301254333); 70,000 PLN; 2007-2010; grant coordinated by Michal Dadlez from Institute of Biochemistry and Biophysics PAN in collaboration with A. Szybinska
- "Role of mTOR-regulated proteins in development of dendritic tree of hippocampal and cortical neurons" (2P04A01530); 220,800 PLN; 2006-2009; J. Jaworski
- 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
- "Biochemical and microscopical characterization of APPL-positive endosomes" (2P04A03828); 390,040 PLN; 2005-2008; M. Miaczynska
- "Investigation of structure of presenilin protein and significance of its mutations in Alzheimer's disease development" (0695/P05/2005/29); 220,000 PLN; 2005-2008; K. Jozwiak
- "STRUF – the novel software for classification and prediction of proteins functions" (3T11C04029); 105,000 PLN; 2005-2007; J. Sasin
- "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
- "Differences in action of stress-induced and constitutively synthesized Hsp70" (KBN-0408/P04/2004/27); 550,200 PLN; 2004-2008; M. Zylicz

Ministerial Habilitation Grants

- "Variation of restriction enzymes sequence specificities by combination of different methods of bioinformatics and protein engineering" (N30110031/3043); 278,000 PLN; 2006-2009; K. Skowronek

Ministerial Doctoral Grants (supervisor/PhD student)

- "Evolutionary, structural and functional methyltransferase classification" (N30110532/3599); 49,000 PLN; 2007-2009; J.M. Bujnicki/K. Tkaczuk
- "Modification of the substrate specificity of Bsp6I restriction endonuclease with novel methods of directed evolution" (N30204532/3598); 50,020 PLN; 2007-2009; J.M. Bujnicki/S. Pawlak
- "A novel method for assessment of global credibility and local correctness of protein structure models" (N30110632/3600); 36,600; 2007-2009; J.M. Bujnicki/M. Pawlowski
- "Studies of agonist and antagonist binding modes in opioid receptors" (N/N401/1401/33); 50,000 PLN; 2007-2009; S. Filipek/M. Kolinski
- "Sequence-structure-function relationships in apoptotic nuclease DNase II" (N40110731/2409); 30,080 PLN; 2006-2007; J.M. Bujnicki/I. Cymerman
- "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/K. Krzysko
- "Investigation of the structure of arrestin-rhodopsin complex by theoretical methods" (0121/P01/2006/30); 33,000 PLN; 2006-2007; S. Filipek/A. Modzelewska

Ministerial Commissioned Grants

- "Ageing of the Polish population – medical, psychological, sociological and economic aspects" (PBZ-MEiN-9/2/2006); 12,178,420 PLN; 2007-2010; Director: P. Bledowski, coordinator M. Mossakowska
- "Novel computer programs for homology modelling and fold recognition of RNA" (PBZ/MNiSW/07/2006/04 POL-POSTDOC III); 240,000 PLN; 2007-2010; M. Boniecki
- "Structural studies of restriction endonucleases generating unusual cleavage patterns" POL-POSTDOC II grant; 160,000 PLN; 2007-2008; H. Czapinska
- "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
- "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

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 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); Director: P. Liberski
- "Role of Hsp90 in regulation of gene expression involved in tumorigenic transformation", (PBZ–KBN–107/P04/2004); 1,048,000 PLN; 2004-2007; M. Zylicz (within ordered grant directed by Jagiellonian University); Director: J. Dulak

Other Research Grants

- Scientific Network "Mechanisms of cellular movements – Mobilitas.pl" coordinated by the Nencki Institute of Experimental Biology Polish Academy of Science (2/E-36/SN-0075/2007); 75,000 PLN; M. Miaczynska
- Scientific Network organized by Institute of Pharmacology Polish Academy of Science – "Looking for systemic targets of potential neurotrophic drugs" (26/E-40/SN-023/2007); 78,636 PLN; M. Wisniewska
- Professorial Grant from Foundation for Polish Science (SP10/04) "Beta-catenin metabolism in health and disease"; 240,000 PLN; 2004-2008; J. Kuznicki

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

- **Mossakowska M**, Barcikowska M, Broczek K, Grodzicki T, Klich-Raczka A, Kupisz-Urbanska M, Podsiadly-Moczydlowska T, Sikora E, **Szybinska A**, Wieczorkowska-Tobis K, Zyczkowska J, **Kuznicki J**. Polish Centenarians Programme – Multidisciplinary studies of successful ageing: Aims, methods, and preliminary results. Experimantal Gerontology, 2008, 43: 238-244
- Baranowska B, Wolinska-Witort E, Bik W, Baranowska-Bik A, Martynska L, Broczek K, **Mossakowska M**, Chmielowska M. Corrigendum to "Evaluation of neuroendocrine status in longevity" Neurobiol Aging 2007, 28:774–783
- Kollajtis-Dolowy A, Pietruszka B, Kaluza J, Pawliniska-Chmara R, Broczek K, **Mossakowska M**. The nutritional habits among centenarians living in Warsaw. Rocz Panstw Zakl Hig. 2007, 58: 279-286
- **Mossakowska M**. Polish Centenarians Programme "PolStu2001" (in Polish). In: Skazani na długowieczność. W poszukiwaniu czynników pomyślnego starzenia. (Destined for longevity: In Search of Determinants of Succesful Aging). Editors: M. Mossakowska, K. Broczek, M. Witt. Ośrodek Wydawnictw Naukowych: 2007, 13-18.
- **Olszewski MB**, Groot AJ, **Dastych J**, Knol EF. TNF trafficking to human mast cell granules: mature chain-dependent endocytosis. J Immunol, 2007; 178:5701-9
- Wojda A, Zietkiewicz E, **Witt M**. Effects of age and gender on micronucleus and chromosome nondisjunction frequencies in centenarians and younger subjects. Mutagenesis 2007; 22: 195-200

Scientific Meetings and Lectures

- Symposium "Advanced Methods in Molecular Hematology", 10.01.2007, Warsaw, Poland, IIMCB
- "Scientific Communication" – practical course for graduate students for whom English is a second language given by Prof. Edward Potworowski (Armand-Frappier Institute, Montreal, Canada), 7-14.05.2007, Warsaw, Poland, IIMCB
- IIMCB Annual Report Session, 25.05.2007, Lacha, Poland
- International Annual Symposium, 01.06.2007, Warsaw, Poland, IIMCB
- 5th International Conference: Inhibitors of Protein Kinases and Workshop Session: Novel Molecular Design and Simulation Methods, 23-27.06.2007, Warsaw, Poland, coorganized by IIMCB
- 2nd Polish Congress of Genetics, 18-20.09.2007, Warsaw, the Warsaw University of Life Sciences (SGGW), Poland, coorganized by IIMCB
- "New Targets and Approaches Toward Neurodegeneration and Neuroprotection", 29.10.2007, Warsaw, Poland coorganized by IIMCB

Seminars of invited speakers

Urszula Hibner (IGMM CNRS UMR5535, Montpellier, France) "Modulation of signal transduction pathways in hepatocarcinogenesis", 13.02.2007

Guy Haegeman (Laboratory for Eukaryotic Gene Expression and Signal Transduction – LEGEST, Department of Molecular Biology, Ghent University, Belgium) "Molecular mechanisms and signal transduction of inflammatory gene expression", 22.03.2007

Grzegorz Kudla (FAS Center for Systems Biology, Harvard University, USA) "Coding sequence determinants of gene expression", 24.04.2007

Alexander Wlodawer (Laboratory of the Macromolecular Crystallography, National Cancer Institute, Frederick, USA) "Structural basis for inhibition of translation by the tumor suppressor Pdcd4", 14.05.2007

Rafal Butowt (Department of Physiology, School of Medicine, University of Nevada, Reno, NV, USA) "Long-distance trafficking of trophic factors in the nervous system: The concept of trophic currencies", 01.06.2007

Arkadiusz Chworos (Department of Physics, University of California, Santa Barbara, CA, USA) "From tecto-RNA nanoarchitectures to AFM and biosensors", 01.06.2007

Wojciech Niedzwiedz (Department of Medicine, Cambridge University/MRC-LMB, Cambridge, UK) "Fanconi anemia: Moving forward in (cross)-linking DNA repair", 01.06.2007

Arkadiusz Welman (Cancer Research UK, Paterson Institute for Cancer Research, University of Manchester, Manchester, UK) "Can a biologist fix a radio? – or my approach to functional oncogenomics", 01.06.2007

Cezary Wojcik (Indiana University School of Medicine, Evansville, IN, USA) "VCP (valosin-containing protein; p97) in ER-associated degradation and beyond", 01.06.2007

Susanne Wolf (Department of Neurosurgery, Stanford University, Stanford, CA, USA) "Regulation of neurogenesis in health and disease", 01.06.2007

Robert D. Wells (Institute of Biosciences and Technology, Texas A&M University System Health Science Center, Texas Medical Center Houston, USA) "The alternate forms of DNA microsatelites in health and disease", 14.06.2007

Michal Hetman (Kentucky Spinal Cord Injury Research Center & Department of Neurological Surgery, University of Louisville, Louisville, USA) "Neuronal response to transcriptional inhibition", 20.06.2007

Paulina Dominiak (Faculty of Chemistry, Warsaw University, Poland) "Electrostatics of biological molecules based on crystallographic data", 08.11.2007

John Moses (University of Nottingham School of Chemistry, UK) "Targeting telomerase: a click chemistry approach towards novel G-Quadruplex inhibitors", 16.11.2007

Maurizio Memo (Faculty of Medicine, University of Brescia, Italy) "p53 involvement in Alzheimer's disease: how fibroblasts may link neurons with lymphocytes", 22.11.2007

Lada Biedermannova (Faculty of Science Charles University in Prague, Czech Republic) "The role of noncovalent interactions of aromatic moiety in proteins", 12.12.2007

IIMCB researchers seminars

Jakub Urbanski (Department of Molecular Biology) "Silence is gold... How to work with the siRNA", 11.01.2007

Kristian Rother (Laboratory of Bioinformatics and Protein Engineering) "Creating publication-quality pictures of molecules. Finding structures – using PyMOL – improving images", 18.01.2007

Magdalena Banach-Orlowska (Laboratory of Cell Biology) "A new face of endocytic proteins", 25.01.2007

Aneta Kaczmarczyk (Laboratory of Structural Biology MPG/PAN) "Not only for face-lift – what else are the glycosaminoglycans good for? ", 08.02.2007

Neli Kachamakova (Laboratory of Neurodegeneration) "Putting presenilins centre stage: Presenilin mutations in Alzheimer disease", 22.02.2007

Marcin Klejman (Department of Molecular Biology) "Microarrays from basics to cancer diagnosis", 01.03.2007

Lukasz Zyla (Department of Molecular Biology) "FRET: long known phenomenon – novel tool for molecular biology", 08.03.2007

Grzegorz Chojnowski (Laboratory of Structural Biology MPG/PAN) "XFEL – new generation of X-ray sources", 15.03.2007

Janusz Bujnicki, Jan Kosinski (Laboratory of Bioinformatics and Protein Engineering) and Matthias Bochtler, Magdalena Kaus-Drobek (Laboratory of Structural Biology MPG/PAN) "Modeling vs. crystallography: Restriction endonuklease MvaI structure study – what can and what can't be predicted", 29.03.2007

Maciej Olszewski (Department of Molecular Biology) "FRAP: FRying And Prying or what the cells will tell you if you ask them", 05.04.2007

Katarzyna Misztal (Laboratory of Neurodegeneration) "ß – catenin, from physiology to pathology", 12.04.2007

Marta Wisniewska (Laboratory of Neurodegeneration) "How to make a human brain of a mouse brain. Beta-catenin in the nervous system", 19.04.2007

Michal J. Gajda (Laboratory of Bioinformatics and Protein Engineering) "Frankenstein3D: a server for template-based modeling of proteins by iterative refinement of alignments, model evaluation, and recombination of fragments", 25.05.2007

Magdalena Lipka (Laboratory of Structural Biology MPG/PAN) "VgbB – streptogramin B lyase", 25.05.2007

Jakub Urbanski (Department of Molecular Biology) "Knockdown of Hsp's and p53 transcriptional activity", 25.05.2007

Jakub Sedzinski (Laboratory of Cortex Mechanics MPG/PAN) "Actin cortex flows and cleavage furrow positioning", 25.05.2007

Bozena Zebrowska (Laboratory of Neurodegeneration) "Alzheimer's disease-linked mutations in presenilins: biological effects in patients lymphoblasts", 25.05.2007

Marcin Feder (Laboratory of Bioinformatics and Protein Engineering) "Combating bacterial resistance to macrolide antibiotics: Virtual screening for identification of inhibitors against RNA methyltransferase ErmC" 25.05.2007

Lukasz Swiech (Laboratory of Molecular and Cellular Neurobiology) "CLIP170 role in dendritic arbor development", 25.05.2007

Iwona Pilecka (Laboratory of Cell Biology) "Post-translational modifications of APPL proteins", 25.05.2007

Monika Sokolowska (Laboratory of Structural Biology MPG/PAN) "Crystal structures of restriction endonuclease BcnI", 25.05.2007

Aleksander Debinski (Laboratory of Biomodelling) "Investigation of stability regions of Rhodopsin by simulated mechanical unfolding", 25.05.2007

Wojciech Michowski (Laboratory of Neurodegeneration) "Brain localisation and stress induced nuclear accumulation of CHORD containing protein-1 (CHP-1) a novel zinc binding protein", 25.05.2007

Lukasz Sadowski (Laboratory of Cell Biology) "Endocytic routes of Platelet Derived Growth Factor (PDGF) and its receptor", 25.05.2007

Urszula Wojda (Laboratory of Neurodegeneration) "What lymphocytes can tell us about Alzheimer disease?", 04.10.2007

Katarzyna Filip (Laboratory of Bioinformatics and Protein Engineering, IIMCB) "DNA repair enzymes", 18.10.2007

Aleksandra Szybinska (Laboratory of Neurodegeneration, IIMCB) "Role of protein kinase Cdk 5 in Alzheimer disease", 15.11.2007

Dario Piano (Laboratory of Structural Biology, IIMCB) "Photosynthesis: structural and functional studies of Photosystem II, a possible role of the PsbS subunit in the xanthophylls cycle as a base for the non photochemical quenching", 29.11.2007

Kristian Rothler (Laboratory of Bioinformatics and Protein Engineering, IIMCB) "Sprint at Genesilico: software for sequence analysis glued together", 06.12.2007

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**
XII	2007	16	**Wojciech Niedzwiedz**

[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 2007, attracted to IIMCB 16 candidates, among them: eight foreigners, five Polish nationals working in USA, two Poles from UK and one from Sweden. Dr. Wojciech Niedzwiedz, who accepted an offered position, will start his research activities at IIMCB in 2008.

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. Dr. Marta Miaczynska, a leader of Laboratory of Cell Biology at the International Institute of Molecular and Cell Biology (IIMCB) in Warsaw, is heading a Partner Group of the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden. **The MPI-CBG/IIMCB Partner Group,** was established based on a contract between the two institutions and has been operating at IIMCB since January 2006 for a fixed term of 3 years, with a possibility of two year extension. From the side of MPI-CBG, Prof. Marino Zerial (Director of MPI-CBG) is the cooperation partner and a scientific mentor of the Partner Group. Dr. Miaczynska has been working in the group of Prof. Zerial in Dresden as a senior postdoctoral fellow in years 2001-2005, before her return to Poland in April 2005. The scientific project of the Partner Group, entitled "Biochemical and microscopical characterization of APPL-positive endosomes", is a continuation of the work that Dr. Miaczynska carried out in the laboratory of Prof. Zerial in Dresden.

With 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, three of them defended their thesis in Utrecht (details see: Educational Activities p. 69).

Visits to IIMCB:

- 28.02.2007: **Prof. Michal Sewerynski**, Minister of Science and Higher Education and a group of journalists visited some research institutes located in Ochota Camups including IIMCB. Guided by Prof. Jacek Kuznicki and Prof. Michal Witt, Minister Sewerynski visited School of Science Festival (pictures below). Further Minister was introduced by Dr. hab. Urszula Wojda to Alzheimer Disease research performed at the Laboratory of Neurodegeneration.

- 14.06.2007: **Prof. Robert D. Wells**, the Director of the Center for Genome Research at the Institute of Biosciences and Technology, Texas Medical Center in Huston.

- 10.09.2007: **representatives of Finnish Summer University and Oulu's University, Finland**
- 17.10.2007: **Prof. Jürgen Mlynek**, President of Helmholtz Association of National Research Centres

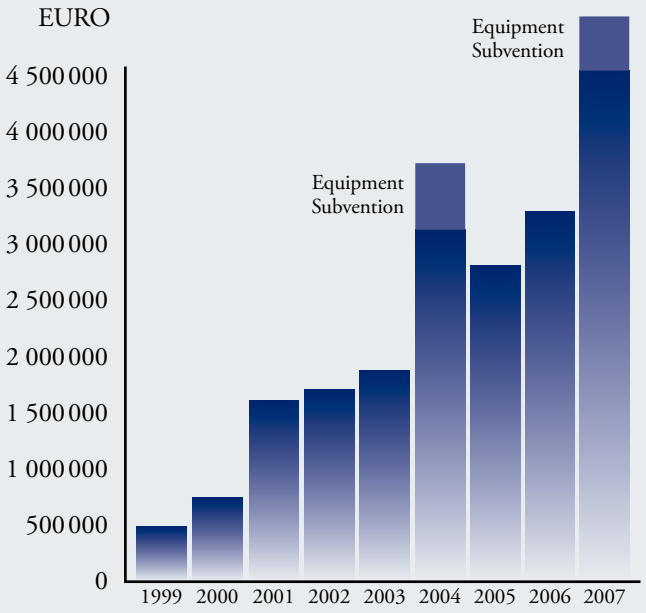
Mid- and long-term research visits of non-Polish scientists (1999-2007)

- **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, 2006-2007 (one year)
- **Laura Lopez Munoz,** BSc (Spain) - MSc student in the Laboratory of Bioinformatics and Protein Engineering 2006-2007 (one semester)
- **Tran Cat Dong,** PhD (Vietnam) – Post-doctoral fellow in the Laboratory of Neurodegeneration, 2007 (2 months)
- **Nguyen Trong Hung,** MD (Vietnam) – PhD student in the Laboratory of Neurodegeneration, 2007 (1 month)
- **Dario Piano,** MSc (Italy) – expert involved in EU grant – MEMPROT, the Laboratory of Structure Biology, since 2007

IIMCB Annual Report Session at Lacha, May 2007

Diversity of Funding IIMCB '2007

Annual budget

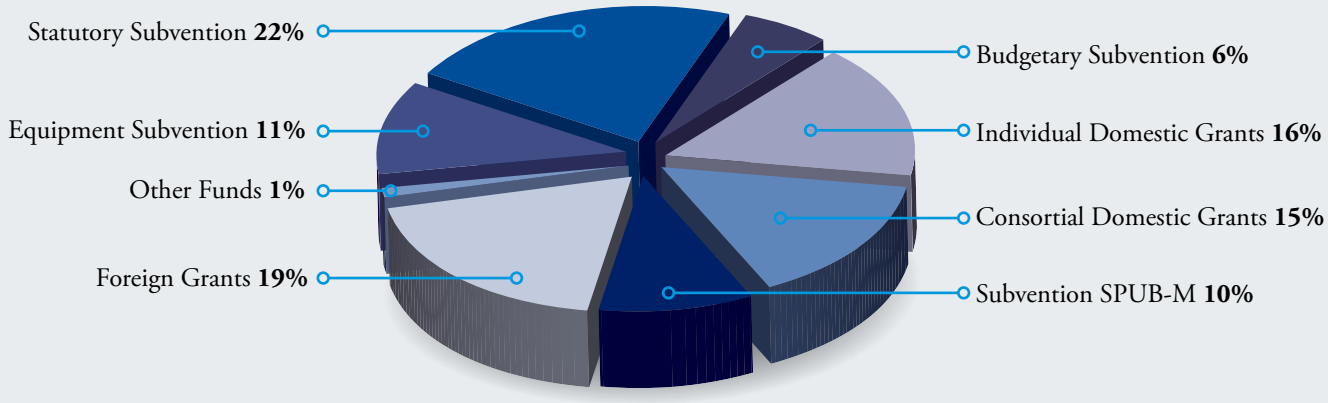


Sources of Funding	amounts in PLN	amounts in EURO*
Statutory Subvention	3 699 000	1 032 663
Budgetary Subvention	1 090 000	304 299
Individual Domestic Grants	2 694 815	752 321
Consortial Domestic Grants	2 624 480	732 686
Subventions SPUB-M	1 772 156	494 739
Foreign Grants	3 301 235	921 618
Other Funds (FNP, Networks)	173 636	48 475
Equipment Subvention	1 845 000	515 075
Total	**17 200 322**	**4 801 877**

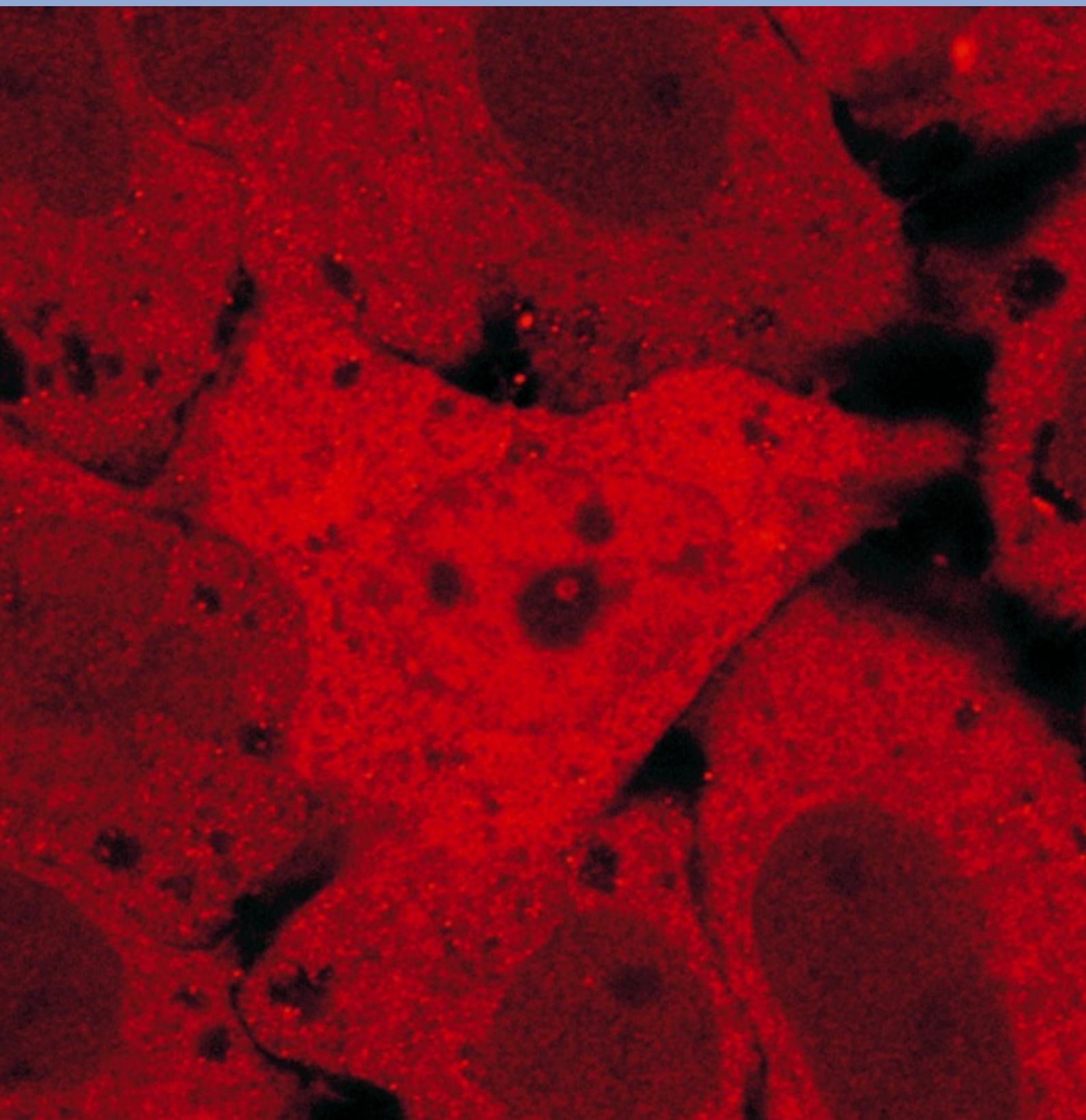
** 1 EURO – 3,582 @31st Dec `2007*

Profit & loss statement

	amounts in PLN
A. net revenue on sales and equivalents	14 769 637
B. operational activity costs:	15 823 950
Depreciation (equipment)	1 518 054
Research materials	5 352 514
Utilities	330 022
Services	981 236
Fees and taxes	551 660
Salaries and wages	4 431 768
Social and health insurance	1 004 434
Other operational expenses, in this:	1 654 262
business trips	440 144
property insurance	14 331
expenditures of indirect costs	696 508
fellowships	503 280
C. other operational income (subventions)	1 055 166
D. financial income:	156 864
Interests	116 222
Others	40 642
E. financial expenses:	56 308
Interests	1 032
Others	55 276
Profit / loss on business activity (A-C+D-B-E)	101 410



Department of Molecular Biology



Lab Leader

Maciej Zylicz, PhD, Professor

Vice Head:
Alicja Zylicz, PhD, Professor

Research Associate:
Pawel Bieganowski, PhD
Marcin Klejman, PhD
Maciej Olszewski, PhD

PhD Students:
Marta Frankowska (since October 2007)
Malgorzata Gutkowska, MSc
Leszek Lipinski, MSc
Jakub Urbanski, MSc
Dawid Walerych, MSc
Anna Zurawska, MSc

Secretary:
Grazyna Orleanska, MSc

Technician:
Wanda Gocal

Picture on the left:
HeLa cells were transfected with p53 and subjected to 42^{0}C heatshock, fixed and stained for Hsp90. The depicted cell exhibits nuclear localization of Hsp90 induced by expression of p53.

