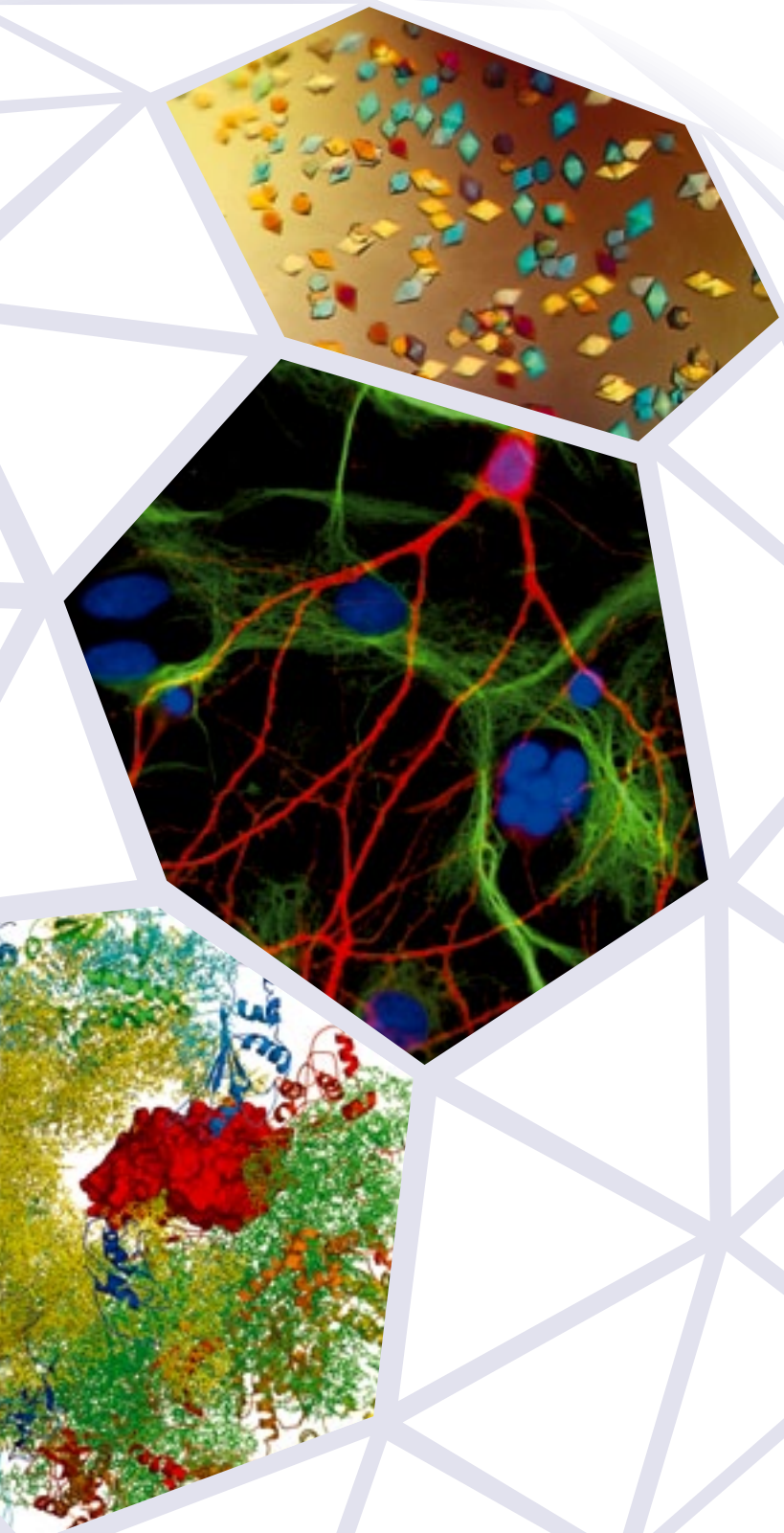


Annual Report 2008

10th Aniversary of IIMCB

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY

Director
Jacek Kuźnicki

Deputy Scientific Director
Michał Witt

Deputy Administrative Director
Jarosław Filiński

Financial Manager
Hanna Iwaniukowicz

Chairman of the International Advisory Board
Angelo Azzi

Deputy Chairman of the International Advisory Board
Leszek Kaczmarek

International Institute of Molecular and Cell Biology
4 Ks. Trojdena Street
02-109 Warsaw, Poland
Tel. (48 22) 59 70 700; Fax (48 22) 59 70 715
e-mail: secretariat@iimcb.gov.pl
internet: www.iimcb.gov.pl

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

Contents

International Institute of Molecular and Cell Biology in Warsaw: its origin and distinctive features

Towards the end of the 1980s, political and mentality changes provided a fertile ground for a timid idea that, perhaps, it would be worthwhile to start thinking about creating in Poland a completely new, independent and modern research institute which would deal with fundamental biomedical research concerning molecular and cell biology - an institute like nobody had seen here before.

Discussions about the subject, involving Maciej J. Nałęcz, Jacek Kuźnicki, and Leszek Kaczmarek – all, at that time, young associate professors of the Nencki Institute of Experimental Biology – led to ideas which had an increased chance of being implemented because they met with a favourable attitude among the scholars of the Institute and the authorities of the Polish Academy of Sciences (Prof. Leszek Kuźnicki – then President of the Academy, and its Presidium), who supported the establishment of various international centres.

The idea was also supported by representatives of UNESCO (Prof. Angelo Azzi), and Prof. Federico Mayor, a biology scholar and UNESCO's Director General at the time, immediately declared its support for such an initiative.

The proposal to create the Institute, drafted by Maciej J. Nałęcz and Angelo Azzi, was published in 1991 (Net-News, Bulletin of the Molecular and Cell Biology Network, UNESCO). The following year, after a wave of change had been initiated, the Polish Network for Molecular and Cell Biology – MCBN-UNESCO-PAN – was created.

In 1993, following multilateral negotiations, one of the new buildings on the Ochota Campus was designated for the international research institute – which, though planned at that time, was still not yet in existence. This idea was approved and officially supported by the 27th Session of UNESCO's General Conference.

In 1994, both the newly established Committee for Scientific Research (KBN) and the Presidium of the Polish Academy of Sciences approved UNESCO's initiative which, in May 1995, resulted in an international agreement signed by Prof. F. Mayor, UNESCO's Director General, and Prof. A. Łuczak, Poland's Deputy Prime Minister and Head of KBN. The agreement established the International Institute of Molecular and Cell Biology in Warsaw (IIMCB).

The newly established Institute – while still not formally in existence - inaugurated its scientific activities in October 1995 by organizing the International Conference on "New Frontiers in Cell and Molecular Biology", featuring world-renowned biologists in Warsaw.

At the same time, the Polish Parliament (Sejm) and Poland's President ratified the international agreement signed in May 1995. Eventually, in 1996, the Presidium of the Polish Academy of Sciences launched the Department of Molecular and Cell Biology, whose sole organisational task was to establish the International Institute for Molecular and Cell Biology.

Maciej J. Nałęcz – the driving force behind the whole enterprise since its conception – became the Head of the Department. It was primarily thanks to his persistence and diplomatic skills that the idea of establishing the International Institute became more and more realistic. At this stage, the core group of people behind the idea of establishing an international research institute was joined by Ryszard Przewłocki from Kraków and Michał Witt from Poznań. This group of five, supported by Małgorzata Mossakowska and Andrzej Śliwowski, began practical work on setting up the Institute.

Invaluable help for this nascent project was provided by the administration staff of the Nencki Institute. It must be emphasized here that the initial stages of organizing the IIMCB and the first activities undertaken by the Institute would not have been possible without the help and support of the Nencki Institute.

The correct legal foundation for the operations of the IIMCB was finally created by the Parliamentary bill of the 26th of June 1997 – until then the Polish legal system had no place for scientific bodies of an international nature. The Sejm passed the new law primarily thanks to effective efforts of Democratic Union (UD) MPs, headed by Krzysztof Dołowy.

In 1998, the Institute's International Advisory Committee met for the first time. Prof. Angelo Azzi was appointed Head of the Institute, with deputies Jacek Kuźnicki and Michał Witt (the former was de facto appointed by Prof. Mirosław Mossakowski, the President of the Polish Academy of Sciences, as acting Director of the Institute).

Finally, on 1 January 1999, after the Department for Molecular and Cell Biology had been dissolved, the International Institute for Molecular and Cell Biology began its independent existence. Its problem was that, at that point, it was staffed exclusively by administrative personnel, with no research staff. Even worse, it was still without any research funding, with only a vague chance of obtaining such funding.

This, however, started to change fast. In the same year, first professorial positions were filled and two research

laboratories were launched: the Laboratory of Molecular Immunology, headed by Dr. Jarosław Dastych, and the Department of Molecular Biology, under the leadership of Prof. Maciej Żylicz.

Since 2002, Prof. J. Kuźnicki has been Director of the Institute, and Prof. M. Witt the Institute's Deputy Scientific Director. These two appointments have officially regularised the situation which had existed in IIMCB since the start of its formal existence.

Since then, the Institute's affairs have started to move even faster. Ongoing dynamic development of the International Institute of Molecular and Cell Biology still remains the main thread of the story about an institution which remains atypical.

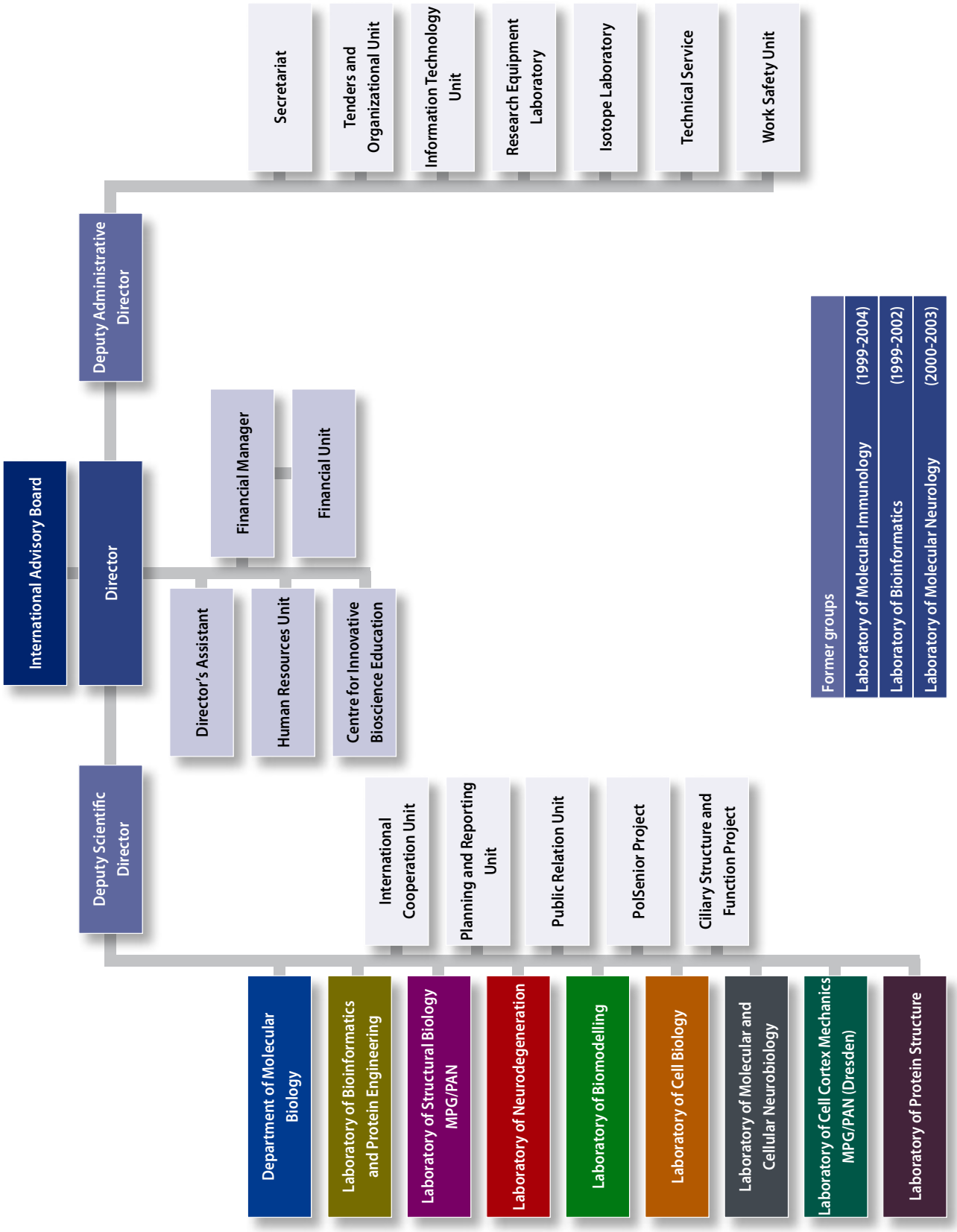
To be in line with such a name several distinctive features of IIMCB should be noted. Involvement of the International Advisory Board (IAB), the rules of recruitment of all faculty members through international competition and lack of tenure employment make it unique among Polish research institutions. The principles of organization of the Institute 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. Based on IAB's recommendation 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 organization 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.

IIMCB is located at the building loaned by the Polish Academy of Sciences. It 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 meeting room for 20 people, 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 – training laboratory for up to 18 students.

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. Kuźnicki appointed as Acting Director
July 1999	Dr. J. Dastych is appointed as a first Lab Leader at IIMCB
Oct. 1999	Prof. M. Żylicz is appointed as Chair of the Department of Molecular Biology
April 2000	An agreement between the Max Planck Society (MPG) and the Polish Academy of Sciences (PAN) to launch a Joint MPG-PAN Junior Research Group
Jan. 2001	The MPG-PAN Junior Research Group commences its activities with Dr. M. Bochtler as a Lab Leader
June 2001	Prof. J. Kuźnicki is elected by the International Advisory Board as Director of the Institute, begins to complete the Laboratory of Neurodegeneration. After consultation with UNESCO, the official nomination was signed by the President of PAN on February 1, 2002
Nov. 2002	New members of the International Advisory Board nominated for 2002-2006 term
Jan. 2003	Status of the Centre of Excellence in Molecular Bio-Medicine is granted by the European Commission within 5th Framework Programme
June 2005	Professor J. Kuźnicki re-elected as Director of the Institute (term 2006-2010)
May 2006	New members of the International Advisory Board nominated for 2006-2010 term
Feb. 2006	Twin MPG-PAN laboratory established at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden with Dr. Ewa Paluch as a Lab Leader.

Structure of the International Institute of Molecular and Cell Biology



Directors and Administration

Jacek Kuźnicki
Director

Michał Witt
Deputy Scientific Director

Jarosław Filiński
Deputy Administrative Director

Hanna Iwaniukowicz
Financial Manager

Zbigniew Przygoda
Director's Advisor

Dominika Dubicka
Director's Assistant

Anna Brzezińska
Secretary (since Jan. 2009)

Monika Kacprzak
Secretary (until Dec. 2008)

Beata Tkacz
Human Resources Specialist

Krystyna Domańska
Human Resources Specialist

Agnieszka Ziemka
Planning and Reporting Specialist

Urszula Białek-
-Wyrzykowska
International Cooperation Manager

Dorota Libiszowska
Foreign Grants Specialist

Magdalena Powierża
International Cooperation Specialist

Marcin Ogonowski
International Cooperation Specialist

Agnieszka Karbowska
Tenders Specialist

Marcin Biedacha
IT Manager

Jakub Skaruz
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

2006-2010 term

Participants of the meeting of the International Advisory Board, June 2008
From left (first row): J. Kuźnicki, A. Azzi, A.A. Bogdanov, M. Witt; (second row) O.A. Krishtal, N. Blin, A. Tramontano; (third row) J. Mallet, L. Kaczmarek, R. Przewłocki, M.J. Nałęcz, I. Dikič; (fourth row) I. Braakman, K. Hahlbrock, I. Baines, M. Żylicz.

Chairman: Angelo Azzi

Deputy Chairman: Leszek Kaczmarek

Members:

Angelo Azzi. 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 Tübingen, Tübingen, Germany; Foreign member of Polish Academy of Sciences

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

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

Jerzy Duszyński. Undersecretary of State, Ministry of Science and Higher Education, formerly 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, 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. Nałęcz. Director, Division of Basic and Engineering Sciences, UNESCO, Paris, France

Ryszard Przewłocki. 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

Directors' note

This year marks the end of the first decade of IIMCB activities. We ourselves are somewhat surprised that it's already been 10 years… On the other hand, it almost feels like we have always been here and that whatever we do here has been going on forever. Simply, perhaps, relativism in the perception of time. In fact, for some of us who are still present here, our adventure with the IIMCB had begun at a much earlier time – in the mid-1990s – at the early stages of its construction and organization. We recall those times and those problems with slightly misty eyes.

Even on the occasion of a jubilee as modest as a 10th anniversary during an economic crisis, it is a natural thing to draw up a balance sheet of achievements and failures. We are aware of the fact that we have succeeded in creating a new organizational standard in Polish science. With no false modesty we can claim that this translates into quality of scientific research, and it is a simple relationship. We know that in a relatively short time we became an important centre of biological sciences in our country, even though we are not always popular with everyone. The organizational rules promoted by us, which still represent a novelty in the Polish scientific research community, remain in line with the government reform plans for the sector. We have succeeded in attracting, as research team leaders, a group of exceptionally talented young scientists who – were it not for the welcoming IIMCB environment – would undoubtedly have become part of other excellent research teams in distinguished laboratories, albeit with no Polish affiliations. We have succeeded in building a team with a very low average age – and to watch the enthusiasm of all these young people is a pleasant sensation in itself.

We keep the research we carry out at IIMCB focused on the latest issues, yet we deliberately avoid uncritically following fashionable trends of no lasting value. Various aspects of protein research have become the leading theme of our activities: from the analysis of molecular ultrastructure to functional research in nervous transmission, carcinogenesis, signal transduction, etc. The equipment we have in our laboratories matches the state-of-the-art equipment at research facilities around the world and the working conditions often surpass those offered elsewhere, even at renowned research centres.

With the most recent competition decided, we have now practically filled up the whole building – and it is no small one. We can boast a large number of various grants , which account for more than half of the Institute's budget. We have developed a very skilled group of administrative staff we can be proud of. Also, due to an increasing number of people working at IIMCB, we are expanding and upgrading our seminar hall.

However, we are still aware of our own shortcomings, and this continually stimulates us to proceed with our efforts. We would like to make the IIMCB even more international than it currently is – our long-term experience to-date has shown that it still is not easy to recruit foreign scientists for research positions in Poland. We believe that various European programmes which facilitate effective hiring practices will be one of the mechanisms which will turn the Institute into a truly international organization. We would like, of course, to obtain more government funding for research, though nowadays such expectations are hard to meet.

We constantly think about the direction in which the IIMCB should be developed in the future. We have to work out a reasonable mechanism of turnover among researchers, to enable the influx of new blood, while providing the feeling of well-earned stability to the best and brightest. We are considering the idea of expanding beyond our building and beyond Warsaw: setting up research units associated with the IIMCB at other research institutes or universities may be one of the ways leading to further development. With all this in mind, we look forward to the second decade with the certainty that it will bring us new challenges, though the nature of these is hard to predict at the moment. We will do our best not to let these challenges take us by surprise.

Description of the Institute's Activities

Relation of IIMCB to PAN

The International Institute of Molecular and Cell Biology is directly subordinate to the President of the Polish Academy of Sciences, who supervises the organization and activities of the Institute. The President of PAN nominates members of IAB and the Institute's Directors. 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 Organization of Research at IIMCB

Nine research groups comprise the structure of IIMCB: Department of Molecular Biology (Żylicz), Laboratory of Bioinformatics and Protein Engineering (Bujnicki), Laboratory of Structural Biology MPG/PAN (Bochtler), Laboratory of Neurodegeneration (Kuźnicki), Laboratory of Biomodelling (Filipek), Laboratory of Cell Biology (Mączyńska), 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 (Żylicz'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 catenin, CHORD containing protein-1) (Kuźnicki's group)
5. The molecular modelling of structure and function (molecular switches) of 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 (Mączynska's group)
7. Molecular processes, including gene transcription, kinase-dependent cell signaling, cytoskeleton dynamics and intracellular trafficking underlying neuronal development and plasticity, as well as central nervous system pathologies (spinal cord injury, epilepsy, neurodegenerative disorders) (Jaworski's group)
8. Mechanics of the actomyosin cortex; study of cortical contractility and of the role of cortical mechanics during cytokinesis and migration (Paluch's group)
9. Structural and biochemical studies of nucleic acid enzymes (Nowotny's group).

Awards, Honors and Titles

- Officer's Cross of the Order of Polonia Restituta awarded by the President of the Republic of Poland to Prof. Jacek Kuźnicki
- Officer's Cross of the Order of Polonia Restituta awarded by the President of the Republic of Poland to Prof. Maciej Żylicz

Jacek Kuźnicki and Maciej Żylicz receiving the Officer's Cross of the Order of Polonia Restituta

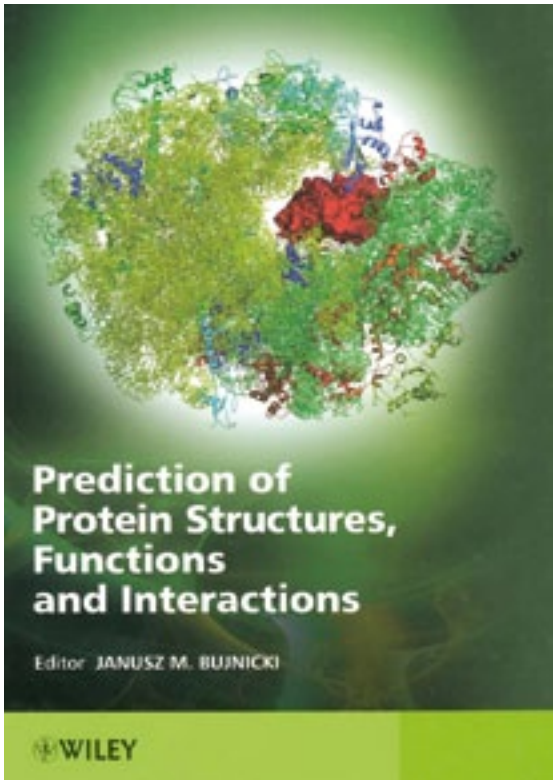
- Award by The Ministry of Health as a coauthor of the publication: „Evaluation of neuroendocrine status in longevity", Neurobiol. Aging 2007, 28:774–783 to Dr. Małgorzata Mossakowska
- Award by The Ministry of Health for the cycle of three papers on molecular and cellular basis of immunological disorders in Multiple Sclerosis to Prof. Alicja Żylicz

Education

IIMCB continues its doctoral program in partnership with other research and educational institutes of the Ochota Campus (40 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 Institute of Biochemistry and Biophysics, the Nencki Institute, the Postgraduate School of Molecular Medicine and of the Foundation for Polish Science continues (see section „Educational Activities" p. 76).

Media Visibility and Popularization of Science

In 2008, IIMCB faculty and staff members actively popularized science in media, reported on activities of IIMCB and commented on various aspects of country's science politics. In the *Public Service Review: European Union* (issue 15), a highly esteemed key source of news and analysis on public sector matters in Europe, **Michał Witt** placed two articles: on reversing the research brain-drain, advocating the importance of gifted young researchers in improving local scientific communities and on new way in Polish science being put into practice at IIMCB. In line with the latter was the editorial article in *Newsweek Polska*, presenting IIMCB as an independent and attractive research institute, calling it (definitely with some exaggeration) …the first exceptional institution of the country's "happiness island archipelago" of Polish science.



The monography edited by Janusz M. Bujnicki, published by John Wiley & Sons, 2008

Gazeta Wyborcza, a leading Polish daily, featured an article on various aspects of aging relating to the Institute's PolSenior Program. The same problem was described in an extensive interview with **Małgorzata Mossakowska** published in *Puls Medycyny*.

Foreign visits of **Jacek Kuźnicki**, IIMCB's Director, were covered in *Gazeta Wyborcza* (National Institutes of Health, Bethesda, USA) and in Ukrainian daily *Zierkalo Tiżnia* (Ukrainian Science Club and Ukrainian Ministry of Science, Kiev, Ukraine).

The Volvox Project, together with achievements of the **Centre for Innovative Bioscience Education** (SFN), was widely described in the leading European teachers' professional journal *Science in School*. The project was also the topic of a commentary in *Nature* (453, May 2008).

Two group visits were hosted at IIMCB, both including a presentation of IIMCB: of gifted youngsters brought here by **National Children's Fund** and of students of biotechnology of **Warmia-Mazury University in Olsztyn**.

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 for the last eight 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. Currently this information is available at www.iimcb.gov.pl/seminars.php.

Computer Network

In 2008 a number of main network services were modernized at IIMCB. This implied installation of the latest system versions and databases which were implemented on brand new and technologically advanced platforms. Based on this, the Institute developed a unique, fully functional and advanced intranet webpage. Apart from this, two new and independent file servers were created. One of them is presently used by the administrative staff, the other was prepared for implementation by researchers. Both servers are controlled by the Linux operating system and equipped with SATA II hard discs functioning in RAID5 configuration. Their capacity is, 1500 GB and 3000 GB respectively. Moreover, a new and efficient application server was implemented, allowing for access to a wide variety of utility software. Also a new server for the accounting unit was launched, which hosts a multiple database system. In future it is planned to install two 24 TB disc arrays, one of them is currently being purchased. Apart from the disc arrays, the Institute is concluding the transaction of advanced firewall, which will contribute highly to a greater network and data safety in the Institute. It is necessary to install a big and highly efficient emergency power pack, which would protect the whole Institute's server room from power black-out related problems. Additionally, two network service servers of great importance are to be launched shortly, that is an electronic mail system and the main webpage server. Data is currently being replaced from the present units into brand new server machines.