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 (Poland)

2008 Panel Chair, Molecular and Structural Biology and Biochemistry (LS1), ERC, Brussels

Membership in Scientific Societies, Organizations and Panels

- Full Member of the Polish Academy of Sciences
- Member of the Polish Academy of Arts and Sciences
- Member of the Academia Europaea
- Member of the American Society of Biochemistry and Molecular Biology
- Member of EMBO
- Member of the Advisory Editorial Board of EMBO Journal, EMBO Reports and IUBMB Life
- Member of EMBO Council (2004-2007)
- 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 the Life Science Committee of ESF (2003-2005)
- Member of the Selection Committee for the special DFG programmes (2001-2005)

Honors, Prizes, Awards

1. Doctor Honoris Causa of University of Wroclaw, 2007
2. Prime Minister Award for Scientific Achievements, 2002
3. "L. Marchlewski" Award from the Biochemistry and Biophysics Committee PAN, 2001
4. Award from the Foundation for Polish Science (FNP) in biological/medical sciences, 1999
5. Awards from the Polish Biochemical Society for the best biochemistry work performed in Polish laboratories, 1996, 2007
6. Award from the Ministry of Education, 1994
7. "Heweliusz" Prize for the Scientific Achievements, awarded by the President of Gdansk, 1993
8. Award from the Polish Academy of Sciences, 1990
9. 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

Over 80 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. The Hirsch parameter, H=37

Selected publications since 2001

- Wawrzynow B, **Zylicz A**, Wallace M, Hupp T, **Zylicz M**. MDM2 chaperones the p53 tumor suppressor. J Biol Chem, 2007; 282:32603-12
- Spiechowicz M, **Zylicz A**, **Bieganowski P, Kuznicki J**, Filipek A. Hsp70 is a new target of Sgt1 – an interaction modulated by S100A6. Biochem Biophys Res Commun, 2007; 357:1148-53
- Schneider G, Nieznanski K, Kilanczyk E, **Bieganowski P, Kuznicki J**, Filipek A. CacyBP/SIP interactions with tubulin in neuroblastoma NB2a cells and induces formation of globular tubulin assemblies. Biochim Biophys Acta, 2007; 1773:1628-36
- Issat T, Nowis D, Legat M, Makowski M, **Klejman MP, Urbanski J**, Skierski J, Koronkiewicz M, Stoklosa T, Brzezinska A, Bil J, Gietka J, Jakobisiak M, Golab J. Potentiated antitumor effects of the combination treatment with statins and pamidronate in vitro and in vivo. Int J Oncol. 2007; 30:1413-25
- **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; 281:22439-45

- 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; 281:33395-402
- 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; 176:1588-99
- **Kudla G, Lipinski L**, Caffin F, **Helwak A, Zylicz M**. High guanine and cytosine content increases mRNA levels in mammalian cells. PLoS Biol, 2006; 4:0933-42
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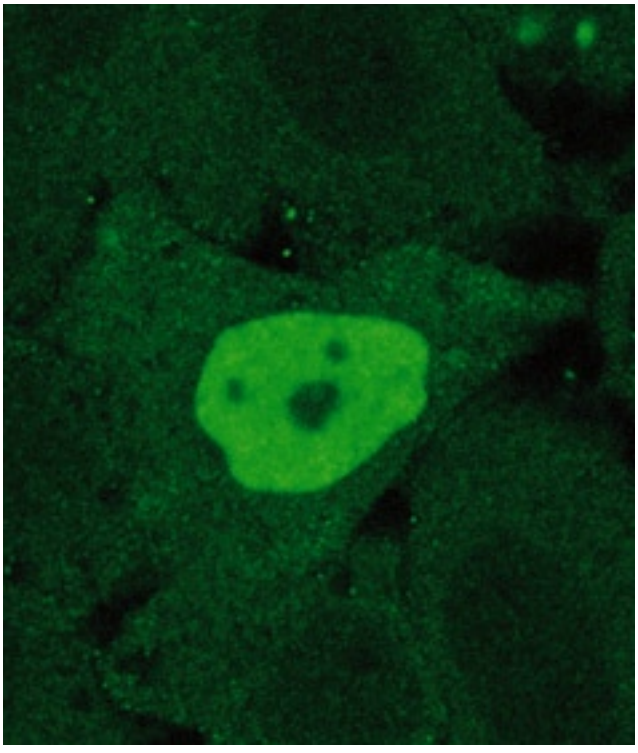


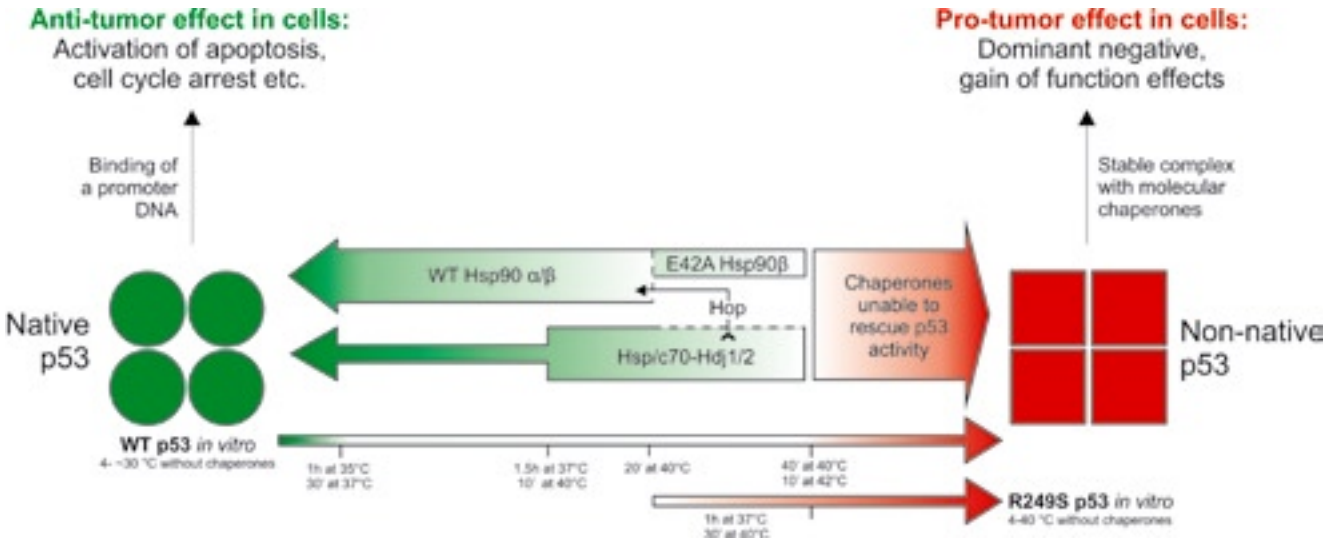
HeLa cells were transfected with p53 and subjected to 42^{0}C heatshock, fixed and stained for p53. The depicted cell exhibits nuclear localization of p53 indicating that transient heatshock is not a factor efficiently changing intracellular transport of wild-type p53.

Current Research

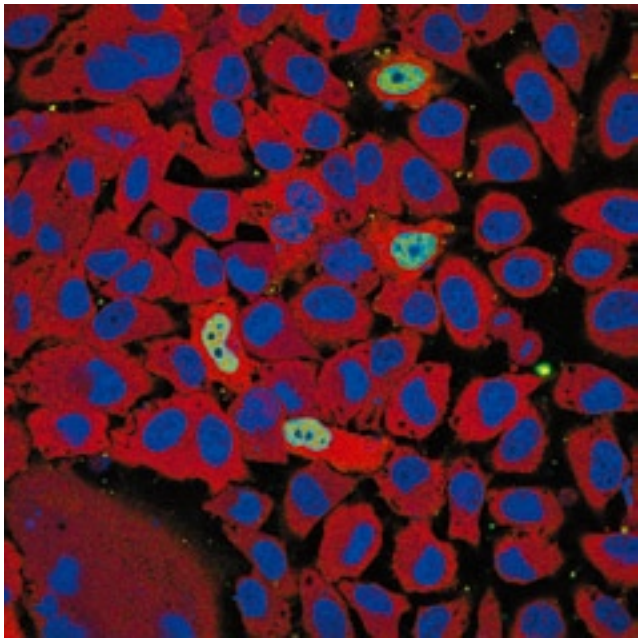
The research conducted in our department is predominantly focused on the role of molecular chaperones in mammalian cells including cell transformation (review Zylicz et al., 2001). Previously, using highly purified recombinant human proteins, we have identified intermediate reactions that lead to the assembly of molecular chaperone complexes with wild type or mutant p53 tumour suppressor protein (King et al., 2001). We have discovered that Hsp90 exhibits 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, consequently, the p21 protein level, while the p53 mRNA and Ser-15P-p53 protein levels were mostly unaffected. Additionally, using Chromatin immunoprecipitation 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.

In the subsequent studies we have shown that at the physiological temperature of 37°C, Hsp90 molecular chaperone alone efficiently rescues the wild-type p53 promoter-DNA binding activity from thermal inactivation. At heat shock conditions of 40°C, the Hsp70 and Hsp40 chaperone machine is required to rescue the wild-type p53. At this temperature, Hsp90 stimulates the reaction by a limited direct action on wild-type p53, and by an indirect chaperoning which requires the presence of Hsp70, Hsp40 and Hop. Thus, for the first time we were able to distinguish two different modes of wt Hsp90 activity on a single substrate. Moreover, the Hsp90β E42A variant, despite its inability to hydrolyze ATP, is more efficient in the direct p53 rescue. Using optimal molecular chaperone variants and reaction conditions (wt or E42A Hsp90β, Hsc70, Hdj1 or Hdj2 and Hop) we tested the ability of molecular chaperones to restore the promoter DNA binding of mutant p53 variants (R175H, R248Q, R249S and R273H). Limited, but detectable recovery was observed mainly in the case of R249S p53. The analogies between wt and mutant rescue reaction requirements suggest that molecular chaperones stably associate with mutant p53 variants as a consequence of an attempt to refold these proteins.

We have proven that MDM2 E3 ligase, in the absence of the E2 and E1 ubiquitylation system, can substitute for the 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 co-transfected 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, in which overexpression is a new independent factor of adverse prognosis in non-small cell



Different modes of wild-type and mutant p53 chaperoning by Hsp90, Hsp70 and co-chaperones.
p53 conformation states are shown as symbols representing native (green circles) and non-native (red squares) tetramers. Most of wt p53 is preserved in the native state, capable of promoter DNA-binding, for more than 1h at 4°C to 30°C, without chaperones. Incubation at higher temperatures leads to a significant decrease in the specific DNA-binding in a time and temperature dependent manner, as indicated by the arrow with time and temperature markers. These are approximate chaperone activity thresholds for different modes and types of used molecular chaperones. Optima of molecular chaperones activity on p53 are shown as box-arrows. R249S oncogenic p53 variant is incapable of the specific DNA-binding at 4°C to 42°C without chaperones. However, its limited fraction may adopt the Hsc/p70-Hdj and Hsp90β E42A substrate state at 37°C and 40°C. Efficient rescue of the p53 activity involves transient associations with molecular chaperones, while ineffective reaction may lead to the stable binding of chaperones to p53 and tumorigenic effects of its stabilization (for more details see text).



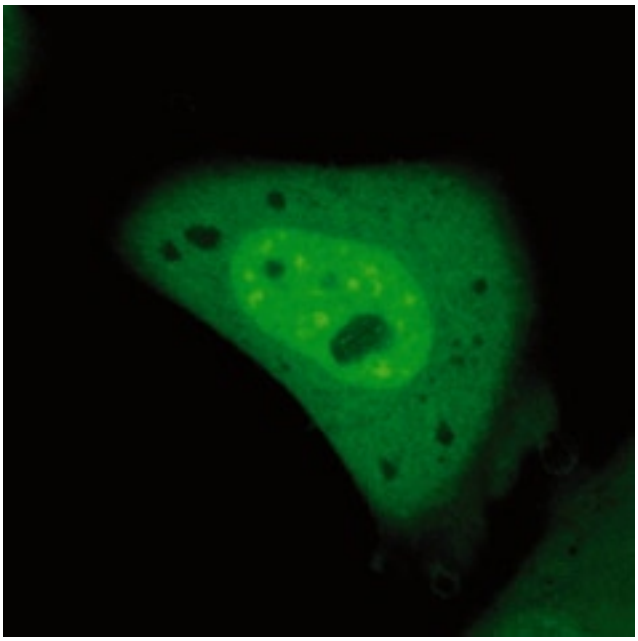
H1299 cell were transfected with p53 and stained for p53 (green) and Hsp70 (red). In cells expressing p53 nuclear localization of Hsp70 is observed. Nuclei were counterstained with Hoechst 33342 (blue).

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 ubiquitylate, 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 (Wawrzynow et al., 2007).

Extensive analysis of human genes, which code for members of the 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 have concluded 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).

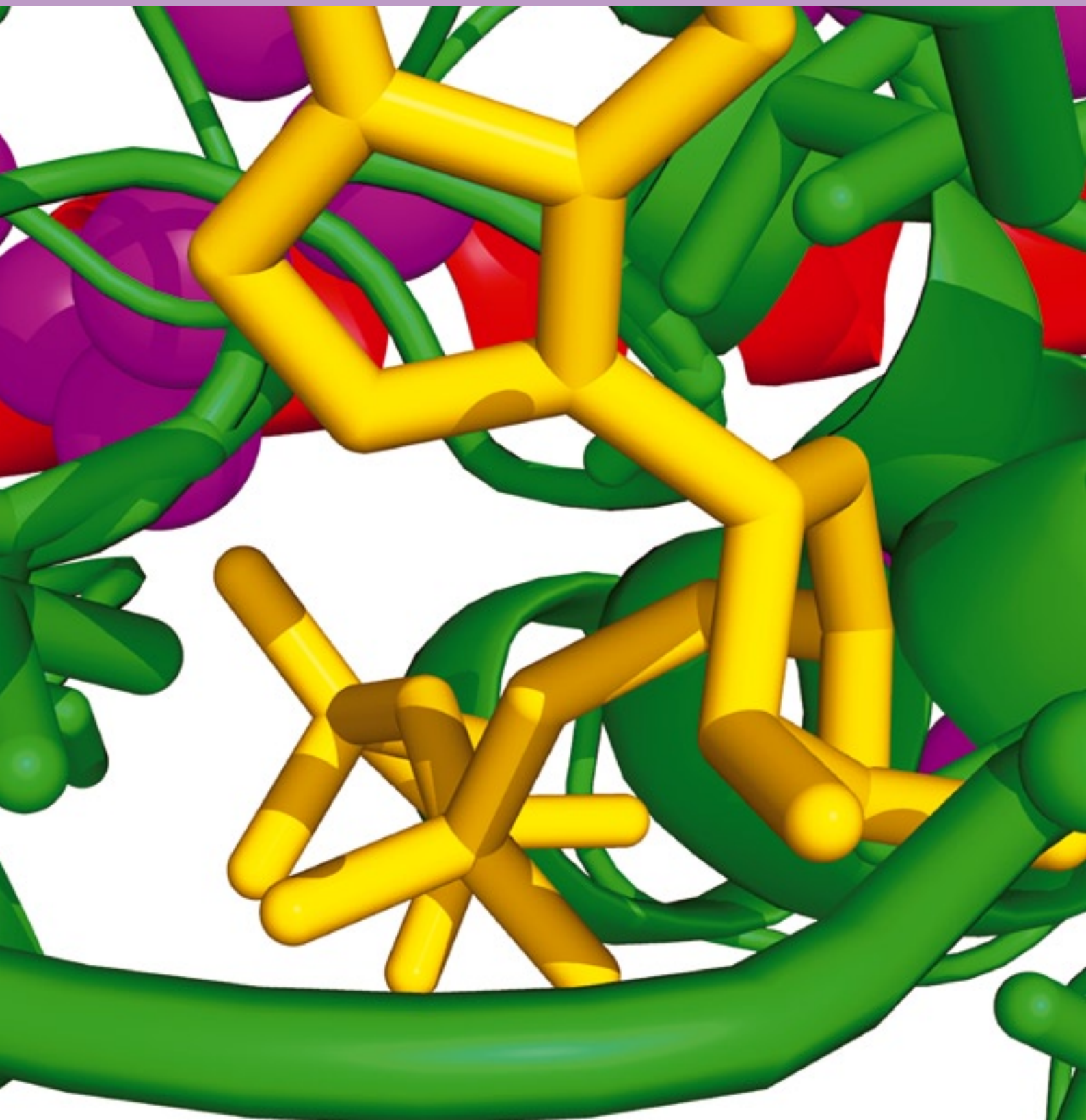
Projects conducted in the laboratory:

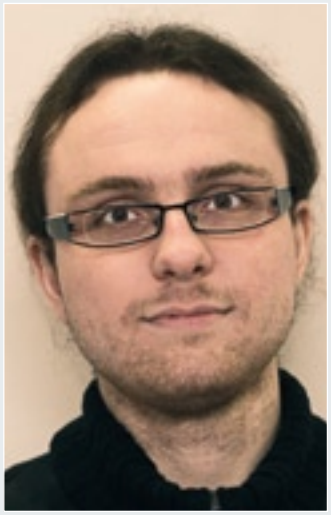
1. Human p53 oligomerization observed by the FRET method.
2. Rescue of human p53 activity by molecular chaperones.
3. Yeast functional assay for testing the interaction between human Hsp90 and p53.
4. Hsp70 family proteins involvement in wild-type and mutant p53 structure and function maintenance under 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. Elucidation of the role of postranslational modifications and co-chaperones binding on the substrate specificity of Hsp90.
7. The molecular process of ΔN p63 α and γ isoforms activation by MDM2 molecular chaperone.
8. Modulation of transcription factors involved in tumorigenesis by MDM2 and other E3 ubiquitin ligases.



H1299 cells were transfected with p53 and Hsp70-EYFP fusion protein and subjected to 42^{0}C heatshock. Small nuclear aggregates visible in YFP (Hsp70) channel also contain p53.

Laboratory of Bioinformatics and Protein Engineering





Lab Leader

Janusz M. Bujnicki, PhD, DSc. Habil.

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Kristian Rother, PhD
Michal Boniecki, PhD

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Undergraduate Students:
Stanislaw Dunin-Horkawicz, BSc, Jan Kaczynski, BSc, Katarzyna H. Kaminska, BSc, Laura Lopez-Munoz, Pawel Lukasz, BSc, Krzysztof Nawara, BSc, Agata Parysz, BSc, Pawel Sztromwasser, BSc, Konrad Tomala, BSc,

Office Manager:
Olga Babicka MSc, Natalia Kalina MSc

Computer Administrator:
Tomasz Jarzynka, Jan Kogut Lukasz Munio

Picture on the left:
Model of ATP-binding site of EcoR124I HsdR subunit. HsdR mutlidomain subunit of Type I Restriction--Modification enzyme EcoR124I was modeled using combination of homology approaches and neutron small angle scattering (SANS).

Janusz Bujnicki, PhD, DSc.Habil.

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 of 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 of 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)

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- Jakubauskas A, Giedriene J, **Bujnicki JM**, Janulaitis A. Identification of a single HNH active site in Type IIS restriction endonuclease Eco31I. J Mol Biol, 2007; 370:157-169
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Current Research

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

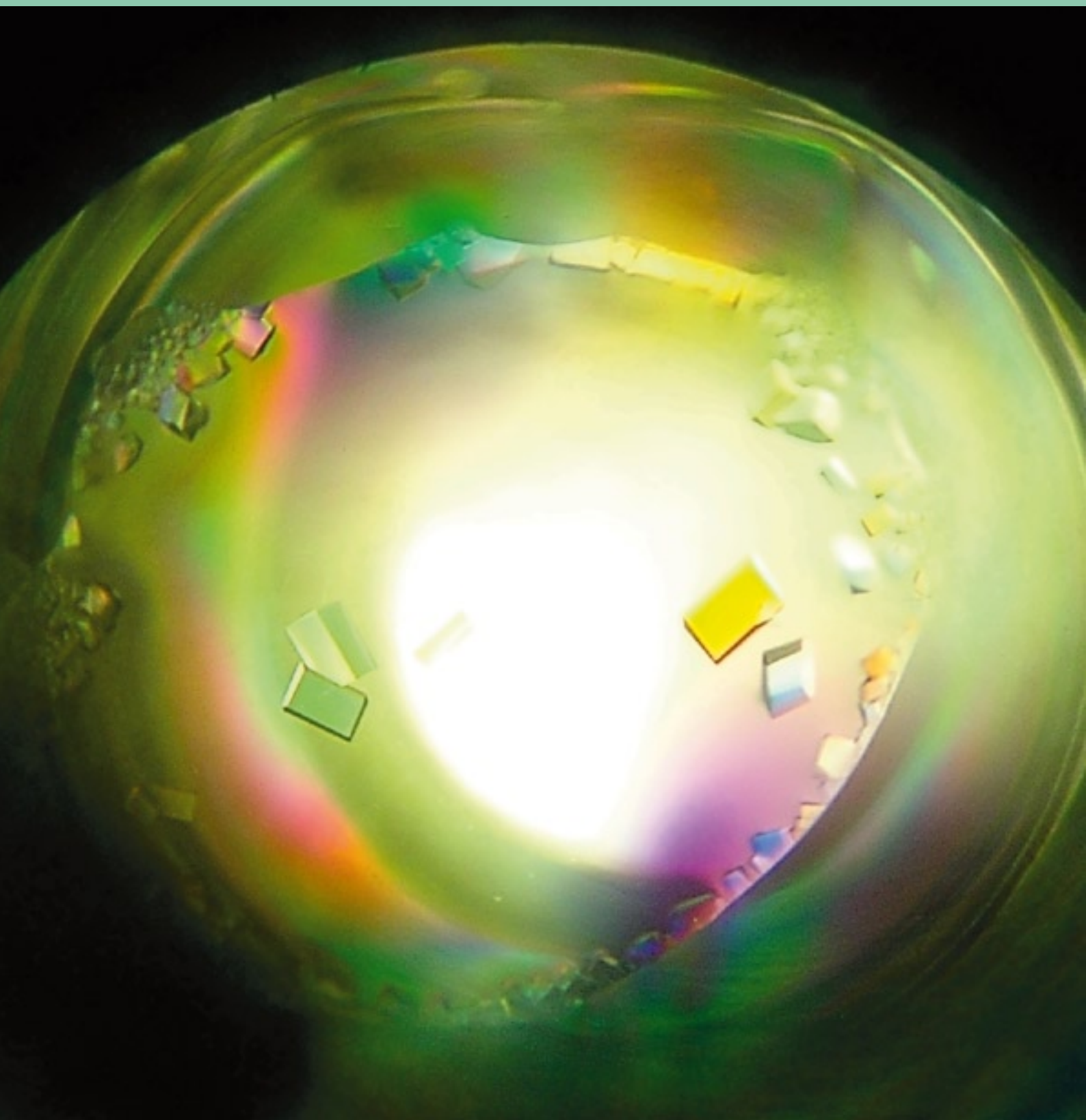
- A section devoted to the development of computer software for analysis of biological macromolecules. The bioinformatics tools include a suite of programs for protein structure prediction and analysis available via the website https://genesilico.pl/toolkit/ (MetaServer for primary, secondary, and tertiary 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 the sequence of 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 2007) include various restriction and homing endonucleases and RNA modification enzymes.
- 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 of 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 structural 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 structures.