Grants

7th Framework Programme

- HEALTH-PROT "Proteins in Health and Disease" (229676); 954,100 EUR; 2009-2012; J. Kuźnicki
- NEURO.GSK3 "GSK-3 in neuronal plasticity and neurodegeneration: basic mechanisms and pre-clinical assessment" (223276); 280,840 EUR; 2008-2011; J. Jaworski
- SBMPs "Structural Biology of Membrane Proteins" (211800); 263,284 EUR; 2008-2012; S. Filipek

6th Framework Programme

- EURASNET "European alternative splicing network of excellence" (LSHG-CT-2005-518238); 120,000 EUR, matching funds 612,792 PLN; 2006-2010; IIMCB participation 2008-2010; J.M. Bujnicki
- MemProt "Structural studies of membrane proteases" (MTKD-CT-2006-042486); 626,800 EUR, matching funds 1,453,851; 2006-2010; M. Bochtler
- EndoTrack "Tracking the endocytic routes of polypeptide growth factor receptor complexes and their modulatory role on signalling" (LSHG-CT-2006-019050); 428,400 EUR; matching funds 1,011,709 PLN; 2006-2010; M. Miączyńska
- 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. Kuźnicki
- 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

Other International Funds

- Polish Norwegian Research Fund "Aberrant synaptic plasticity in epilepsy" (PNRF-96-AI-1/07); 362,200 EUR; 2008-2010; J. Jaworski
- EMBO Installation Grant "Structural and biochemical studies of UvrA DNA repair protein" (1476); 250,000 EUR; 2007-2012; M. Nowotny
- Wellcome Trust International Senior Research Fellowship "Structural and functional studies of two members of integrase superfamily – type 2 RNase H and Ruvc resolvase – from substrate recognition to catalysis" (081760); 4,106,806 PLN; 2007-2012; M. Nowotny
- 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. Miączyńska
- Wellcome Trust International Senior Research Fellowship "Communication between intracellular organelles in trafficking and signalling: The role of APPL proteins" (076469); 4,315,706 PLN; 2006-2010; M. Miączyńska
- NIH Grant "High-accuracy protein models derived from lower resolution data" subcontract (430-46-22 B) within a collaborative grant coordinated by A. Kloczkowski, Iowa State University, USA; 60,000 USD; 2007-2010; J.M. Bujnicki
- NIH Grant "Kinetoplastid SL RNA biogenesis", subcontract (2301 G EN541) within a collaborative grant coordinated by D.A. Campbell, University of California, USA; 100,440 USD; 2004-2009; J.M. Bujnicki
- The MPI-CBG/IIMCB Partner Group at the IIMCB; 109,000 EUR; 2006-2010; M. Miączyńska
- 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, 2001-2010; M. Bochtler
- Utrecht University fellowships for five PhD students (M. Witt's lab, IIMCB and Institute of Human Genetics PAN, Poznań; M. Żylicz'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 2009

Ministerial Research Grants

- International grant "Development and implementation of methods for improving protein's crystals quality by engineering of protein-protein contacts" (DWM/N2/DFG/2008); 940,000 PLN; 2008-2011; J.M. Bujnicki
- "Modultion of activity of transcription factors involved in tumorigenesis, by MDM2 and other E3 ubiquitin ligases" (N N301 032534); 750,000 PLN; 2008-2011; M. Żylicz
- "Structural and biochemical studies of restriction enzymes specific for pseudopalindromic sequences" (N N301 029534); 344,400 PLN; 2008-2011; M. Bochtler
- "Functional characterization of Exonuclease G - the role in the apoptosis and diabetes" (N N401 061535); 290,400 PLN; 2008-2011; I. Cymerman
- Polish-German Special Grant "Relationship between dysregulated calcium homeostasis and synaptic

pathology in Alzheimer's disease as a target for therapy" (P-N/001/2006); 1,050,000 PLN; 2007-2010; J. Kuźnicki

- 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
- "Experimental characterization of the complete set of RNA methyltransferases in the model organisms and identification of their counterparts in sequenced genomes" (N N301 239633); 460,000 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" (N30110932/3854); 303,000 PLN; 2007-2010; U. Wojda
- "Role of dendritic mRNA transport and local protein synthesis in development of dendritic arbor of neurons" (N N301 314733); 300,000 PLN; 2007-2010; J. Jaworski
- "Investigations of activation of GPCRs by theoretical methods" (N N301 203833); 205,000 PLN; 2007-2010; S. Filipek
- "S-nitrosylation and CDK5 dependent phosphorylation of proteins – proteomic analysis of synaptosomal fractions from transgenic mice Alzheimer disease models" (N N301 254333); 70,000 PLN; 2007-2010; grant coordinated by Michal Dadlez from Institute of Biochemistry and Biophysics PAN in collaboration with A. Szybińska
- "Role of mTOR-regulated proteins in development of dendritic tree of hippocampal and cortical neurons" (2 P04A 015 30); 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; 2006-2009; E. Paluch
- "Biochemical and microscopical characterization of APPL-positive endosomes" (2P04A03828); 390,040 PLN; 2005-2008; M. Miączyńska
- "Investigation of structure of presenilin protein and significance of its mutations in Alzheimer's disease development" (2 P05A 129 29); 220,000 PLN; 2005-2008; K. Jóźwiak
- "Identification of natural substrates for enzymes from the HINT family of phosphoroamidases and identification of enzymes that synthesize these substrates" (2 P04A 050 29); 352,000 PLN; 2005-2008; P. Bieganowski
- "Differences in action of stress-induced and constitutively synthesized Hsp70" (2 P04A 010 27); 550,200 PLN; 2004-2008; M. Żylicz

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

- "Structural and biochemical characterization of two restriction enzymes: BcnI recognizing asymmetric DNA and AvaII cutting RNA-DNA hybrids" (N N301 028934); 30,600 PLN; 2008-2009; M. Bochtler/M. Sokołowska
- "MvaI restriction-modification system: different ways to recognize the same DNA sequence" (N N301 030634); 31,080 PLN; 2008-2009; M. Bochtler/M. Kaus-Drobek
- "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. Pawłowski
- "Studies of agonist and antagonist binding modes in opioid receptors" (N N401 1401 33); 50,000 PLN; 2007-2009; S. Filipek/M. Koliński

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. Błędowski, 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" (PBZ/MEiN/01/2006/24 POL-POSTDOC II); 160,000 PLN; 2007-2009; H. Czapińska
- "Mechanism of biosynthesis of unusual protein-glycosaminoglycan linkage that plays key role in inflammatory processes" (PBZ/MEiN/01/2006/50 POL-POSTDOC II); 106,560 PLN; 2007-2008; A. Kaczmarczyk
- "Advanced molecular methods in haematology. Development and implementation of standarized research procedures for minimal residual disease, posttransplantation chimerism and marker translocations" (PBZ-KBN-120/P05/2004); 3,027,500 PLN; 2005-2009; 13 groups in Poland; Director: M. Witt

Ministerial Commissioned Grants coordinated by other institutions

- Three tasks within an commissioned 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. Kamińska-Kaczmarek from Nencki Institute of Experimental Biology; within the commissioned grant: „Application of functional genomics and bioinformatics for characterization and modeling of biological processes of critical importance for medicine and agriculture" (3/0-PBZ-MNiI-2/1/2005); 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 commissioned grant PBZ-KBN-124/P05/2004 directed by Medical University of Lodz); Director: P. Liberski

Other Research Grants

- Scientific Network "Visualization of biomedical phenomena" – BIOWIZJA coordinated by the Institute of Fundamental Technological Research (63/E-89/BWSN-0142/2008); 200,000 PLN; J. Bujnicki/M. Nowotny
- Scientific Network "Mechanisms of cellular movements – Mobilitas.pl" coordinated by the Nencki Institute of Experimental Biology PAN (2/E-36/SN-0075/2007); 75,000 PLN; 2007-2008; M. Miączyńska
- Scientific Network organized by Institute of Pharmacology PAN – "Looking for systemic targets of potential neurotrophic drugs" (26/E-40/BWSN-0023/2008); 54,680 PLN; J. Kuźnicki/M. Wiśniewska
- Grant from Foundation for Polish Science (Homing Programme) "Post-translational modifications and nuclear functions of endosomal APPL proteins" (HOM/ed2007/126); 80,000 PLN; 2007-2009; I. Pilecka
- Professorial Grant from Foundation for Polish Science (SP10/04) "Beta-catenin metabolism in health and disease"; 240,000 PLN; 2004-2008; J. Kuźnicki

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

1. **Mossakowska M**, Pawlinska-Chmara R, Broczek KM. Asthma, allergy, and respiratory symptoms in centenarians living in Poland. J Physiol Pharmacol. 2008; 56 Suppl 6:483-489
2. Geremek M, Schoenmaker F, Zietkiewicz E, Pogorzelski A, Diehl S, Wijmenga C, **Witt M**. Sequence analysis of 21 genes located in the Kartagener syndrome linkage region on chromosome 15q. Eur J Hum Genet, 2008; 16:688-695
3. Dawidowska M, Jółkowska J, Szczepański T, Derwich K, Wachowiak J, **Witt M**. Implementation of the standard strategy for identification of Ig/TCR targets for minimal residual disease diagnostics in B-cell precursor ALL pediatric patients: Polish experience. Arch Immunol Ther Exp, 2008; 56:409-418
4. Loges NT, Olbrich H, Fenske L, Mussaffi H, Horvath J, Fliegauf M, Kuhl H, Baktai G, Peterffy E, Chodhari R, Chung EM, Rutman A, O'Callaghan C, Blau H, Tiszlavicz L, Voelkel K, **Witt M**, Zietkiewicz E, Neesen J, Reinhardt R, Mitchison HM, Omran H. DNAI2 mutations cause primary ciliary dyskinesia with defects in the outer dynein arm. Am J Hum Genet, 2008; 83:547-558

Scientific degrees in 2008

Academic Habilitation:

- **Marta Miączyńska,** habilitation thesis: „Effector proteins of Rab5 GTPase in the regulation of endocytosis and signal transduction", Nencki Institute of Experimental Biology PAN, Warsaw, 27.06.2008
- **Paweł Bieganowski**, habilitation thesis: „New developments in biosynthesis of NAD and NADP", Institute of Bioorganic Chemistry PAN, Poznań, 4.11.2008

PhD:

- **Magdalena Błażejczyk**, PhD thesis: „Neuronal characterization of two calcium binding proteins, calmyrin 1 and calmyrin 2 belonging to „EF-hand" superfamily", Mentor: U. Wojda, Nencki Institute of Experimental Biology PAN, Warsaw, 20.02.2008
- **Walerych Dawid**, PhD thesis: „Rescue of human p53 activity by molecular chaperones", Mentor: A. Żylicz, Institute of Biochemistry and Biophysics PAN, Warsaw, 6.05.2008
- **Aleksandra Helwak**, PhD thesis: „Comparative analysis of the Hsc70 and Hsp70 molecular chaperones", Mentor: M. Żylicz, Nencki Institute of Experimental Biology PAN, Warsaw, 27.05.2008
- **Małgorzata Gutkowska**, PhD thesis: „Human Hsp90, its isoforms and postulated mechanism of function", Mentor: A. Żylicz, Nencki Institute of Experimental Biology PAN, Warsaw, 29.09.2008
- **Adam Sobczak**, PhD thesis: „ Identification and characterisation of interction between Calmyrin 1 - calcium binding protei, and SCG10 - tubulin cytoskeleton regulator in neuronal cells", Mentor: U. Wojda, Nencki Institute of Experimental Biology PAN, Warsaw, 4.11.2008
- **Leszek Lipiński**, PhD thesis: „Functional analysis of serine-threonine protein kinase TSSK3", Mentor: M. Żylicz, Institute of Biochemistry and Biophysics PAN, Warsaw, 25.11.2008
- **Łukasz Bojarski**, PhD thesis: „Presenilin 1 related deregulation of cellular calcium homeostasis in Alzheimer's disease", Mentor: J. Kuźnicki, Nencki Institute of Experimental Biology PAN, Warsaw, 9.12.2008

Other consortial projects coordinated at IIMCB

Molecular haematology project

The 2008 was a third 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 Poznań, Warszawa, Kraków, 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. Michał Witt and coordinated by Dr. Małgorzata Mossakowska.

Currently a monography book "Molecular haematology" edited by M. Witt, T. Szczepański and M. Dawidowska is being completed as one of final results of the project.

PolSenior project

The IIMCB was one of the major initiators of multidisciplinary projects on ageing and is currently a coordinator of the ministerial commissioned project *"Medical, psychological and economical aspects of ageing in Poland"*. The country's largest project in this area of research, with a budget of over 12 million PLN, aims to conduct an interdisciplinary study of various ageing-related problems encompassing diverse research disciplines concerning the ageing process in the Polish society. About 6,000 patients will participate in a survey and over 1,500 patients in eight regions will be examined by geriatricians within the framework of this project. Biological material (a DNA bank) will be deposited at the IIMCB, and our Institute will also provide the whole consortium with a database of the assembled tests results. Four sub-projects are focussed on large-scale epidemiological studies, while two are examining physical and architectural barriers for the elderly population. Specialists involved range from sociologists, psychologists, economists and demographers to geriatricians, cardiologists, nephrologists, neurologists, epidemiologists and molecular biologists from major research centers of Katowice, Kraków, Wrocław, and Warszawa. The final target is an ultimate definition of the needs of the ageing population in terms which are usable in social policy, administrative care, etc. to shape future decision-making in this area. The need to ensure geriatric medicine training for physicians in primary care is being emphasized within the project. The project is being conducted by the International Institute of Molecular and Cell Biology in Warsaw with Prof. Piotr Błędowski (President of Polish Gerontological Society) as head of the project and Dr. Małgorzata Mossakowska as coordinator.

Scientific Meetings and Lectures

- International Symposium: "Molecular approach to the diagnosis and monitoring of blood neoplasia", 9.01.2008, Warsaw, Poland, coorganized by IIMCB
- Spring School of Polish Neuroscience Society on: "Plasticity of neuronal connectivity: morphological background and molecular mechanisms", 4.06.2008, Warsaw, Poland, coorganized by IIMCB
- The First Poland-Taiwan Conference on: „Molecular Biology & Molecular Neuroscience: Focus on RNA", 21-27.04.2008, Warsaw-Białowieża-Kraków, Poland, coorganized by IIMCB
- III and IV Promemoria Workshops in Warsaw: "1. RNA interference for neuroscience; 2. Building your career and your first laboratory",11-15.05.2008, 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), 12-19.05.2008, Warsaw, Poland, IIMCB
- IIMCB Annual Report Session, 16.05.2008, Rynia, Poland
- The Annual Meeting of the ENI-NET European Neuroscience Institutes Network, 19-21.05.2008, Warsaw, Poland, coorganized by IIMCB
- International Annual Symposium (13th Lab Leader Competition), 6.06.2008, Warsaw, Poland, IIMCB

Seminars of invited speakers

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

Andrzej Dziembowski (Dept. of Genetics and Biotechnology, Warsaw University, Poland, EMBO Installation Grantee) "The mechanism of action of the major eukaryotic exonuclease, the exosome complex", 28.02.2008

Katarzyna Kotulska (Department of Neurology, The Children's Memorial Health Institute, Warsaw, Poland, Recipient of L'Oreal Poland Habilitation Fellowship 2007) "Experimental approach to peripheral nerve repair", 27.03.2008

Włodzimierz Krzyżosiak (Institute of Bioorganic Chemistry, Polish Academy of Sciences Poznań, Poland, Laureate of FNP Prize 2007) "Silencing triplet repeat expansion diseases with RNA interference", 10.04.2008

Alicja Józkowicz (Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland, Recipient of the Wellcome Trust International Senior Fellowship) "Heme oxygenase-1 in tumors – is it a false friend?", 26.06.2008

Joanna Kufel (Institute of Genetics and Biotechnology, Faculty of Biology, Warsaw University, Poland, Recipient of the Wellcome Trust International Senior Fellowship) "The termination tale: mechanisms of transcription termination by RNA polymerases in yeast", 27.11.2008

- **Lab Leader Competition seminars:**

Agnieszka Chacińska (Institute of Biochemistry and Molecular Biology, University of Freiburg, Germany) "Versatility and dynamics of mitochondrial protein import machineries", 6.06.2008

A. Arockia Jeyaprakash (Max-Planck Institute of Biochemistry, Martinsried, Germany) "Chromosomal passengers: Structural insights into a journey through mitosis", 6.06.2008

Erica Kreimann-Strobl (International Institute of Nano and Molecular Medicine, Dept. of Radiology, School of Medicine, University of Missouri, Columbia, MO, USA) "The role of NHERF1 in the regulation of cell adhesion and transformation", 6.06.2008

Michał Minczuk (Dunn Human Nutrition Unit, Medical Research Council, Cambridge, UK) "Engineered zinc finger enzymes for manipulation of mammalian mitochondrial DNA", 6.06.2008

Werner L. Vos (National University of Ireland, Maynooth, Ireland) "Pharmacological chaperones for membrane proteins related to Retinitis Pigmentosa - Is there light at the end of the tunnel?", 6.06.2008

Tomasz Wilanowski (Rotary Bone Marrow Research Laboratories, Royal Melbourne Hospital, Melbourne, Australia) "The grainyhead Odyssey", 6.06.2008

- **Regular IIMCB seminars**

Raphael Bauer (Structural Bioinformatics Group, Charite Universitätsmedizin, Berlin, Germany) "From similarity to superposition - research and web-services at the Structural Bioinformatics Group Berlin", 25.02.2008

Marek Cieplak (Institute of Physics, Polish Academy of Sciences, Warsaw, Poland) "Genetic interaction networks from microarray data", 13.03.2008

Michał Jaźwiński (Dept. of Medicine, Tulane University Health Sciences Center, New Orleans, USA) "Understanding the role of LAGs and LASSes in aging: a case of ceramidipity", 6.05.2008

Rob Hooft (Geneva Research Center, Merck Serono International S.A., Switzerland) "Drug target discovery

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

in theory and practice: control of the growth hormone receptor signaling and systemic growth by protein tyrosine phosphatase H1", 8.05.2008

Arkadiusz Ciesielski (Institute of Biochemistry and Biophysics, Warsaw, Poland) "Regulation of kinase activity by SnRK2 calcium sensor (SCaS)", 29.05.2008

Fabio Tanfani (Institute of Biochemistry, Faculty of Sciences, Universita Politecnica delle Marche, Ancona, Italy) "Proteins from thermophilic organisms: Strategies of adaptation to high temperatures", 13.06.2008

Enzo Tramontano (Dept. of Experimental Biology, University of Cagliari, Italy) "HIV-1 reverse transcriptase associated RNase H activity: implications for drug treatment", 30.06.2008

Michał Hetman (Molecular Signaling Kentucky Spinal Cord Injury Research Center and Dept. of Neurological Surgery, University of Louisville, USA) "Survival signaling networks that involve neuronal ERK1/2 MAP kinases", 10.07.2008

Jacek Lubkowski (Macromolecular Crystallography Laboratory, National Cancer Institute at Frederick, USA) "Structure-function relationship in human defensins", 9.09.2008

Titia Sixma (Netherlands Cancer Institute, Amsterdam, The Netherlands) "Dimerization of RING domains regulates DNA damage tolerance and chromatin modification", 21.10.2008

Harald Stenmark (Centre for Cancer Biomedicine University of Oslo, Norway) "Endocytic downregulation of signaling receptors as a mechanism of tumour suppression", 23.10.2008

Michal Novak (Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia) "Disease modifying pathways of misfolded tau protein in Alzheimer's disease", 30.10.2008

Izabela Wagner (Center of Sociology of Work and Organization Institute of Sociology, Warsaw University CEMS/EHESS Paris) "The specificity of work in the international lab team", 13.11.2008

IIMCB researchers seminars

Magdalena Lipka (Laboratory of Structural Biology) "*Staphylococcus cohnii* virginiamycin B lyase – Vgb", 10.01.2008

Sajid Rashid (Laboratory of Cell Biology) "Regulation of B-catenin signaling by APPL proteins", 17.01.2008

Grzegorz Papaj (Laboratory of Bioinformatics and Protein Engineering) "Divide and conquer or the introduction to ontologies", 24.01.2008

Maciej Olszewski (Department of Molecular Biology) "To screen is to believe! HTS in microscopy", 7.02.2008

Anna Skibińska-Kijek (Laboratory of Neurodegeneration) "Biochemistry in neurobiology of behavior: how cortical plasticity influence synaptic proteins and what does it mean?", 14.02.2008

Marcin Nowotny (Laboratory of Protein Structure) "Structural studies of RNases H", 6.03.2008

Matylda Macias (Laboratory of Molecular and Cellular Neurobiology) "Injury related dendritic plasticity in the mature central nervous system", 17.04.2008

Marta Frankowska (Department of Molecular Biology) "The French connection: Hsp90 and HCV", 24.04.2008

Dawid Walerych (Department of Molecular Biology) "From cells *to in vitro* or *vice versa*: The cooperation of Hsp90 and Hsp70 systems in the chaperoning of p53", 16.10.2008

Adam Sobczak (Laboratory of Neurodegeneration) "Hidden net of cellular connections. Calcium – a cryptic informer", 4.12.2008

Anna Malik (Laboratory of Molecular and Cellular Neurobiology) "mTOR kinase in human disease", 11.12.2008

Beata Pyrzyńska (Laboratory of Cell Biology) "APPL proteins as multifunctional adaptors: Do they participate in tumor formation?", 18.12.2008

- **Retreat Report Session Seminars (Rynia)**

Maciej Olszewski (Department of Molecular Biology) "Interactions of Hsp70 protein family members and p53 in vivo", 16.05.2008

Elżbieta Purta (Laboratory of Bioinformatics and Protein Engineering) "Identification and chracterization of new RNA methyltransferases by combination of theoretical and experimental approaches", 16.05.2008

Roman Szczepanowski (Laboratory of Structural Biology MPG/PAN) "PspGI: Degenerate specificity at the atomic level", 16.05.2008

Katarzyna Misztal (Laboratory of Neurodegeneration) "Beta-catenin in the nucleus of thalamic neurons - why and how?", 16.05.2008

Wojciech Puławski (Laboratory of Biomodelling) "Modelling of stability and interactions of membrane proteins using simplified forcefields", 16.05.2008

Anna Hupałowska (Laboratory of Cell Biology) "Life on the move: Characterization of APPL-positive endosomes in living cells", 16.05.2008

Małgorzata Urbańska (Laboratory of Molecular and Cellular Neurobiology) "Dendritic arbor development of hippocampal neurons is regulated by mTORC2", 16.05.2008

Alba Diz Munoz (Laboratory of Cell Cortex Mechanics MPG/PAN in Dresden) "The role of protrusion formation for cell migration in vivo" 16.05.2008

Małgorzata Figiel (Laboratory of Protein Structure) "Structural studies of reverse transcriptases and their complexes with nucleic acids", 16.05.2008

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 scientific 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.

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 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 2008, attracted to IIMCB 14 candidates, among them: six foreigners, two Polish nationals working in USA, two Poles from UK and one from: Australia, Finland, Germany and Poland. Dr. Agnieszka Chacińska, who accepted an offered position, will start her research activities at IIMCB in 2009.

Dr. Agnieszka Chacińska will join IIMCB in the early fall of 2009, after completion of her postdoctoral fellowship in the laboratory of Prof. Nikolaus Pfanner, Institute for Biochemistry and Molecular Biology, University of Freiburg, Germany. She will continue work on dynamic events resulting in the formation and maintenance of complex functional elements such as multimeric protein assemblies and whole organelles in the cell. Specifically she will focus on mechanisms employed by Mia40, the sulfhydryl oxidase Erv1, membrane-embedded transporters, isomerases and reductases of disulfides, as well as their spatial and temporal coordination in order to facilitate the sorting of proteins residing in the mitochondrial intermembrane space.

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

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

[2)]no result

[3)]the winner did not accept the offer

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 side. The Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (MPICBG), 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 MPICBG/IIMCB Partner Group was established based on a contract between the two institutions in January 2006 initially for a fixed term of 3 years and has been recently extended for two more years, i.e. until December 2010. 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. Miączyńska 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 dealing with the characterization of APPL-positive endosomes is a continuation of the work that Dr. Miączyńska carried out in the laboratory of Prof. Zerial in Dresden.

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 (details see: Educational Activities p. 76).

Visits to IIMCB:

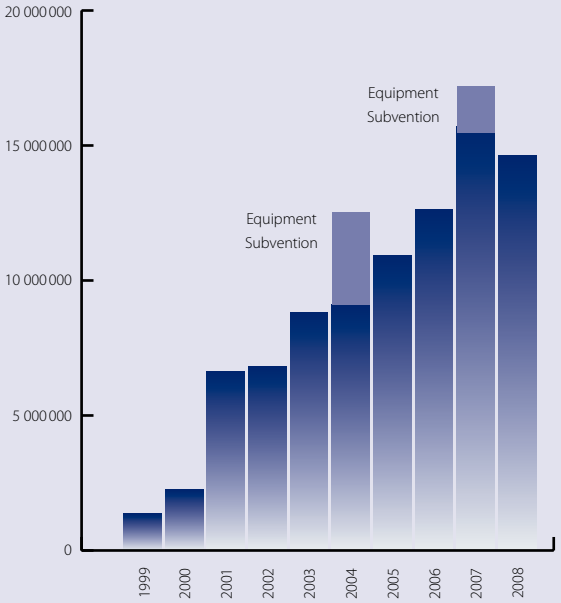
- 24.04.2008: **Prof. Janet Thornton**, Director, EMBL Outstation, European Bioinformatics Institute, Hinxton, UK
- 2.06.2008: **Prof. Annette Schavan**, the Federal Minister of Education and Research of Germany and **Rita Süssmuth**, the President of German-Polish Foundation for Science
- 11.07.2008: French delegation consisting of Director General of the French Institute of Health and Medical Research (INSERM) **Prof. Andre Syrota**, Deputy Director for European Affairs of INSERM **Philippe Arrhets** and Deputy Scientific Attache of French Embassy in Warsaw **Guillame Giraudet**
- 4.11.2008: **Prof. Peter Gruss**, President of the Max Planck Society and **Prof. K. Hahlbrock**, **Dr. Sabine Zimmermann** and **Mrs. Kristina Schmucker**
- 6.11.2008: **Prof. Tim Hunt**, Cell Cycle Control Laboratory of London Research Institute, the Nobel Prize Laureate in Physiology and Medicine in 2001.