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. "Functional specialization of domains tandemly duplicated within 16S rRNA methyltransferase RsmC" and "Human lysosomal DNase IIalpha contains two requisite PLD-signature (HxK) motifs: Evidence for a pseudodimeric structure of the active enzyme species" 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: Structure – function relationships in rRNA methyltransferase RsmC

Bioinformatic analyses of RsmC methyltransferase suggested that this protein contains a duplication of the catalytic domain. In the course of evolution each copy has specialized, one being responsible for substrate rRNA binding and the other for binding of the methyl group donor S-adenosylmethionine (SAM) and catalysis of the methylation reaction. To test this prediction, a series of point substitutions were designed and introduced. Biochemical analysis of mutated proteins confirmed the prediction: mutations in C-terminal catalytic domain led to a decrease in catalytic activity without interfering with the substrate binding, whereas mutations in the selected positions in the N-terminal domain severely impaired interactions with the substrate RNA. We have also constructed truncated recombinant proteins limited to each of the two postulated domains. Their biochemical properties further confirmed the subfunctionalization hypothesis.

Laboratory of Structural Biology MPG/PAN



Lab Leader

Matthias Bochtler, PhD, DSc. Habil.

Post-doctoral Fellows:
Honorata Czapinska, PhD
Renata Filipek, PhD
Malgorzata Firczuk, PhD
Aneta Kaczmarczyk, PhD
Izabela Sabala, PhD

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

EU visiting expert:
Dario Piano, 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:
Crystals of AvaII restriction endonuclease.

Matthias Bochtler, PhD, DSc.Habil.

Degrees

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

PhD in biochemistry, Technical University of Munich, Germany, 1999

MSc in experimental physics, Munich University, Germany, 1995

Research Training

1999-2000 Max Planck Institute of Biochemistry, Martinsried, Germany

1996-1999 Research Assistant, MPI of Biochemistry, 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 the 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 of Biochemistry in Martinsried, Germany

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

Recent publications

- **Kaus-Drobek M, Czapinska H, Sokolowska M**, Tamulaitis G, **Szczepanowski RH**, Urbanke C, Siksnys V, **Bochtler M**. Restriction endonuclease MvaI is a monomer that recognizes its target sequence asymmetrically. Nucleic Acids Res, 2007; 35:2035-46
- Tamulaitis G, Zaremba M, **Szczepanowski RH, Bochtler M**, Siksnys V. Nucleotide flipping by restriction enzymes analyzed by 2-aminopurine steady-state fluorescence. Nucleic Acids Res, 2007; 35:4792-9
- **Sokolowska M, Kaus-Drobek M, Czapinska H**, Tamulaitis G, **Szczepanowski RH**, Urbanke C, Siksnys V, **Bochtler M**. Monomeric restriction endonuclease BcnI in the apo-form and in an asymmetric complex with target DNA. J Mol Biol, 2007; 369:722-34
- **Sokolowska M, Kaus-Drobek M, Czapinska H**, Tamulaitis G, Siksnys V, **Bochtler M**. Restriction endonucleases that resemble a component of the bacterial DNA repair machinery. (review) Cell Mol Life Sci, 2007; 64:2351-7
- **Chojnowski G, Bochtler M**. The statistics of the highest E value. Acta Crystallogr A, 2007; 63:297-305
- **Firczuk M, Bochtler M**. Folds and activities of peptidoglycan amidases. (Review) FEMS Microbiol Rev, 2007a; 31:676-91
- **Bochtler M, Chojnowski G**. The highest reflection intensity in a resolution shell. Acta Crystallogr A, 2007; 63:146-155
- **Firczuk M**, **Bochtler M**. Mutational analysis of peptidoglycan amidase MepA. Biochemistry, 2007b; 46:120-128
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- **Firczuk M**, Mucha A, **Bochtler M**. Crystal structures of active LytM. J Mol Biol, 2005; 354:578-590
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- Azim MK, Goehring W, Song HK, Ramachandran R, **Bochtler M**, Goettig P. Characterization of the HslU chaperone affinity for HslV protease. Protein Sci, 2005; 14:1357-62
- **Szczepanowski RH, Filipek R, Bochtler M**. Crystal structure of a fragment of mouse ubiquitin-activating enzyme. J Biol Chem, 2005; 280:22006-11
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Current Research

The Laboratory of Structural Biology MPG/PAN is mostly focused on the determination of atomic resolution structures of proteins. In 2007, we have studied membrane embedded peptidases, restriction endonucleases, and some theoretical problems in protein crystallography.

Peptidases:

We are shifting from peptidoglycan amidases (Firczuk and Bochtler, 2007a, Firczuk and Bochtler, 2007b) to membrane-embedded peptidases. These play key roles in biology: γ-secretase is a key player in Notch-signalling and Alzheimer disease; site2P peptidase is involved in lipid signalling and the control of cholesterol metabolism; CAAX peptidases are required for the correct processing of farnesylated and geranylgeranylated proteins; and rhomboid peptidases play key roles in development. The eukaryotic membrane-embedded peptidases continue to elude structure determination, but prokaryotic rhomboid and Site2P peptidase structures have recently been published. With EU-ToK support, we are trying to crystallize prokaryotic representatives of some other families of structurally uncharacterized membrane-embedded peptidases.

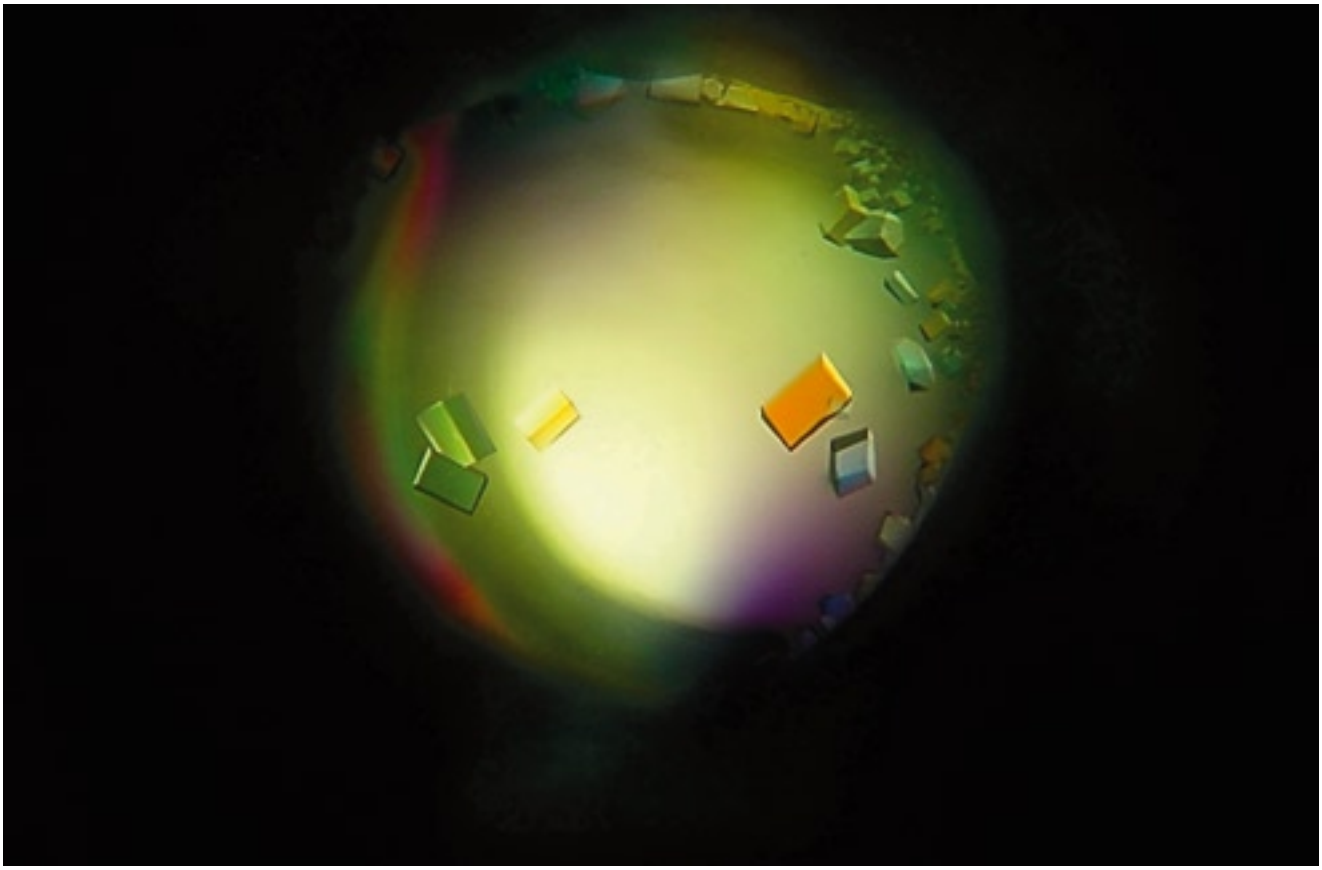
Restriction endonucleases; protein-DNA interactions:

Many DNA-binding proteins match the two-fold symmetry of the DNA backbone and assemble into functional dimers.

Therefore, the target sequences of these proteins are often two-fold symmetric. The interactions of proteins with symmetric (palindromic) DNA are well understood. We have studied how proteins read out the base sequence in pseudo-symmetric (pseudopalindromic) DNA. Simple arguments show that recognition is always degenerate at the centre and difficult to explain by conventional hydrogen bonding. We have attempted to understand how this degenerate recognition works at the molecular level, using restriction endonucleases and methyltransferases as our test systems. Starting with work in 2006, we have shown that some restriction endonucleases solve the pseudosymmetry problem by flipping the "offending" bases from the inside of the DNA into extrahelical positions (Bochtler et al., 2006, Tamulaitis et al., 2007). We have also shown that this strategy is far from universal: other restriction endonucleases, such as BcnI and MvaI, simply ignore the approximate symmetry of their targets and interact with them as monomers (Sokolowska et al., 2007, Kaus-Drobek et al., 2007, Sokolowska et al., 2007b).

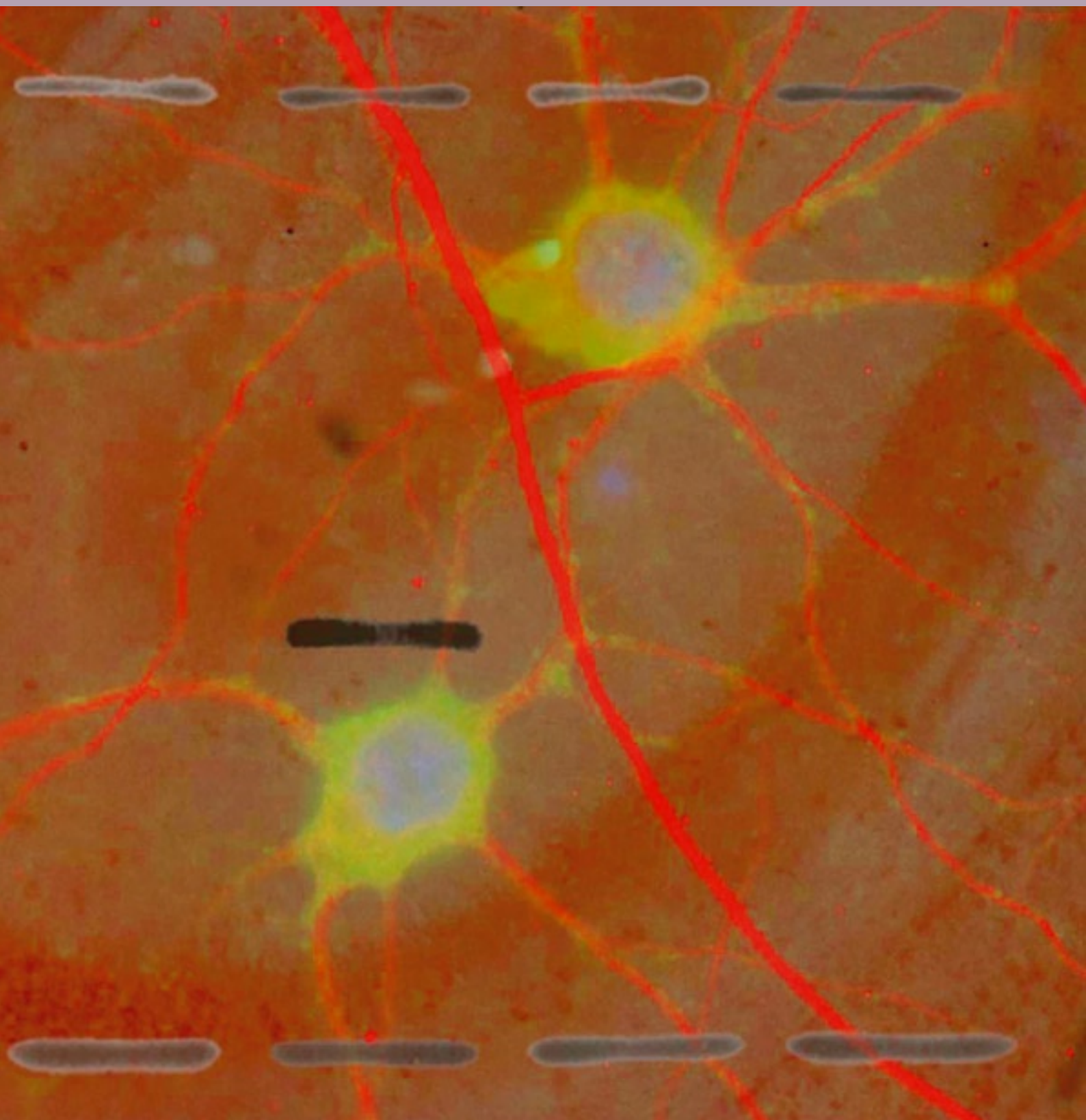
Method development:

Protein crystallography investigates molecular structure with the help of the diffraction phenomenon. In essence, a diffraction experiment reveals information about the distribution of electrons in a molecule, but unfortunately, this information is not directly accessible. Instead, the raw data of a diffraction experiment are structure factors, which can be used for the calculation of the electron density by Fourier transformation only if additional phase information has been obtained from other sources. There are several known ways to fill in the missing information: they either rely on differences in scattering, as additional atoms are added (SIR, MIR), or made to react to changes in wavelength in distinctive ways (SAD, MAD). Alternatively, phases for an unknown protein structure can be calculated from a related known structure, provided the similarity of the known and unknown structures is sufficiently high. Could one use small, "generic" secondary structure elements for molecular replacement? The conventional answer is no, because these fragments cannot be reliably positioned. We asked ourselves if perhaps a more favorable answer was possible – at least in was some cases – if 3D-diffraction patterns of protein crystals was searched for characteristic fibre diffraction "signatures" of secondary structure elements. The main problem with this approach is to distinguish signal from noise. Therefore, we have started with a very careful analysis of the fluctuations of structure factors in the absence of characteristic secondary structures (Bochtler and Chojnowski, 2007, Chojnowski and Bochtler, 2007). We hope that the detailed results of the detection of fiber diffraction features in 3D-diffraction data of proteins will be ready for publication next year.



Crystals of AvaII restriction endonuclease.

Laboratory of Neurodegeneration



Lab Leader

Jacek Kuznicki, PhD, Professor

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

Post-doctoral Fellows:
Monika Klejman, PhD
Marta Wisniewska, PhD
Anna Skibinska-Kijek, PhD
Joanna Gruszczynska, PhD

PhD Students:
Magdalena Blazejczyk, MSc (until March 2008, PhD defense Feb. 2008)
Emilia Bialopiotrowicz, MSc
Lukasz Bojarski, MSc
Katarzyna Debowska, MSc
Wojciech Michowski, MSc
Katarzyna Misztal, MSc
Adam Sobczak, MSc
Aleksandra Szybinska, MSc

Office Manager:
Dominika Dubicka, MSc

MSc Student:
Mirosław Drab, Kamila Skieterska, Bozena Zebrowska

Picture on the left:
Study on neurodegeneration: from brain tissue to protein analysis.

Jacek Kuznicki, PhD, Professor

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, National Institute of Health, 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 Sciences (PAN), since December 2004
- Member of the American Society for Biochemistry and Molecular Biology, since 2003
- Head of the 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), 2004-2008
- Prime Minister Award for the scientific achievements, 2003
- Award from the Division of Biological Sciences of 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 2007

- Lanni C, Racchi M, Mazzini G, Ranzenigo A, Polotti R, Sinforiani E, Olivari L, Barcikowska M, Styczynska M, **Kuznicki J, Szybinska A**, Govoni S, Memo M, Uberti D. Conformationally altered p53: a novel Alzheimer's disease marker? Mol Psychiatr, 2007; Aug 7 [Epub ahead of print]
- Filipek A, **Michowski W, Kuznicki J**. Involvement of S100A6 (calcyclin) and its binding partners in intracellular signaling pathways. Adv Enzyme Regul, 2007; Nov 19 [Epub ahead of print]
- **Mossakowska M**, Barcikowska M, Broczek K, Grodzicki T, Klich-Raczka A, Kupisz-Urbanska M, Podsiadly-Moczydlowska T, Sikora E, **Szybinska A**, Wieczorowska-Tobis K, Zyczkowska J, **Kuznicki J**. Polish Centenarians Programme – Multidisciplinary studies of successful ageing: Aims, methods, and preliminary results. Exp Gerontol, 2008; 43:238-244
- **Bojarski L, Lewandowicz A, Blazejczyk M, Sobczak A, Kuznicki J, Wojda U**. Biochemical properties of endogenous presenilin 1 and presenilin 2 in cultured human B-lymphocytes. Clin Chem Lab Med, 2007; 45:1273-6
- **Bojarski L**, Herms J, **Kuznicki J**. Calcium dysregulation in Alzheimer's disease. Neurochem Int, 2008; 52:621-633
- Lesniak W, Slomnicki LP, **Kuznicki J**. Epigenetic control of the S100A6 (calcyclin) gene expression. J Invest Derm, 2007; 127:2307-14
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- Spiechowicz M, **Zylicz A, Bieganowski P, Kuznicki J**, Filipek A. Hsp70 is a new target of Sgt1 – an interaction modulated by S100A6. Biochem Biophys Res Commun, 2007; 357:1148-53
- Schneider G, Nieznanski K, Kilanczyk E, **Bieganowski P, Kuznicki J**, Filipek A. CacyBP/SIP interacts with tubulin in neuroblastoma NB2a cells and induces formation of globular tubulin assemblies. Biochim Biophys Acta, 2007; 1773:1628-36.

Current Projects

We are interested in molecular mechanisms involved in learning and memory, as well as in neurodegeneration; we 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 disease
3. Studies on the cyclin-dependent kinase 5 involvement in pathogenesis of Alzheimer disease
4. Analysis of Ca^{2+}-binding proteins under normal and pathological conditions in neurons
5. Analysis of proteins involved in Ca^{2+} homeostasis in neurons
6. Regulation and role of β-catenin/Lef1 complex in mature neurons
7. 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 in collaboration with the group of Maria Barcikowska and Cezary Zekanowski at the Medical Research Center, PAN)*

More than 100 mutations linked to early-onset familiar Alzheimer disease (FAD) have been identified in presenilin proteins. Presenilin 1 and presenilin 2 are the catalytic components of the gamma-secretase enzymatic complex, which also comprises of nicastrin, Aph-1 and Pen-1 proteins. Gamma-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 beta-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 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 lymphoblast lines including those obtained during the Polish Centenarian Project. We examined the previously recognized pathogenic mutation in PSEN1 gene and novel, mutations identified by us, on beta-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. Beta-amyloid 1-40 and 1-42 levels in serum-free culture media were estimated by ELISA. Cells with mutated presenilins produced higher amounts of beta-amyloid than control cells and the beta-amyloid 42/beta-amyloid 40 ratio was significantly increased versus controls, indicating that novel mutations identified in Polish patients are likely responsible for FAD.

2. Search for functional bio-markers and potential therapeutic targets of Alzheimer disease *(Emilia Bialopiotrowicz, Lukasz Bojarski, Miroslaw Drab, Aleksandra Szybinska, Urszula Wojda, Bozena Zebrowska in collaboration with other laboratories)*

In this area, several projects were carried out:

2.1. In cooperation with Prof. Mauricio Memo and Dr. Daniela Uberti (University of Brescia) the conformational mutant p53 as a new putative marker to discriminate AD from non-AD patients was analyzed. Conformation of p53 protein was studied in cell lysates from our immortalized 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., Mol Psychiatr, 2007; in press).

2.2. We have been analyzing FAD mutations in PS1 effects on basic cellular functions, such as cell cycle progression and apoptosis. These studies are performed using immortalized lymphocytes from patients with FAD PS1 mutants, from SAD patients and from the control groups. Effects of PS1 mutations are also being tested in HEK cells transfected with PS1 mutant constructs. We are searching for molecular mechanism(s) underlying changes observed in PS1 mutant cells in comparison to cells expressing wild type PS1.

2.3. In collaboration with Dr. Jochen Herms (Ludwig Maximilians University), we have also been analyzing lymphocytes from patients with PS1 mutations showing similar alterations in the calcium homeostasis to neurons from transgenic animal models of familial AD. We are performing cell-imaging screens for new potential therapeutic targets for AD and also analyzing 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 in collaboration with Aleksandra Wyslouch--Cieszynska from laboratory of Mass Spectrometry, 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 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, using the 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. Using different methods of samples of preparation and fractionation, we identified over 1500 synaptic proteins. Preliminary statistical analysis of mass spectrometry data obtained from wild type and transgenic animals synaptosomes revealed a set of differential proteins, some of which are known to be dysregulated in Alzheimer disease.

4. Analysis of Ca^{2+}-binding proteins under normal and pathological conditions in neurons *(Magdalena Blazejczyk, Katarzyna Debowska, Bozena Zebrowska, Adam Sobczak, under the supervision of Urszula Wojda and in collaboration with other laboratories)*

Ca^{2+}-binding proteins in neurons regulate neuronal development, plasticity, and neurodegeneration and draw much attention due to implications in multiple brain pathologies including Alzheimer disease. Genomic databases indicated the existence of a novel family of Ca^{2+}-binding proteins called calmyrins (CaMy, known also as KIP or CIB). Calmyrins are evolutionarily conserved from Nematoda to humans. In humans, four genes encode calmyrin proteins (CaMy1 – CaMy4) but until now the only member of the CaMy family that has been analyzed is CaMy1. We have previously demonstrated that CaMy1 is implicated in Alzheimer disease and that it interacts specifically with Alzheimer disease associated presenilin 2 (PS2) 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). Our results indicate, however, that the interaction of CaMy1with PS2 in neurons is limited and does not account for the involvement of CaMy1 in Alzheimer disease. Therefore, we have undertaken the search for other possible binding protein partners of CaMy1 and using several biochemical methods, we identified a new potential target of CaMy1. Currently, we characterize CaMy1 interaction with its novel protein ligand. Moreover, we pursued first studies on rat calmyrin 2 (CaMy2). We cloned rat recombinant CaMy2 protein and obtained polyclonal anti-CaMy2 antibodies. We demonstrated CaMy2 Ca^{2+}-sensor properties, neuronal pattern of brain expression, and subcellular localization in Golgi apparatus and dendrites. Moreover, regulation of CaMy2 expression has been studied in primary cultures of rat neurons. We have also searched for protein ligands of CaMy2 in rat brain and identified several new potential targets of CaMy2. These interactions were confirmed by several in vitro methods, and their physiological significance has become the aim of further studies. In addition, we are analyzing the changes in CaMy1 and CaMy2 biochemical properties, protein ligands binding and in involvement in neuronal functions as a result of neurodegenerative processes.

5. Analysis of proteins involved in Ca^{2+} homeostasis in neurons *(Lukasz Bojarski, Monika Klejman, Joanna Gruszczynska, Anna Skibinska-Kijek, in collaboration with partners from PROMEMORIA 6th FP of EU and from the Polish-German grant)*

We study STIM and Orai that are involved in the process of store operated Ca^{2+} entry. STIM is localized in the ER membrane where it serves as a Ca^{2+} sensor. Upon ER Ca^{2+} depletion STIM redistributes into punctuate structures, moves closer to the plasma membrane and activates Orai channels that refill Ca^{2+} stores. In our study we focus on analysis of STIM and Orai proteins in the brain: cellular localization and function. Using immunocytochemistry and other immunofluorescent methods, as well as biochemical analyses of the brain protein extracts, we describe expression pattern of STIM1 and STIM2 in mouse brain. We have developed rabbit polyclonal antibodies recognizing Orai1 protein and are currently optimizing conditions for application in immunohistochemical staining and immunoblotting.

6. Role and regulation of β-catenin in mature neurons *(Monika Klejman, Katarzyna Misztal, Anna Skibinska-Kijek, 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 suggest it might be involved in learning and memory formation, as well as in some brain pathology. β-catenin and Lef1 expression levels were analyzed in the forebrain of adult mice and rats using immunocytochemical staining and immunofluorescent methods, as well as biochemical analysis of the brain protein extracts. Currently, we are exploring the mechanism of constant stabilization of β-catenin in mature thalamic neurons both in vivo and in vitro and are looking for β-catenin/Lef1 target genes in mature neurons.

7. Characterization of biological function of CHORD containing proteins in the nervous system *(Wojciech Michowski, Anna Skibinska-Kijek, 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 have identified melusin as a novel protein target of the S100 Ca^{2+}-signal-sensing proteins. Chp-1 is an 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 have decided to explore its function in neurons. We are currently focused on elucidating the mechanisms involved in stress dependent nuclear accumulation of the CHP-1 protein. Using point and deletion mutants we characterize structural elements of CHP-1 that regulate cellular localization of the protein. By means of mass spectrometry we are trying to identify posttranslational modifications occurring in CHP-1 after exposure of cells to stress conditions. We are also determining the chaperoning activity of CHP-1 applying in vitro assays on purified proteins. These studies are complemented by looking at changes of CHP-1 expression pattern in brains of animal models of neurological diseases.

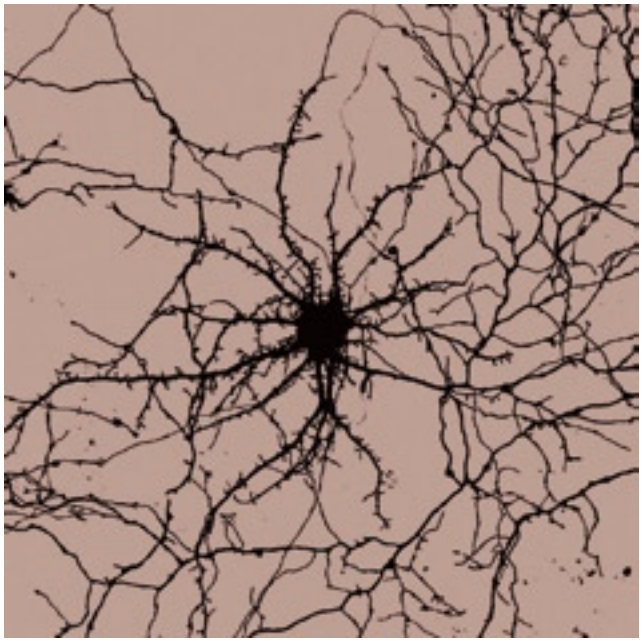
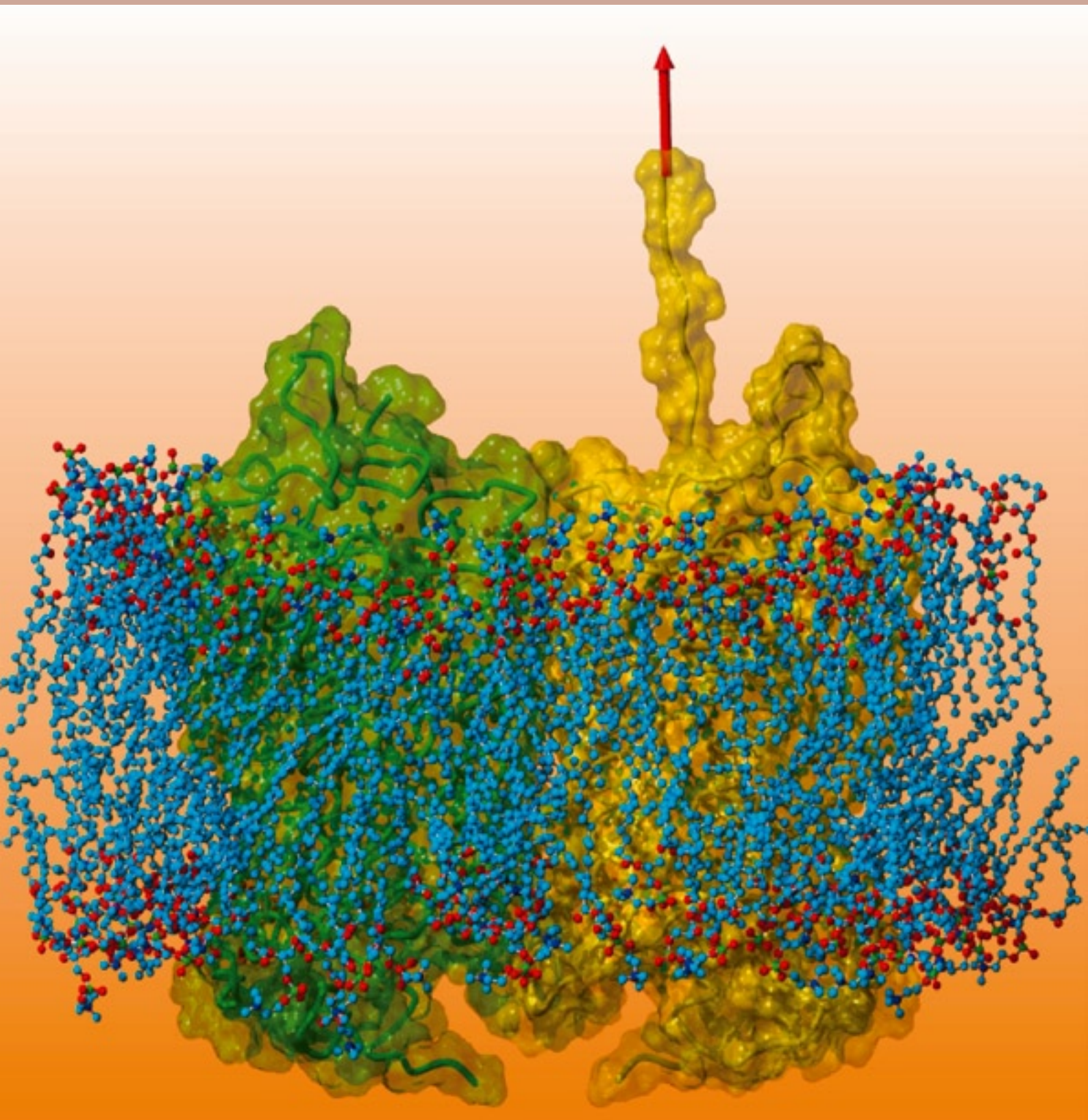


Figure 1.
Primary rat hippocampal neuron transfected with CaMy2-EGFP.

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
Aleksander Debinski, MSc
Wojciech Pulawski, MSc

Undergraduate students:
Anna Zwolinska

Picture on the left:
The principle of Single-Molecule Force Spectroscopy (SMFS) on pulling of one rhodopsin molecule from a dimer in the membrane.

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
Polish Society of Medicinal Chemistry

Editorial Board Member

Journal of Bionanoscience
The Open Structural Biology Journal

Publications

over 60 publications in primary scientific journals
over 1300 citations
over 1000 citations with IIMCB affiliation (years 2003-2007)

Selected publications

- **Jozwiak K**, Zekanowski C, **Filipek S**. Linear patterns of Alzheimer's disease mutations along alpha-helices of presenilins as a tool for PS-1 model construction. J Neurochem, 2006; 98:1560-72
- Sapra KT, Park PS, **Filipek S**, Engel A, Muller DJ, Palczewski K. Detecting molecular interactions that stabilize native bovine rhodopsin. J Mol Biol, 2006; 358:255-269
- **Modzelewska A, Filipek S**, Palczewski K, Park PS. Arrestin interaction with rhodopsin: conceptual models. Cell Biochem Biophys, 2006; 46:1-15
- **Filipek S**. Organization of rhodopsin molecules in native membranes of rod cells – old theoretical model compared to new experimental data. J Mol Model, 2005; 11:385-391
- Park PS-H, **Filipek S**, Wells JW, Palczewski K. Oligomerization of G protein-coupled receptors: past, present, and future. Biochemistry-US, 2004; 43:15643-56
- **Filipek S, Krzysko KA**, Fotiadis D, Liang Y, Saperstein DA, Engel A, Palczewski K. A concept for G protein activation by G protein-coupled receptor dimers: the transducin / rhodopsin interface. Photochem Photobiol Sci, 2004; 3:628-638
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- Liang Y, Fotiadis D, Maeda T, Maeda A, **Modzelewska A, Filipek S**, Saperstein DA, Engel A, Palczewski K. Rhodopsin signaling and organization in heterozygote rhodopsin knockout mice. J Biol Chem, 2004; 279:48189-96
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- Liang Y, Fotiadis D, **Filipek S**, Saperstein DA, Palczewski K, Engel A. Organization of the G Protein-coupled Receptors Rhodopsin and Opsin in Native Membranes. J Biol Chem, 2003; 278:21655-62
- Fotiadis D, Liang Y, **Filipek S**, Saperstein DA, Engel A, Palczewski K. Rhodopsin dimers in native disc membranes. Nature, 2003; 421:127-128
- Schädel SA, Heck M, Maretzki D, **Filipek S**, Teller DC, Palczewski K, Hofmann KP. Ligand channeling within a G-protein-coupled receptor: The entry and exit of retinals in native opsin. J Biol Chem, 2003; 278:24896-903
- 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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*Papers marked with an asterisk have no IIMCB affiliation of the authors

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- **Jozwiak K, Krzysko KA, Bojarski L**, Gacia M, **Filipek S**. Molecular models of the interface between anterior pharynx--defective protein 1 (APH-1) and presenilin involving GxxxG motifs. ChemMedChem, accepted in 2007
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Description of Current Research

1. Studies of molecular interactions that stabilize membrane proteins

Atomic force microscopy (AFM) allows high-resolution imaging of individual membrane proteins in their native environment. Single-molecule force spectroscopy (SMFS), which is based on pulling individual protein out of the membrane using AFM tip, is a powerful tool to dissect molecular interactions that govern the stability and function of 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. SMFS was applied to understand the effect of Zn^{2+} ions on the molecular interactions underlying the structure of rhodopsin. Force-distance curves obtained from SMFS assays revealed that zinc ions increased the stability of most structural segments of rhodopsin. This effect was not mimicked by Ca^{2+}, Cd^{2+}, or Co^{2+}. Thus, zinc ions stabilize the structure of rhodopsin in a specific manner.

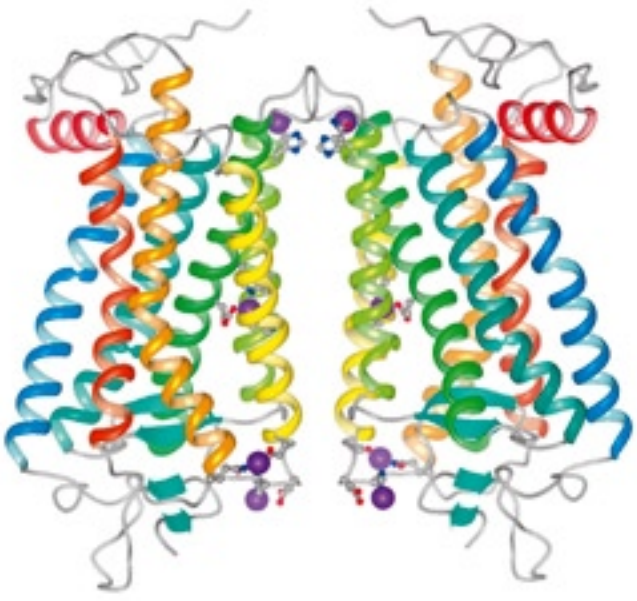

Figure 1.
Side view of a rhodopsin dimer model. The position of Zn^{2+} in binding sites observed in crystal structures of rhodopsin are highlighted as purple spheres. Helices of rhodopsin are coloured as follows: TM1 in blue, TM2 in blue-green, TM3 in green, TM4 in green-yellow, TM5 in yellow, TM6 in orange, TM7 and H8 in red. Amino acid residues that coordinate Zn^{2+} are shown in ball-and-stick representations.

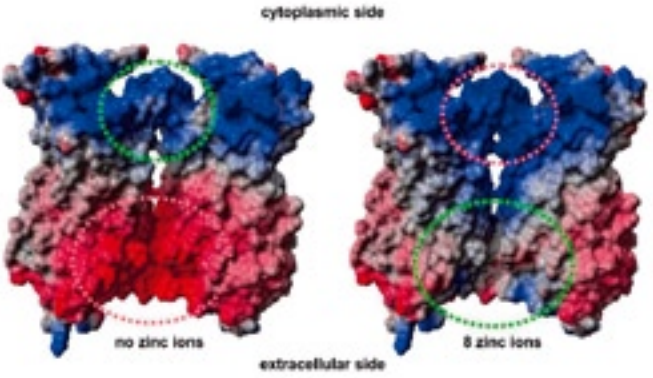

Figure 2.
The electrostatic potential of rhodopsin dimer model. (Left) Rhodopsin dimer without bound Zn^{2+}; (Right) Rhodopsin dimer with 8 bound Zn^{2+}. The electrostatic potential is coloured as follows: blue – positive, red – negative, white – neutral. Green and red dashed ellipses indicate favourable and unfavourable electrostatic interactions, respectively.

Upon close examination of the Zn^{2+}-binding sites considered in our study, it is apparent that these sites line up along the putative dimer interface of rhodopsin, which has been proposed based on packing constraints from AFM studies (Fig. 1). Oligomerization of rhodopsin and other GPCRs has only recently become an appreciated concept and likely plays a central role in the signaling process. The binding of Zn^{2+} to rhodopsin changes the electrostatic potential of the putative dimer interface of the receptor. Electrostatic interactions at the dimer interface must be optimized for dimerization to occur. In the absence of bound Zn^{2+} (Fig. 2, Left), the contact area at the cytoplasmic side is characterized by a slightly positive electrostatic potential that would not prevent the dimerization of rhodopsin. In contrast, the contact area at the extracellular side has a strong negative potential that would introduce a strong repulsion and thereby hinder dimerization. Binding of Zn^{2+} to all eight sites observed in crystal structures eliminates the repulsive negative electrostatic potential at the extracellular contact area (Fig. 2, Right). However, the electrostatic potential at the cytoplasmic contact area becomes strongly positive and therefore repulsive. The optimal electrostatic potential at the dimer interface of rhodopsin will occur when all Zn^{2+}-binding sites are occupied except for the site at the cytoplasmic surface.

Rhodopsin is a prototypical member of the GPCR superfamily, so the potential physiological role of Zn^{2+} in rhodopsin signaling may extend to other members of the GPCR family. Many GPCRs modulate activities in the central nervous system. The brain, similarly to the eye, contains high concentrations of Zn^{2+}. The concentration of Zn^{2+} in the brain has been estimated to be 150 μM and it has been suggested that the release of Zn^{2+} into the synapses of hippocampal neurons can result in local concentrations as high as 300 μM. Several GPCRs residing in the brain reportedly bind Zn^{2+} with micromolar binding affinities and modulate the binding properties of agonists, antagonists, and inverse agonists. Similarly, the dissociation constant of Zn^{2+} for rhodopsin in disc membrane and purified rhodopsin in the dark has been

estimated to be 2-10 μM, and the maximal regeneration of rhodopsin by its chromophore is reduced in the presence of Zn^{2+}. These observations suggest that Zn^{2+} can bind to rhodopsin and other GPCRs under physiological conditions and may play a role in signaling processes.

2. G protein-coupled receptors and their 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 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 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 the endoplasmic reticulum with the help of dimer-probing cytosolic chaperons to the internalization of these receptors. Dimerization, and more generally oligomerization, of G protein-coupled receptors (GPCRs) is experimentally proven and possibly all GPCRs act in oligomeric form. The coupling with G protein, phosphorylation by kinase and binding to arrestin, which starts the internalization process, have also been shown to be influenced by the oligomeric state of the receptors. Cooperative interactions within homo- and heterodimers of GPCRs may be critical for the propagation of an external signal across the cell membrane, activation of a G protein and passing the signal down to effector proteins. We performed simulation of pulling rhodopsin using Steered Molecular Dynamics (Fig. 3). Obtained results are in agreement with the experiment and allow for molecular interpretation of SMFS data.

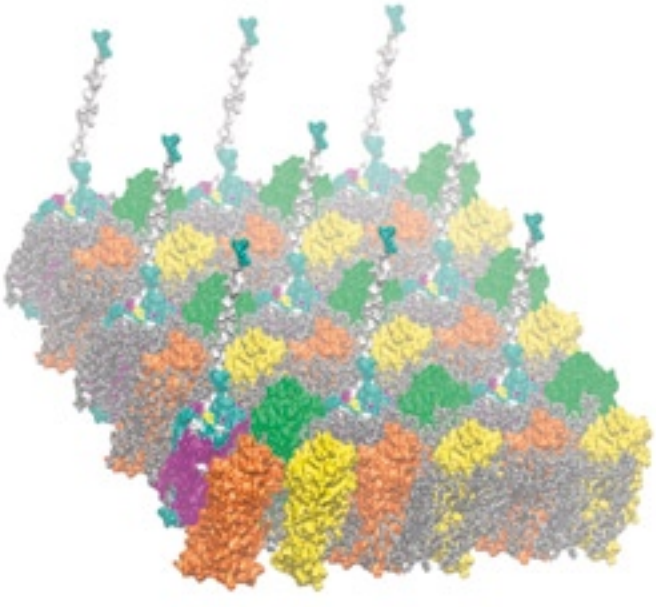

Figure 3.
Simulation of pulling of rhodopsin molecule from oligomeric assembly. A single periodic box consists of four rhodopsin molecules. This real box containing the whole investigated system is surrounded by its exact images.

In the paper [Lee et al. PNAS 2007] six putative chromosomal regions critical for clonal expansion of intraurothelial neoplasia and development of bladder cancer were identified by using genetic mapping. Focusing on one of the regions, which includes the model tumour suppressor RB1, allelotyping of single-nucleotide polymorphic sites was performed and identified a 1.34-Mb segment around RB1 characterized by a loss of polymorphism associated with the initial expansion of in situ neoplasia. This segment contains several positional candidate genes referred to as forerunner genes that may contribute to such expansion. Efforts were concentrated on the two neighbour genes flanking RB1, namely ITM2B and CHC1L, as well as P2RY5, which is located inside RB1. ITM2B and P2RY5 modulated cell survival and were silenced by methylation or point mutations, respectively, and thus by functional loss may contribute to the growth advantage

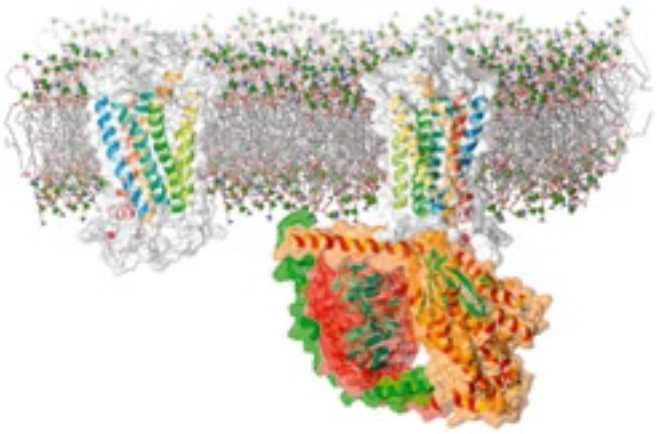

Figure 4.
The model of inactive P2RY5 receptor containing seven-transmembrane (TM1–TM7) and one cytoplasmic (H8) helix structures showing the position of polymorphism in codon 307 located within the cytoplasmic domain of the protein (Left) that may affect its interaction with the Gαβγ trimeric protein complex (Right).

of neoplasia. A 1722 G-T polymorphism resulting in the substitution of tryptophan for cysteine at position 307 was of potential importance because it was detected in several bladder tumours and nontumour DNA from the same patient. In addition, molecular models of P2RY5 protein (Fig. 4) developed by using its homology to rhodopsin suggest that this substitution involving the cytoplasmic domain of the protein may affect its interaction with the G protein complex and compromise its biological activity.

3. Alzheimer Disease mutations and γ-secretase membrane protease

γ-Secretase is an integral membrane protease, which is a complex of four membrane proteins. Improper functioning of γ-secretase was found to be critical in the pathogenesis of Alzheimer disease. Despite numerous efforts, the structure of the protease as well as its proteolytic mechanism remains poorly understood. We constructed a model of interactions between two proteins forming γ-secretase: APH-1 and presenilin. This interface is based on a highly conserved GxxxGxxxG motif in the APH-1 protein. It can form a tight contact with a small-residue AxxxAxxxG motif in presenilin. We proposed and verified four binding modes based on similar structures involving GxxxG motifs in glycophorin and aquaporin. The resulting best model employs antiparallel orientations of interacting helices and is in agreement with the currently accepted topology of both proteins. All three hydrophobic regions (HRs) (8-10) in PS-1 CTF bear the GxxxG motifs. However, HR8 is directly forming the catalytic site and HR10, containing the PAL region, is also recognized as being essential for the catalytic activity of γ-secretase. Furthermore, HR10 is not involved in the formation or stabilization of the γ-secretase complex. Taking into account the fact that HR8 and HR10 are involved in substrate processing, HR9 seems to be the most plausible one to form an interface for interaction with APH-1 (Fig. 5).

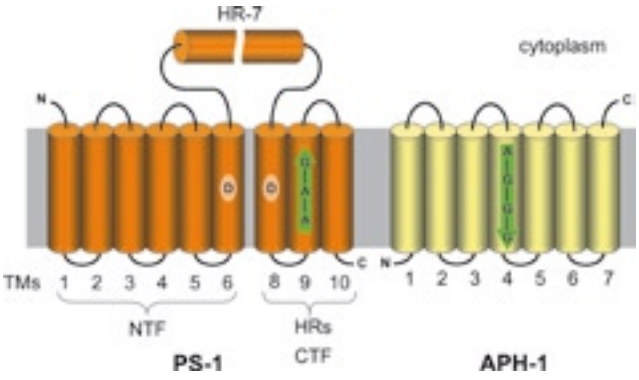

Figure 5.
The scheme of topologies of PS-1 and APH-1. The proposed interface between these proteins is highlighted by arrows. Location of two catalytic aspartic acid residues of PS-1 is shown.