Foreign scientists at IIMCB

- **Frank King**, MSc (USA) – PhD student in the Department of Molecular Biology, 1999-2001; graduated in Oct., 2001
- **Sanne Mikkelsen**, MSc (Denmark) – involved in Polish Centenarians Program PolStu99, then in the Laboratory of Neurodegeneration, 1999-2001
- **Sophie Chiron** (France) – senior technican at Cell and DNA Bank, Polish Centenarians Program PolStu99, 1999-2000
- **Matthias Bochtler**, PhD (Germany) – Head of the Laboratory of Structural Biology MPG/PAN Junior Research Group, 2000-present
- **Sergey Odintsov**, MSc (Belarus) – SMM's PhD student in the Laboratory of Structural Biology MPG/PAN, 2001-2004
- **Ahmad Noor Jalili**, MD (Iran) – PhD student in the Laboratory of Molecular Neurology, 2002-2003
- **Tiziana Cacciamani**, PhD (Italy) – Post-doctoral fellow in the Laboratory of Department of Molecular Biology, 2003 (2 months)
- **Gang Zhao**, PhD (China) – Post-doctoral fellow in the Laboratory of Neurodegeneration, 2003-2004
- **Michael Kreutz**, PhD (Germany) – Senior researcher in the Laboratory of Neurodegeneration, 2004 (2 months)
- **Rashid Sajid**, PhD (Pakistan) – Post-doctoral fellow in the Laboratory of Cell Biology, 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
- **Laura Lopez Munoz**, BSc (Spain) - MSc student in the Laboratory of Bioinformatics and Protein Engineering 2006-2007 (one semester)
- **Tran Cat Dong**, PhD (Vietnam) – Post-doctoral fellow in the Laboratory of Neurodegeneration, 2007 (2 months)
- **Nguyen Trong Hung**, MD (Vietnam) – PhD student in the Laboratory of Neurodegeneration, 2007 (1 month)
- **Dario Piano**, PhD (Italy) – expert involved in EU grant – MEMPROT, the Laboratory of Structure Biology, since 2007
- **Elisa Tomat**; PhD (Italy) -visiting researcher (Dept. of Chemistry, MIT) in the Laboratory of Molecular and Cellular Neurobiology, July 7-25, 2008
- **El Alaoui Sabah**, PhD (Spain) – expert involved in EU grant – MEMPROT, the Laboratory of Structure Biology, since 2008

Diversity of Funding IIMCB'2008

Annual budget (in PLN)



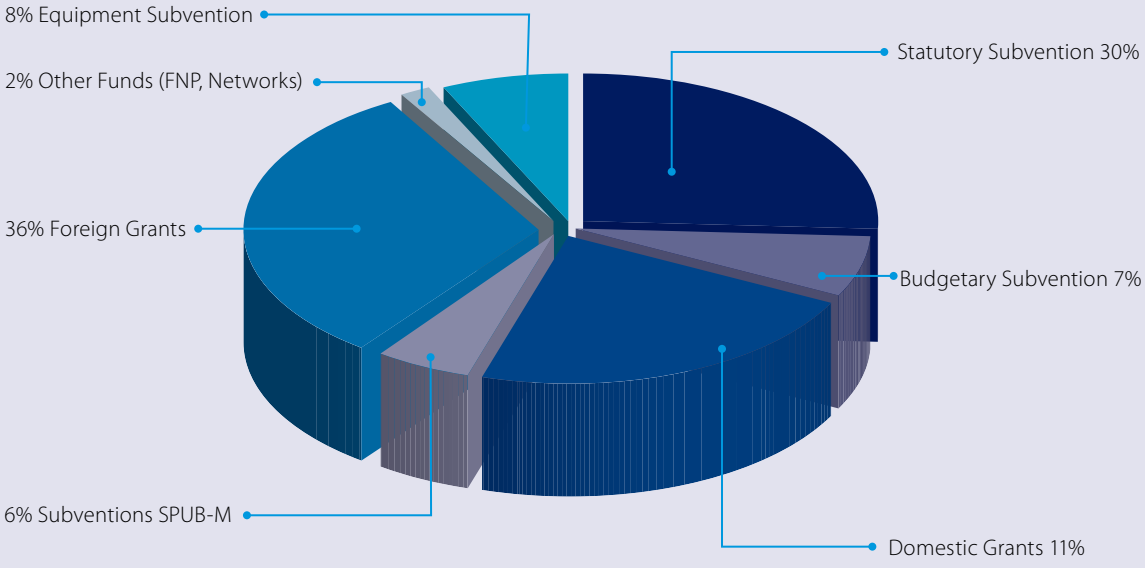
Sources of Funding	amounts in PLN	amounts in EUR*
Statutory Subvention	4 351 600	1 042 949
Budgetary Subvention	1 090 000	261 241
Individual Domestic Grants	2 674 675	641 040
*Consortial Domestic Grants	-1 031 210	-247 150
Subventions SPUB-M	884 662	212 027
Foreign Grants	5 249 734	1 258 205
Other Funds (FNP, Networks)	294 727	70 637
Equipment Subvention	1 120 516	268 554
Total	**14 634 704**	**3 507 503**

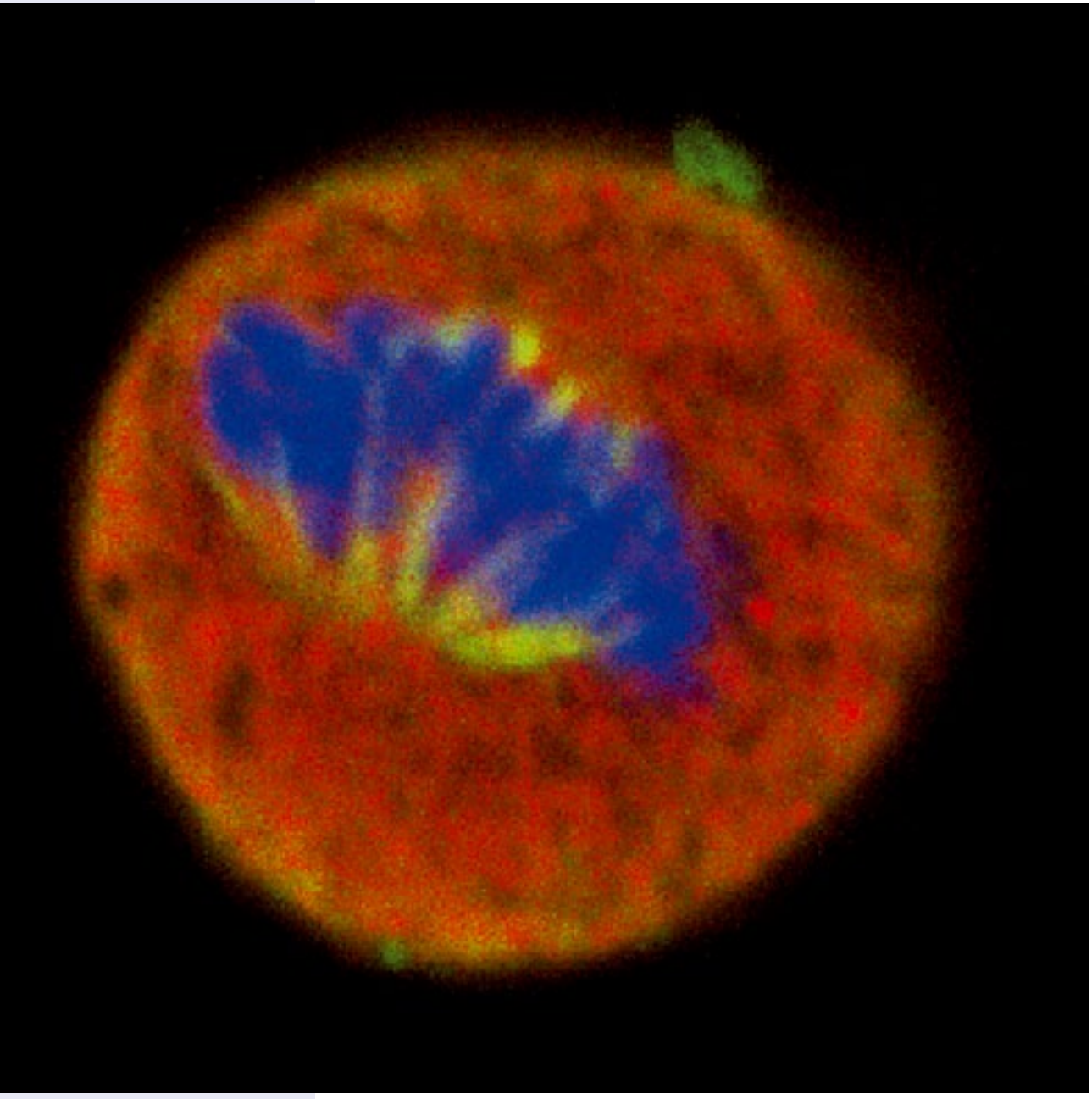
*1EUR – 4,1724 @ 31st Dec' 2008

Profit & loss statement

	amounts in PLN
A. net revenue on sales and equivalents	15 366 397*
B. operational activity costs:	16 836 142
Depreciation (equipment)	2 329 401
Research materials	3 811 995
Utilities	323 569
Services	1 389 785
Fees and taxes	571 495
Salaries and wages	5 146 251
Social and health insurance	1 134 191
Other operational expenses, in this:	2 129 454
business trips	534 186
property insurance	19 647
expenditures of indirect costs	834 457
fellowships	740 902
C. other operational income (subventions)	1 471 056
D. other operational expenses:	40 645
E. financial income:	193 207
Interests	135 889
Others	57 317
F. financial expenses:	277
Interests	175
Others	102
Profit / loss on business activity (A-B+C-D+E-F)	**153 596**

*About 1 mln PLN (≈200,000 EUR) obtained in Dec. 2007 was distributed to the partners of PolSenior in 2008.





HeLa cell arrested in mitosis stained for DNA (DAPI, blue), tubulin (green) and NudC (red) (author: Marcin Klejman).

Department **of Molecular Biology**

Lab Leader:

Maciej Żylicz, PhD, Professor

Vice Head:

Alicja Żylicz, PhD, Professor

Research Associates:

Paweł Bieganowski, PhD

Marcin Klejman, PhD

Maciej Olszewski, PhD

Dawid Walerych, PhD (from June 2008)

Junior Researchers:

Marta Frankowska, MSc

Aleksandra Helwak, MSc (graduated in May 2008)

Małgorzata Gutkowska, MSc (graduated in September, 2008)

Leszek Lipiński, MSc (graduated in November, 2008)

Zuzanna Szymańska, MSc

Jakub Urbański, MSc

Dawid Walerych, MSc (graduated in May, 2008)

Anna Żurawska, MSc

Milena Ostrysz, MSc (from October, 2008)

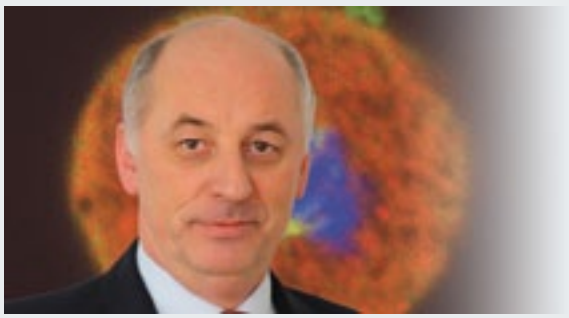
Paweł Krawczyk, undergraduate student

Secretary:

Grażyna Orleańska, MSc

Technician:

Wanda Gocal



Maciej Żylicz
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 Gdańsk, Poland, 1979
- MSc in physics, University of Gdańsk, 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 and Stanford University, Department of Biochemistry, USA

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

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 Gdańsk

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

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

1990-1993 Vice President, University of Gdańsk

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

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

OTHER PROFESSIONAL ACTIVITIES

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

2008-present 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 (2004-2008) 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. Officer's Cross of the Order of Polonia Restituta awarded by the President of the Republic of Poland (2008)
2. Doctor Honoris Causa of University of Wrocław, 2007
3. Prime Minister Award for Scientific Achievements, 2002
4. "L. Marchlewski" Award from the Biochemistry and Biophysics Committee PAN, 2001
5. Award from the Foundation for Polish Science (FNP) in biological/medical sciences, 1999
6. Awards from the Polish Biochemical Society for the best biochemistry work performed in Polish laboratories, 1996, 2007
7. Award from the Ministry of Education, 1994
8. "Heweliusz" Prize for the Scientific Achievements, awarded by the President of Gdańsk, 1993
9. Award from the Polish Academy of Sciences, 1990
10. Individual Award from the Polish Academy of Sciences for Scientific Achievements, 1986

DOCTORATES

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

ACADEMIC HABILITATIONS

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

PROFESSOR TITLES RECEIVED

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

PUBLICATIONS

Over 80 publications in primary scientific journals including: two papers published in Cell, six in EMBO J, six in PNAS and 25 in J Biol Chem. These papers were cited more than 5000 times. The Hirsch parameter, H=40

Selected publications

- Wawrzynow B, Pettersson S, **Zylicz A**, Bramham J, Worral E, Hupp TR, and Ball K. A function for the RING finger domain in the allosteric control of MDM2 conformation and function. J Biol Chem, 2009; in press
- **Szymanska Z**, **Zylicz M**. Mathematical Modeling of Heat Shock Proteins Synthesis in Response to Temperature Change. J Math Biol, 2009; in press
- **Zurawska A**, **Urbanski J**, **Bieganowski P**. Hsp90n - An accidental product of a fortuitous chromosomal translocation rather than a regular Hsp90 family member of human proteome. Biochim Biophys Acta, 2008; 1784:1844-6
- Stevens C, Pettersson S, **Wawrzynow B**, Wallace M, Ball K, **Zylicz A**, Hupp T.R. ATP stimulates MDM2-mediated inhibition of the DNA-binding function of E2F1. FEBS J, 2008; 275:4875-86
- **Szymanska Z**, **Urbanski J**, Marciniak-Czochra A. Mathematical modelling of the influence of heat shock proteins on cancer invasion of tissue. J Math Biol, 2008; DOI 10.1007/s00285-008-0220-0
- **Wawrzynow B**, **Zylicz A**, Wallace M, Hupp T, **Zylicz M**. MDM2 Chaperones the p53 Tumor Suppressor. J Biol Chem, 2007; 282:32603-12
- Issat T, Nowis D, Legat M, Makowski M, **Klejman MP**, **Urbanski J**, Skierski J, Koronkiewicz M, Stoklosa T, Brzezinska A, Bil J, Gietka J, Jakobisiak M, Golab J. Potentiated antitumor effects of the combination treatment with statins and pamidronate *in vitro* and *in vivo*. Int J Oncol, 2007; 6:1413-25
- Spiechowicz M, **Zylicz A**, **Bieganowski P**, Kuznicki J, Filipek A. Hsp70 is a new target of Sgt1- an interaction modulated by S100A6A. Biochem Biophys Res Commun, 2007; 357:1148-53
- **Kudla G**, **Lipinski L**, **Caffin F**, **Helwak A**, **Zylicz M**. High guanine and cytosine content increases mRNA levels in mammalian cells. PLoS Biology, 2006; 4:0933-42
- **Walerych D**, **Kudla G**, **Gutkowska M**, **Wawrzynow B**, Muller L, **King G**, **Helwak A**, **Boros J**, **Zylicz A**, **Zylicz M**. Hsp90 chaperones wild-type p53 tumor suppressor protein. J Biol Chem, 2004; 279: 48836-45
- Dworakowska D, Jassem E, Jassem J, Peters B, Dziadziuszko R, **Zylicz M**, **Jakobkiewicz-Banecka J**, Kobierska-Gulida G, Szymanowska A, Skokowski J, Roessner A, Schneider-Stock R. MDM2 gene amplification: new independent factor of adverse prognosis in non-small cell lung cancer (NSCLC) Lung Cancer, 2004; 43:285-295
- **Kudla G**, **Helwak A**, **Lipinski L**. Gene conversion and GC-content evolution in mammalian Hsp70. Mol Biol Evol, 2004; 21:1438-54
- **Zylicz M**, **King FW**, **Wawrzynow A**. Hsp70 interactions with the p53 tumour suppressor protein. EMBO J, 2001; 20:4634-8
- **King FW**, **Wawrzynow A**, Hohfeld J, **Zylicz M**. Co-chaperones Bag-1, Hop and Hsp40 regulate Hsc70 and Hsp90 interactions with wild-type or mutant p53. EMBO J, 2001; 20:6297-305

Summary of work

The research conducted in our department is mainly focused on activities of molecular chaperones in mammalian cells, including cell transformation (review Zylicz *et al.*, 2001, also see recently published system biology papers: Szymanska *et al.*, 2008, Szymanska and Zylicz, 2009). Using highly purified recombinant human proteins we had previously identified intermediate reactions leading to the assembly of molecular chaperone complexes with the wild type or mutant p53 tumour suppressor protein (King *et al.*, 2001). More recently we have demonstrated that Hsp90 molecular chaperone is required for binding of wt p53 to the promoter sequences under physiological temperature, 37°C and that this chaperoning activity is ATP-dependent (Walerych *et al.*, 2004). Currently we provide *in vivo* evidence that Hsp90 and Hsp70 chaperone machines are required for proper folding of wt p53, its specific binding to chromatin and transcription of p53-dependent genes.

p53 tumor suppressor protein is a thermodynamically unstable, largely unstructured transcription factor. Applying conformation-specific immunoprecipitation, we showed that in H1299 p53 -/- cells, transfected with human wild-type p53, both Hsp90 and Hsp70 molecular chaperones were required for maintenance and stabilization of native conformation of p53 under physiological and heat-shock conditions and for refolding of WT p53 at 37°C, during the recovery from heat-shock. Using FRAP analysis we demonstrated that at physiological temperature inhibition of both Hsp70 and Hsp90 systems by expression of the dominant negative Hsp70K71S variant and 17-AAG, respectively, increased the nuclear mobility of WT p53, most likely reflecting changes of its specific interactions with chromatin. Using chromatin immunoprecipitation (ChIP) we confirmed that interaction of WT p53 with *WAF1* promoter indeed was sensitive to Hsp70 and Hsp90 inhibition at the physiological temperature of 37°C and further decreased upon heat-shock. The influence of Hsp90 and Hsp70 on p53 binding to the *WAF1* promoter sequence have also been confirmed *in vitro*, using highly

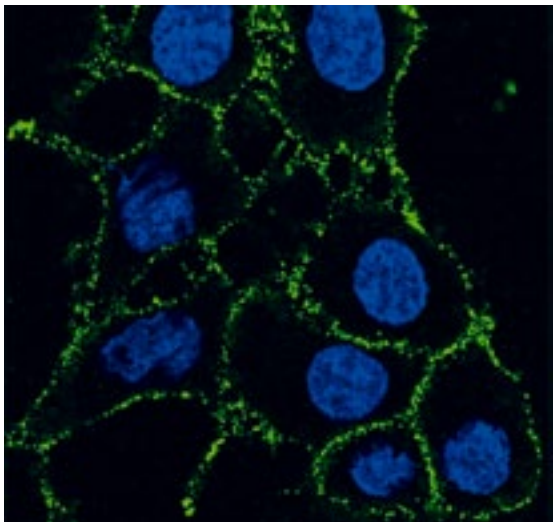
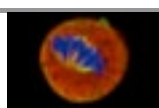


Fig. 1. Living MCF-7 cells were surface stained with anti-Hsp90 antibodies on ice, followed by fixation, permeabilization and DAPI stain of cell nuclei (author: Marcin Klejman).



purified recombinant proteins. Hsp90 stabilized binding of p53 to the promoter sequence at 37°C, however, during prolonged incubation at 37°C or under heat-shock conditions the requirement for Hsp70-Hsp40 system and its cooperation with Hsp90 increased. These interactions were additionally stimulated by Hop co-chaperone. In conclusion, we showed that both *in vivo* and *in vitro*, Hsp90 and Hsp70 chaperones were not only important for WT p53 function during and after heat-shock but also for the suppression of intrinsic WT p53 instability under physiological conditions.

Surprisingly, the Hsp90 protein possessing a single amino acid substitution E42A (a variant protein that can bind ATP but not catalyze its hydrolysis), still efficiently supported binding of p53 to the *WAF1* promoter. Directed mutagenesis of Hsp90 in conjunction with selective inhibition of Hsp90 activity by radicicol allowed us to elucidate the mechanism of Hsp90 molecular chaperone activity directed towards its natural substrate, p53. We have also proved that Hsp90N, a truncated form of human Hsp90 described in the literature as being involved in cell transformation, in fact was inexistent and its reported presence was an experimental artifact (Zurawska *et al.*, 2008).

In search for novel Hsp90-interacting proteins we have identified human NudC (nuclear distribution protein C homolog) protein family as *bona fide* Hsp90 cochaperones (Klejman *et al.*, manuscript in preparation). NudC was previously suggested to be involved in mitosis control via Plk1 and microtubules regulation. Hsp90 binds NudC in an ATP dependent fashion and Hsp90 inhibition with 17AAG diminishes the interaction. However, NudC levels remain stable, indicating that it is not a direct substrate of Hsp90. NudC protein family contains p23/Hsp20/CS-like domain. We have shown that, similarly to p23, NudC inhibits Hsp90 ATPase activity *in vitro*. Interestingly, isolated CS domain does not seem to interact with Hsp90 *in vitro*. This is in contrast with CS domains from other Hsp90 cochaperones, namely p23, and Sgt1. We speculate that mitotic checkpoint control by Hsp90 may be at least in part exerted by NudC.

In collaboration with Prof. Jacek Jassem, a clinician at Medical University of Gdańsk, we had previously demonstrated that MDM2 overexpresion was a new independent factor of adverse prognosis in non-small cell lung cancer (Dworakowska *et al.*, 2004). Recently we have discovered that MDM2, besides its E3-ubiquitin ligase activity, also possessed a molecular chaperone activity. We demonstrated that MDM2 mutant protein defective in ATP binding (K454A) lacked the chaperone activity both *in vivo* and *in vitro*. Wt MDM2 coexpressed with wild-type p53 stimulated efficient p53 protein folding *in vivo* and this effect was abrogated in case of ATP-binding defective form of MDM2 (Wawrzynow *et al.*, 2007). In collaboration with Prof. Ted Hupp laboratory we have developed a system for the analysis of the molecular chaperone function of MDM2 towards its target proteins, e.g. the transcription factor E2F1. In the absence of ATP, MDM2 was able to catalyse the inhibition of the DNA-binding function of E2F1. However, the inhibition of E2F1 by MDM2 was stimulated by ATP, and a mutation in the ATP-binding domain of MDM2 (K454A) prevented the ATP-stimulated inhibition of E2F1. Further, ATP stabilized the binding of E2F1 to MDM2 using in the same conditions in which ATP destabilized the MDM2:p53 complex. However, the ATP-binding-deficient mutant of MDM2 was active as an E3 ubiquitin ligase on E2F1 and p53, highlighting a specific function for the ATP-binding domain of MDM2 in altering substrate protein folding (Stevens *et al.*, 2008).

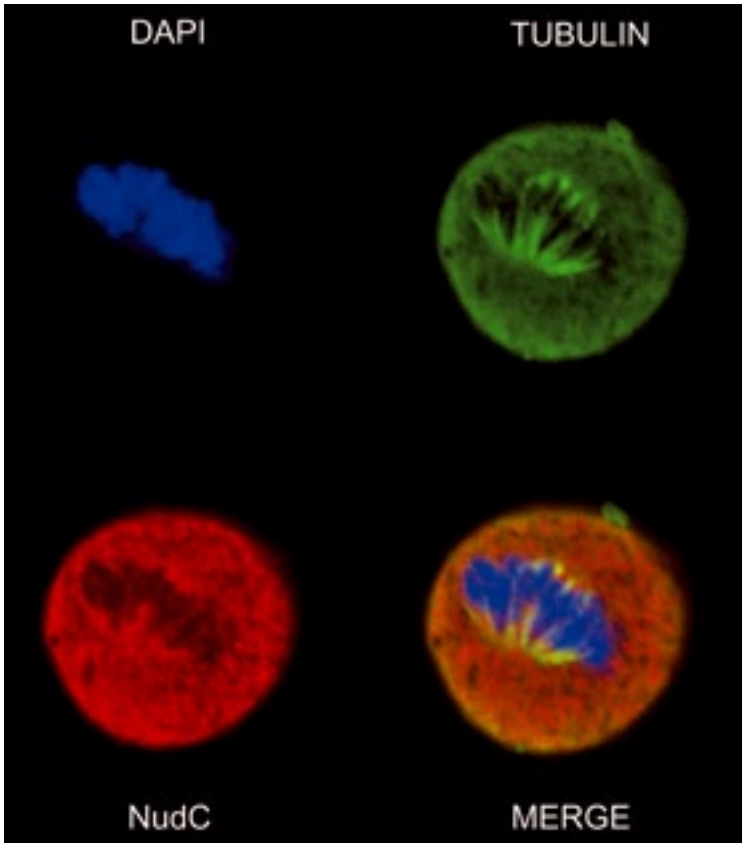


Fig. 2. HeLa cell arrested in mitosis stained for DNA (DAPI, blue), tubulin (green) and NudC (red) (author: Marcin Klejman).

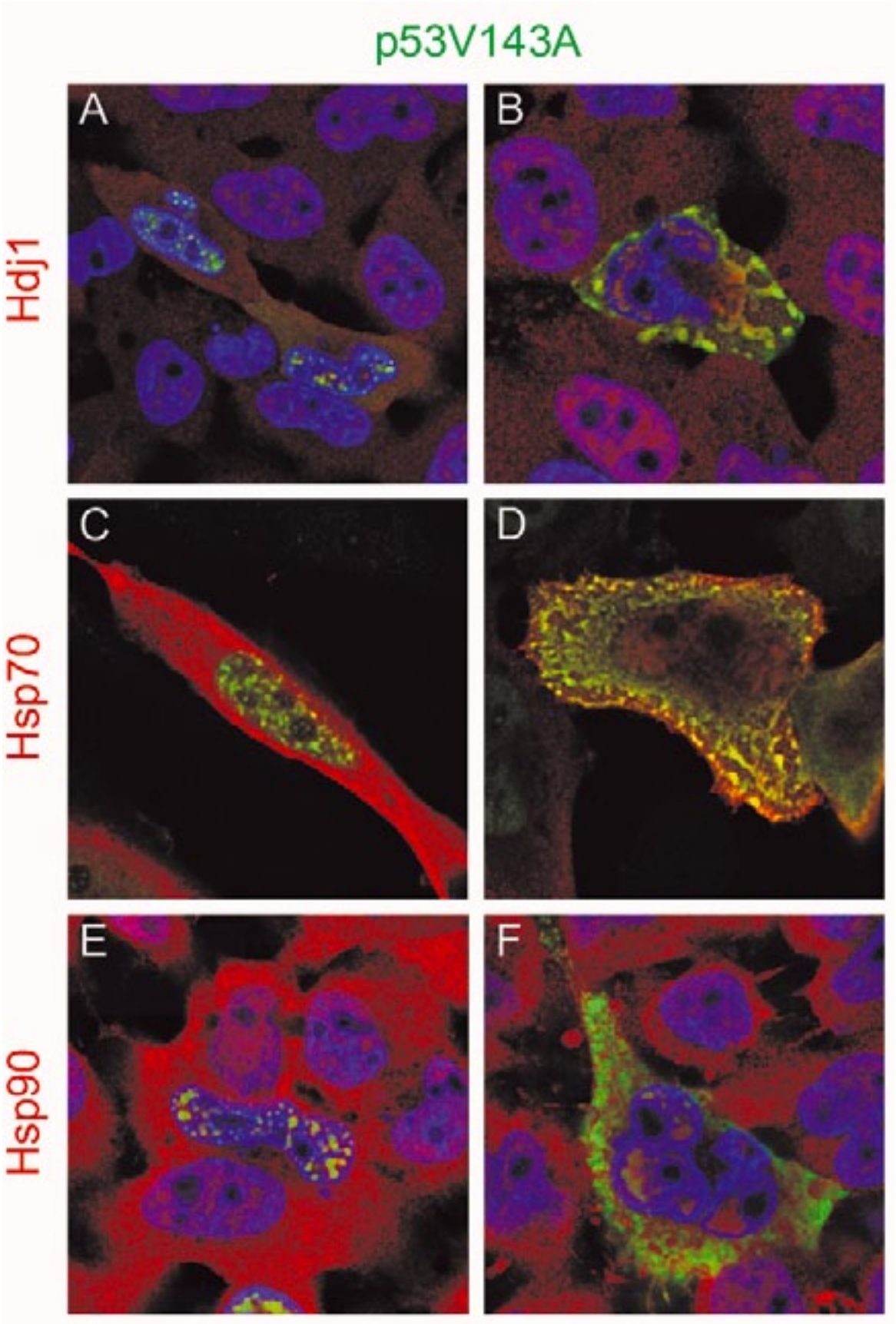
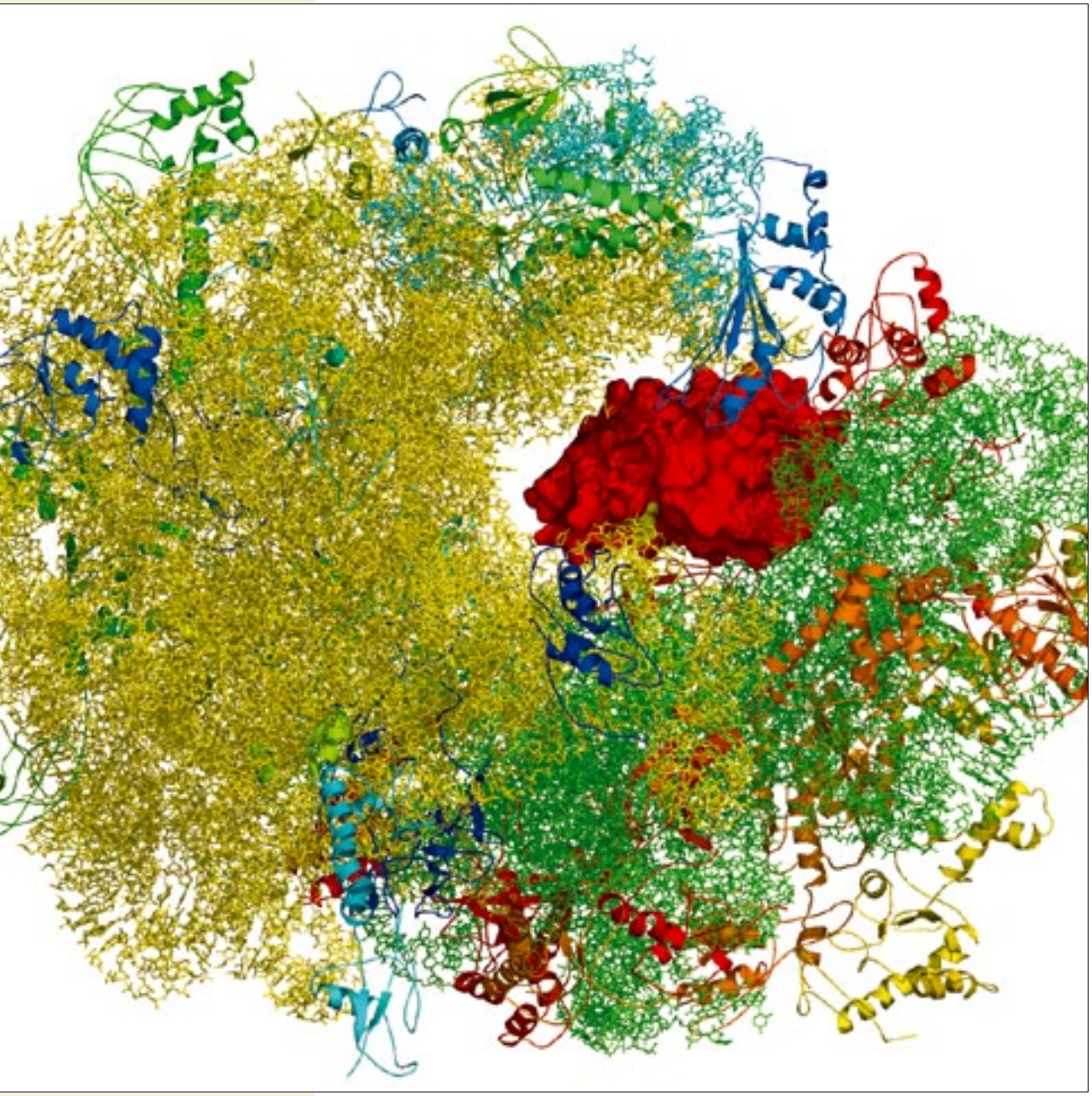


Fig. 3. Massive overexpression of Hsp70 induces formation of cytoplasmic aggregates containing mutant p53, Hsp90, Hsp70 and Hdj1 (author: Maciej Olszewski).
H1299 cells were cotransfected with p53 V143A and Hsp70, incubated at 37°C for 24 hours and immunostained for p53, Hdj1, Hsp70 and Hsp90, as indicated. Cells forming nuclear and more rare cytoplasmic aggregates are shown.



Computational docking model of m3Psi methyltransferase RlmH, bound to the bacterial ribosome. RlmH is shown in red. This image is also featured on the cover of the book „Prediction of Protein Structures, Functions, and Interactions", edited by Janusz M. Bujnicki for John Wiley & Sons (published in December 2008) – see page 10.

Laboratory of Bioinformatics and Protein Engineering

Lab Leader:

Janusz M. Bujnicki, PhD, DSc. Habil.

Post-doctoral Fellows:

Krzysztof J. Skowronek, PhD

Kristian Rother, PhD

Michał Boniecki, PhD

Junior Researchers & Research Assistants:

Małgorzata Durawa, MSc; Marcin Feder, MSc; Agata Kamaszewska, MSc; Katarzyna H. Kamińska, MSc; Andrzej Kamiński, MSc; Jan Kosiński, MSc; Łukasz Kozłowski, MSc; Agnieszka Obarska-Kosińska, MSc; Jerzy Orłowski, MSc; Grzegorz Papaj, MSc; Sebastian Pawlak, MSc; Marcin Pawłowski, MSc; Dariusz Pianka, MSc; Michał J. Piętal, MSc; Katarzyna Poleszak, MSc; Wojciech Potrzebowski, MSc; Elżbieta Purta, MSc; Wojciech Siwek, MSc; Karolina L. Tkaczuk, MSc; Ewa Tkalińska, MSc; Irina Tuszyńska, MSc; Maria Werner, MSc

Undergraduate Students:

Natalia Borkowska, Justyna Lesiak, Paweł Łukasz, BSc, Magdalena Mika, Krzysztof Nawara, BSc, Agata Nawara, BSc, Ewelina Osińska, Konrad Tomala, BSc

Office Manager:

Agnieszka Faliszewska, MSc (since December 2008), Natalia Kalina, MSc (until December 2008),

Computer Administrators:

Jan Kogut, MSc, Tomasz Jarzynka, Łukasz Munio

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)

PROFESSIONAL AFFILIATIONS

- Polish Society for Bioinformatics, PTBI (founding member and vice-president, since 2007)
- Society of Bioinformatics in Northern Europe, SocBiN (board member, since 2004)
- Member of the ELIXIR committee for building the Bioinformatics Training Strategy and the committee on global collaboration
- Member of International Society for Computational Biology and RNA Society
- Series editor, Nucleic Acids and Molecular Biology (Springer Verlag, since 2009)
- Editorial Board of Nucleic Acids Research, Advances in Bioinformatics, Journal of Applied Genetics, Biotechnology Journal, the Database Journal, Journal of Nucleic Acids

AWARDS

2008 Adam Mickiewicz University Rector Award for Research Achievement (Individual work)
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 Trends Biochem Sci. 2001 Jan; 26(1): 9-11)
2001 Award from the Polish Biochemical Society (the best Polish publication on nucleic acid biochemistry in the year 2000: FASEB J. 2000 Nov; 14(14): 2365-2368)

Publications in 2008

- **Orlowski J**, Mebrhatu MT, Michiels CW, **Bujnicki JM**, Aertsen A. Mutational analysis and a structural model of methyl-directed restriction enzyme Mrr. Biochem Biophys Res Commun 377, 2008; 862–866
- Gyrd-Hansen M, Darding M, Miasari M, Santoro MM, Zender L, Xue W, Tenev T, da Fonseca PCA, Zvelebil M, **Bujnicki JM,** Lowe S, Silke J, Meier P. IAPs contain an evolutionarily conserved ubiquitin-binding domain that regulates NF-kappaB as well as cell survival and oncogenesis. Nature Cell Biol, 2008; 10:1309-17
- **Purta E**, O'Connor M, **Bujnicki JM**, Douthwaite S. YccW is the m5C methyltransferase specific for 23S rRNA nucleotide 1962. J Mol Biol, 2008; 383:641-651
- **Obarska-Kosinska A**, Taylor JE, Callow P, Orlowski J, **Bujnicki JM**, Kneale GG. HsdR subunit of the Type I restriction-modification enzyme EcoR124I: biophysical characterisation and structural modelling. J Mol Biol, 2008; 376:438-452
- Sunita S, **Tkaczuk KL, Purta E**, Kasprzak J, Douthwaite S, **Bujnicki JM**, Sivaraman J. Crystal structure of the Escherichia coli 23S rRNA:m5C methyltransferase RlmI (YccW) reveals evolutionary links between RNA modification enzymes. J Mol Biol, 2008; 383:652-666
- **Pawlowski M, Gajda MJ, Matlak R, Bujnicki JM**. MetaMQAP: a meta-server for the quality assessment of protein models. BMC Bioinformatics, 2008; 9:403
- **Purta E, Kaminska KH**, Kasprzak J, **Bujnicki JM**, Douthwaite S. YbeA is the m3Psi methyltransferase RlmH that targets nucleotide 1915 in 23S rRNA. RNA, 2008; 14:2234-44
- **Kosinski J**, Plotz G, Guarne A, **Bujnicki JM**, Friedhoff P. The PMS2 subunit of human MutLalpha contains a metal ion binding domain of the iron-dependent repressor protein family. J Mol Biol, 2008; 382:610-627
- White J, Li Z, Sardana R, **Bujnicki JM**, Marcotte EM, Johnson AW. Bud23 methylates G1575 of 18S rRNA and is required for efficient nuclear export of pre-40S subunits. Mol Cell Biol, 2008; 28:3151-61
- **Orlowski J, Bujnicki JM**. Structural and evolutionary classification of Type II restriction enzymes based on theoretical and experimental analyses. Nucleic Acids Res, 2008; 36:3552-69
- Roovers M, **Kaminska KH, Tkaczuk KL**, Gigot D, Droogmans L, **Bujnicki JM**. The YqfN protein of Bacillus subtilis is the tRNA:m1A22 methyltransferase (TrmK). Nucleic Acids Res, 2008; 36:3252-62
- Khiang CT, **Bujnicki JM**, Chye TT, Huynh F, Patel BK, Sivaraman J. Mechanism of action and binding mode revealed by the structure of sucrose phosphate synthase from Halothermothrix orenii. Plant Cell, 2008; 20:1059-72
- Sen TZ, Kloster M, Jernigan RL, Kolinski A, **Bujnicki JM**, Kloczkowski A. Predicting the complex structure and functional motions of the outer membrane transporter and signal transducer FecA. Biophys J, 2008; 94:2482-91
- Cymerman IA, Chung I, Beckmann BM, Bujnicki JM, Meiss G. EXOG, a novel paralog of Endonuclease G in higher eukaryotes. Nucleic Acids Res, 2008; 36:1369-79
- Carpenter MA, **Bujnicki JM**, Bhagwat AS. Is AID a monomer in solution? DNA Repair, 2008; 7:349-350. Letter to the Editor.
- **Feder M, Purta E, Koscinski L**, Cubrilo S, Vlahovicek G, **Bujnicki JM**. Virtual screening and experimental verification to identify potential inhibitors of the ErmC methyltransferase responsible for bacterial resistance against macrolide antibiotics. ChemMedChem, 2008; 3:316-322
- Mittra B, Zamudio JR, **Bujnicki JM**, Stepinski J, Darzynkiewicz E, Campbell DA, Sturm NR. The TbMTr1 spliced leader RNA cap 1 2'-O-ribose methyltransferase from Trypanosoma brucei acts with substrate specificity. J Biol Chem, 2008; 283:3161-72
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Current Research

The Laboratory of Bioinformatics and Protein Engineering is involved in theoretical and experimental research on the sequence-structure-function relationships in proteins and nucleic acids and in macromolecular complexes. The laboratory is comprised of 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://iimcb.genesilico.pl/toolkit/ (MetaServer for primary, secondary, and tertiary structure prediction, methods for template-based and de novo modeling of three-dimensional protein structures, 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://iimcb.genesilico.pl/protmap2d.htm); the MODOMICS database for systems biology of RNA modification (http://iimcb genesilico.pl/modomics/) and the REPAIRTOIRE database for systems biology of DNA repair (http://iimcb.genesilico.pl/repairtoire/).
- A section devoted to the application of bioinformatics software to make biologically and biomedically relevant predictions. Recently published research includes phylogenomic analyses of various nuclease and methyltransferase superfamilies, and detailed structure prediction and modeling of individual proteins that are of wide interest (e.g. EXOG, a mitochondrial 5'–3' exonuclease potentially involved in apoptosis). 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 2008) 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 by gene cloning, protein expression, purification, development of in vitro and in vivo functional assays and biochemical and cellular characterization.
2. Experimental testing of structural predictions by application of low-resolution structural probing methods, such as mutagenesis, chemical modification,

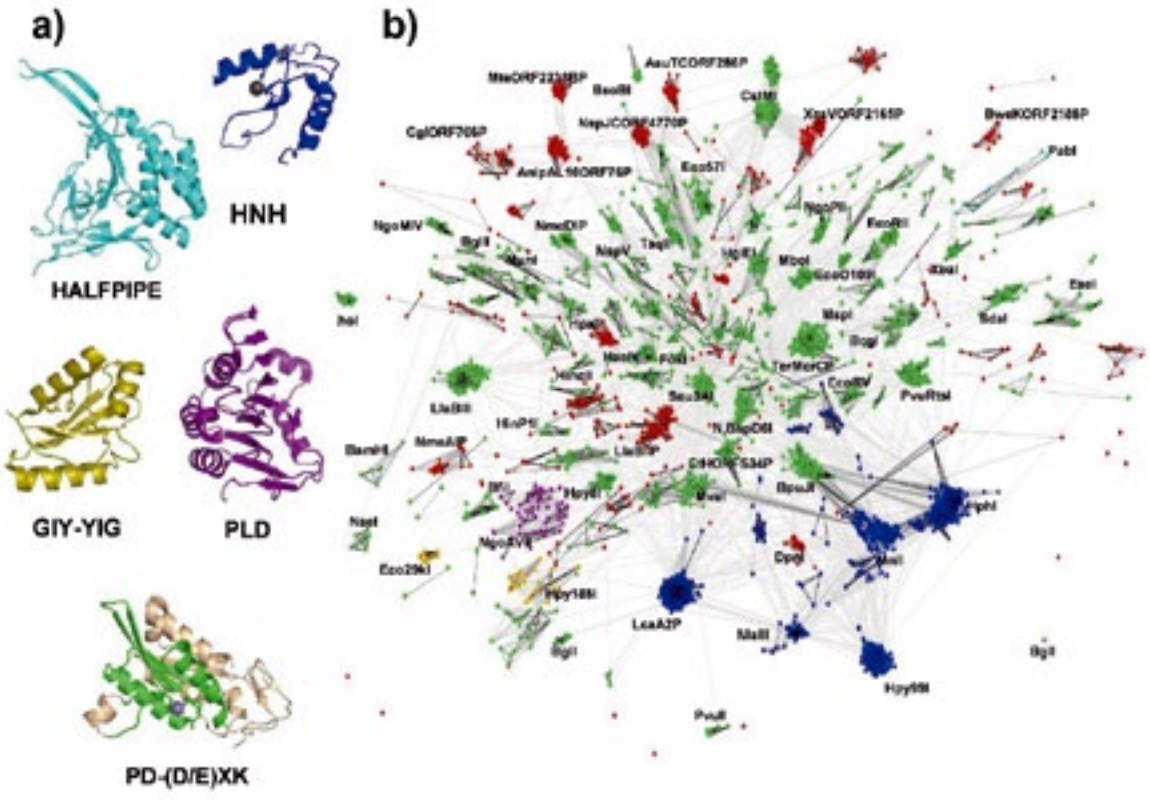


Fig. Distribution of Type II restriction enzyme sequences among known superfamilies/folds (published in Nucleic Acids Res 2008 Jun;36(11):3552-69).
a) Representative structures of nucleases with catalytic domains of different folds: PD-(D/E)XK – restriction enzyme BamHI (PDB: 3bam), HNH – T4 endonuclease VII (PDB: 1en7), GIY-YIG – restriction enzyme BfiI (PDB: 2c1l), PLD – homing endonuclease I-TevI (PDB: 1mk0), HALFPIPE – restriction enzyme PabI (PDB: 2dvy)
b) Results of clustering of nuclease domains of Type II REases from REBASE and their homologues in the nr and env_nr database with CLANS. Sequences and structures are colored according to the their assignment to superfamilies: green – PD-D(E)XK, blue – HNH, yellow – GIY-YIG, violet – PLD, light blue – HALFPIPE, red – unclassified. Connections between dots represent the degree of pairwise sequence similarity, as quantified by BLAST P-value (the darker the line, the higher similarity). The original figure is accessible from (Nucleic Acid Research (http://nar.oxfordjournals.org/cgi/content/full/36/11/3552) (author: Jerzy Orłowski).

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. prediction and characterization of new RNA methyltransferases. 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 highlights:

Protein Structure Prediction

The GeneSilico human predictors' group and several servers developed in our laboratory achieved high positions in rankings of the 8th Community-Wide Experiment on the Critical Assessment of Techniques for Protein Structure Prediction (CASP8), organized by the Protein Structure Prediction Center. Servers for protein structure prediction and model quality assessment achieved respectable positions in their respective categories. The most successful was the GeneSilico metaserver for protein disorder prediction, which ranked as number 1 in its category. These successful servers are freely available to all academic researchers via the GeneSilico toolkit.

New rRNA modification enzymes predicted and confirmed experimentally

Researchers from our laboratory predicted (with bioinformatics) and then experimentally confirmed that two so far uncharacterized open reading frames encode methyltransferases acting on 23S rRNA. YbeA is the m3Psi methyltransferase RlmH that targets nucleotide 1915, while YccW is the m5C methyltransferase RlmI specific for nucleotide 1962. Computational docking analysis revealed the potential way how RlmH may interact with the ribosome (as visualized on the figure p. 26). The experimental analysis was done in collaboration with a group in Odense headed by Prof. Stephen Douthwaite (University of Southern Denmark). Moreover, researchers from our laboratory analyzed the crystal structure of YccW/RlmI solved by a collaborating group (prof. Jayaraman Sivaraman, University of Singapore) and found that it provides a missing link in the evolutionary history of several different families of methyltransferases that modify cytosine in DNA or RNA as well as uridine in RNA. They have also identified a new putative RNA-binding domain dubbed „EEHEE". This domain is common to RlmI and several other RNA-modification enzymes, including methyltransferases involved in m5U formation and in Wye base biosynthesis.

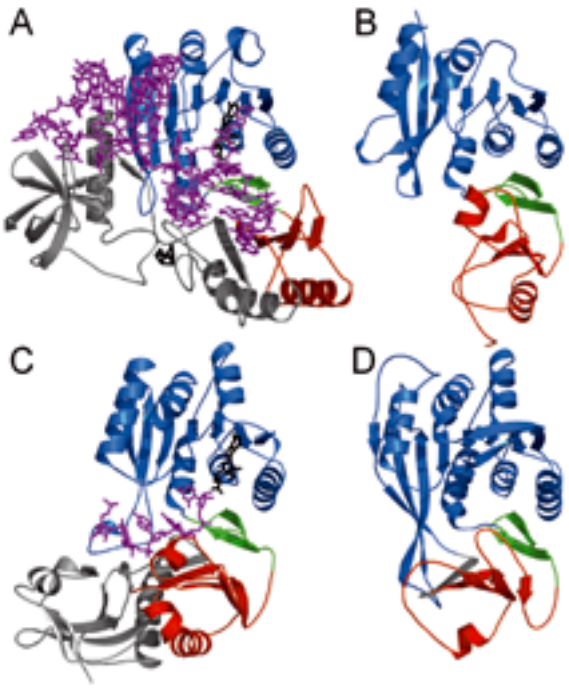
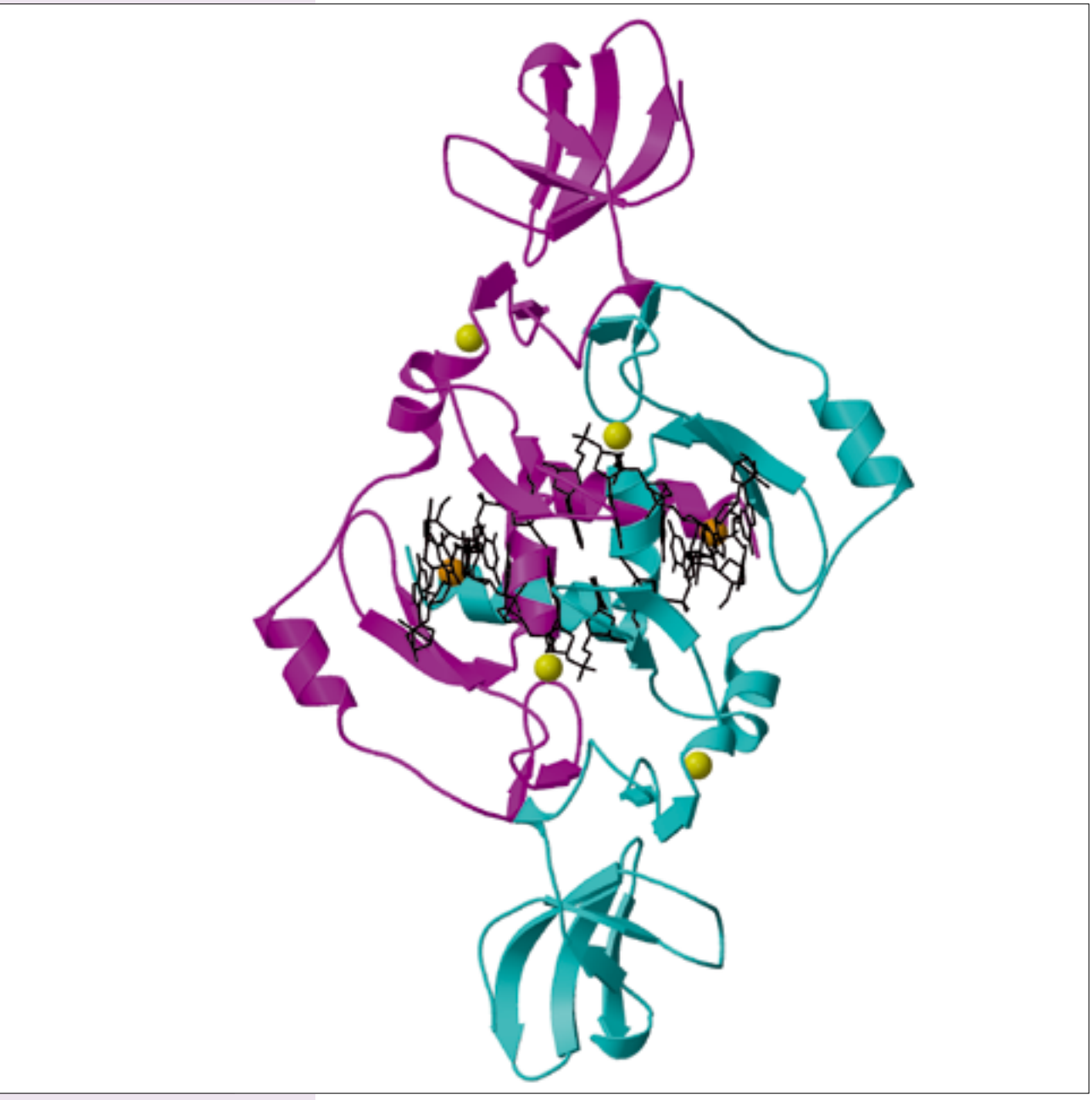


Fig. A novel putative RNA-binding domain discovered to be frequently associated with the Rossmann-fold methyltransferase domains in various RNA-modifying enzymes (Sunita et al., J Mol Biol 2008 Nov 14;383(3):652-66.).
A) rRNA:m5U methyltransferase RlmD in complex with the substrate RNA (PDB id: 2bh2),
B) tRNA:yW-86 synthesizing enzyme Trm12/Tyw2 (PDB id: 2frn),
C) rRNA:m5C methyltransferase RlmI (discovered and characterized with coworkers from Dennmark and Singapore) modeled as a complex with a fragment of the substrate RNA; PDB id: 3c0k)
D) hypothetical RNA modification enzyme (PDB id: 2igt) The catalytic Rossmann-fold methyltransferase domain is shown in blue.
The newly discovered domain nick-named „EEHEE" (abbreviation for the description of its secondary structure: extended-extended-helix-extended-extended") is shown in red. A beta-hairpin linker is shown in green. Other protein parts are in grey. RNA is shown in magenta, small-molecule ligands are shown in black (author: Janusz M. Bujnicki).