In antiparallel orientation the $_{409}$AxxxAxxxG$_{417}$ motif located on the PS-1 HR9 helix can interact with an extended $_{118}$AxxxGxxxGxxxG$_{130}$ motif found in APH-1 TM4. This is because of a much smaller helix-helix angle compared to the parallel models. Paradoxically this extension diminishes the contact surface, but also improves greatly the binding energy. This strongly suggests that the antiparallel models better approximate the interface. The framework of adjacent side-chains is essential for creating stable helix interaction, so each case of (small)xxx(small) motif must be tested independently. In the case of analyzed PS-1 – APH-1 interface there is a

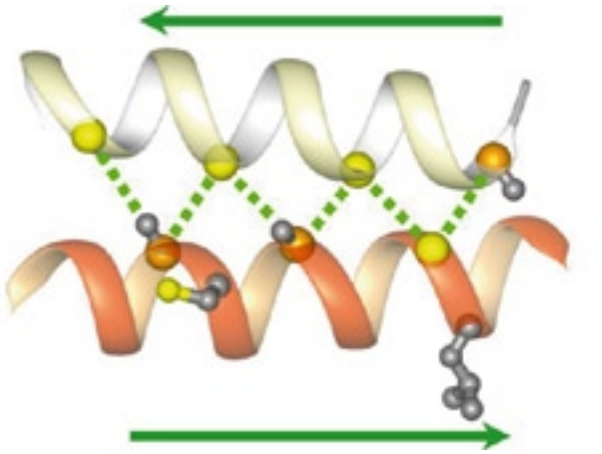

Figure 6.
The molecular model of the interface between APH-1 and PS-1 based on a double GxxxG motif. C_α of Gly and Ala forming the interface are shown as yellow and orange spheres, respectively. Green arrows indicate directions of helices.

long network of small residues (Fig. 6) and, additionally, a hydrogen bond between interacting helices is cementing them. Such an arrangement of residues in antiparallel model provides the lowest binding energy suggesting the most probable mode of interaction. This model can be used for further structural characterization of γ-secretase and its components. Nevertheless, subsequent experimental data are required to reveal all binding details and especially to provide information how adjacent helices of both interacting proteins affect the interface.

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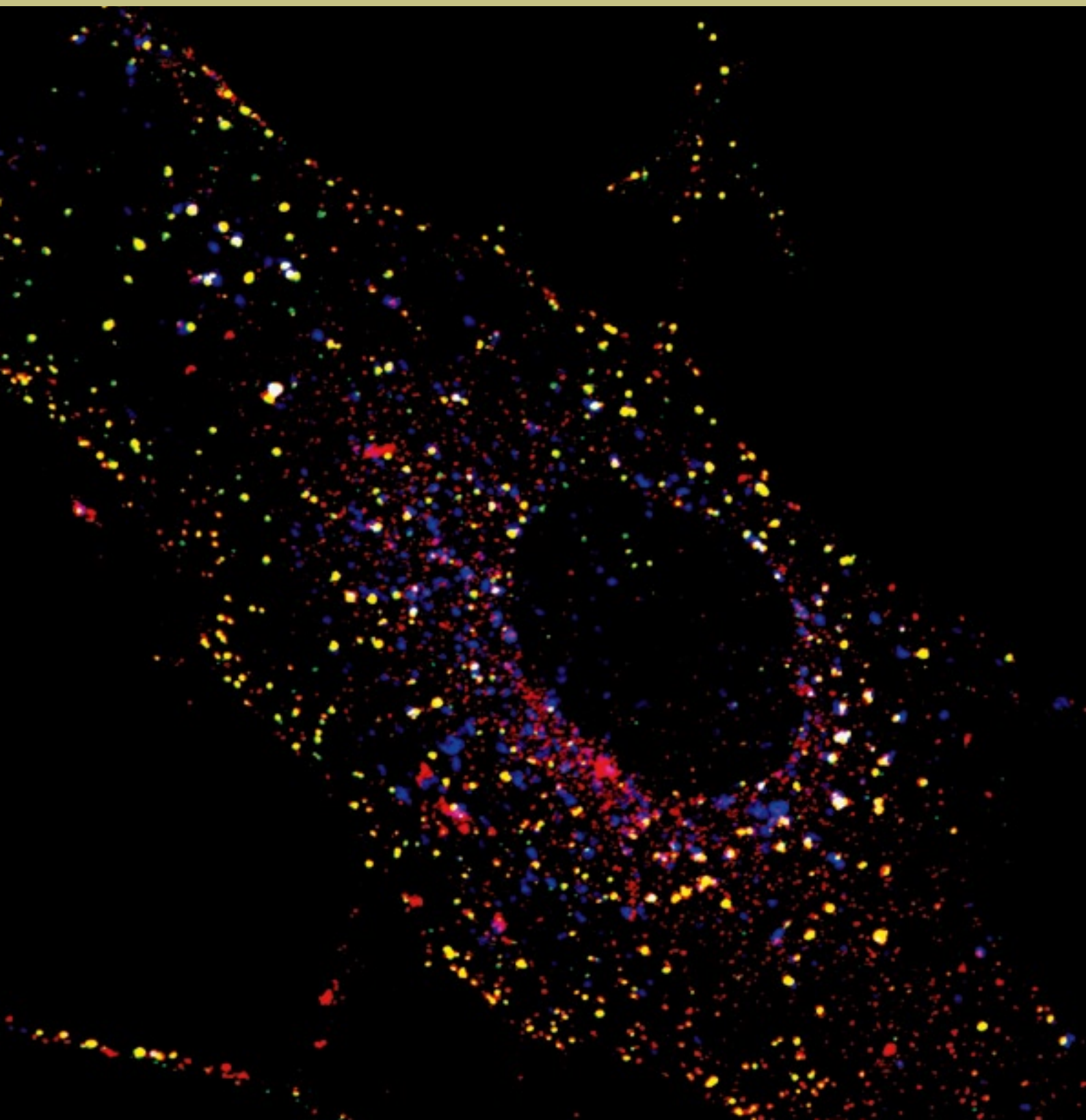

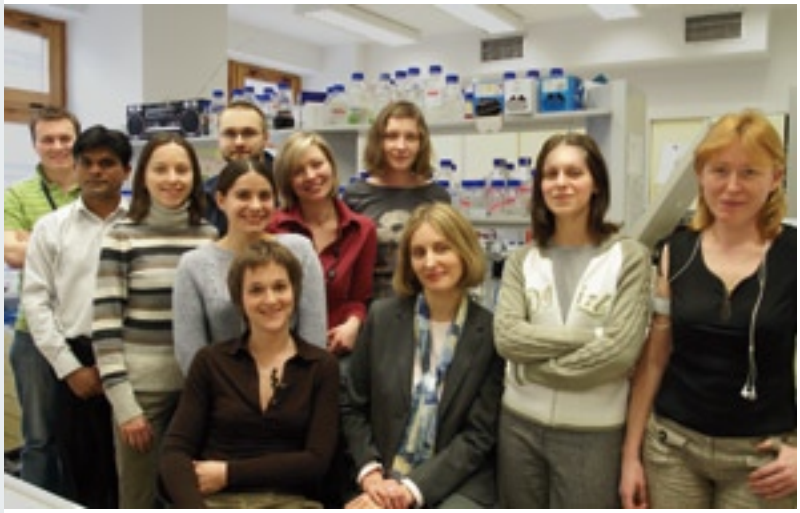

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Trainees & Undergraduate Students
Anna Torun, MSc (since October 2007)
Michal Mlacki (since December 2007)

Grant Administrator:
Vanessa Formas, MA (until December 2007)

Picture on the left:
A human foreskin fibroblast was stimulated with platelet-derived growth factor (PDGF) for 15 min. Internalized PDGF (green) and its receptor (red) are present in intracellular endosomal vesicles. Early endosomes are marked with EEA1 (Early Endosome Antigen 1) in blue.

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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

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Fellowships and awards

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Selected publications

- **Miaczynska M**, Stenmark H. Mechanisms and functions of endocytosis. J Cell Biol, 2008; 80:7-11
- **Pilecka I, Banach-Orlowska M, Miaczynska M**. Nuclear functions of endocytic proteins. Eur J Cell Biol, 2007; 86:533–547
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*Papers marked with an asterisk have no the IIMCB affiliation of the authors

Description of Current Research

The unifying theme of our research is the relationship between the processes of intracellular membrane transport and signal transduction in response to extracellular stimuli. We would like to understand the molecular mechanisms by which endosomal compartments and their resident proteins play an active role in transmitting intracellular signals. To address these questions we use cultured mammalian cells as models and employ a variety of biochemical and microscopy methods, as well as cell-based functional assays.

An increasing number of studies, including our own, indicate that intracellular compartmentalization of signal transduction processes may play an important role in modulating the overall cellular response. It is well established that signals initiated at the plasma membrane, e.g. by binding of ligands to their receptors, are transmitted to the nucleus through the cytoplasm via a series of protein-protein interactions. At first, endocytosis was viewed merely as a mechanism for signal termination by downregulation of surface receptors and their degradation. However, more recent data strongly argue that endosomal compartments and their resident proteins play an important role in transmitting intracellular signals by transporting ligand-receptor complexes and affecting their activity inside the cell. Moreover, the relaying of signals from the plasma membrane via endosomes to the nucleus requires signal mediators to be transported between different cellular locations. Again, a growing number of clathrin adaptors and endosomal proteins are reported to undergo nucleocytoplasmic shuttling. This process is often based on intrinsic sequence motifs and requires active transport mechanisms. Endocytic proteins can associate with nuclear molecules, changing their localization and activity, and may modulate the levels and specificity of gene transcription. As a consequence, certain endocytic genes affect cell proliferation, act as tumour suppressors or change their expression in human cancers. We made an attempt to summarize current knowledge of nuclear functions of endocytic proteins in a recent review (Pilecka et al., 2007).

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. This raised a possibility that APPL-harbouring endosomes may represent a specialized endosomal compartment, potentially involved in signalling. Interestingly, APPL proteins can undergo nucleocytoplasmic

shuttling and interact with nuclear proteins, among them 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 trafficking and signalling pathway 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 confocal microscopy to characterize the properties of APPL endosomes and the transport pathways leading through them, in

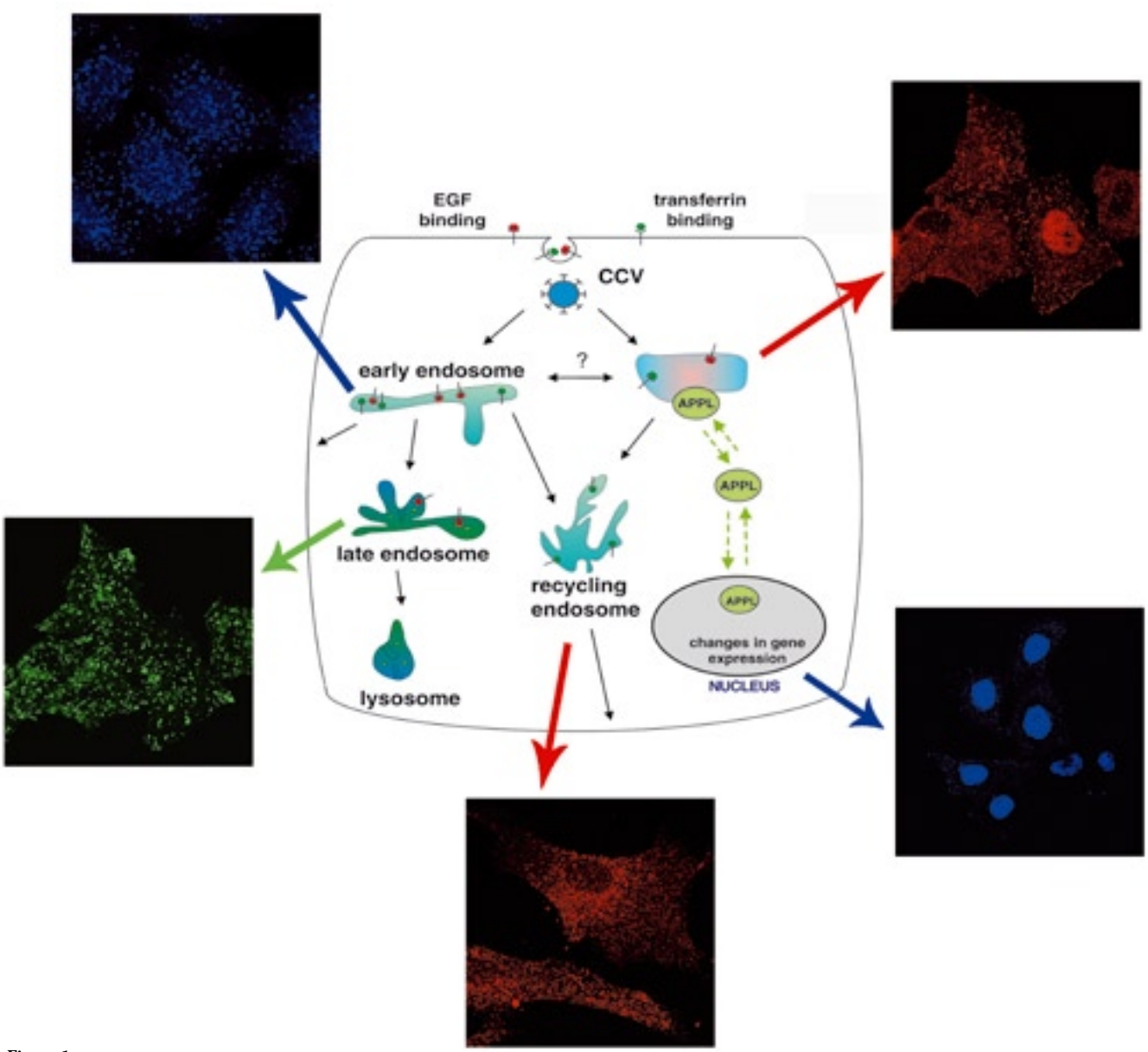


Figure 1.
Schematic representation of cargo trafficking pathways via APPL-positive endosomes – a working model.
APPL-harbouring compartment receives cargo directly from the plasma membrane and is capable of differential cargo sorting towards the canonical early and recycling endosomes. The exact flow of cargo between canonical early endosomes and APPL vesicles, indicated with the question mark, is being investigated. In addition to its endosomal localization, APPL proteins can undergo nucleocytoplasmic shuttling and interact with nuclear proteins, possibly modulating gene expression.
Microscopical panels indicate organelles stained for: EEA1 (canonical early endosomes), APPL1 (APPL-positive endosomes), Lamp1 (late endosomes), transferrin receptor (recycling endosomes) and MTA2 protein, a component of the NuRD complex (the nucleus).

comparison with the canonical early endosomes labelled by EEA1. The quantitative analyses of confocal images are performed in collaboration with Drs. Yannis Kalaidzidis and Marino Zerial (MPI Dresden).To this end, APPL endosomes were visualized by antibody staining and their morphometric features, such as average size of vesicles, their number and fluorescence intensity, were quantified using vesicle tracking algorithms developed by Dr. Kalaidzidis. By internalizing fluorescent cargo (transferrin destined for recycling, epidermal growth factor (EGF) destined for degradation) into HeLa cells using a pulse-chase protocol, a pattern of cargo transport through APPL endosomes was established. These studies demonstrate that both transferrin and EGF are trafficked through APPL and EEA1 compartments but with different kinetics. We concluded that APPL-positive membranes represent a distinct and stable subpopulation of early endosomes, receiving cargo directly from the plasma membrane and capable of differential cargo sorting towards the canonical early and recycling endosomes (Fig. 1). At the moment, we are extending these observations to determine the properties of APPL endosomes, such as their motility and the frequency of fusion events with other endosomal compartments, in living cells. This will be achieved by recording dynamics of APPL endosomes, in comparison with other endosomal markers or cargo, in HeLa cells. Cumulatively, these biochemical and microscopical studies should uncover 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) and we are searching for compartment-specific determinants localising APPL1 to various organelles. We have determined that APPL proteins undergo a number of posttranslational modifications and we are currently clarifying whether differential modifications could be responsible for the partitioning of APPL proteins into various pools. 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.

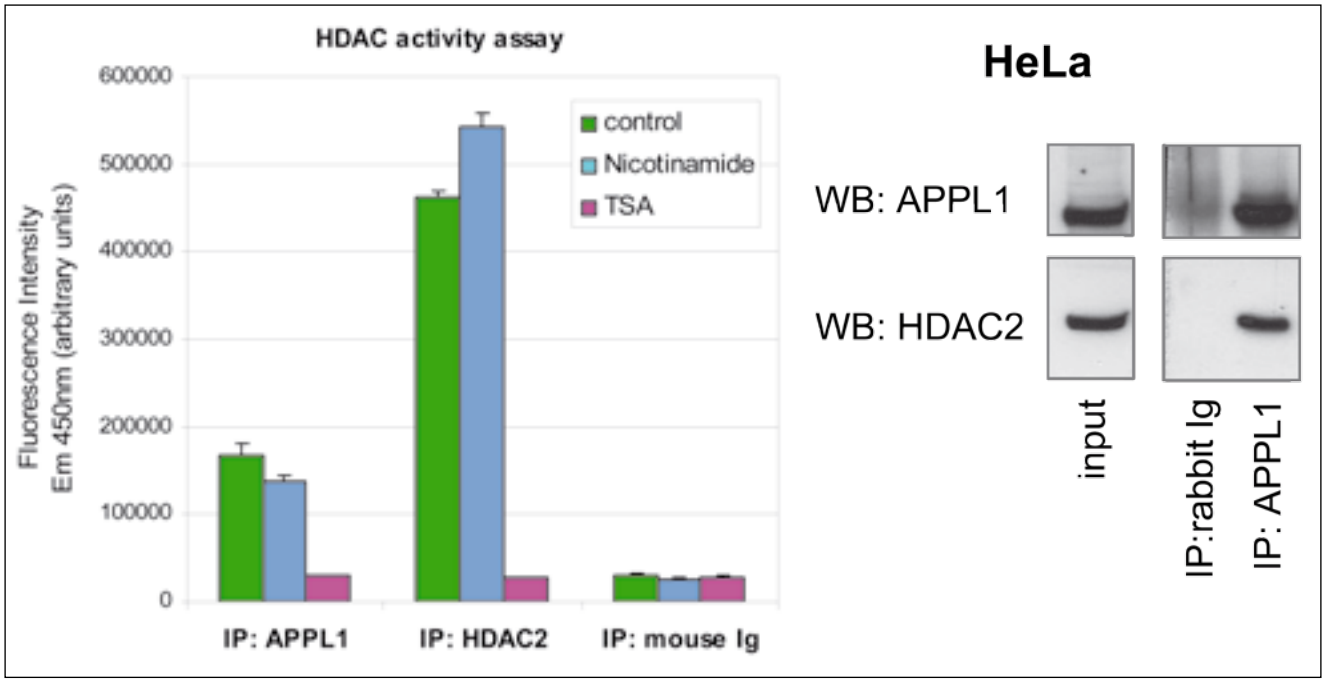


Figure 2.
Immunoprecipitates (IP) of APPL1 contain active histone deacetylase (HDAC) which is sensitive to Trichostatin A (TSA), an inhibitor of class I and II but not to nicotinamide (an inhibitor of class III HDAC), measured in a fluorimetric assay (left panel). Class I HDAC2 was used as a positive control. Western blot analyses, demonstrating the presence of HDAC2 protein in the immunoprecipitates of APPL1 are shown in the right panel.

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 pulldown). In particular, we are interested in the interaction of APPL proteins with the histone deacetylase and chromatin remodelling complex NuRD. This is a multiprotein complex involved in a number of processes, among them transcriptional silencing. We have confirmed that histone deacetylase (HDAC) activity of class I/II can be detected in the immunoprecipitates of APPL1 (Fig. 2). We are also clarifying 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 endocytic transport, including 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), in particular with the group of Prof. Carl-Henrik Heldin (Ludwig Institute for Cancer Research, Uppsala, Sweden). To this end, we have established methods for detecting the internalized platelet-derived growth factor (PDGF), both biochemically in cell extracts and under the microscope. A biochemical method is based on the quantification of internalized biotinylated PDGF, captured on a streptavidine-coated electrode (Fig. 3). Subsequent detection is performed employing the primary anti-PDGF antibody and a secondary antibody coupled with a ruthenium compound, capable of emitting light when electrochemically stimulated. The light emission is quantitatively measured using an electrochemiluminescence plate reader. The assay was established together with Dr. Patrick Keller from Meso Scale Discovery. We will employ this assay along with the microscopy-based colocalization analyses to measure the amount of endocytosed PDGF in cells under various conditions and to delineate its trafficking pathways. The ultimate goal is to establish whether and by which mechanisms the signal transduction downstream of PDGF depends on its endocytic trafficking.

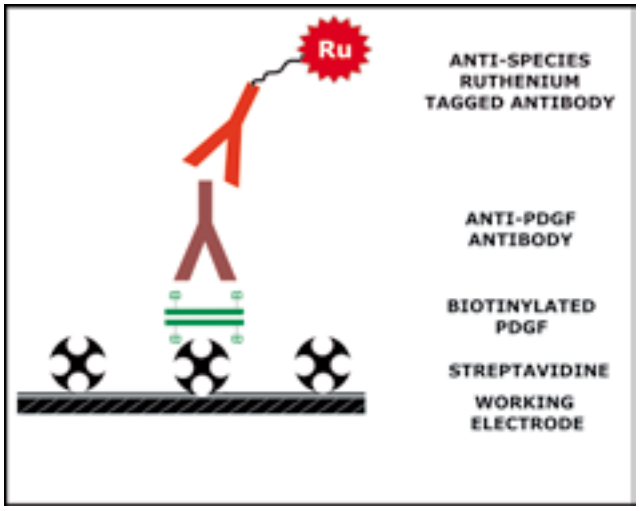
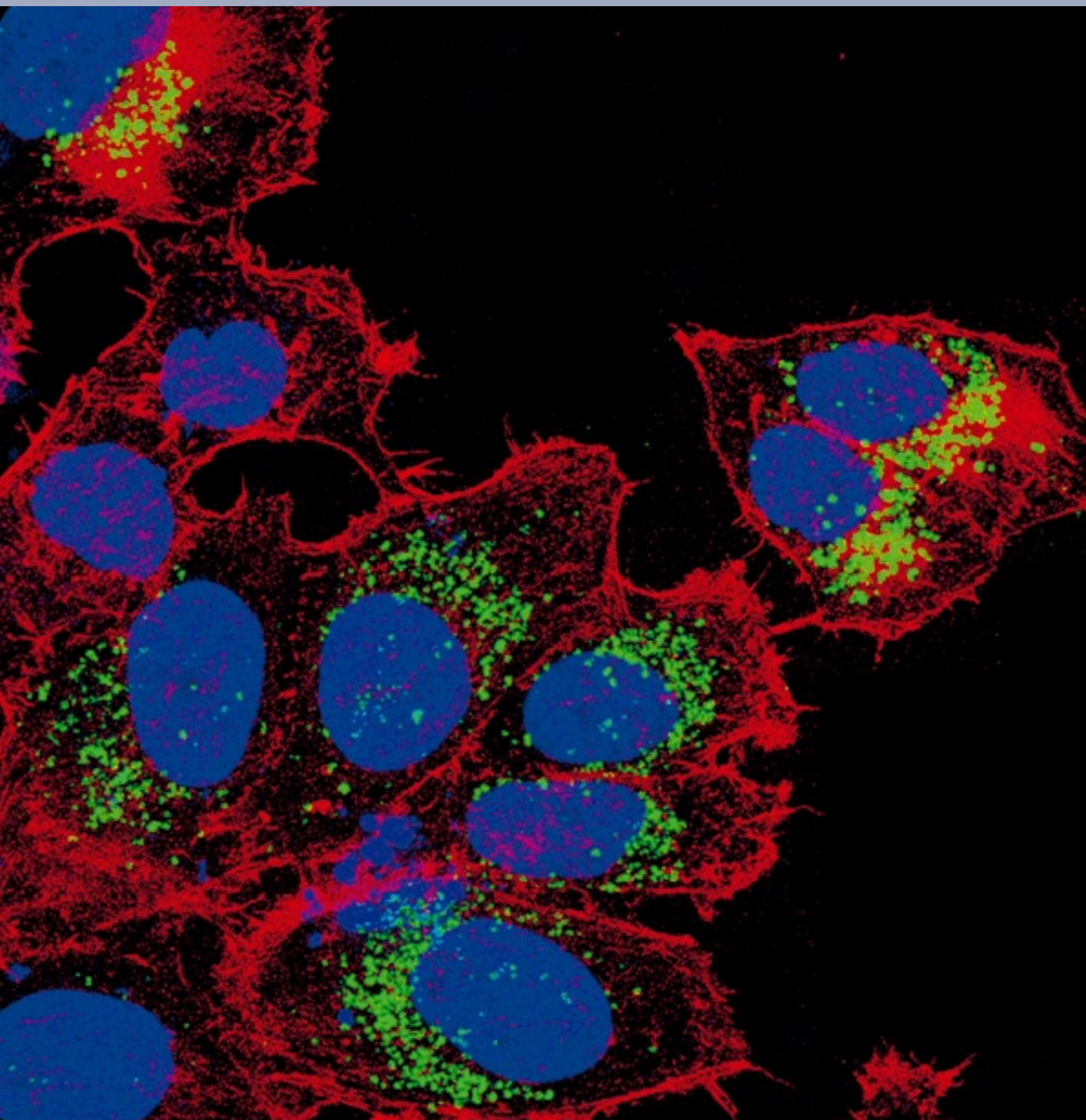


Figure 3.
Principle of an assay to detect biotinylated PDGF in cell extracts. See text for description.

Laboratory of Molecular and Cellular Neurobiology



Lab Leader

Jacek Jaworski, PhD

Post-doctoral Fellow
Matylda Macias, PhD

PhD Students
Anna Malik, MSc
Malgorzata Perycz, MSc
Lukasz Swiech, MSc

Undergraduate Students
Malgorzata Urbanska
Patrycja Pietruszka
Kamil Parobczak

Technician:
Monika Dudek

Picture on the left:
Increased autophagy in HeLa cells after mTOR inhibition visualized with LC3-GFP reporter protein (other channels: F-actin – red, DAPI – blue). mTOR regulates several cellular processes including transcription, translation and autophagy. Inhibition of mTOR leads to dramatic increase in autophagy in various cell types. Since disregulation of autophagy seems to be one of important factors in Poly-Q brain diseases, one of our aims is to find mTOR downstream effectors involved in this process.

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, research visit, one 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 (one month), research training

1997-2001 Laboratoire de Genetique Moleculaire de la Neurotransmission et des Processus Neurodegeneratifs (LGN.), Prof. J. Mallet, UMR 9923 CNRS, Paris, France (seven months total), research training

1996-2002 Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Prof. L. 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 The Prime Minister Award for PhD thesis

2001	Foundation for Polish Science National Scholarship for Young Investigators, one-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

- **Jaworski J**, Sheng M. The growing role of mTOR in neuronal development and plasticity, Mol. Neurobiol, 2006; 34:205-219
- *__Jaworski J__, Spangler S, Seeburg DP, Hoogenraad CC, Sheng M. Control of dendritic arborization by the PI3-kinase – Akt – mTOR pathway. J Neurosci, 2005; 25:11300-12
- *Dunah AW, Hueske E, Wyszynski M, Hoogenraad CC, **Jaworski J**, Pak DT, Simonetta A, Liu G, Sheng M. LAR receptor protein tyrosine phosphatases in the development and maintenance of excitatory synapses in hippocampal neurons. Nat Neurosci, 2005; 8:458-467
- *Chang CJ, **Jaworski J**, Nolan EM, Sheng M, Lippard SJ. A tautomeric zinc sensor for ratiometric fluorescence imaging: Application to nitric oxide-induced release of intracellular zinc. Proc Natl Acad Sci USA, 2004; 101:1129-34
- *__Jaworski J__, Mioduszewska B, Sanchez-Capelo A, Figiel I, Habas A, Gozdz A, Proszynki T, Hetman H, Mallet J, Kaczmarek L. Inducible cAMP Early Repressor (ICER), an endogenous antagonist of cAMP responsive element binding protein (CREB) evokes neuronal apoptosis in vitro. J Neurosci, 2003; 23:4519-26
- *__Jaworski J__, Biederman I, Lapinska J, Szklarczyk AW, Figiel I, Konopka D, Nowicka D, Filipkowski RK, Hetman M, Kowalczyk A, Kaczmarek L. Neuronal excitation-driven and AP-1-dependent activation of timp1 gene expression in rodent hippocampus. J Biol Chem, 1999; 274: 28106-12.

Publications in 2007

- **Swiech L, Perycz M, Malik A, Jaworski J**. Role of mTOR in physiology and pathology of the nervous system. Biochim Biophys Acta, 2008; 1784:116-132
- **Jaworski J**, Hoogenraad CC, Akhmanova A. Microtubule plus-end tracking proteins in differentiated mammalian cells. Int J Biochem Cell Biol, 2007; Epub ahead of print
- **Jaworski J**. ARF6 in the nervous system. Eur J Cell Biol, 2007; 86:513-524
- Michaluk P, Kolodziej L, Mioduszewska B, Wilczynski GM, Dzwonek J, **Jaworski J**, Gorecki DC, Ottersen OP, Kaczmarek L. Beta-dystroglycan as a target for MMP-9, in response to enhanced neuronal activity. J Biol Chem, 2007; 282:16036-41
- Mioduszewska B, **Jaworski J**, Szklarczyk AW, Klejman A, Kaczmarek L. Inducible cAMP early repressor (ICER)-evoked delayed neuronal death in the organotypic hippocampal culture. J Neurosci Res, 2007; 86:61-70
- 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; 62:359-362
- **Perycz M, Swiech L, Malik A, Jaworski J**. mTOR in physiology and pathology of the nervous system (in Polish). Postepy Biologii Komorki, 2007; 34: 511-526.

*Papers marked with an asterisk have no the IIMCB affiliation of the authors

Description of Current Research

The main scientific objective of the Laboratory of Molecular and Cellular Neurobiology is a role of the mTOR protein kinase in physiological brain development as well as in the course of neurodevelopmental disorders. We mostly focus 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 work demonstrated

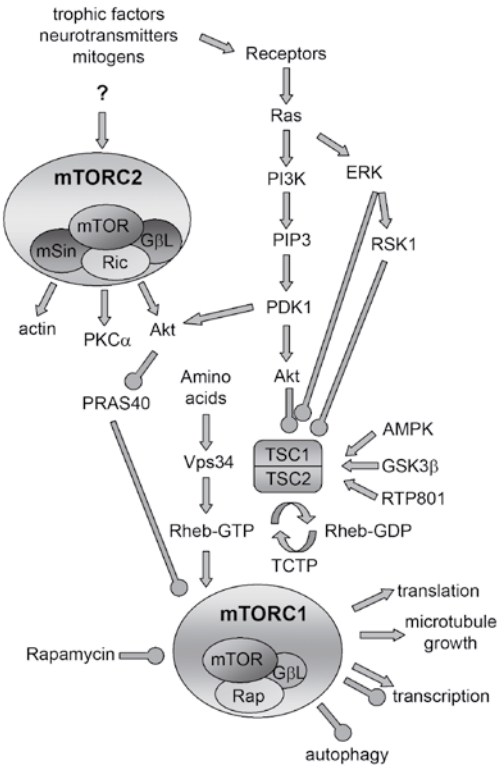


Figure 1. Schematic diagram of mTOR activity control. Stimulation of several receptors at the plasma membrane by mitogens, trophic factors and neurotransmitters leads to mTOR activation via Ras and class I PI3K- and ERK-dependent pathways. An increased level of amino acids (AA) induces mTOR activity in PI3K class III-dependent manner. AMPK, GSK3β and RTP801 inhibit mTOR activating TSC2. mTOR bound to Raptor protein forms the TORC1 complex that is responsible for the control of protein translation, transcription, autophagy and microtubule dynamics. mTOR forms also TORC2 complex with Rictor, which regulates actin dynamics and phosphorylate Akt and PKC. Ric – Rictor; Rap – Raptor; arrows and oval arrows – activation and inhibition of target protein or cellular process, respectively.

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. Its major role is to merge extracellular instructions with information about cellular metabolic resources and to control the rate of anabolic and catabolic processes accordingly (Fig. 1). Not surprisingly, this kinase controls cell size in both non-neuronal and neuronal cells. In neurons, however, the 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 (4EBP) and p70 ribosomal S6 protein kinase (p70S6K), which are important regulators of protein translation. In the context of mTOR involvement in local protein synthesis in neuronal dendrites, our recent data describing mTOR-4EB-P1 and p70S6 kinase involvement in dendritic branching raises an interesting question whether it is local or general mTOR signaling that 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 suggests that mTOR might be involved in cellular functions other than translation such as transcription, membrane turnover, mitochondrial function, autophagy and microtubule stability. However, in mammalian cells, mTOR forms two heteromeric and functionally distinct protein complexes called mTORC1 and mTORC2, respectively. mTORC1 is rapamycin-sensitive and consists of mTOR bound to Raptor. This complex is involved in the control of a wide variety of cellular processes discussed already above (see also Figure 1). On the other hand, rapamycin-insensitive mTORC2, containing mTOR and Rictor regulates actin cytoskeleton dynamics and controls the activity of two protein kinases – Akt and PKC (Figure 1). Taking into account the key role that mTOR plays in neuronal physiology, it is not surprising that under various neuropathological conditions mTOR signaling is disturbed. Changed mTOR activity has been reported in brain tumors, tuberous sclerosis, cortical displasia and neurodegenerative disorders such as Alzheimer's, Parkinson and Huntington diseases.

Yet, in cases of either physiological processes or neuropathology, our knowledge of molecular events downstream of mTOR is rather rudimentary. It raises a general question that should be answered first – what are the mTOR dependent proteins and cellular processes involved in the dendritogenesis process, and which of them are particularly disturbed in brain pathologies? In our quest to answer these questions our main goals are:

1) Identification of mTOR partners and regulated proteins involved in the process of dendritic branching.
2) Establishing a link between local protein translation and physiological dendritic arbor development.
3) Characterization of both mTOR-regulated cellular processes and local protein synthesis role in pathologies of central nervous system.

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

Our major effort towards identification of mTOR regulated proteins involved in dendritic arborization is to design siRNA library against mRNA encoding those proteins and perform a screen in neurons cultured in vitro. During the last two years, we have selected over 100 proteins potentially regulated by mTOR-Raptor complex, based on the bioinformatic approach, and have designed a library of siRNAs against all selected candidates. In 2007 we finished preparation of siRNA-pSUPER and pSUPER-GFP-plasmid based libraries. Each library consists of over 300 plasmids encoding individual siRNAs against selected genes (3 hairpins per sequence) and currently we are preparing high purity DNA to start library screens in neurons.

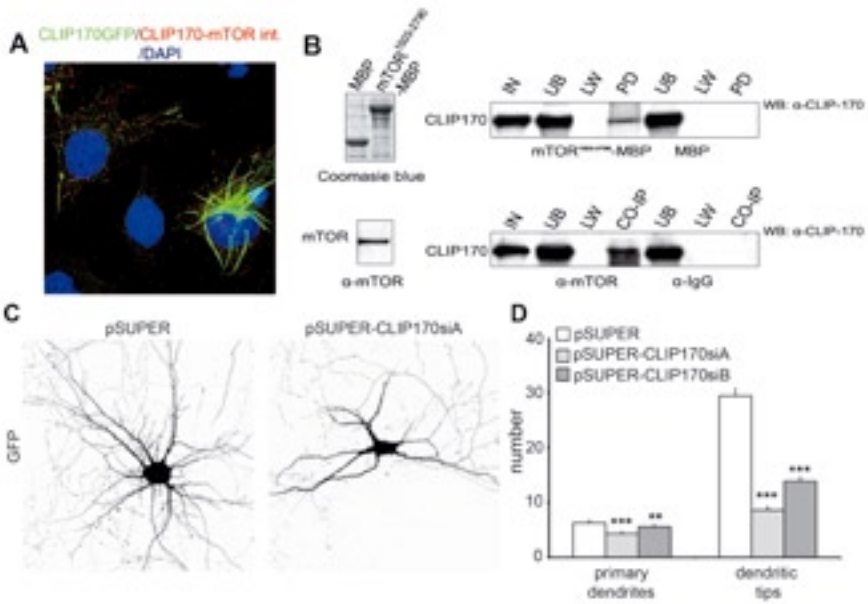


Figure 2. CLIP170 as potential target for mTOR in dendritogenesis. A) CLIP170 and mTOR interact directly in Hela cells. HeLa cells were transfected with GFP-CLIP170 and myc-mTOR. Direct interaction of those two proteins were visualized with use of Duolink system (Olink AB) (red chanel).B) CLIP170 and mTOR interact in rat brain protein extracts. CLIP170 was pulled down and co-immunoprecipitated from rat brain lysate with use of mTOR1503-2790 fragment tagged with MBP (upper panel) and antibody against mTOR, respectively (lower panel); IN - input, UB – unbound fraction, LW – last wash, PD – pull-down, CO-IP – co-immunoprecipitate, **C) Knock-down of CLIP170 in neurons results in dendritic arbor simplification.** Rat hippocampal neurons cultured for 7 days in vitro were transfected with siRNAs against CLIP170. GFP was contransfected for visualization of cell morphology. Cells were fixed 5 days after transfection. **D) Quantification of effects of siRNA mediated CLIP170 knock down on number of primary dendrites and dendritic tips.**

As an alternative strategy to siRNA library screen, we address the question of which cellular processes are controlled by mTOR during dendritic arbor development and stabilization, focusing on selected, already well described mTOR regulated proteins and mTOR interactors. Our studies on 4E-BP1 and protein translation can serve as an example of such approach. It is well documented that regulation of cytoskeleton dynamics is one of the crucial events for proper dendritogenesis. Since mTOR was shown to be important for cytoskeleton dynamics in non-neuronal cells, a possibility arises that it controls dendritogenesis also in this manner. mTOR is potentially one of the kinases capable of regulating CLIP170 activity. CLIP170 belongs to a group of microtubule plus-end tracking proteins (+TIPs) and is believed to regulate MT dynamics at plus-end during polymerization by promoting the rescue-phase. It was shown that activity of CLIP170 depends on its phosphorylation status. Our extensive research performed in 2007 shows that mTOR and CLIP170 can interact in non-neuronal cells as well as in brain extracts (Figure 2). Furthermore, inhibition of mTOR activity prevents full phosphorylation of CLIP170. Introduction of small interfering RNA (siRNA) against CLIP170 into rat hippocampal neurons in the dissociated and organotypic primary cultures resulted in the significant reduction of the number of dendrites, a decrease in the complexity of dendritic arbors and shrinkage of dendritic fields (Figure 2). Moreover, CLIP-170 knock-down exerts a strong effect on the shape of dendritic arbor even under conditions promoting dendritogenesis such as overexpression of constitutively active forms of PI3K and Akt kinases, which are crucial upstream components of mTOR signaling pathway. Taken together, these data strongly suggests the role of CLIP170 in the development of dendritic arbor, which may be regulated in mTOR-dependent manner.

So far, our research focused mostly on proteins regulated via mTORC1 complex (p70S6K, 4E-BP1, CLIP170), underinvestigating potential involvement of mTORC2 in dendritogenesis. Recently, we have separated activities of TORC1 and TORC2 by use of RNA interference-mediated Raptor and Rictor knockdown in developing rat hippocampal neurons in culture. Introduction of small interfering RNA against either one of those proteins into neurons resulted in the significant reduction of the complexity of dendritic arbor. Furthermore, negative effects of Rictor knockdown on dendritic arbor were reversed by over expression of dominant negative form of RhoA, strongly suggesting, that mTORC2 exerts its effect on dendrites by controlling actin dynamics.

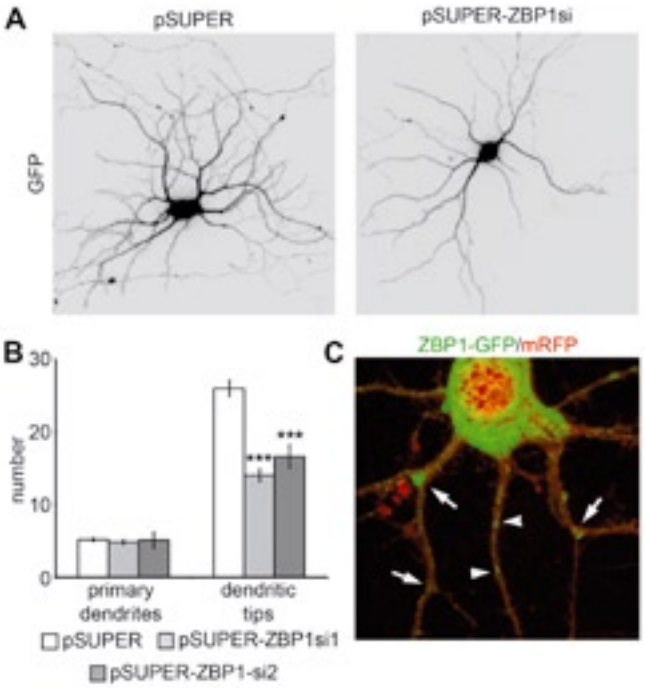
Establishing a link between local protein translation and physiological dendritic arbor development

To study a role of local protein translation in dendritic arbor development, we have proceeded with experiments aiming at knockdown of mRNA dendritic transport machinery that allow for selective silencing of local translation in dendrites. With use of siRNA technology we targeted major components of mRNA transport machinery such as β-actin zip-code binding protein 1 (ZBP-1) and Staufen in hippocampal neurons. ZBP1 plays a role in β-actin mRNA transport to the cell's periphery, including dendrites and axons, and by this means, enables local translation of actin and contributes to local cytoskeleton maintenance. In neurons expressing EGFP-ZBP1, this protein is characteristically distributed in close vicinity of dendritic branching points (Figure 3), which may suggest that local translation of actin is one of the bases for either new branch formation and dendritic tree development, or sustaining already existing ones and dendritic arbor stabilization. Indeed, ZBP-1 knockdown with short interfering RNAs results in a decrease in the total number of dendrites and dendritic tree complexity of rat hippocampal neurons cultured in vitro (Figure 3). These effects are reversed with two siRNA-resistant ZBP-1 mutants as well as by treatment with the actin polymerizing drug – jasplakinolide. We observed that ZBP-1 knockdown had bigger impact on younger neurons, actively developing dendritic arbors when compared to the cells transfected in

more matured cultures (two weeks in vitro). These data suggest that ZBP-1 dependent mRNA transport and most probably local translation of actin are indispensible for proper dendritic arborization of hippocampal neurons.

Characterization of both mTOR-regulated cellular processes and local protein synthesis role in pathologies of central nervous system

In addition to our main research activities described above, our group is involved in a research project with several Polish groups (Commissioned Grant of the Ministry of Science and Higher Education), with an 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). Among the hallmarks of the TSC that are brain related, are hypertrophy of neuronal cells and development of subependymal giant cell astrocytomas (SEGA, 5-15% of cases). Indeed, silencing tuberin at the early stage of neuron development (3-8 days in vitro) with short interfering RNA resulted in an increase in neuron soma size. Additionally, we observed increased number of primary dendrites and overall simplification of the dendritic tree. Interestingly, while inhibition of mTOR activity in transfected neurons with rapamycin reversed the increase in cell soma size, it did not affect changes in the dendritic arbor. Thus, our data suggests that dendritic branching might be regulated by tuberin independently of mTORC1, which is rapamycin-senstitive. Such alternative pathways may involve rapamycin-insensitive mTORC2, or tuberin targets distinct from Rheb, including other small GTPases – Rap1 and Rab5, or independent actions of hamartin. Currently, we are focusing our efforts on understanding the effects of tuberin knockdown in primary astrocytes and glioma derived cell lines. Most of the TSC patients have seizures (90% of affected individuals), and experimental evidence supports causative role of mTOR in these forms of epilepsy, since application of mTOR inhibitors in animal models and in clinic results in significant reduction of symptoms. So far, however, links of mTOR to other types of epilepsy are underinvestigated. Our initial data shows overactivation of mTOR in the rat hippocampus after kainic acid induced seizures that are one of the models of temporal lobe epilepsy. Since mTOR plays an important role in synaptic transmission, this observation suggests that mTOR might be involved in aberrant neuronal plasticity, one of the major causes of epileptogenesis.



Collaborative projects

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. Our research focused mostly on +TIP protein EB3. Together, we showed that growing microtubule plus-ends decorated by EB3 penetrate into dendritic spines and can modulate spine morphology. Inhibition of microtubule dynamics, as well as knockdown of either EB3 modulates spine shape by exerting an effect on the actin cytoskeleton. These data are further corroborated by our observation that knockdown of another +TIP – CLIP170 also leads to spine deterioration.

Finally, due to our group expertise in neuronal physiology and siRNA technology, we are involved in several collaborations at the IIMCB (Prof. J. Kuznicki; Dr. M. Miaczynska; Dr. U. Wojda, grant # N30110932/3854) and at the Ochota Campus (Prof. L. Kaczmarek; Dr. G. Wilczynski, grant NN301314733; Dr. W. Klopocka, grant # N303017933).

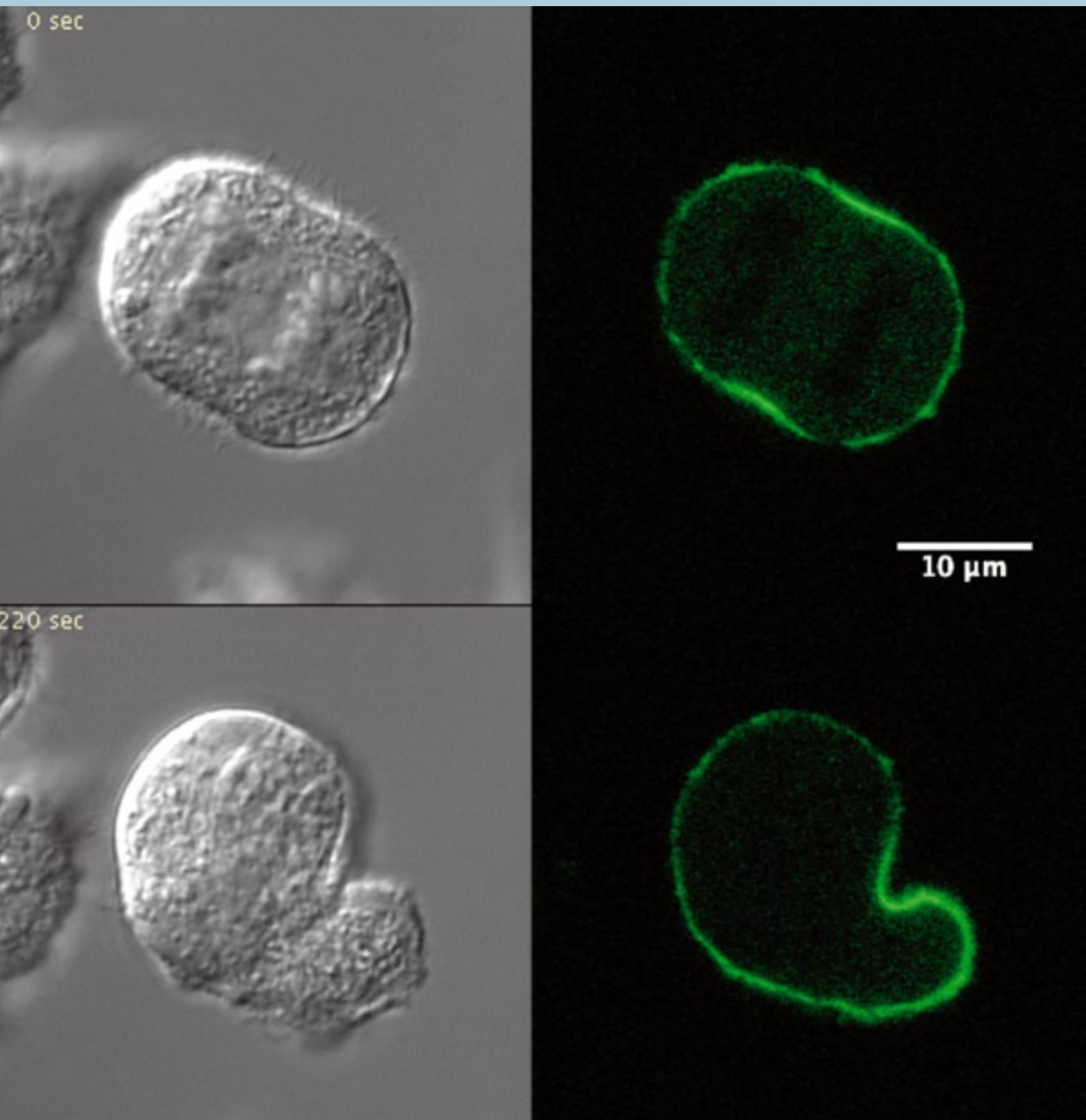
Our research plans for 2008 include:

- finalizing research on topics including involvement of Rictor, tuberin, CLIP170 and ZBP1 in dendritic branching of hippocampal neurons
- further investigation on the role of mRNA binding proteins: Staufen1, Staufen2 and hnRNPA in dendritogenesis
- launching into siRNA library screen in neurons
- conducting the kick off proteomic screens for mTOR interacting partners in neurons under physiological and pathological (epilepsy) conditions.