ββα-Me restriction endonuclease Hpy99I in complex with DNA. The two protein subunits are shown as ribbons and the DNA in all atom representation. The metal ions of the ββα-Me motifs are shown in orange, and structural Zn^{2+} ions in yellow.

Laboratory
of Structural Biology
MPG/PAN

Lab Leader:

Matthias Bochtler, PhD, DSc. Habil.

Post-doctoral Fellows:

Honorata Czapińska, PhD

Renata Filipek, PhD

Małgorzata Firczuk, PhD (until March 2009)

Izabela Sabała, PhD

Junior Researchers:

Grzegorz Chojnowski, MSc

Magdalena Kaus-Drobek, MSc

Henryk Korza, MSc

Magdalena Lipka, MSc (until March 2009)

Patrycja Kubajek, MSc

Monika Sokołowska, MSc

Roman Szczepanowski, MSc

Marek Wojciechowski, MSc

EU visiting experts:

Dario Piano, PhD

Sabah El Alaoui, PhD

MAX-PLANCK-GESELLSCHAFT

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY

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

Matthias Bochtler
PhD, 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 2007	part time Director of Structural Biology, Cardiff University, United Kingdom
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

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

Recent publications

Protein-DNA interactions

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- **Odintsov SG**, **Sabala I**, Bourenkov G, Rybin V, **Bochtler M**. Staphylococcus aureus aminopeptidase S is a founding member of a new peptidase clan. J Biol Chem, 2005; 280:27792-9
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Method development

- **Chojnowski G**, **Bochtler M**. The statistics of the highest E value. Acta Crystallogr A, 2007; 63:297-305
- **Bochtler M**, **Chojnowski G**. The highest reflection intensity in a resolution shell. Acta Crystallogr A, 2007; 63:146-155

Other

- Breer K, Wielgus-Kutrowska B, Hashimoto M, Hikishima S, Yokomatsu T, **Szczepanowski RH**, **Bochtler M**, Girstun A, Starón K, Bzowska A. Thermodynamic studies of interactions of calf spleen PNP with acyclic phosphonate inhibitors. Nucleic Acids Symp Ser (Oxf), 2008; 52:663-664.

Current Research

Protein-DNA interactions

Two-fold symmetry is a recurrent theme in protein-DNA interactions. Classic examples are the interactions between type II restriction endonucleases and their two-fold symmetric (palindromic) target sequences. In most cases, two-fold symmetry governs sequence recognition and DNA cleavage. Exact two-fold symmetry is only possible in DNA duplexes that consist of an even number of base pairs. In duplexes that consist of an odd number of base pairs, the requirements of hydrogen bonding and two-fold symmetry conflict for the central base pair. Therefore, such sequences can at best be pseudosymmetric (pseudopalindromic). At the center, the possibilities for base recognition are limited: either the bases in this position are not read out at all, or A:T pairs (W) are distinguished from G:C pairs (S), irrespective of which DNA strand contains the purine and which strand contains the pyrimidine base. The latter type of recognition poses difficulties for the "typical" major groove readout of the DNA base sequence: in the major groove, the hydrogen bonding patterns of a G:C and T:A are similar, but differ from the patterns for C:G and A:T. The research group has systematically studied restriction endonucleases that recognize and cleave pseudopalindromic DNA sequences.

The nucleotide flippers Ecl18kI and PspGI: The related PD-(D/E)XK restriction endonucleases Ecl18kI and PspGI are specific for the sequences /CCNGG and /CCWGG respectively. Our crystal structures of these enzymes show that both enzymes form functional dimers that extrude the central bases of their recognition sequences from the DNA and flip them into pockets of the enzymes.

"Nucleotide flips" have been observed before, especially in the contexts of DNA base modification and DNA repair. However, the nucleotide flips in the complexes of Ecl18kI and PspGI with DNA are unusual in several ways: (a) the enzymes flip intact bases, which are not modified chemically. (b) the enzymes flip both bases of the DNA stack. (c) the void left behind by the flipped bases is not filled by DNA intercalating residues as in most other cases of nucleotide flipping, but is instead closed by "compressing" the DNA, so that the base pairs that flank the flipped base pair get into almost direct contact. Ecl18kI simply "skips" the flipped bases for recognition, but PspGI distinguishes A:T pairs from G:C pairs. Is there a mechanism that can explain this distinction without the need to identify individual bases? We reasoned that nucleotide flipping might serve as a "test" of the strength of the hydrogen bonding interactions. According to this model, PspGI would be "strong" enough to break the two hydrogen bonds that hold A:T/T:A ("W, weak"), but not G:C/C:G ("S, strong") pairs together. Kinetic experiments show that base pair strength plays a role for base pair discrimination, but is alone insufficient to explain the high specificity, as was previously concluded for DNA repair enzymes that recognize lesions in DNA.

The monomeric restriction endonucleases MvaI and BcnI: The PD-(D/E)XK restriction endonucleases MvaI (CC/WGG) and BcnI (CC/SGG) recognize similar sequences as

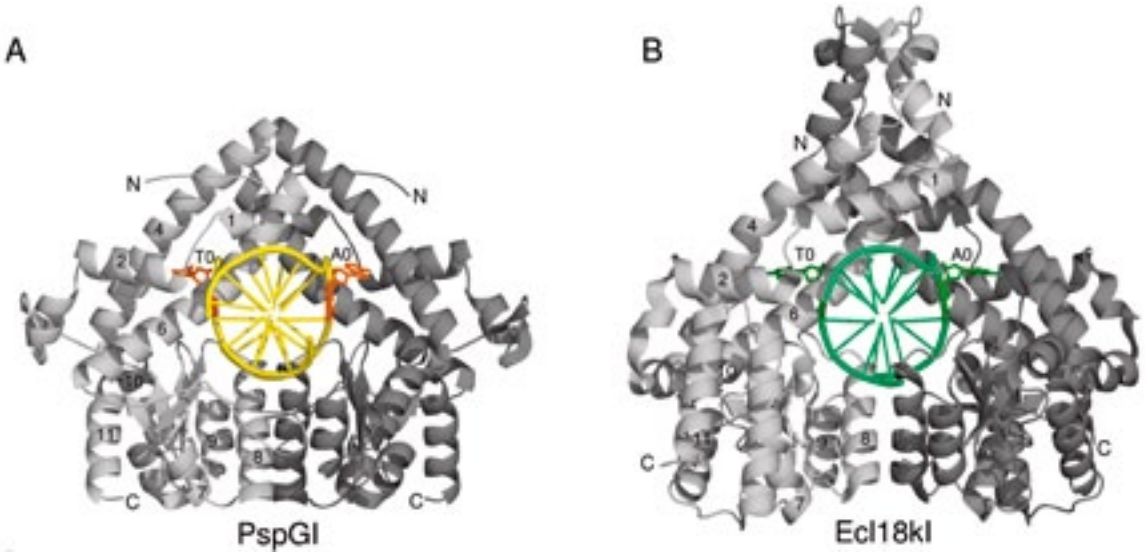


Fig. 1. Co-crystal structures of (A) PspGI and (B) Ecl18kI with DNA. The flipped nucleotides are shown in color in all-atom representation. The figure was taken from Szczepanowski et al., Nucleic Acids Res. 2008, 36:6109-17.

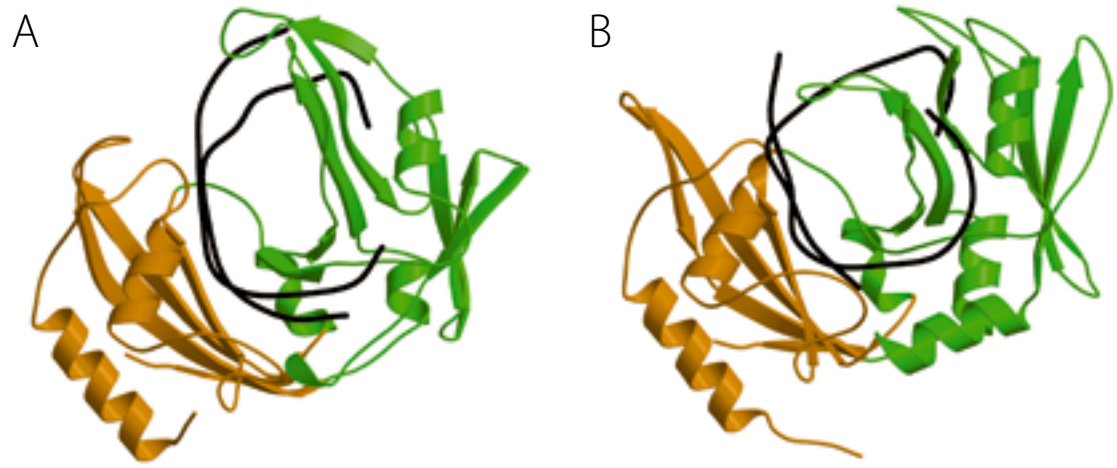


Fig. 2. Comparison of the DNA co-crystal structures of (A) BcnI and (B) MvaI. The figure was adapted from Sokolowska et al., Cell Mol Life Sci 2007, 64: 2351-2357.

Ecl18kI and PspGI, but cleave them with a different stagger. Nevertheless, MvaI and BcnI have evolved a very different strategy to deal with the asymmetries of their substrates: both enzymes bind their substrates as monomers. As there is only one active site per monomer, the implication is that MvaI and BcnI must cleave the two DNA strands one after another, with an intermittent DNA rebinding event to bring the uncleaved strand of the nicked intermediate into a position proximal to the active site. Support for a nicked intermediate in the DNA cleavage reactions by MvaI and BcnI is also provided by comparison of the MvaI and BcnI structures with all structures in the Protein Data Bank, because it turns out the MvaI and BcnI are more similar to the DNA nickase MutH, a component of the mismatch repair machinery, than to any other DNA restriction endonuclease of known structure (Fig. 2).

The ββα-Me restriction endonuclease Hpy99I: This restriction endonuclease is specific for the sequence CGWCG/ and cuts DNA into fragments with highly unusual 5 nucleotide long 3'-overhangs. Our recent crystal structure of this enzyme represents the first structure of a ββα-Me restriction endonuclease and allows detailed comparisons with previously determined structures of ββα-Me endonucleases that play no role in restriction biology, but are involved in unspecific DNA degradation (such as the Serratia nuclease), in homing (such as I-PpoI) or Holliday junction resolution (T4 endonuclease VII). Hpy99I distinguishes between W and S at the center of its target sequence by exclusive minor groove readout.

Unlike major readout, minor groove readout is perfectly suitable to distinguish S and W. The presence of an amino group (of guanine) in the central minor groove position signals a G:C/C:G pair, its absence (which is verified by two Hpy99I arginines) confirms the presence of an A:T/T:A pair. Based on mutagenesis data alone, this mechanism has been suggested earlier for methyltransferases. To our knowledge, our Hpy99I-DNA co-crystal structure provides its first crystallographic demonstration.

Peptidoglycan amidases

Bacterial peptidoglycan amidases are a diverse group of enzymes. Some are metallopeptidases, others serine peptidases, and yet others cysteine peptidases. Only aspartic (and threonine) peptidases have so far not been found. In each catalytic group, different folds can be discerned,

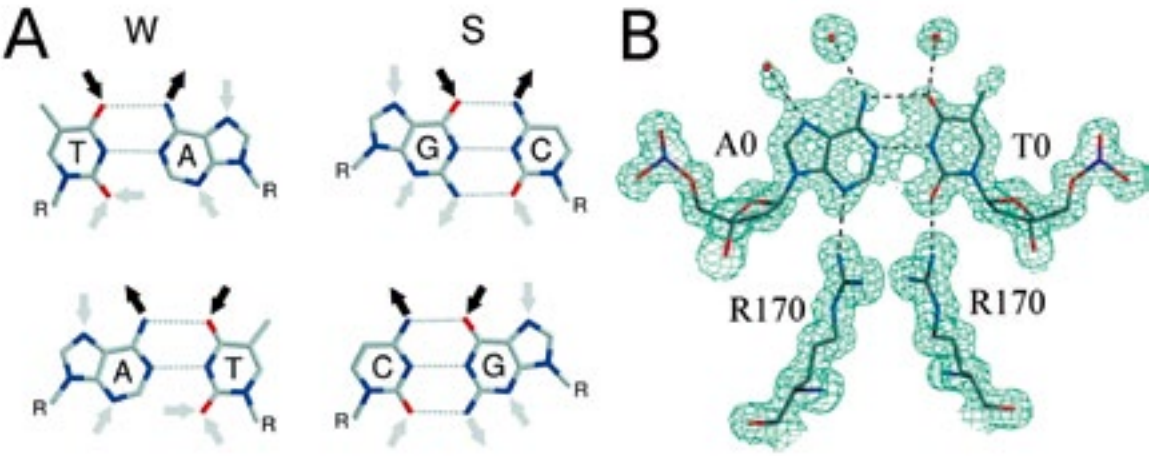


Fig. 3. (A) Hydrogen bonding patterns of T:A/A:T pairs (W) and G:C/C:G pairs (S). The figure highlights the similarity of T:A and G:C pairs on the major groove side, as well as the similarity of A:T and C:G pairs, which makes the distinction between W and S by major groove readout difficult. (B) Recognition of the symmetry violating A:T/T:A pair by Hpy99I. This enzyme "verifies" the absence of a guanine amino group in the central minor groove position, which could clash with the two arginine residues of the protein. The figure has been adapted from Szczepanowski et al., Nucleic Acids Res. 2008, 36:6109-17 and from Sokolowska et al., Nucleic Acid Research 2009, in press.

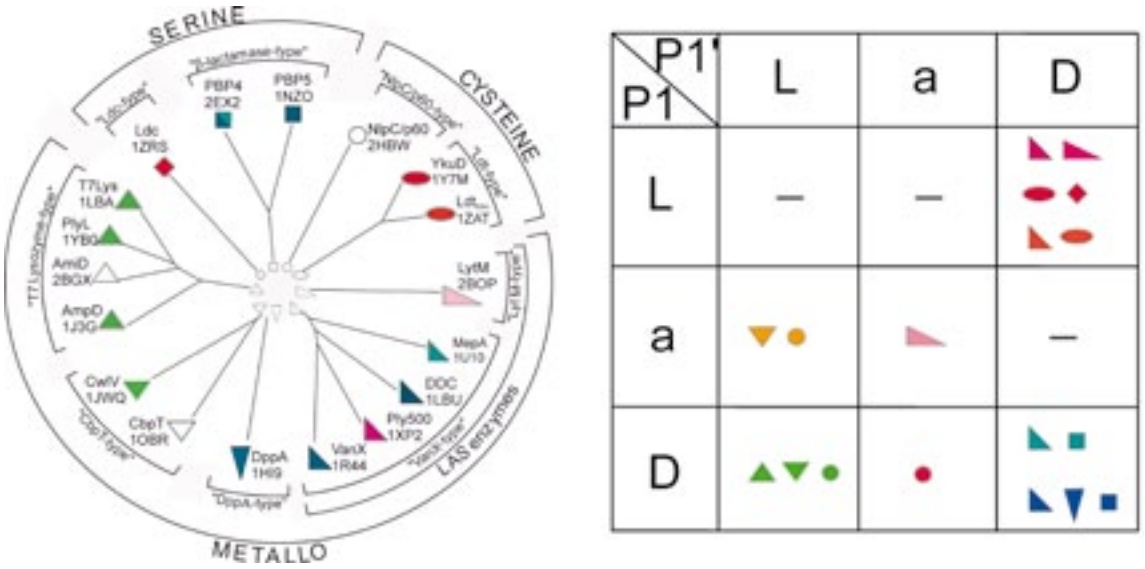


Fig. 4. Overview of peptidoglycan amidase fold groups (left) and their catalytic activities, ordered according to the chiral configuration of the amino acids immediately upstream (P1) and downstream (P1') of the scissile amide bond. Geometric shapes represent fold groups and colors code for different peptide bonds in peptidoglycan. The figure is adapted from Firczuk and Bochtler, FEMS Microbiol Rev. 2007 31:676-91.

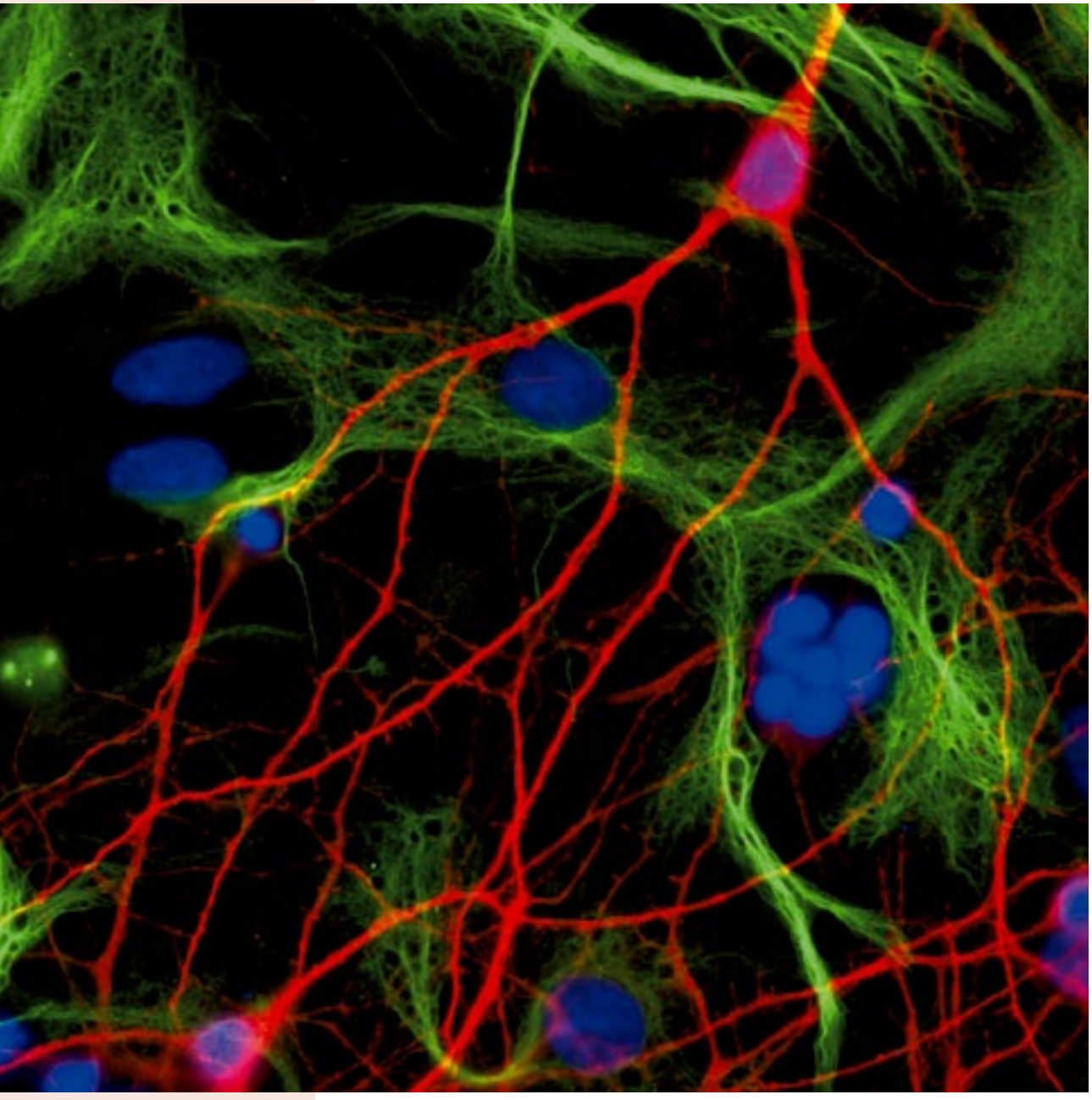
strongly suggesting that different peptidase clans have independently acquired the ability to cleave peptidoglycan. Most peptidoglycan amidases are highly specialized enzymes and cleave only one type of amide bond in bacterial cell walls. However, the connection between enzyme folds and activities has been largely unclear, in part because "founder" structures for many peptidoglycan amidase clans had not yet been resolved crystallographically.

Our group has systematically attempted the determination of crystal structures of peptidoglycan amidases that we predicted to have new folds. In some cases, our quest for "founder" structures has been successful (LytM, MepA, Ldc). Altogether, our contributions to the Protein Data Bank have expanded the "fold space" of peptidoglycan amidases by more than 10%.

What has been learned from these studies? Some of the individual structures that we solved were revealing. In the case of LytM, we found the first example of an "asparagine switch", a variant of the well-known "cysteine switch" that keeps standard HEXXH metallopeptidases inactive. Together with the MepA structure, the LytM structure also prompted the definition of the LAS group of peptidoglycan amidases and related enzymes. Despite negligible sequence similarity, these enzymes share a core folding motif and similar active sites. In the serine peptidase LD-carboxypeptidase we found an unusual catalytic triad with Ser-His-Glu instead of the usual Ser-His-Asp. The active site serine residue is located (in a strained Ramachandran forbidden conformation) at the N-terminus of a 3/10-helix that leads into a regular α-helix. This so-called "nucleophilic elbow" arrangement is essentially identical to the arrangement of the active site nucleophile in the αβ-hydrolases. As LD-carboxypeptidases and αβ-hydrolases have dissimilar overall folds, the recurrence of the nucleophilic elbow motif represents an example of convergent evolution of a catalytically useful module. Together with the work of others, our structures also shed light on the link between peptidoglycan amidase structure and function: they reveal that related enzymes (in the same fold group) often cleave different bonds in peptidoglycan, but have usually identical or similar stereochemical preferences for the chiral centers upstream and downstream of the scissile peptide bond (Fig. 4).

Method development

X-ray fiber diffraction photographs of proteins and nucleic acids show characteristic peaks that reflect simple repeats of these structures. In the case of B-DNA, the most prominent peaks are the so-called "meridional" 3.4 Å reflections which arise due to the constructive interference of scattering from base pairs at van der Waals distance. For proteins, peaks of similar shape at 1.5 Å and of more complex shape at lower resolution are due to the presence of the α-helices and β-sheets. If DNA or protein is present in 3D-crystals, the characteristic fiber diffraction pattern is sampled by the reciprocal lattice, but because cell constants are typically large compared to characteristic distances in secondary structure, not too much information is lost by the sampling. We have developed software that looks for the traces of fiber diffraction peaks in 3D diffraction data. Our first tool is the program DIBER, which helps the user to decide whether a user dataset contains only protein, only DNA or a mixture of both. Despite its conceptual simplicity, the program outperforms sophisticated molecular replacement programs such as PHASER in this simple task. A CCP4 and CCP4I compatible version of DIBER will be made available under GNU Public Licence.



Thalamic cultures. Thalamic neurons (red), glial cells (green), nuclei (blue) (author: Katarzyna Misztal).

Laboratory **of Neurodegeneration**

Lab Leader:

Jacek Kuźnicki, PhD, Professor

Associate Professor:

Urszula Wojda, PhD, DSc. Habil.

Post-doctoral Fellows:

Joanna Gruszczyńska, PhD

Monika Klejman, PhD

Anna Skibińska-Kijek, PhD

Marta Wiśniewska, PhD

Junior researchers who defended their PhD thesis in 2008

Łukasz Bojarski

Magdalena Blażejczyk (until March 2008)

Adam Sobczak

Junior researchers:

Emilia Białopiotrowicz, MSc

Katarzyna Dębowska, MSc

Bożena Kuźniewska, MSc

Wojciech Michowski, MSc

Katarzyna Misztal, MSc

Andrzej Nagalski, MSc

Aleksandra Szybińska, MSc

Office Manager:

Dominika Dubicka, MSc (until July 2008)

MSc Students:

Mirosław Drab (thesis defended in July 2008)

Kamila Skieterska

Bożena Żebrowska (until June 2008)

Jacek Kuźnicki
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 European Calcium Society Board, since 2008
- Member of Prime Minister Award Committee, 2007 - 2009
- Member of Health Research Advisory Group the 7th FP European Commission, since 2006
- Member of the Polish Academy of Sciences (PAN), since 2004
- Member of the American Society for Biochemistry and Molecular Biology, since 2003
- Head of the Advisory Board of the Centre for Innovative Bioscience Education (SFN), 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

- Officer's Cross of the Order of Polonia Restituta awarded by the President of the Republic of Poland, 2008
- Professorial Subsidy Program Award from Foundation for Polish Science (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
- Knight's Cross of the Order of Polonia Restituta awarded by the President of the Republic of Poland, 1998
- Polish Anatomical Society Award for the article on calcium binding proteins published in "Advances in Cell Biology", 1987
- Skarżyński 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
- Mozołowski Award, Polish Biochemical Society for outstanding Polish young biochemists, 1977
- MSc, Magna cum laude, University of Warsaw, 1976

Publications in 2008

- **Bojarski L**, Pomorski P, **Szybinska A, Drab M, Skibinska-Kijek A, Gruszczynska-Biegala J, Kuznicki J.** Presenilin-dependent expressions of STIM proteins and dysregulation of capacitative Ca(2+) entry in familial Alzheimer's disease. Biochim Biophys Acta MCR, 2008 Dec 6; [Epub ahead of print]
- **Klejman ME, Gruszczynska-Biegala J, Skibinska-Kijek A, Wisniewska MB, Misztal K, Blazejczyk M, Bojarski L, Kuznicki J.** Expression of STIM1 in brain and puncta-like colocalization of STIM1 and ORAI1 upon depletion of Ca^{2+} store in neurons. Neurochem Int, 2009; 54:49-55
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- **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. Exp Gerontol, 2008; 43:238-244
- **Bojarski L**, Herms J, **Kuznicki J**. Calcium dysregulation in Alzheimer's disease. Neurochem Int, 2008; 52:621-633
- **Wojda U**, Salinska E, **Kuznicki J.** Calcium ions in neuronal degeneration. IUBMB Life, 2008; 60:575-590
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- Zabka M, Lesniak W, Prus W, **Kuznicki J,** Filipek A. Sgt1 has co-chaperone properties and is up-regulated by heat shock. Biochem Biophys Res Commun, 2008; 370:179-183
- Filipek A, **Michowski W, Kuznicki J.** Involvement of S100A6 (calcyclin) and its binding partners in intracellular signaling pathways. Advan Enzyme Regul, 2008; 48:225-239
- **Blazejczyk M, Wojda U.** Factfile: Calmyrin 1 (review). Calcium Binding Proteins, 2008; 3:1-3

Current Projects

We are interested in molecular mechanisms involved in learning and memory, as well as neurodegeneration; we study these processes at the genomic, proteomic and cellular levels.

Our major projects are:

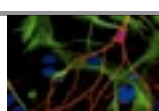
1. Search for bio-markers and potential therapeutic targets for Alzheimer's disease (AD).
2. Analysis of proteins involved in Ca^{2+} homeostasis in neurons and non-neuronal cells.
3. Analysis of Ca^{2+}-binding proteins calmyrin1 and calmyrin2 in neurons.
4. Regulation and role of β-catenin/Lef1 complex in mature neurons.
5. Characterization of biological functions of CHORD containing proteins in the nervous system.
6. Studies on the cyclin-dependent kinase 5 involvement in pathogenesis of Alzheimer's disease.

1. Search for functional bio-markers and potential therapeutic targets of Alzheimer's disease *(Emilia Białopiotrowicz, Łukasz Bojarski, Mirosław Drab, Aleksandra Szybińska, Bożena Kuźniewska, Urszula Wojda, in collaboration with other laboratories)*

In this area, several projects were carried out:

1.1. In cooperation with Prof. Maurizio 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 10 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 Psych, 2008). Since p53 conformational tertiary structure is influenced by redox status of the cells, we also evaluated the oxidative profile of these patients. We found that among the markers of oxidative stress, hydroxy-transnonenal-modified proteins were significantly increased in FAD patients. Furthermore it is interested to note that, besides increased levels of oxidative markers, the antioxidant defence mechanisms were compromised in these patients. These results supported and enhanced the first evidences of peripheral unfolded p53 associated with AD pathology and highlighted the identification of oxidative stress markers in peripheral, immortalised cells derived from FAD patients.

1.2. It has been suggested that the aberrant expression of cell cycle molecules in the brain contributes to the development of Alzheimer's disease (AD) and causes neuronal death. The aim of the current study was to determine whether the alterations in cell cycle progression can be observed in lymphocytes from SAD and FAD patients. Immortalized B-lymphocytes from 17 SAD and 6 FAD patients (bearing distinct PS1 mutations) were studied in comparison to lymphocytes from 18 healthy individuals.



Additionally, cell cycle analysis was performed in transiently and stably transfected HEK293 cells with wild type and mutated PS1 constructs. The cell cycle was analyzed by flow cytometry after staining of cells with propidium iodide. Moreover, expression level of cell-cycle related proteins was assessed by immunoblotting. The obtained data has shown cell cycle disturbances in lymphocytes from AD patients. Moreover, our results reveal a relationship between PS1 and the cell cycle regulation. Finally, this data indicates that human lymphocytes sustain an easily accessible material that can be used in studies on AD pathogenesis, and in search for possible diagnostic markers and therapeutic targets (Bialopiotrowicz et al., in preparation).

1.3. 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.

2. Analysis of proteins involved in Ca^{2+} homeostasis in neurons and non-neuronal cells *(Łukasz Bojarski, Monika Klejman, Joanna Gruszczyńska-Biegała, Anna Skibińska-Kijek, in collaboration with partners from PROMEMORIA 6th FP of EU and from the Polish-German grant)*

Store Operated Calcium Entry (SOCE) is well known in non-excitable cells. It is based on the interaction of ER calcium sensors STIM1 or STIM2 with the plasma membrane calcium channel protein ORAI1. Although SOCE is ubiquitous in non-excitable cells, it is also crucial for the nervous system. Its alterations cause deregulation of calcium homeostasis in the cell and may lead to pathology like Alzheimer's and Huntington's disease.

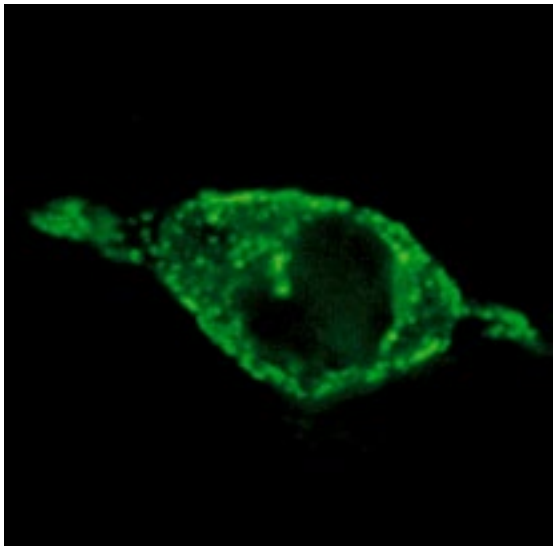


Fig. 1. STIM1 forms puncta upon thapsigargin-induced ER depletion in cortical neurons. Cortical neurons were co-transfected with ORAI1 and YFP- STIM1 and treated with 3 μM thapsigargin for 15 min. Neurons were analyzed using a confocal microscope and image represents 0.25 μM thick confocal scan (author: Joanna Gruszczyńska-Biegała).

We analyzed and compared the distribution of STIM1 and STIM2 in mice brains and in cultured cortical and hippocampal neurons using various techniques. We showed that the protein and mRNA levels of STIM1 and STIM2 vary in different brain regions. Immunohistochemistry of brain sections shows a distinct distribution of both proteins mostly in the hippocampus, cerebellum and the amygdala (Skibinska-Kijek et al., submitted). We also demonstrate that STIM1 and STIM2 are present in cultured neurons and their expression is accumulated mainly in the cell bodies.

Our data revealed that depletion of calcium stores in cultured cortical neurons induces a change in the localization of YFP-STIM1, YFP-STIM2 and ORAI1 from disperse, in untreated, to puncta-like in thapsigargin treated cells. We propose that, in neurons, just as in non-excitable cells, the ORAI1 and STIM proteins are involved in store operated calcium entry (Klejman et al., Neurochem Int 2008). We also investigated the role of STIM proteins in presenilin dependent alterations of capacitative Ca^{2+} entry that are observed in AD (Bojarski et al., BBA MCR, 2008).

3. Analysis of Ca^{2+} - binding proteins calmyrin 1 and calmyrin 2 in neurons *(Magdalena Błażejczyk, Katarzyna Dębowska, Adam Sobczak, under the supervision of Urszula Wojda and in collaboration with the Laboratory of Molecular and Cellular Neurobiology headed by Dr. Jacek Jaworski)*

Ca^{2+}-binding proteins in neurons regulate neuronal development, plasticity, and neurodegeneration. They also draw much attention due to implications in multiple brain pathologies including Alzheimer's disease. Our research concentrated on a novel family of Ca^{2+}-binding proteins called calmyrins (CaMy, known also as KIP or CIB). In humans, four genes encode calmyrin proteins (CaMy1 – CaMy4). The aim of our studies is to elucidate functions of CaMy1 and CaMy2 in neurons by analysis of CaMy1 and CaMy2 localization, biochemical properties and protein ligands in the brain.

We have previously demonstrated that CaMy1 is implicated in Alzheimer's disease and that it interacts specifically with Alzheimer's disease associated presenilin 2 (PS2) in vitro and in vivo (Bernstein et al., Neuropathol Appl Neurobiol. 2005; Blazejczyk et al., Biochim Biophys Acta. 2006). Our results indicate, however, that the interaction of CaMy1 with PS2 in neurons is limited and does not account for the involvement of CaMy1 in Alzheimer's disease. Therefore, we have undertaken the search for other possible protein ligands of CaMy1. Using several biochemical methods, we identified a new potential target of CaMy1 in neurons and characterized CaMy1 interaction with its novel protein ligand in vitro. Currently, we are investigating the functional role of this new CaMy1 interaction using cultured primary hippocampal neurons (Sobczak et al., in preparation).

Moreover, we pursued 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 the Golgi apparatus and dendrites. Moreover, we showed that CaMy2 expression

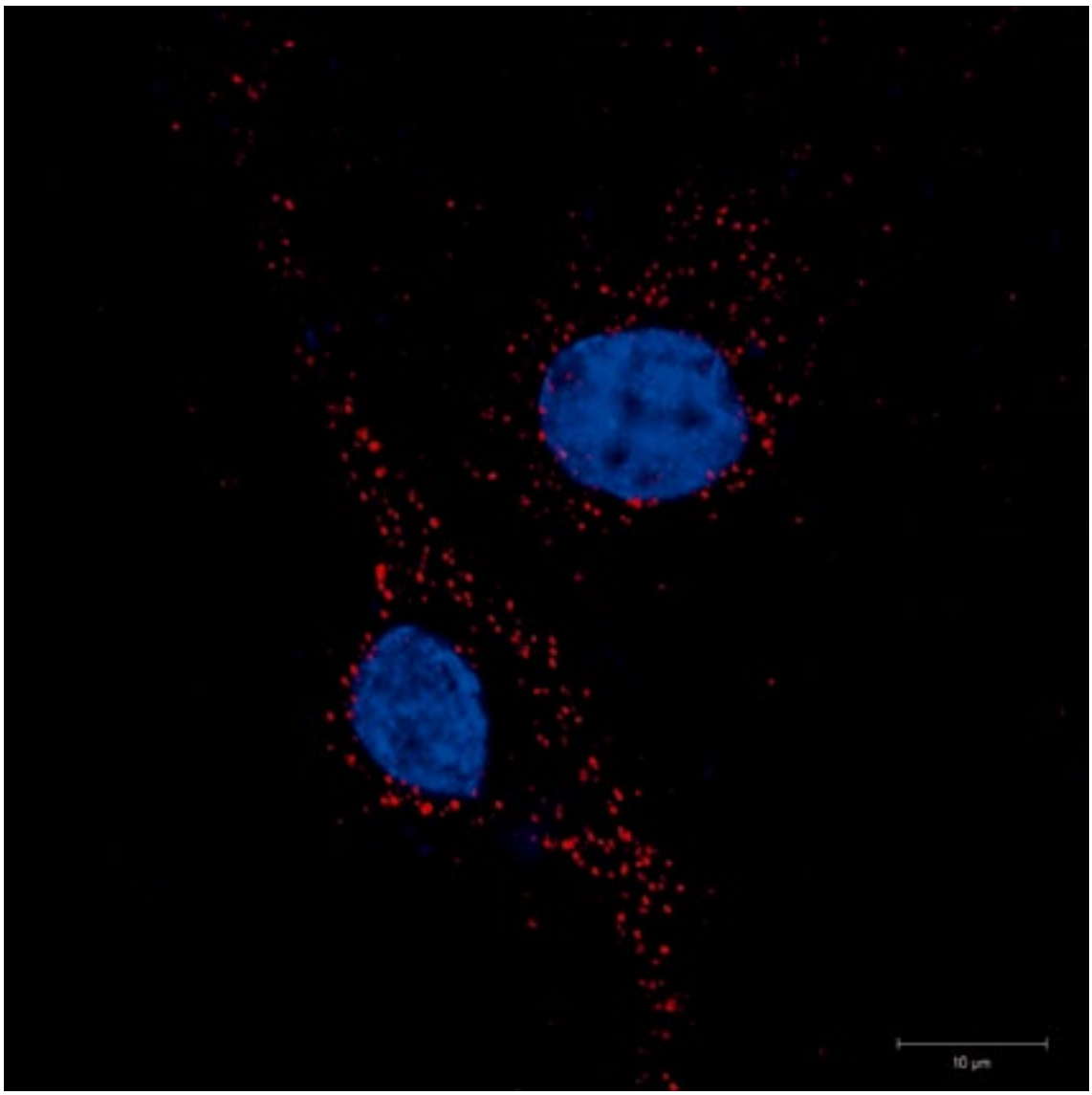


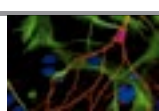
Fig. 2. 21 days *in vitro* hippocampal culture from rat embryos E19; stimulated with NMDA; Duolink in situ Proximity Ligation Assay for Calmyrin 2 and NSF interaction (red dots); nucleus (blue) (author: Magdalena Błażejczyk).

in primary cultures of rat neurons is regulated by NMDA receptor activation and associated Ca^{2+} signaling (Blazejczyk et al., submitted). We have also identified several new potential targets of CaMy2 in rat brains and confirmed these interactions by several methods in vitro. Physiological significance of these interactions in primary neurons is currently under investigation.

4. Role and regulation of β-catenin in mature neurons
(Katarzyna Misztal, Wojciech Michowski, Andrzej Nagalski, Marta Wiśniewska in collaboration with partners from PROMEMORIA 6th FP of EU)

β-catenin is involved in the regulation of proliferation and differentiation of neuronal precursor cells as an activator of the Lef1/Tcf transcription factor and component of the cadherin cell-adhesion complex. In mature neurons β-catenin participates in synaptogenesis and synaptic function in the cadherin complex. However, its transcriptional activity in mature neurons remain elusive. We are interested in the function of β-catenin in the adult brain, since new data suggests it might be involved in learning and memory formation, as well as in some brain pathology. We look for β-catenin/Lef1 target genes in mature neurons **i)** and explore the mechanism of stabilization of β-catenin in mature thalamic neurons **ii)**.

i)Using Real Time PCR, immunohistochemistry and Western blot techniques we demonstrated that β-catenin and Lef1 are expressed at high levels in neurons of the adult thalamus, in contrast to other regions of the forebrain. Moreover, both proteins are present in the cell nuclei, implying their involvement in gene expression. To answer the question about the role of the Lef1/β-catenin complex in the adult thalamic neurons we investigated a possible involvement of the complex in regulating genes encoding proteins indispensable for thalamic functions. We hypothesized that the Lef1/β-catenin transcription complex enhances expression of Cacna1G encoding Cav3.1 in mature



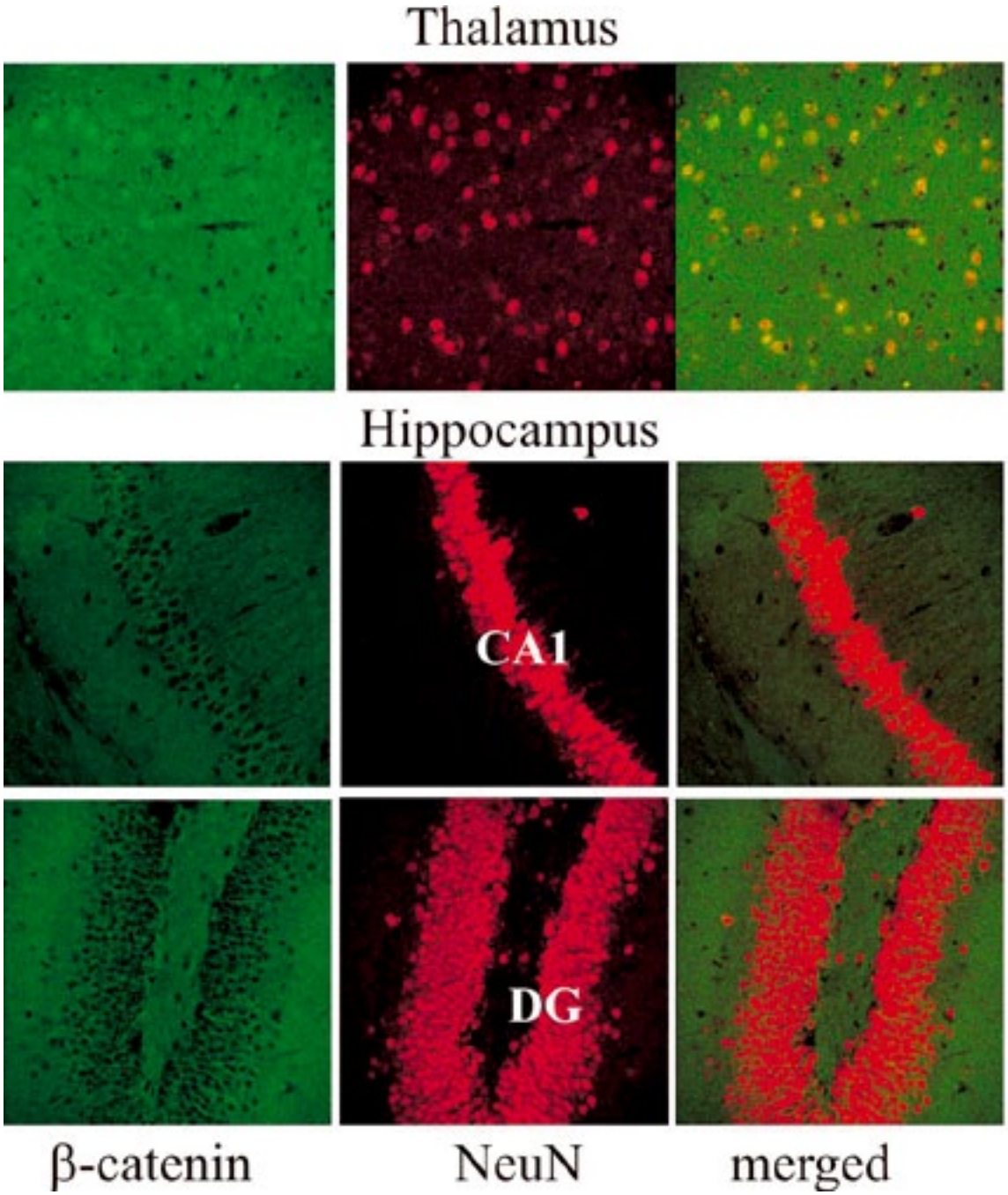


Fig. 3. β-catenin accumulation in thalamic neurons – immunohistochemistry. The sections were labeled with β-catenin specific (in green) and neuronal marker NeuN specific antibodies (in red) (author: Marta Wiśniewska).

thalamic neurons. We collected evidence in silico, in vitro and in vivo that corroborates with our assumption (Wisniewska et al., submitted).

ii) We established thalamic cultures and observed that 30%-40% of the cultured thalamic neurons contain nuclear β-catenin, in sharp contrast to cultures of cortical and hippocampal neurons that exhibit β-catenin only in membranes. This confirms an unusual characteristic of thalamic neurons regarding the subcellular distribution of β-catenin. We want to answer the question whether nuclear localization of β-catenin in thalamic cultures depends on extrinsic factors and which signaling pathway is engaged in this process (Misztal et al., in preparation).

5. Characterization of biological function of CHORD containing proteins in the nervous system *(Wojciech Michowski, Anna Skibińska-Kijek, Kamila Skieterska in collaboration with Prof. Guido Tarone from University of Turin)*

CHORD (Cys and His Rich Domain) domains contain a novel type of zinc fingers. In plants these domains are

involved in defence against pathogens. In human genomes there are two genes encoding CHORD containing proteins, melusin and CHP-1. Melusin is present exclusively in cardiac and skeletal muscles. It protects the heart from the consequences of chronic aortic hypertension. The highest level of CHP-1 is found in the brain but it is also present in other tissues. The biological role of this protein remains unknown but it is believed that CHP-1 might be a chaperon responsible for maintaining proper cell function under stress conditions. CHP-1 gene is regulated by HSF-1 (Heat Shock Factor 1) and the protein interacts directly with the major cellular chaperon, HSP90 (Sbroggiò et al., FEBS Lett, 2008). We characterize CHP-1 expression pattern in rodents brains under normal conditions and after insults causing stress. We are studying the CHP-1 chaperon activity, using an in vitro assay which is based on monitoring of an aggregation of a heat liable proteins, citrate synthase. We are interested in changes of subcellular distribution of CHP-1 under different stress conditions. We also investigated the susceptibility of cells with different CHP-1 levels (overexpression and RNAi) to stress induced apoptosis (Michowski et al., in preparation).

6. Studies on cyclin-dependent kinase 5 involvement in pathogenesis of Alzheimer's disease *(Aleksandra Szybińska in collaboration with Aleksandra Wysłouch-Cieszyńska and Prof. Michał Dadlez 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 brains and adult brains. In an adult brain, cdk5 via its interactions with different synaptic, cytoskeletal and cellular adhesion proteins as well as NMDA receptors and calcium channels, is involved in synaptic plasticity, memory and learning processes impaired in Alzheimer's disease. It was shown recently that in AD patients, the brain expression and activation of cdk5 is upregulated. That upregulation results in MAP tau overphosphorylation together with that caused by GSKβ kinase. Other consequences of cdk5 activity impairment regarding AD are poorly understood. Using the proteomics methods we analyse protein expression and modifications in synaptosomes of transgenic mice, AD models bearing human mutated presenilin 1 and APP genes. Using different methods of samples of preparation and fractionation, we identified almost 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's disease but expression changes of some other proteins are being shown for the first time. Additionally, we have made an attempt to increase the efficiency of identification of membrane proteins which are known to be underrepresented in different proteomic analyses.