Figure 3. Role of dendritic mRNA transport in shaping neuronal dendritic arbor. A) Knock-down of ZBP-1 in neurons results in dendritic arbor simplification. Rat hippocampal neurons cultured for 7 days in vitro were transfected with siRNAs against ZBP-1, an β-actin mRNA binding protein. GFP was contransfected for visualization of cell morphology. Cells were fixed 5 days after transfection and number of primary dendrites and dendritic tips were analyzed. **B) ZBP-1 protein preferentially localizes to dendritic branching points.** Rat hippocampal neurons cultured for 7 days in vitro were transfected with ZBP1-GFP. mRFP was contransfected for visualization of cell morphology. Cells were fixed 2 days after transfection. Arrows point to ZBP1-GFP granules in dendrite branching points. Arrowheads show ZBP1-GFP in dendritic segments.

Laboratory of Cell Cortex Mechanics MPG/PAN

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



Lab Leader

Ewa Paluch, PhD

PhD Students:
Jakub Sedzinski, MSc
Maté Biro, MSc
Alba Diz Muñoz, MSc

MSc Student:
Ulrike Schulze, BSc

Technician:
Julia Roensch, BSc

MAX-PLANCK-GESELLSCHAFT

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY

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

Picture on the left:
Cytokinesis defect induced by laser ablation in a L929 fibroblast. Green: anillin-GFP.

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; 175:687-692
- *__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.

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, 2čme année, collection Puissance Prépas, publisher: Bréal (methods and corrected exercises for 2nd year Physics students

*Papers marked with an asterisk have no the IIMCB affiliation of the authors

Research

The cell cortex is a network of actin, myosin and associated proteins that underlies the plasma membrane and determines the shape of the cell body. The cortex enables the cell to resist externally applied stresses and to exert mechanical work. As such, it plays a role in normal physiology during events involving cell deformation such as mitosis, cytokinesis, and cell locomotion, and in the pathophysiology of diseases such as cancer where cortical contractility is upregulated. Despite its importance, little is known about how the cortex is assembled and regulated.

As the cortex is an intrinsically mechanical structure (its biological activity results from its ability to contract and to exert forces), its biological properties cannot be understood in isolation from its mechanics. The main focus of the group is to understand how these mechanical properties are determined by the molecular components of the cortex and how these properties are regulated, locally and globally, to allow the cell to undergo deformations such as the ingression of the cleavage furrow during cytokinesis. To this aim, the staff composed of biologists and physicists combine biophysical and molecular approaches. Our main lines of research are:

1. Role of cortical proteins in cortex mechanics

We aim to characterize the role of the various cortical components in cortex mechanics. For this we have chosen two readouts: cortical tension, which characterizes the cell mechanical state and cortex flows, which reveal cortex dynamics.

Cortical tension is measured by micropipette aspiration. We have shown that it is a well-defined quantity for a given cell line. We are currently assessing the effects of various cortical protein depletion on the tension of the cortex.

We have also developed an assay for the effect of protein depletion on cortical dynamics. When cortical contractility is enhanced by depolymerization of microtubules, suspension cells form a constriction ring, which oscillates across the cell with a well-defined period. These oscillations are driven by concerted flows of the cortex. Such flows result from gradients in cortical tension. Strikingly, similar flows have been observed during cytokinesis or during polarity proteins segregation in asymmetric cell divisions. We use the oscillations as a reporter of the cortex ability to dynamically contract and flow.

A long-term project will be to pin down the minimal ingredients necessary for cortex activity, paving the way for a biomimetic system of the cell cortex.

2. Blebs as a probe of cortex mechanics

We have shown that laser ablation of the cell cortex with a UV pulsed laser leads to the formation of a bleb, a spherical

membrane protrusion initially devoid of an actin cortex. After about 30 seconds, a cortex reassembles and the bleb retracts. Similar blebs are known to spontaneously form in cells during cytokinesis, migration and spreading. Bleb formation has been proposed to correlate with elevated levels of cortical tension.

We have taken advantage of the laser ablation-induced blebs to thoroughly study the relation between blebbing and tension. We have shown that bleb size and growth dynamics are indeed governed by the tension of the actomyosin cortex and have proposed a theoretical model that accounts for our observations (collaboration with the group of Prof. Joanny, Paris). Our findings support the view that bleb growth is driven by hydrodynamic pressure of the cytoplasm against the cell membrane, and that this pressure is generated by the contractile cortex.

3. Cortical flows and stability of the cytokinetic furrow

Strikingly, we have discovered that ablation of the actin cortex during cytokinesis leads to oscillations of the cleavage furrow and results in division failure. Several groups have observed similar furrow oscillations after depletion of different actin binding proteins. We are currently investigating the mechanism of this oscillation using both laser ablation and RNAi treatments. This will help in understanding how the cleavage furrow remains stable at the cell equator during normal division.

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

– the role of blebs and cortical dynamics during cell migration in tissues in developing embryos.

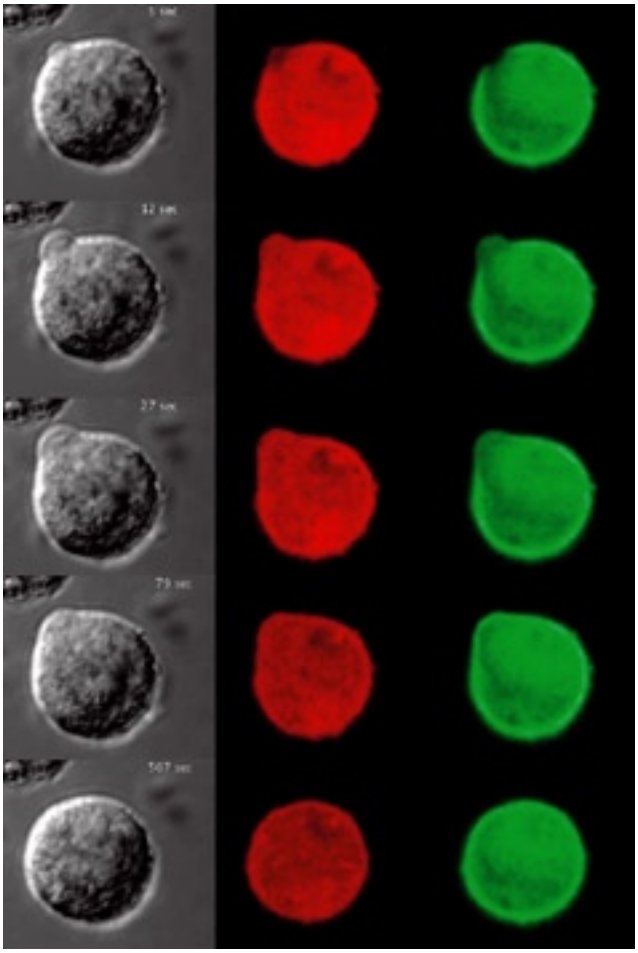
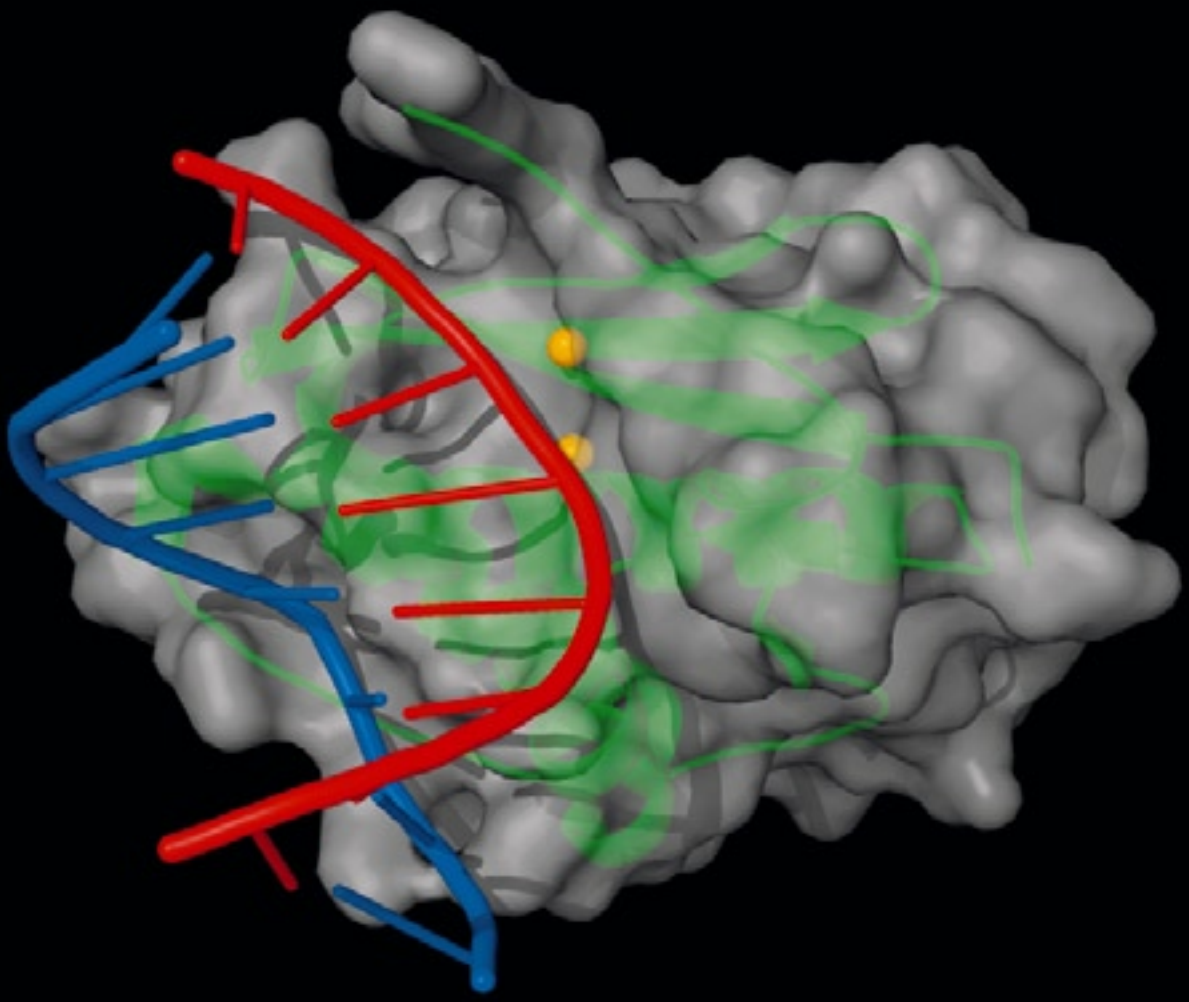


Figure 1.
Life cycle of a bleb induced by laser ablation in a L929 fibroblast.
Red: actin-RFP, green: myosin-GFP.

Laboratory of Protein Structure



Lab Leader

Marcin Nowotny, PhD

PhD Students:
Malgorzata Figiel, MSc
Paulina Klimek, MSc
Anna Tanska, MSc

Lab manager/Technician:
Magdalena Cybulska, MSc

Picture on the left:
Figure 1.
Structure of Bacillus halodurans RNase H1 in complex with RNA/DNA hybrid (RNA in red and DNA in blue). Two magnesium ions involved in catalysis are shown as yellow spheres. The protein is shown as cartoon and surface representations.

Marcin Nowotny, PhD

Degrees

PhD *magna cum laude* in biochemistry, Nencki Institute of Experimental Biology PAN, Department of Molecular and Cellular Neurobiology, Warsaw, 2002

MSc in organic chemistry and biochemistry, Warsaw University, Department of Chemistry 1998

Postdoctoral training

2003-2008 Postdoctoral Fellow, Wei Yang laboratory, National Institutes of Health, National Institute of Diabetes & Digestive & Kidney Diseases, Bethesda MD, USA

Professional Employment

Since 2008 Head of the Laboratory of Protein Structure, IIMCB

Honors, Prizes, Awards

2003 Prime Minister's award for PhD thesis

2001, 2002 Annual Stipend for Young Scientists of the Foundation for Polish Science

1999 Fellowship of the Kronenberg Bank Foundation

Selected publications

- *__Nowotny M__, Cerritelli SM, Ghirlando R, Gaidamakov SA, Crouch RJ, Yang W. Specific Recognition of RNA/DNA hybrid and Enhancement of Human RNase H1 Activity by HBD. EMBO J, 2008; in press
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- *__Nowotny M__, Yang W. Stepwise Analyses of Metal Ions In RNase H Catalysis: From Substrate Destabilization To Product Release. EMBO J, 2006; 25:1924-33
- *Yang W, Lee JY, **Nowotny M**. Making and Breaking Nucleic Acids: Two-Mg2+-ion Catalysis and Substrate Specificity, (review). Mol Cell, 2006; 22:5-13
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- *Filipek A, Jastrzebska B, **Nowotny M**, Kwiatkowska K, Hetman M, Surmacz L, Wyroba E, **Kuznicki J**. Ca2+-dependent translocation of the calcyclin-binding protein in neurons and neuroblastoma NB-2a cells. J Biol Chem, 2002; 277:21103-9
- *__Nowotny M__, Bhattacharya S, Filipek A, Krezel AM, Chazin W, **Kuznicki J**. Characterization of the interaction of calcyclin (S100A6) and calcyclin-binding protein. J Biol Chem, 2000; 275:31178-82.

*Papers marked with an asterisk have no the IIMCB affiliation of the authors

Description of Current Research

Our laboratory will focus on structural and biochemical studies of nucleic acid enzymes. Our primary method will be protein crystallography. Our projects can be subdivided into three groups:

1. Structural studies of substrate complexes of members of integrase superfamily.
2. Structural studies of reverse transcriptases.
3. Structural studies of UvrA DNA repair protein.

1. Integrase superfamily

Integrase superfamily (ISF) comprises an important and interesting nucleic acid processing enzyme family, containing transposases, integrases, and various nucleases. They are involved in a wide range of processes such as transposition, replication, and repair of DNA, homologous recombination and the action of siRNAs. One of the best characterized members of ISF is RNase H. It is a nuclease that binds RNA/DNA hybrids in a sequence, non-specific manner and degrades the RNA strand. Two types of RNases H have been identified – type 1 (RNase H1) and type 2 (RNase H2). Type 1 enzymes are present in all forms of life from bacteria to animals. They are also an integral part of reverse transcriptases. In HIV reverse transciptase the RNase H activity is essential for viral progression and is, at the same time, one of the least explored and most promising drug targets for the treatment of AIDS.

Type 1 RNase H is the only member of the ISF family for which the structures of its complexes with nucleic acids that are bona fide substrates for the enzymatic reaction are currently available (Nowotny et al. Cell 2005, Nowotny et al. Mol Cell 2007). The mechanism of RNA/DNA recognition was revealed and it was shown that the mechanism of catalysis relies on two metal ions. Members of ISF share the same fold of the catalytic core and very similar architecture of the active site, yet they act on a wide range of nucleic acids. In order to see how these different substrates are recognized and reveal the mechanism for their processing, we plan to solve crystal structures of substrate complexes of two ISF enzymes – RNase H2 and RuvC.

In biochemical properties, RNase H2 differs from type 1 enzyme. For example, it can cleave out single ribonucleotides embedded in DNA and is therefore thought to participate in DNA repair. Recently, it was shown that mutations in human RNase H2 result in Aicardi-Goutières syndrome (AGS) – an autosomal recessive genetic disorder with symptoms similar to in utero viral infection that severely affects the nervous system. The human enzyme is thus essential. The apo structures of bacterial and archeal are known but there is no structural information about substrate binding and cleavage. To reveal

these mechanisms we plan to co-crystallize RNase H2 with RNA/DNA hybrids and solve the structure of this complex.

RuvC cleaves Holliday junctions – 4-way DNA structures which are intermediates in homologous recombination. We would like to learn how specific binding of Holliday junctions is achieved and to reveal the molecular details of the assembled pre-reaction active site. We would also like to find out how the sequence-specific cleavage is achieved. To this end we plan to solve crystal structures of RuvC in complex with Holliday junctions. Together with known RNase H1 structures, crystallographic studies of RuvC and RNase H2 will allow us to generalize the mode of ISF members' action and to predict the detailed mechanism for such important enzymes as HIV integrase and Argonaute.

2. Reverse transcriptases

Reverse transcriptases are multifunctional enzymes catalyzing the conversion of single-stranded RNA to dsDNA. This process is essential for the life cycle of certain viruses e.g. retroviruses (HIV) or hepadnaviruses (hepatitis B virus). Although the RNase H domain of HIV RT is an important drug target, efforts to develop its efficient inhibitors failed. One line of our research will be to use novel approaches for the identification of the next generation of inhibitors of HIV RNase H. One of the main problems with known RNase H inhibitors is their lack of specificity. We will exploit the structural differences between human and HIV RNase H to find new inhibitors using the combination of Virtual Screening and protein crystallography.

Crystal structures of only two reverse transcriptases have been solved. Only for HIV RT the structures of its complexes with the nucleic acids are available. There is a significant variability of RT architecture between different viruses and several important aspects of the mechanism of RT action remain unclear, e.g. the way in which the polymerase and RNase H activities are coordinated. No structural information is available for hepatitis B virus RT (HBV RT) which is an important drug target. This enzyme cannot be produced in an active form in sufficient quantities to allow structural studies. Therefore, we plan to use bioinformatics to identify its close homologues, crystallize them and next solve their structures. Based on these structures an accurate homology model of HBV RT will be built in collaboration with the bioinformatics group of Dr. Janusz Bujnicki. We will also undertake co-crystallization experiments of these new RTs with their nucleic acid substrates. We hope to identify proteins that will readily form crystals with various nucleic acids corresponding to particular stages of reverse transcription. These snapshots will allow us to reconstruct the detailed mechanism of the reaction.

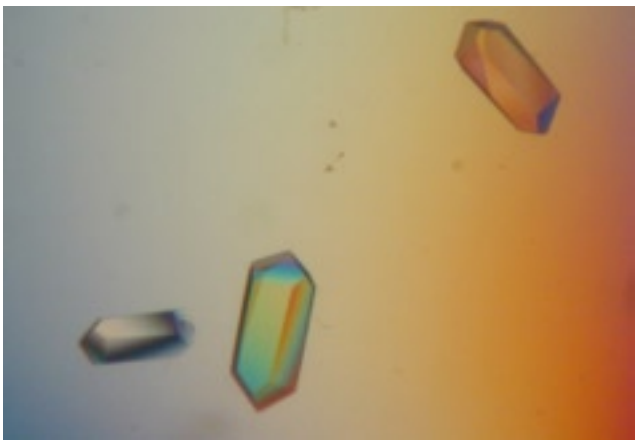


Figure 2.
Crystals of the apo form of Bacillus halodurans RNase H1.

3. Structural and biochemical studies of UvrA DNA repair protein

DNA molecules – the carriers of genetic information – are susceptible to chemical damage. One of the primary pathways to remove these modifications is nucleotide excision repair (NER), in which a stretch of bases harboring the lesion is cleaved out and the resulting gap is filled by a DNA polymerase. The remarkable feature of NER is the fact that it can act on a wide spectrum of unrelated DNA lesions, varying greatly in chemical structure. In bacteria one of its key components is UvrA protein which is thought to be the first to detect the DNA damage. It then recruits other components of NER. Recently, a crystal structure of apo UvrA has been reported but the detailed information about the mechanism of damaged DNA recognition is still lacking. By solving a crystal structure of UvrA with different types of damaged DNA we would like to learn how the remarkably wide specificity of NER system is achieved. We would like to reveal what features of different lesions are used by UvrA to recognize the damage. The enzyme contains two ATPase domains and ATP hydrolysis is essential for damage recognition. Co-crystallization of UvrA with ATP analogues, ADP and without the nucleotide should reveal the conformational changes during ATP hydrolysis and their consequences for DNA binding. These studies should help explain the central question in the DNA repair – the mechanisms of damage recognition.

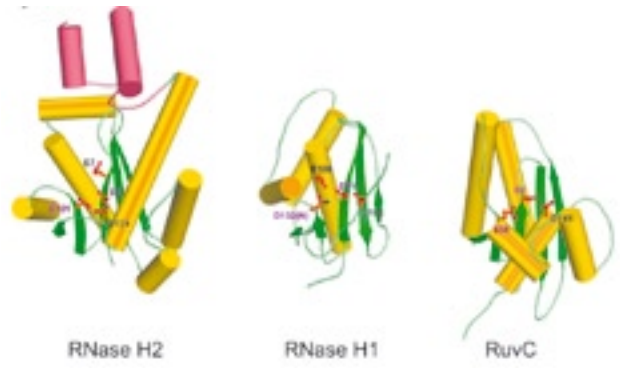
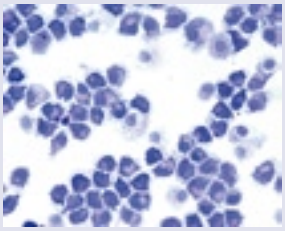


Figure 3.
Comparison of the structures of three members of integrase superfamily – RNase H1, RNase H2, and RuvC. The common secondary structure element – the central β-sheet is shown in green. The active site residues are shown as an orange ball-and-stick. Two carboxylates that are spatially conserved among the members of integrase superfamily are labeled in purple. PDB codes: B. halodurans RNase H – 1ZBI, A. fulgidus RNase H2 – 1I39, E. coli RuvC – 1HJR.

Other consortial projects coordinated at IIMCB

Molecular haematology project



The 2007 was a second year of running of the ministerial commissioned project *Advanced molecular methods in haematology. Work out and implementation of standards of minimal residual disease, posttransplant chimerism and marker translocations analysis.* The project with the overall budget of about 3 mln PLN links elements of pediatric and adult molecular haematology related to basic research and practical applications. The consortium is composed of the major Polish centers of molecular haematology in Poznan, Warsaw, Cracow, Lublin and Zabrze what constitutes the core of a future reference laboratories network active in this area in Poland. Haematological disorders selected for the study by the consortium are: pediatric acute lymphoblastic leukemia (ALL), pediatric and adult acute myeloid leukemia (AML), chronic myeloid leukemia (CML) and non-Hodgkin lymphomas (NHL); they were chosen as model disorders being already analysed on a molecular level in some of the Polish centers. A posttransplant chimerism and a minimal residual disease (MRD) are being analysed in connection to the disorders listed above. The project also aims at constructing the database linking available clinical and molecular data of patients recruited to the program and also on establishing the multicenter collection of relevant biological material. The program was initiated at IIMCB and is being conducted by Prof. Michal Witt and coordinated by Dr. Malgorzata Mossakowska.