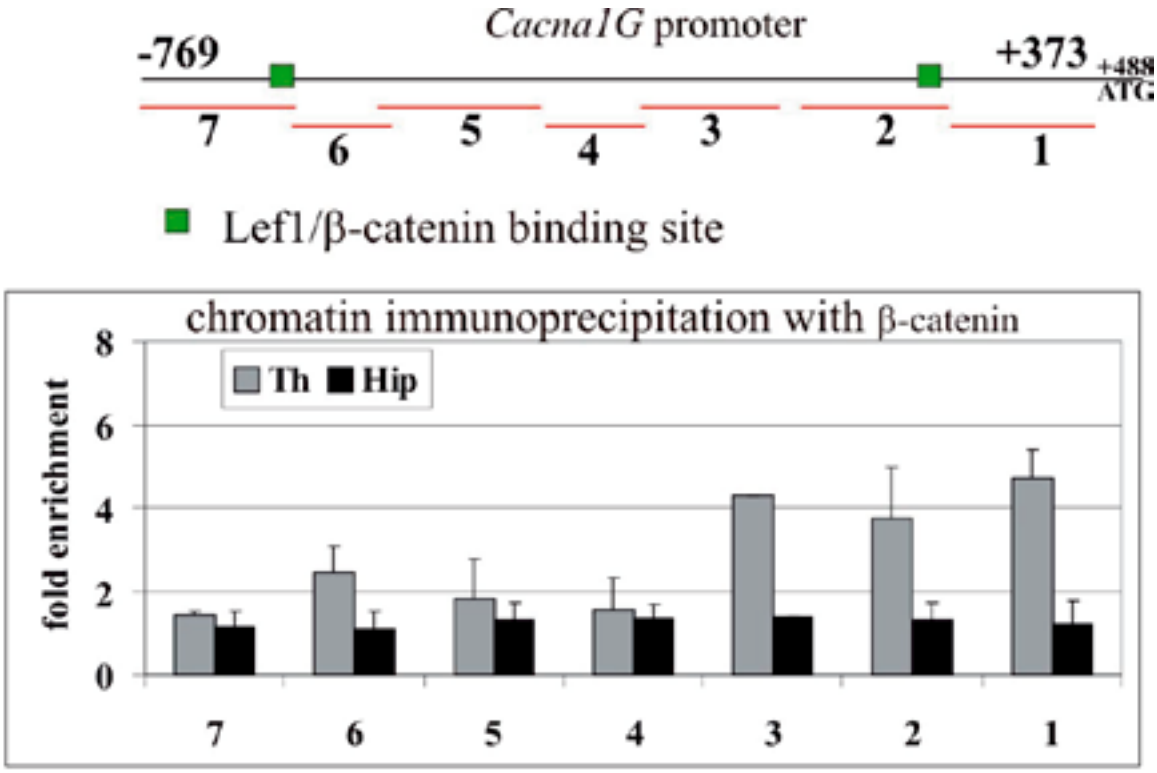
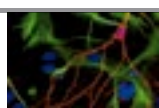
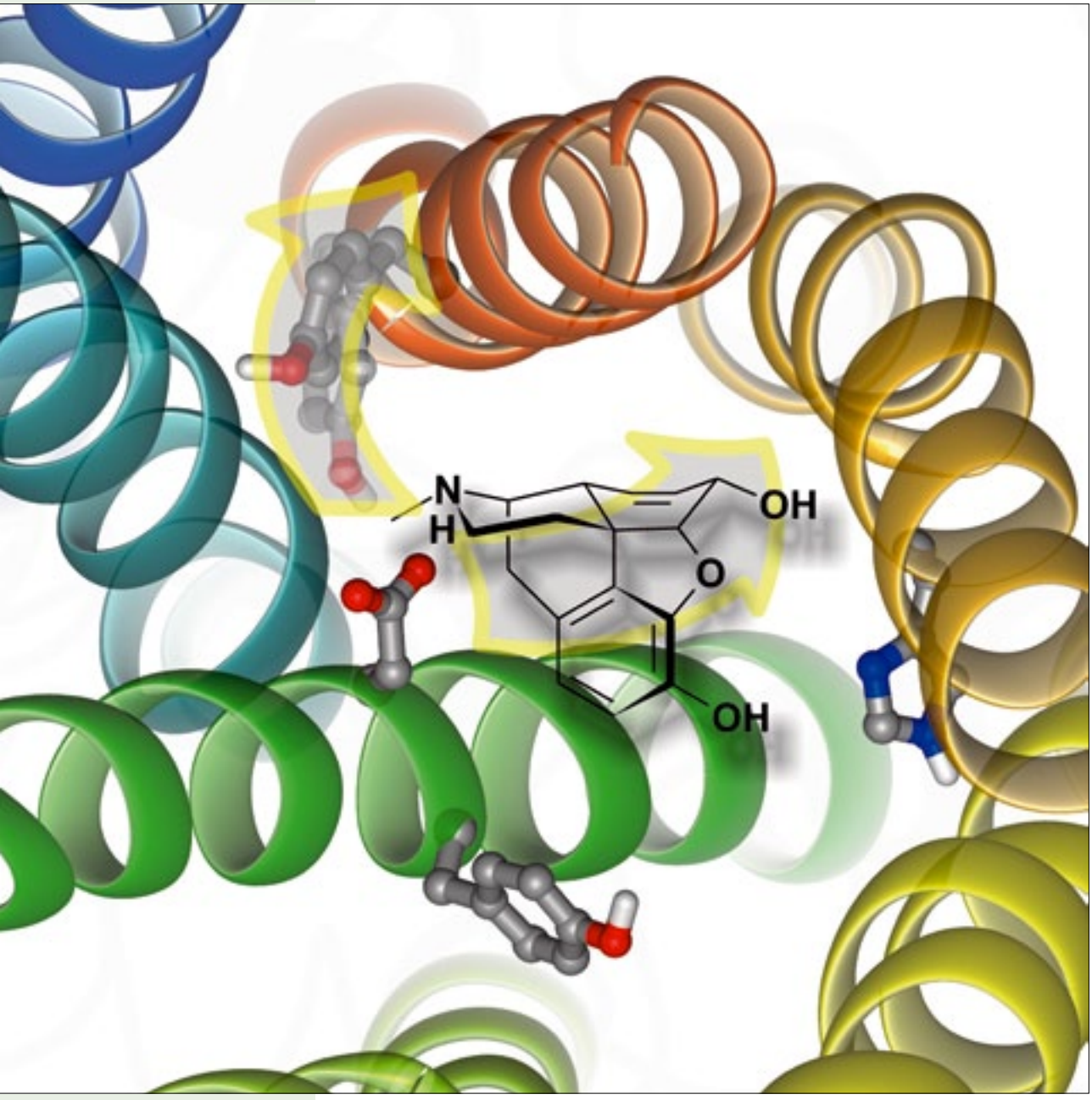


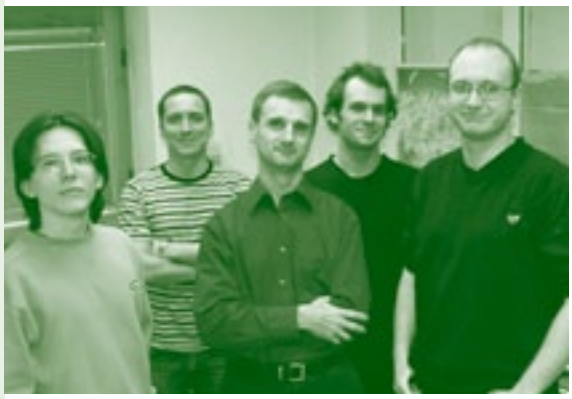
Fig. 4. β-catenin occupancy along Cacna1G promoter - chromatin immunoprecipitation (ChIP). Real Time PCR analysis of the ChIP products. The results are shown as fold enrichment above background (IgG) (author: Marta Wiśniewska).





A scheme of initial steps during μ opioid receptor (GPCR family) activation by the agonist morphine (shown as a structural formula). The arrows indicate motion of a ligand (wider arrow) and breaking of a connection (narrow arrow) between transmembrane helices TM3 (green) and TM7 (red) which constitutes the "3-7 lock" molecular switch (author: Sławomir Filipek).

Laboratory **of Biomodelling**



Lab Leader:

Sławomir Filipek, PhD, DSc. Habil.

Junior Researchers:

Michał Koliński, MSc

Aleksander Dębiński, MSc

Wojciech Puławski, MSc

Undergraduate student:

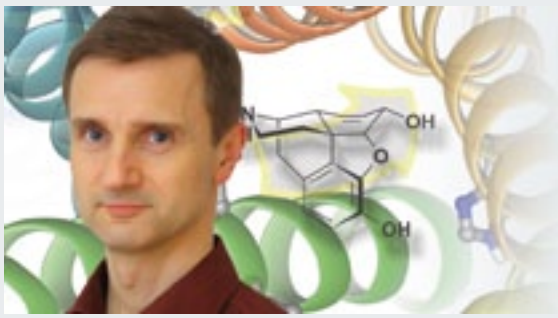
Krzysztof Młynarczyk

Left the Laboratory in 2008:

Krzysztof Jóźwiak – Post-doctoral Fellow

Krystiana Krzyśko – PhD defense, April 2008

Anna Zwolińska – MSc defense, Sep. 2008



Sławomir 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
- Polish Bioinformatics Society

EDITORIAL BOARD MEMBER

- Journal of Bionanoscience
- The Open Structural Biology Journal

PUBLICATIONS

- about 70 publications in primary scientific journals
- about 1700 citations
- about 1400 citations with IIMCB affiliation (years 2003-2008)

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
- **Modzelewska A**, **Filipek S**, Palczewski K, Park PS. Arrestin interaction with rhodopsin: conceptual models. Cell Biochem Biophys, 2006; 46:1-15
- **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
- Suda K, **Filipek S**, Palczewski K, Engel A, Fotiadis D. The supramolecular structure of the GPCR rhodopsin in solution and native disc membranes. Mol Membr Biol, 2004; 21:435-446
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- Fotiadis D, Liang Y, **Filipek S**, Saperstein DA, Engel A, Palczewski K. Rhodopsin dimers in native disc membranes. Nature, 2003; 421:127-128
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Publications in 2008-2009

- Austermann J, Nazmi AR, Heil A, Fritz G, **Kolinski M**, **Filipek S**, Gerke V. Generation and characterization of a novel, permanently active S100P mutant. Biochim. Biophys. Acta – Mol Cell Res, 2009; accepted
- Kannan AM, Renugopalakrishnan V, **Filipek S**, Li P, Audette GF, Munukutla L. Bio-batteries and bio fuel cells: Leveraging on electronic charge transfer proteins. J Nanosci Nanotechnol, 2009; 9:1665-78
- Thavasi V, Lazarova T, **Filipek S**, **Kolinski M**, Querol E, Kumar A, Ramakrishna S, Padros E, Renugopalakrishnan V. Study on the feasibility of bacteriorhodopsin as bio-photosensitizer in excitonic solar cell: A first report. J Nanosci Nanotechnol, 2009; 9:1679-87
- Bhatia P, **Kolinski M**, Moaddel R, Jozwiak K, Wainer IW. Determination and modelling of stereoselective interactions of ligands with drug transporters: a key dimension in the understanding of drug disposition. Xenobiotica, 2008; 38:656-675
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Current Research

1. Studies of activation switches in opioid receptors

G protein coupled receptors (GPCRs) interact with very diverse sets of ligands which bind to the transmembrane (TM) segments and sometimes also to the receptor extracellular domains. Each receptor subfamily undergoes a series of conformational rearrangements leading to the binding of a G protein during the activation process. All GPCRs preserved the 7-TM scaffold during evolution but adapted it to different sets of ligands by structure customization. Binding of structurally different agonists requires the disruption of different intramolecular interactions, leading to different receptor conformations and differential effects on downstream signaling proteins. The dynamic character of GPCRs is likely to be essential for their physiological functions, and a better understanding of this molecular plasticity could be important for drug discovery.

Experiments suggest that agonist binding and receptor activation occur through a series of conformational intermediates. Transition between these intermediate states involves the disruption of intramolecular interactions that stabilize the basal state of a receptor. Such profound changes are evoked by the action of molecular switches. The switches proposed so far for different GPCRs include the "rotamer toggle switch" involving the CWxPxF sequence on helix TM6, the switch based on the NPxxY(x)(5,6)F sequence linking helices TM7 and H8, the "3-7 lock" interaction connecting TM3 and TM7 (involving the Schiff base-counterion interaction in rhodopsin), and the "ion lock" linking transmembrane helices TM3 and TM6 and employing the E/DRY motif on TM3. Although, in the rhodopsin structure, all these switches are closed (inactive state), in recent crystal structures of β_1- and β_2-adrenergic receptor complexes with antagonists and inverse agonists the "ion lock" is open while the "rotamer toggle switch" remains closed.

Opioid receptors belong to the family of GPCRs. They are located in the membranes of neurons of the central nervous system and of some types of smooth muscle cells. Due to the important role they play in the human body in controlling pain and stress, modulating immune responses and developing addiction, opioid receptors have been the subject of numerous investigations. There are four types of opioid receptors: μOR, δOR, κOR and the nociceptin/opioid receptor-like 1. There are also additional, pharmacologically classified, subtypes of opioid receptors, though it is believed that they may, at least partly, originate from homodimerization of the four main opioid receptor types and their heterodimerization with other GPCRs. Knowledge of the structural details of receptor activation is absolutely necessary in order to design new drugs with precise action and negligible side effects.

Opioid receptors, like other GPCRs, undergo specific structural rearrangements upon activation by agonists. Such processes proceed via several steps ruled by different molecular switches. The first event in opioid receptor activation includes sensing of agonists and antagonists. Agonist binding is the first step in ligand-induced receptor activation. To investigate the relationship between the final movements of a ligand in a receptor binding site and the first steps of the activation process in opioid receptors, we chose a set of rigid ligands with the structural motif of tyramine (p-hydroxyphenethylamine) so that the two parts - the "message" (tyramine) and the "address" - are well distinguished. We used antagonists - naltrexone and β-FNA, and two closely related agonists - morphine and N-methyl-morphine.

Using homology modeling, simulated annealing and molecular dynamics of the μ opioid receptor complexes, we proposed distinct binding modes of opioids carrying the same structural motif – tyramine. Although they bind to the same binding pocket and the protonated amine interacts with D3.32, the antagonist's phenolic OH group tends to bind Y3.33 whereas the agonist's phenolic OH group tends to bind H6.52 (numbers according to the Ballesteros-Weinstein numbering scheme). All the studied agonists broke the "3-7 lock" (the hydrogen bond D3.32-Y7.43 between TM3 and TM7). Moreover, the antagonist naltrexone, when restrained to bind H6.52, was also able to break this connection (Fig. 1a,b) and,

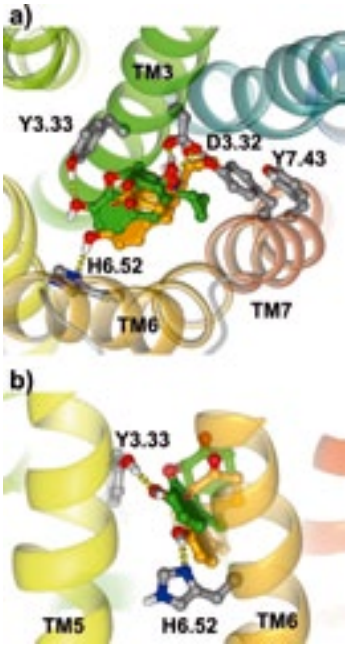


Fig. 1. The structure of naltrexone-μOR complex from unrestrained (ligand in green) and restrained (ligand in orange) MD simulations. During the restrained simulation (a bond between the phenolic OH group of the ligand and H6.52 was fixed) the "3-7 lock" (a hydrogen bond D3.32-Y7.43 between TM3 and TM7) is breaking. (**a**) view from the extracellular side. (**b**) a side view of the same structure (author: Sławomir Filipek).

additionally, to induce a rotamer toggle switch. Because of small temporal difference between these events, both switches may be interdependent or even constitute a larger multicomponent switch. The nanosecond timescale used in the simulations is small compared to the full activation time of the receptor. However, we investigated only the specific action of switches and the ligands were already located in the binding site, so usage of this timescale is justified.

2. Ca^{2+}-dependent regulation of phototransduction

Phototransduction is initiated with absorption of a photon by the chromophore 11-*cis*-retinal which is covalently linked to G-protein-coupled receptors known as opsins. Isomerization of 11-*cis*-retinal to all-trans-retinal and dissociation of the chromophore produces a conformational change in the opsin and consequent activation of the coupled heterotrimeric G-protein transducin. The activated transducin then activates a retina-specific phosphodiesterase which cleaves cGMP, depleting cytoplasmic cGMP and closing cGMP-gated cation channels. The level of cGMP is then restored to dark levels by activation of retina-specific guanylate cyclases (transmembrane proteins located in the disk membranes of photoreceptor cells). Both disruption of normal phototransduction and recovery of the dark state by photoreceptors exposed to light are associated with a variety of cone-rod retinopathies. retGCs are regulated by Ca^{2+}, which plays a crucial role in the regulation of phototransduction in photoreceptors. The concentration of free Ca^{2+} in the cytoplasm drops from 550 to 50 nM during activation, due to both the closing of cGMP-gated cation channels that block the influx of Ca^{2+} and to the action of Na^{+}/Ca^{2+}-K^{+} exchangers which export Ca^{2+} from the cell.

A decrease in free Ca^{2+} concentration in the photoreceptor cytosol is sensed by Ca^{2+}-binding proteins that modulate phototransduction and activate the recovery phase to reestablish the photoreceptor dark potential. Guanylate cyclase-activating proteins (GCAPs) belong to the neuronal calcium sensor (NCS) family which, in turn, belongs to the EF-hand superfamily of Ca^{2+}-binding proteins. They are expressed in neurons and have four EF-hands but only two or three of them are able to bind Ca^{2+}. Most NCS proteins are myristoylated at the N-terminus. Two classes of myristoylated NCS proteins are expressed in photoreceptors and are active in phototransduction: guanylate cyclase-activating proteins (GCAPs) which regulate retGCs in response to Ca^{2+} and mediate the restoration of dark levels of cGMP; and recoverin - which plays a role in prolonging the photoresponse.

The Ca^{2+}-bound structure of recoverin has the myristoyl group exposed whereas the Ca^{2+}-free structure has the myristoyl group bound in a hydrophobic cleft on the surface of the protein. The exposure of the myristoyl group in Ca^{2+} is essential for anchoring the protein to the membrane. Upon Ca^{2+} release, the myristoyl group is sequestered in a deep hydrophobic cleft of the protein, allowing free movement of recoverin through the cytoplasm. This behavior has been termed the "Ca^{2+}-myristoyl switch". However, some NCS proteins, including GCAPs, do not appear to have a canonical myristoyl switch. Therefore, the role of the N-terminal acylation in these proteins has remained obscure. The chimera experiments suggest that the N- and C-terminal helices of GCAP1 are the most important for retGC1. The structure of myristoylated GCAP1 - in the Ca^{2+}-bound, inhibitory conformation of the protein - shows these regions in close proximity to each other and clustered with the myristoyl group (Fig. 2a). Any rotation of the N- and C-terminal domains relative to each other would necessarily pull the terminal helices apart (Fig. 2b). We propose that this separation of the N- and C-terminal helices in GCAP1 is crucial to induce the activated conformation of retGC1.

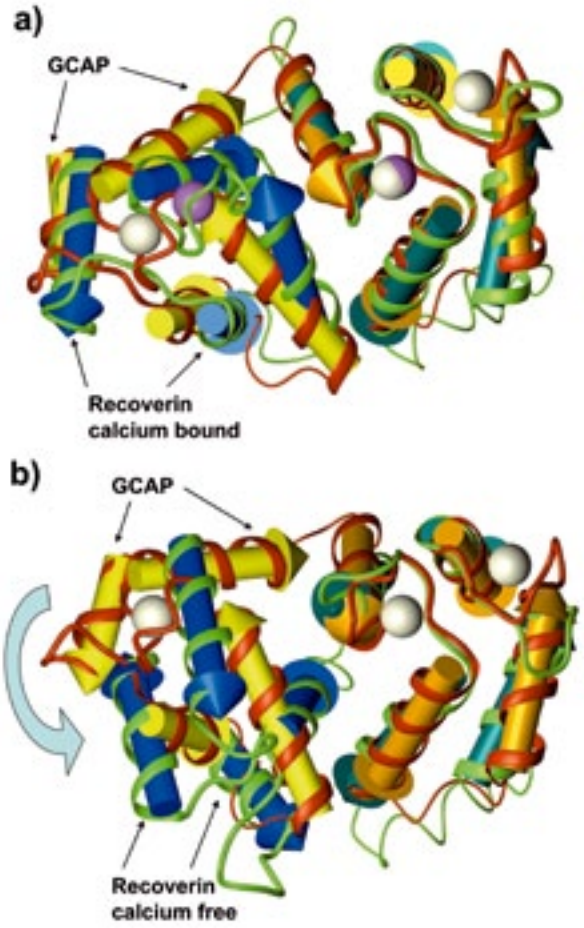


Fig. 2. Model of the Ca^{2+}-induced conformational change in GCAP1. (**a**) EF-hands of Ca^{2+}-bound GCAP, represented by yellow and orange arrowed cylinders, red ribbon and white calcium ions, superimposed on Ca^{2+}-bound recoverin, represented by blue and green-blue arrowed cylinders, green ribbon and violet Ca^{2+} ions. N- and C-terminal parts of the structure were removed for clarity. Superimposition was done on C-terminal EF-hand pairs (orange and green-blue cylinders).
(**b**) EF-hands of Ca^{2+}-bound GCAP superimposed on Ca^{2+}-free recoverin. Superimposition was done on C-terminal EF-hand pairs. Coloring as in (a) (author: Sławomir Filipek).

3. Studies on Ca^{2+}-independent mutants of S100 family protein

S100 proteins function as Ca^{2+} signal transducers by regulating cellular targets in their Ca^{2+}-bound conformation. They are small proteins and are composed of only two EF-hand domains. S100P is a member of the family that can activate the membrane and F-actin binding protein ezrin in a Ca^{2+}-dependent manner, at least *in vitro*. Here we studied a S100P derivative that contained mutations in the two EF-hand loops predicted to lock the protein in a permanently active state. In line with this permanent complex formation, S100P pa mutant colocalized with ezrin to plasma membrane

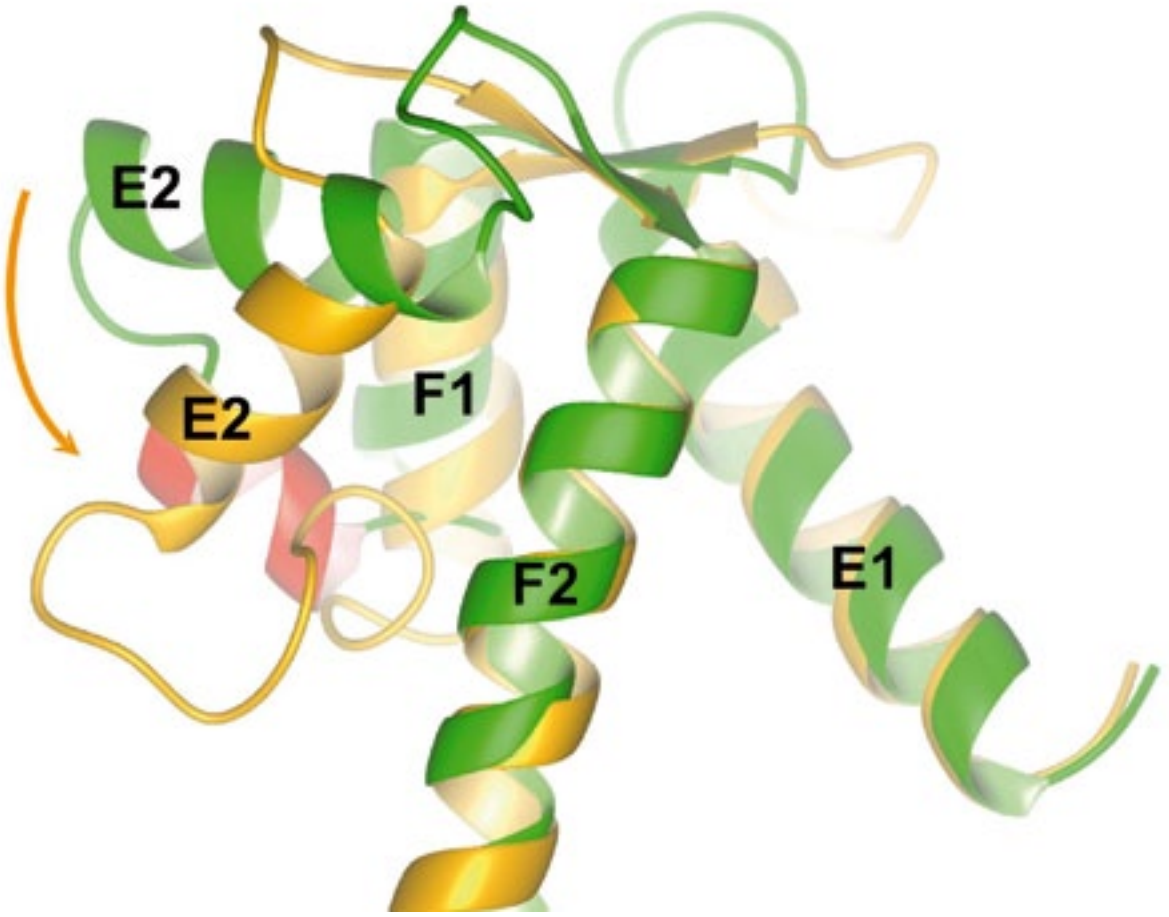
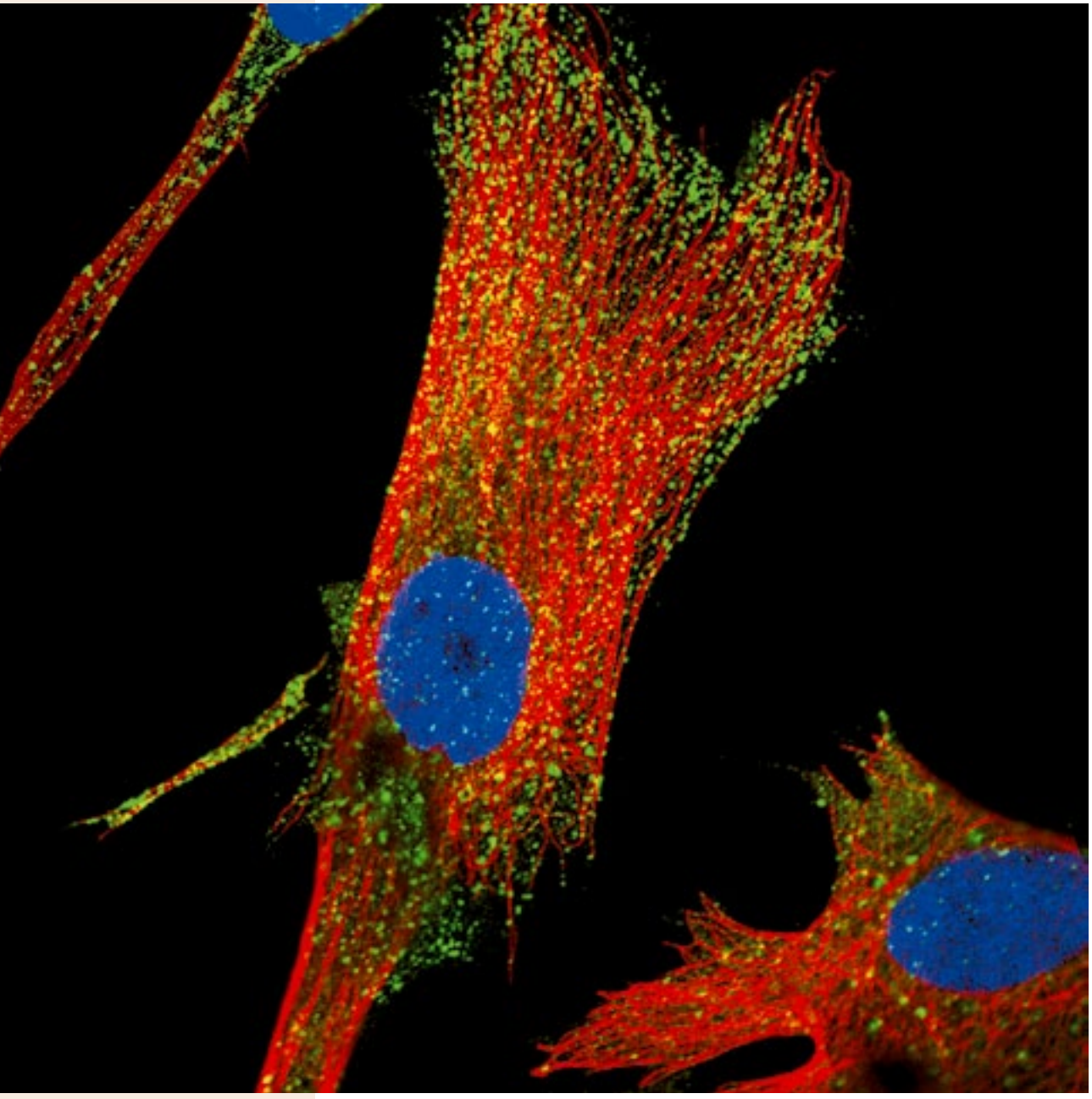


Fig. 3. Superimposition of EF-hand structures of representative S100P Ca^{2+} (S100P pa is nearly identical) (green) and apo S100P (orange) after molecular dynamics simulation. A helix between helices F1 and E2 in S100P Ca^{2+} is shown in red. This helix does not exist in the apo S100P structure (author: Sławomir Filipek).

protrusions of mammary epithelial cells, even in the absence of intracellular Ca^{2+} transients. Thus, S100P pa is a novel type of S100 protein mutant which is locked in a permanently active state that shows unregulated complex formation with its cellular target ezrin.