Polsenior project

IIMCB was one of the major initiators of multidisciplinary projects on ageing and currently is a coordinator of the ministerial commissioned project *Medical, psychological and economical aspects of aging in Poland.* The largest country's project in this area of research with its budget over 12 mln PLN aims at conducting an interdisciplinary study of various aging-related problems. About 6000 patients will participate in a survey and over 1500 patients in 8 regions will be examined by geriatricians within the framework of this project. Biological material (DNA bank) will be deposited at IIMCB, also our Institute will provide the whole consortium with an database gathering all tests results. Four subprojects focus on a large-scale epidemiological studies, two on physical and architectural barriers for the old population. Specialists involved range from sociologists, psychologists, economists and demographers to geriatrists, cardiologists, nephrologists, neurologists, epidemiologists and molecular biologists. Final target is an ultimate definition of needs of aging population in terms usable in social policy, administrative care etc. that can shape future decision-making in this area. The project is conducted at the International Institute of Molecular and Cell Biology in Warsaw with Prof. Piotr Bledowski (President of Polish Gerontological Society) as a head of the project and Dr. Malgorzata Mossakowska as coordinator.

Educational Activities

Utrecht University International 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, former Rector of the Utrecht University, 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 doctoral thesis are being defended in Utrecht in front of the dissertation committee of the Utrecht Medical Center. As a result till now three students M. Bucko-Justyna

(M. Zylicz lab, IIMCB) in 2005, K. Starowicz (R. Przewlocki lab, Institute of Pharmacology PAN, Cracow) in 2006 and M. Olszewski (former Dastych's lab, IIMCB) defended their theses in a due course; the latter was defended in December 2007 on *Regulation of cytokine expression in mast cells: pro- and antiinflammatory potential,* with J. Dastych and E. Knol as co-promoters. Currently four students are still enrolled in the program: M. Geremek (M. Witt lab, IIMCB and Institute of Human Genetics PAN, Poznan: *Genetic analysis of primary ciliary dyskinesia/Kartagener Syndrome [PCD/KS]),* M. Lukowiak (A. Lipkowski 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 lab, Nencki Institute PAN, Warsaw: *Role of MMP-9 in neuronal plasticity*), Jakub Urbanski (M. Zylicz lab, IIMCB: *Molecular chaperones in tumor invasiveness*). IIMCB is a general coordinator of the entire program on the Polish site.

Postgraduate School of Molecular Medicine (SMM)

(www.iimcb.gov.pl/smm.php)

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 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 the 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. Ten groups of students were accepted during the period of 1998-2007, including six foreign individuals.

Successful candidates accomplish their scientific program, under the supervision of their tutors, in home laboratories throughout Poland. The members of the SMM Scientific Council evaluate student progress annually. The tutorial program offered to the students includes theoretical (lectures and 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, the 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) and National Center for Scientific Research (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 2007, the following courses were organized:

- Theoretical course "Glycobiology", 12-16.02.2007, Kracow, organized by Prof. Claudine Kieda (CNRS, France and SMM) and Prof. Jozef Dulak (Collegium Medicum, Jagiellonian University) – open to all SMM students.
- SMM Spring School lecture course "From gene to protein, from structure to function and dysfunction", 16-20.04.2007, Warsaw. This annual course, obligatory for first-year students, was organized by prof. Liliana Konarska. The lectures were given by twenty-six outstanding scientists and academic teachers from the top clinical and research institutions in Poland.
- Practical course "Scientific communication", 14-18.05.2007, 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 laboratory course "Molecular methods applied in medicine", 25-29.06.2007, Poznan, outsourced at the Summer School "Progress in Molecular Biology"- obligatory for first-year students.
- 9th Annual Inaugural and Research Report SMM Session, 15-16.10.2007, Warsaw, organized by Prof. Liliana Konarska, Medical University of Warsaw and SMM students. Inaugural lecture "Cancer Biomarkers: from Genomics to Proteomics. The Example of Menin, a Nuclear Oncosuppressor Protein at the Cross-road Between Regulation of Transcription and Genome Stability" was given by Prof. Patrick Gaudray (FRE CNRS et Universite Francis Rabelais, Tours). During the session, 26 SMM students presented their research results obtained during the academic year 2006/2007, and 6 new students presented their research projects. The presentations were divided into seven subsessions: protein engineering and biomodelling, molecular diagnosis of cancer and human disorders, neurobiology, immune system in disease, cancer cell biology and signaling, miscellaneous and new project presentations.
- 7th Integrated course "Molecular medicine in the diagnosis and treatment of diabetes", 4-6.10.2007, Lodz, organized by SMM and Medical University of Lodz. This course, directed to second, third and fourth-year students was organized by Prof. Liliana Konarska and Prof. Wojciech Mlynarski. Over fifty physicians, researchers and PhD students attended the course consisting of the symposium, workshop and clinical sessions.

Currently SMM is in the process of general changes in its organizational status. Final new arrangements (affiliation, rules of operation, etc.) will be completed in 2008.

9th Annual Inaugural and Research Report SMM Session, 15-16.10.2007, Warsaw

Science Festival School (SFN) Popularization of Science

(current name: Centre for Innovative Bioscience Education)

Science Festival School – Society meetings with Science

The aim of the Science Festival School (SFN) is 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 awareness of biology in society. The co-founders of the Science Festival School are four institutions: International Institute of Molecular and Cell Biology (IIMCB), Nencki Institute of Experimental Biology PAN (IBD), Institute of Biochemistry and Biophysics PAN (IBB), Warsaw University of Life Sciences (SGGW) and Warsaw Science Festival. IIMCB hosts SFN laboratory, office and administration. Additionally, Science Festival School leads another laboratory at Warsaw University of Life Sciences. In 2007, total number over 1500 young participants visited laboratory workshops. At the same time, over 100 biology teachers and about 800 students attended our lectures.

The Price of Polish Society of Genetics

The award of the Polish Society of Genetics was given to the Science Festival School for popularizing modern biology in the period of 2004 – 2006, specifically for developing interest of young students in biology and for encouraging teachers to incorporate molecular biology into biology school curricula.

Researchers' Night 2007

'European Researchers' Night' is a pan-European event involving a wide range of scientific and research organizations – including museums, laboratories and academic institutions – hosting a variety of entertaining and fun events planned to run late into the Friday night. The aim is to give the public, and in particular young people, the opportunity to meet researchers within the context of festive and 'fun' activities and to highlight the appeal of pursuing a career in research. The 2007 Researchers' Night, held on 28 September was a big success. Within the framework of the Polish edition Science Festival School had organized two events, first for broad public, second a workshop for journalists.

The name of the first event proposed by Science Festival School was "Fair – let's like Europe". During this event we had been presented results of the project named Volvox. One of the main goals of Volvox is to invent new and interesting simple biology experiments for schools and exchange of these ideas among all partners. Each of participating countries had its own exposition booth where experiments developed in this particular country were presented. The second important aim of Volvox project is to introduce basics of scientific thinking and methodology in the classroom.

During the event we presented related teaching materials with a presentation of the cultural, scientific and educational offerings, with a special focus on scientific achievements of the leading scientific centers and opportunities for higher education for foreigners, of each of grant partner countries. These materials we obtained from embassies of countries such as: UK, Denmark, Portugal, Italy and Germany. The event organized by Science Festival School was also supported by one of the oldest sweets factory's in Poland "Wedel".

The workshop for specific target group: journalists

One of the major aims of the event was to prove that even complicated knowledge can be delivered to the audience at adequate level. Topics related to DNA technology that are often misunderstood by journalists were presented, such as the structure of DNA, the flow of information from DNA to proteins, technologies to utilize information contained in DNA, etc.

Ist Scientific Workshop "DNA – the Encyclopaedia of Life"(25-29 April 2007)

Students from the Biotechnology Department of the University of Life Sciences, the Science Festival School and the Foundation of BioEducation organized in Warsaw several meetings in form of lectures, discussions and workshops, dedicated to genetics, biotechnology and molecular biology. During five days over 2400 people from all over Poland participated in this event.

XIth Science Picnic (25 May 2007)

Like in previous years, BioEducation Foundation and Science Festival School organized exhibition and science show. The year 2007 motto was "The mathematics and we".

- The chocolate challenge – how to investigate and to compare the taste between people
- The mystery of butterflies in a meadow – the basics of statistics for non biologists
- Family genetics – the basics of inheritance
- Isolation DNA from onion and how to explore power of your enzymes

XIth Warsaw Science Festival (22-30 September 2007)

The goal of the Science Festival is to make people to realize that the future of our country depends on our ability to use achievements of science as well as their practical applications in our social life. The Science Festival is organized by scientists, not by government. About 140 scientific institutes and about 1000 scientists are involved every year in organization of this event. The Science Festival School in 2007 organized such events as:

- Laboratory workshop: Explore your own DNA – **Joanna Lilpop, Michal Mlacki**
- Laboratory workshops: "Do you know, what you eat?" – **Monika Ostaszewska** and **Justyna Wasil**
- Laboratory workshop: "The shared history – evolution recorded in genes" – **Damian Graczyk, Agnieszka Choluj, Michal Mlacki**
- "The meeting with biology" – XI Science Festival in Jabłonna Palace **Agnieszka Choluj**

Laboratory workshops

The participants of workshops performed real live experiments and learned how to use laboratory techniques and equipment. The practical experiments were supported by lectures presenting the theoretical basis of molecular biology, genetics and its techniques. The workshops were one day events covering such topics as:

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

Courses for biology teachers

During our workshops for teachers we try to build a connection between them and scientists so they can feel being part of science community. Since we strongly encourage teachers to implement practical protocols at schools, we not only train them but also equip them with classroom scenarios

and affordable experimental kits that can be used at school laboratories. In 2007, as a part of teachers' education following events were organized:

- *"Modern biology – challenge for biology teachers" 12-13.X.2007*
- *"The faces of modern biology" 9-10.XI.2007*
- *"The VIth conference for biology teachers" 1.XII.2007*
- *"Modern biology in school" 14-15.XII.2007 and 8-9.III.2007*

Laboratory training for talented secondary school students

Two weeks laboratory training for gifted secondary school student was organized during 2007 summer holidays. Katarzyna Drzewicka joined for two weeks two research groups in IIMCB: Laboratory of Cell Biology and Laboratory of Molecular and Cellular Neurobiology. SFN organized initial training in laboratory practice.

Educational projects:
"VOLVOX" project under FP6, activities in 2007

Science Festival School started in 2005 the implementation of the Volvox Specific Support Action project funded by the European Commission within FP6 and 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 9 partners from Denmark, Estonia, Germany, Italy, Luxembourg, Poland, Portugal, Sweden and UK. New attractive resources should encourage more young people to develop positive attitudes towards studying science and to consider a scientific career.

During the third year of the operation of Volvox project, Science Festival School organized a small meeting to plan the project's web sites. The bulk of Volvox's educational resources will become available towards the end of the project. Volvox now has a substantial body of materials to be offered to teachers. The purpose of the meeting was to determine the requirements that partners would have to meet, to define the overall tone of the sites and to describe their appearance, and to determine the functionality that would be required to meet the needs of teachers and other users of the sites.

The Science Festival School in 2007 developed such new protocols as: amylase; urease; PCR of D1S80 DNA, how to design a scientific experiment and a comparative fish dissection. We also translated several partner's protocols into Polish.

Cooperation with a European journal for teachers "Science in School"

Workers and co-workers of the Science Festival School translated almost all biology-based articles (10 from 11), which are published on the web site ***http://www.scienceinschool.org/polish***. Science in School aims to promote inspiring science teaching by encouraging communication between teachers, scientists, and everyone else involved in European science education. It addresses science teaching both across Europe and across disciplines: highlighting the best in teaching and cutting-edge research. It covers not only biology, physics and chemistry, but also mathematics, earth sciences, engineering and medicine, focusing on interdisciplinary work.

The contents include teaching materials; cutting-edge science; education projects; interviews with young scientists and inspiring teachers; European education news; reviews of books and other resources; and announcements about European events for teachers. The journal is available free online where articles are published in several European languages. Also free printed English version is distributed across Europe.

Open lectures

Besides our offer for schools and teachers, in 2007 SFN presented theoretical issues of modern biology to the more general audience. Every two weeks during a summer semester and once a month during a winter semester, SFN organized open lectures on modern biology given by top Polish scientists. These lectures were accessible to everyone with basic knowledge of biology. Among topics were: genomics, evolution, genetic diseases, genetically modified organisms, gene expression regulation, immunology, stem cells. In 2007 we presented two Nobel Prize lectures: "siRNA – small molecule but big hope. The express Nobel XXI century" given by Dr. Jacek Jaworski and "Why embryonic stem cells – Nobel 2007" by Prof. Maria Anna Ciemerych–Litwinienko. In 2007 the lectures attracted audience of 500 people.

Staff at IIMCB

(as of April 2008)

Administration

Name		Funding
Jacek Kuznicki	Director	IIMCB
Michal Witt	Deputy Scientific Director	IIMCB(1/2)
Jaroslaw Filinski	Deputy Administrative Director	IIMCB
Hanna Iwaniukowicz	Financial Manager	IIMCB
Monika Nowicka	Payroll Specialist	IIMCB
Renata Knyziak	Accounting Specialist	IIMCB
Sylwia Adamiec	Accounting Specialist	IIMCB
Krystyna Domanska	Human Resources Specialist	IIMCB
Beata Tkacz	Human Resources Specialist	IIMCB
Urszula Bialek-Wyrzykowska	International Cooperation Manager	IIMCB(1/2)
Dorota Wasiak-Libiszowska	Foreign Grants Specialist	IIMCB
Magdalena Glogowska	PR Specialist	IIMCB
Agnieszka Wagner-Ziemka	Planning and Reporting Specialist	IIMCB
Agnieszka Karbowska	Tender Specialist	IIMCB
Iwona Wiesik	Director's Assistant	IIMCB
Monika Kacprzak	Secretary	IIMCB
Marcin Biedacha	IT Manager	IIMCB
Jakub Skaruz	IT Specialist	IIMCB
Robert Banasiak	Maintenance Specialist	IIMCB

Department of Molecular Biology

Name		Funding
Maciej Zylicz	Head	IIMCB
Alicja Zylicz	Vice Head	IIMCB
Marcin Klejman	Research Assistant	IIMCB
Pawel Bieganowski	Research Assistant	IIMCB/Ministerial grant
Maciej Olszewski	Research Assistant	Ministerial grant
Leszek Lipinski	PhD Student	IBB
Magdalena Gutkowska	PhD Student	IIMCB/Nencki PhD School
Dawid Walerych	PhD Student	Ministerial grant
Marta Frankowska	PhD student	IBB PhD School/Ministerial grant
Anna Zurawska	PhD Student	IBB PhD School/Ministerial grant
Jakub Urbanski	PhD Student	Utrecht Fellowship
Grazyna Orleanska	Secretary	IIMCB
Pawel Krawczyk	Undergraduate Student	Volunteer

Laboratory of Bioinformatics and Protein Engineering

Name		Funding
Janusz M. Bujnicki	Head	IIMCB
Krzysztof Skowronek	Research Coordinator	IIMCB
Kristian Rother	Post doctoral Fellow	EU grant
Michal Boniecki	Post doctoral Fellow	Ministerial grant
Malgorzata Durawa	PhD Student	Ministerial funds*/IBB PhD School
Marcin Feder	PhD Student	NIH UCLA grant
Andrzej Kaminski	PhD Student	NIH WSU grant/IBB PhD School
Jan Kosinski	PhD Student	NIH WSU grant/IBB PhD School
Grzegorz Papaj	PhD Student	Ministerial grant/IBB PhD School
Sebastian Pawlak	PhD Student	Ministerial funds*/IBB PhD School
Marcin Pawlowski	PhD Student	IIMCB/IBB PhD School
Elżbieta Purta	PhD Student	Ministerial funds*/IBB PhD School
Karolina Tkaczuk	PhD Student	NIH WSU grant
Irina Tuszynska	PhD Student	Ministerial grant
Katarzyna Filip	PhD Student	Ministerial grant/IBB PhD School
Agnieszka Obarska-Kosinska	PhD Student	Ministerial funds*/IBB PhD School
Jerzy Orlowski	PhD Student	NIH UCLA grant/IBB PhD School
Agata Kamaszewska	PhD Student	Ministerial grant/IBB PhD School
Dariusz Pianka	PhD Student	Ministerial grant/IBB PhD School
Wojciech Potrzebowski	PhD Student	Ministerial grant/IBB PhD School
Wojciech Siwek	PhD Student	Ministerial funds*/IBB PhD School
Maria Werner	PhD Student	Ministerial grant/IBB PhD School
Lukasz Kozlowski	Research Assistant	NIH UCLA grant
Ewa Tkalinska	Research Assistant	Ministerial grant
Jan Kaczynski	Msc Student	Volunteer
Katarzyna Kaminska	Msc Student	Volunteer
Pawel Lukasz	Msc Student	Volunteer
Konrad Tomala	Msc Student	Volunteer
Agata Parysz	Msc Student	Volunteer
Krzysztof Nawara	Msc Student	Volunteer
Natalia Kalina	Office Manager	Ministerial grant
Jan Kogut	Computer Administrator	NIH WSU grant
Tomasz Jarzynka	Computer Administrator	Volunteer
Lukasz Munio	Computer Administrator	Ministerial grant

Laboratory of Structural Biology MPG/PAN

Name		Funding
Matthias Bochtler	Head	Max Planck Society/Cardiff University
Izabela Sabala	Post doctoral Fellow	EU grant/Ministerial funds*
Honorata Czapinska	Post doctoral Fellow	Ministerial grant
Renata Filipek	Post doctoral Fellow	EU grant/Ministerial funds*
Aneta Kaczmarczyk	Post doctoral Fellow	Ministerial grant
Malgorzata Firczuk	Post doctoral Fellow	Max Planck Society
Grzegorz Chojnowski	PhD Student	Warsaw University
Henryk Korza	PhD Student	EU grant/Ministerial funds*
Magdalena Lipka	PhD Student	EU grant/Ministerial funds*
Monika Sokolowska	PhD Student	Max Planck Society
Roman Szczepanowski	PhD Student	Max Planck Society
Magdalena Kaus-Drobek	PhD Student	Max Planck Society/Nencki PhD School
Marek Wojciechowski	PhD Student	Max Planck Society/Nencki PhD School
Dario Piano	PhD Student	EU grant

*Ministerial matching funds to EU grant

Laboratory of Biomodelling

Name		Funding
Slawomir Filipek	Head	IIMCB
Krzysztof Jozwiak	Post doctoral Fellow	Ministerial grant
Michal Kolinski	PhD Student	IIMCB/IBB PhD School
Aleksander Debinski	PhD Student	Warsaw University
Wojciech Pulawski	PhD Student	IIMCB/IBB PhD School
Krystiana Krzysko	PhD Student	Volunteer
Anna Zwolinska	MSc Student	Volunteer
Krzysztof Mlynarczyk	MSc Student	Volunteer

Laboratory of Neurodegeneration

Name		Funding
Jacek Kuznicki	Head	IIMCB
Urszula Wojda	Associate Professor	IIMCB
Monika Klejman	Post-doctoral Fellow	Ministerial funds*
Marta Wisniewska	Post-doctoral Fellow	EU grant
Joanna Gruszczynska	Post-doctoral Fellow	Polish-German grant
Anna Skibinska - Kijek	Post-doctoral Fellow	EU grant
Emilia Bialopiotrowicz	PhD Student	Ministerial grant/Nencki PhD School
Lukasz Bojarski	PhD Student	IIMCB/Nencki PhD School
Katarzyna Debowska	PhD Student	Ministerial grant/Nencki PhD School
Wojciech Michowski	PhD Student	Nencki PhD School
Katarzyna Misztal	PhD Student	Ministerial funds*/Nencki PhD School
Adam Sobczak	PhD Student	IIMCB/Nencki PhD School
Aleksandra Szybinska	PhD Student	IIMCB
Miroslaw Drab	MSc Student	Volunteer
Kamila Skieterska	MSc Student	Volunteer
Bozena Zebrowska	MSc Student	Volunteer
Dominika Dubicka	Office Manager	Ministerial funds*

Laboratory of Cell Biology

Name		Funding
Marta Miaczynska	Head	Wellcome Trust grant
Iwona Pilecka	Post-doctoral Fellow	Wellcome Trust grant
Magdalena Banach-Orlowska	Research Assistant	Wellcome Trust grant
Beata Pyrzynska	Post-doctoral Fellow	HHMI grant
Sajid Rashid	Post-doctoral Fellow	EU grant
Marta Olchowik	PhD Student	HHMI grant/Nencki PhD School
Anna Urbanska	PhD Student	IIMCB/Nencki PhD School
Anna Hupalowska	PhD Student	EU grant/Nencki PhD School
Lukasz Sadowski	PhD Student	EU grant/Nencki PhD School
Anna Torun	Trainee	Volunteer
Michal Mlacki	MSc Student	Volunteer

Laboratory of Molecular and Cell Neurobiology

Name		Funding
Jacek Jaworski	Head	IIMCB
Magda Blazejczyk	Post-doctoral Fellow	EU grant/Nencki
Matylda Macias	Post-doctoral Fellow	EU grant/Nencki
Anna Malik	PhD Student	Ministerial grant/Nencki PhD School
Malgorzata Perycz	PhD Student	IIMCB/Nencki PhD School
Lukasz Swiech	PhD Student	IIMCB/Nencki PhD School
Malgorzata Urbanska	PhD Student	Volunteer
Patrycja Pietruszka	MSc Student	Volunteer
Kamil Parobczak	MSc Student	Volunteer
Malgorzata Zarebska	MSc Student	Volunteer

*Ministerial matching funds to EU grant

Laboratory of Protein Structure

Name		Funding
Marcin Nowotny	Head	Wellcome Trust grant
Malgorzata Figiel	PhD student	Ministerial grant
Paulina Klimek	PhD Student	IIMCB/EMBO grant
Anna Tanska	PhD Student	IIMCB/EMBO grant
Magdalena Cybulska	Lab Manager/Technician	Wellcome Trust grant

Laboratory of Cell Cortex Mechanics MPG/PAN

Name		Funding
Ewa Paluch	Head	Ministerial grant
Jakub Sedzinski	PhD Student	Ministerial grant
Maté Biro	PhD Student	Ministerial grant
Alba Diz Muñoz	PhD Student	Ministerial grant
Ulrike Schulze	MSc Student	Volunteer
Julia Roensch	Technician	Ministerial grant

Molecular Haematology Project

Name		Funding
Michal Witt	Project Director	IIMCB
Malgorzata Mossakowska	Coordinator	IIMCB
Przemyslaw Slusarczyk	IT Specialist	Ministerial grant

Polsenior Project

Name		Funding
Malgorzata Mossakowska	Coordinator	IIMCB
Aleksandra Szybalska	Project Assistant	Ministerial grant
Przemyslaw Slusarczyk	IT Specialist	Ministerial grant

Research Equipment Laboratory

Name		Funding
Alicja Zylicz	Head	IIMCB
Pawel Bieganowski	Fellow	IIMCB(1/2)
Iwona Cymerman	Fellow	IIMCB
Malgorzata Durawa	Fellow	IIMCB (1/2)
Wanda Gocal	Technician	IIMCB
Monika Dudek	Technician	IIMCB
Ewa Blazewicz	Technician	IIMCB

School of the Science Festival

Name		Funding
Agnieszka Choluj	Head	IIMCB/Nencki/IBB
Marta Badurek	Organizer	Volvox Project
Joanna Lilpop	Organizer	Volunteer
Jaroslaw 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
Michał Mlacki	Teacher, Technician	Volunteer
Roman Szczesny	Teacher	Volunteer
Monika Hejnowicz	Teacher	Volunteer
Justyna Rudzka	Teacher	Volunteer
Izabela Szczupakowska	Teacher	Volunteer
Justyna Wasil	Teacher	Volunteer
Monika Ostaszewska	Teacher	Volunteer
Damian Graczyk	Teacher	Volunteer
Grzegorz Olszewski	Teacher	Volunteer

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

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