To identify, within human S100P, residues whose mutation could induce a fold similar to that of the Ca^{2+}-bound (i.e. active) protein, we compared the sequences of S100P and S100A10. The latter was chosen since it is the only Ca^{2+}-insensitive S100 protein which is locked in a permanently active conformation resembling that of a Ca^{2+}-bound S100 protein. The comparison identifies, within S100A10, a deletion of three amino acids in the first EF hand loop and three substitutions of Ca^{2+}-coordinating residues in the second EF hand. To evaluate whether similar mutations in S100P would also result in a protein fold resembling the Ca^{2+}-bound conformation, we modeled the structure of such a mutant, herein referred to as S100P pa, which was constructed by homology modeling of the published molecular structure of Ca^{2+}-bound S100P and subsequent molecular dynamics simulation. This template was also used for construction of wild-type apo and Ca^{2+}-bound S100P proteins. Therefore, all changes in the shapes of these proteins resulted from the molecular motions calculated by the molecular dynamics procedure. The superimposition of simulated apo S100P and S100P pa reveals a substantial deviation, in particular in the position of helix E2 (Fig. 3). This shift of helix E2 resembles that seen upon Ca^{2+} binding, as shown in a superimposition of apo S100P and Ca^{2+}-bound S100P.

Molecular dynamics simulations also show that the angle between helices F1 and E2, a characteristic feature distinguishing apo and Ca^{2+}-bound S100 proteins, is very similar for Ca^{2+} S100P and S100P pa. As revealed by simulations, the F1-E2 angle in apo S100P is very flexible and fluctuates between open (~90°) and closed (~45°) states. Furthermore, a small helix between helices F1 and E2, which is present in active S100 proteins (Ca^{2+}-bound S100P and S100A10) and facilitates binding of a ligand, is also present in S100P pa but is unfolded in apo S100P. Thus, modeling and molecular dynamics predict that the folding of S100P pa differs from apo S100P and more closely resembles Ca^{2+}-bound S100P. S100P pa is also predicted to expose a large hydrophobic cavity on its surface, a feature seen with several other S100 proteins in their Ca^{2+} conformation.



Human skin fibroblasts stained with antibodies against α-tubulin (red) and APPL2 (green). The nuclei (Hoechst staining) are labeled in blue (author: Łukasz Sadowski).

Laboratory **of Cell Biology**

Lab Leader:

Marta Miączyńska, PhD, DSc. Habil.

Post-doctoral Fellows:

Iwona Pilecka, PhD

Beata Pyrzyńska, PhD

Sajid Rashid, PhD

Research Assistants:

Magdalena Banach-Orłowska, PhD

Junior Researchers:

Anna Hupałowska, Msc

Marta Olchowik, MSc (former Brewińska)

Łukasz Sadowski, MSc

Anna Toruń, MSc

Anna Urbańska, MSc (former Zarębska)

Undergraduate Students:

Michał Mlącki

Marta Miączyńska, PhD, DSc. Habil.

DEGREES

2008	DSc. Habil. in cell biology, the Nencki Institute of Experimental Biology PAN, Warsaw, Poland
1997	PhD in genetics, University of Vienna, Austria
1993	MSc in molecular biology, Jagiellonian University, Cracow, Poland
1991	BSc in biological sciences, University of Wolverhampton, UK

RESEARCH TRAINING

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

FELLOWSHIPS AND AWARDS

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

Selected publications

- **Sadowski L, Pilecka I, Miaczynska M.** Signaling from endosomes: Location makes a difference. Exp Cell Res, 2008 Oct 7. [Epub ahead of print]
- **Miaczynska M,** Stenmark H. Mechanisms and functions of endocytosis. J Cell Biol, 2008; 80:7-11
- **Pilecka I, Banach-Orlowska M, Miaczynska M.** Nuclear functions of endocytic proteins. Eur J Cell Biol, 2007; 86:533–547
- **Pilecka I, Miaczynska M.** Clathrin-dependent endocytosis – mechanisms and significance. (in Polish), chapter of a book „At the frontiers of chemistry and biology", edited by J. Barciszewski, the Publishing House of Mickiewicz University in Poznan, 2006
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- ***Miaczynska M,** Pelkmans L, Zerial M. Not just a sink: endosomes in control of signal transduction. (Review) Curr Opin Cell Biol, 2004; 16:400-406
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- *Nielsen E, Christoforidis S, Uttenweiler-Joseph S, **Miaczynska M**, Dewitte F, Wilm M, Hoflack B, Zerial M. Rabenosyn-5, a novel Rab5 effector is complexed with hVPS45, and is recruited to endosomes through a FYVE finger domain. J Cell Biol, 2000; 151:601-612
- *Rubino M, **Miaczynska M**, Lippe R, Zerial M. Selective membrane recruitment of EEA1 suggests a role in directional transport of clathrin-coated vesicles to early endosomes. J Biol Chem, 2000; 275:3745-48
- *Christoforidis S, **Miaczynska M**, Ashman K, Wilm M, Zhao L, Yip SC, Waterfield MD, Backer JM, Zerial M. Phosphatidylinositol-3-OH kinases are Rab5 effectors. Nat Cell Biol, 1999; 1:249-252

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

Description of Current Research

The main research objective of the Laboratory of Cell Biology is to study the relationship between the processes of intracellular membrane transport and signal transduction in response to extracellular stimuli in mammalian cells. We aim to investigate the molecular mechanisms underlying this mutual interdependence. In particular, the specific projects developed in the group follow two general lines of investigation and focus on studying:

I. **the role of endosomal compartments in trafficking and signaling of growth factors**
II. **the involvement of endocytic proteins in the regulation of gene expression in the nucleus.**

At a general level both topics deal with the problem of molecular communication between intracellular organelles in endocytic membrane transport and signal transduction. This is a novel problem of increasing significance in the field of cell biology, as many recent studies, including our own, indicate that intracellular compartmentalization of signal transduction processes may play an important role in modulating the overall cellular response. 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 argues that endosomal compartments and their resident proteins play an important role in transmitting intracellular signals, by transporting ligand-receptor complexes and affecting their activity inside the cell (Miaczynska et al., 2004a). The proposal of endosomes as signaling compartments, initially postulated in the mid-nineties, has gained increasing experimental support in the last few years (Sadowski et al., 2008).

Moreover, the relaying of signals from the plasma membrane via endosomes to the nucleus requires signal mediators to be transported between different cellular locations. Intriguingly, a growing number of clathrin adaptors and endosomal proteins are reported to undergo nucleocytoplasmic shuttling. Endocytic proteins can

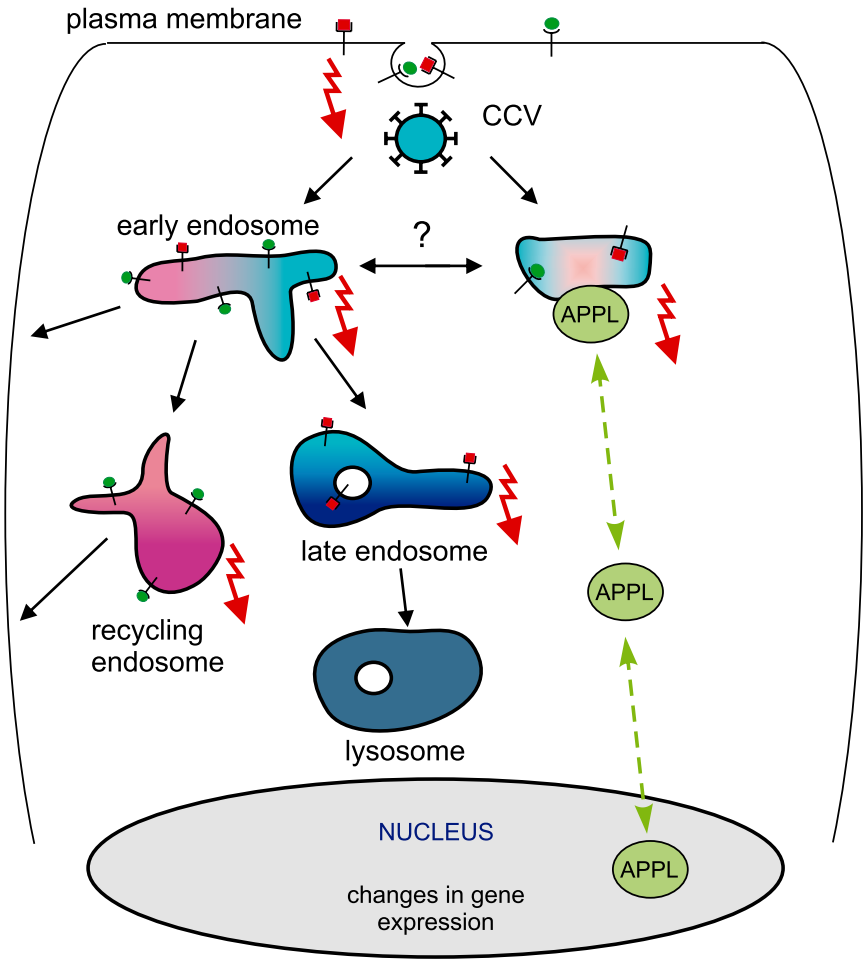


Fig. 1. Scheme of cargo trafficking and signaling along the endocytic pathway (author: Marta Miączyńska).
According to the classical view, ligand-receptor complexes are internalized from the plasma membrane via clathrin-coated vesicles (CCV) to early endosomes from where they are sorted either towards recycling endosomes back to the plasma membrane or to late endosomes and lysosomes for degradation. Our work indicates that APPL-harboring compartment represents a distinct subpopulation of early endosomes and receives cargo from the plasma membrane via CCV and exchanges it with the canonical early endosomes. In addition to its endosomal localization, APPL proteins can undergo nucleocytoplasmic shuttling and interact with nuclear proteins, modulating gene expression. Signal transduction, initiated by signaling ligands binding to their receptors at the plasma membrane, can continue intracellularly from endosomal compartments during trafficking (signaling events marked with red arrows).

interact with nuclear molecules involved in transcription or chromatin remodeling, changing their localization and/or activity - and thus may directly modulate the levels or specificity of gene transcription. Certain endocytic proteins translocate to the nucleus in response to extracellular signals in order to exert a specific biological effect, thus serving as a vehicle of molecular communication between intracellular organelles. In most other cases it is unclear to what extent the endocytic and nuclear functions are related or represent disparate tasks, so called moonlighting (Pilecka et al., 2007).

Our direct entry point to both themes were the previous studies of adaptor proteins APPL1 and APPL2. These homologous endosomal proteins act also as signal transducers capable of nuclear translocation, thus providing an example of both phenomena: the involvement of endosomes in signaling and the activity of endocytic proteins in the nucleus (Miaczynska et al., 2004b). 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. Interestingly, APPL proteins can be released from the endosomal membrane, undergo nucleocytoplasmic shuttling and interact with nuclear proteins, among them the histone deacetylase and chromatin remodeling complex NuRD. 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 remodeling complex NuRD as interacting partners of both APPL proteins, these data suggested for the first time a molecular link between the processes of endocytosis and chromatin remodeling. Moreover, APPL-harboring endosomes appeared as an intermediate in signaling between the plasma membrane and the nucleus. Our initial research efforts concentrated on APPL1 and APPL2 as example proteins involved in endocytic trafficking and nuclear signaling, while more recently we have been extending our studies towards other dual-function endocytic proteins.

The following projects are currently ongoing in the Laboratory:

Within the general theme I: the role of endosomal compartments in trafficking and signaling of growth factors

1. Biochemical characterization of APPL-positive endosomes and APPL-interacting proteins
2. Microscopical characterization of cargo transport via APPL-positive endosomes and its impact on signaling (collaboration within a European consortium "EndoTrack")
3. Tracking the endocytic routes of platelet-derived growth factor (PDGF) and PDGF receptor and their impact on signaling (collaboration within a European consortium "EndoTrack")
4. The role of APPL proteins in cell physiology and tumorigenesis

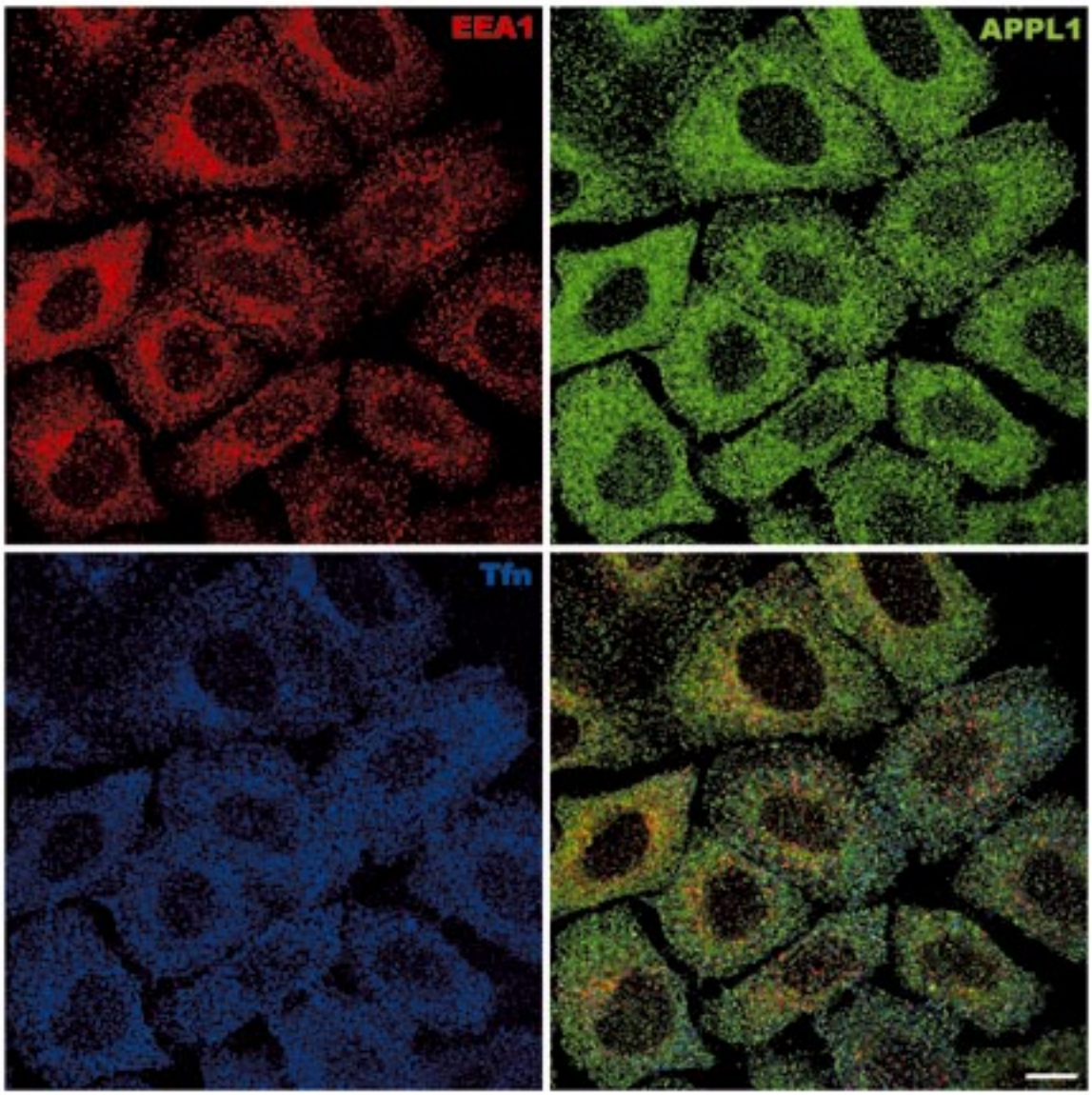
Within the general theme II: the involvement of endocytic proteins in the regulation of gene expression in the nucleus

1. The role of APPL proteins in the regulation of β-catenin/TCF-mediated transcription via their interaction with a transcriptional repressor Reptin
2. Functional characterization of the interactions between endosomal adaptor protein APPL1 and the NuRD co–repressor complex
3. Post-translational modifications as possible determinants of subcellular localization of APPL proteins
4. Testing candidate endocytic proteins for their involvement in transcriptional regulation

Among our recent results within the general theme I, we could demonstrate by cell fractionation experiments that endosomes harboring APPL proteins are distinct from the canonical early endosomes bearing a marker protein EEA1. By quantitative microscopy methods we characterized transport pathways leading cargo via APPL-positive endosomes, in comparison to the canonical EEA1-harboring early endosomes. We showed that APPL endosomes are involved in early trafficking of cargo molecules internalized via clathrin-mediated endocytosis and destined for recycling (transferrin) or degradation (epidermal growth factor, EGF). We also established assays to track platelet-derived growth factor (PDGF) after its internalization in cells, both by microscopy- and electrochemiluminescence-based methods.

Within the general theme II, we have demonstrated that the endosomal proteins APPL1 and APPL2 are novel activators of β-catenin/TCF-mediated transcription. This function of APPL proteins is related to their interaction with Reptin, a transcriptional repressor binding to β-catenin. We proposed a mechanism by which APPL proteins could exert their stimulatory effects on β-catenin/TCF-dependent transcription. Moreover, we characterized biochemically the binding between APPL1 and the nuclear co-repressor complex NuRD, containing nucleosome remodeling and histone deacetylase activities. We further showed that these interactions regulate the nucleocytoplasmic distribution of APPL1. Our data revealed a surprising complexity of APPL1 interactions with histone deacetylases, with functional consequences for the modulation of gene expression.

With respect to the methodology used in the Laboratory, our main experimental system are cultured mammalian cells, but we have also initiated collaborative studies carried out in primary neurons (with the group of Dr. J. Jaworski at IIMCB) and in mice, in order to broaden the impact of our cell-based observations in the context of cell-cell communication or of a whole organism. In our research we use a variety of methods, including cell fractionation and purification of endosomal compartments, confocal microscopy followed by quantitative image analyses, biochemical characterization of proteins and their post-translational modifications, identification of protein interacting partners, cell-based assays for endocytosis, transcription and proliferation.



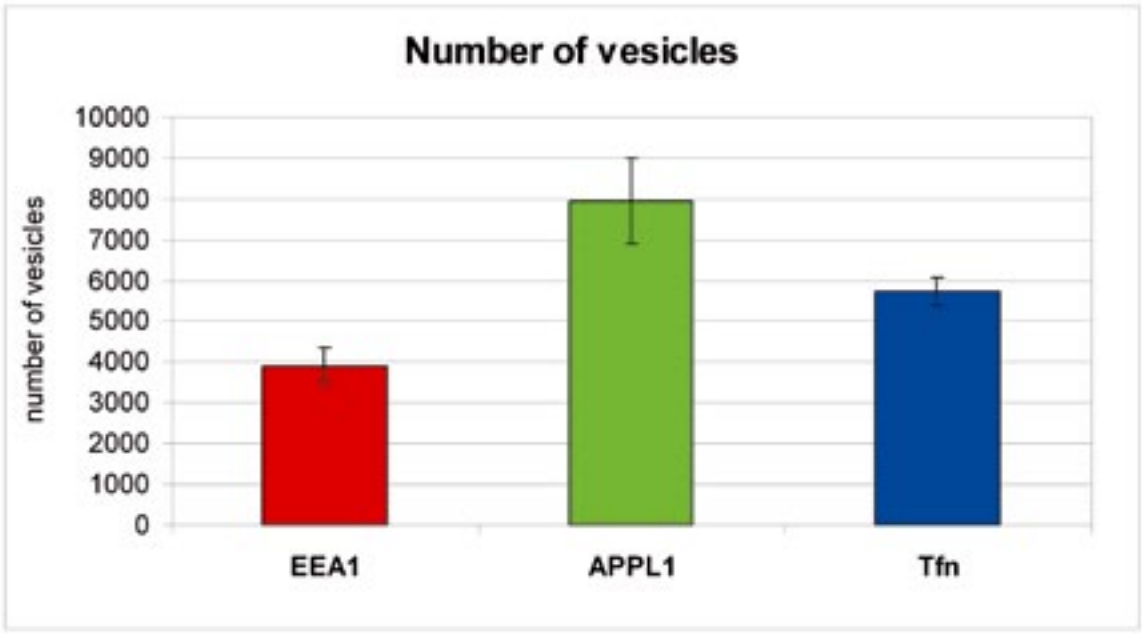
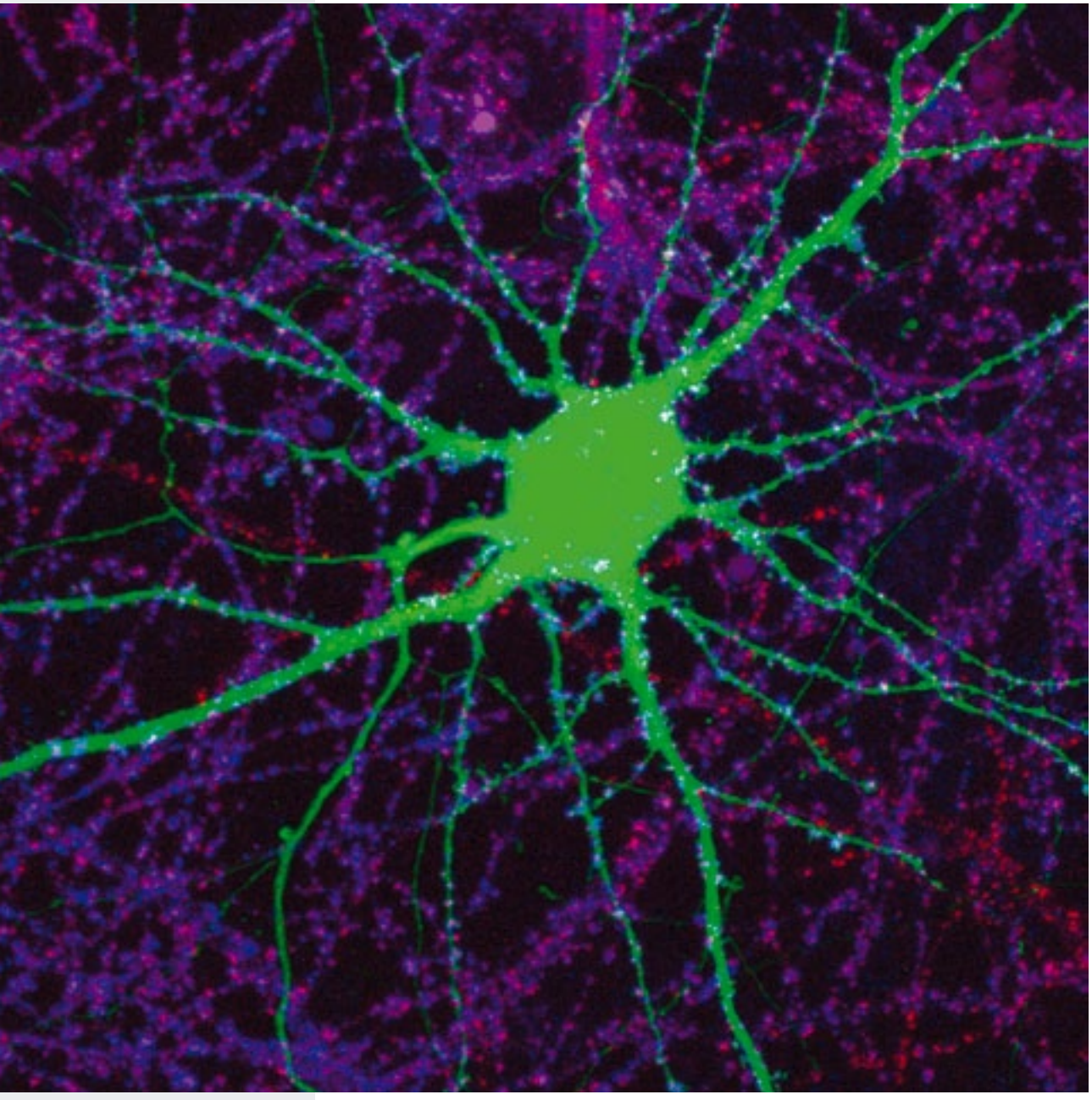


Fig. 2. Endocytosis of transferrin in Hela cells (authors: Marta Olchowik and Anna Hupałowska).
HeLa cells were fixed after 2 minutes of labeling with transferrin (Tfn) conjugated with Alexa647 (blue) and immunostained for EEA1 (red) and APPL1 (green). The chart indicates a number of EEA1, APPL1 and Tfn-positive vesicles in the presented images.



Surface staining for presence of AMPA-type glutamate receptor subunits GluR1 (red) and GluR2 (blue). Magenta indicates colocalization of the two subunits. White color indicates colocalization of AMPA-R subunits on the surface of GFP transfected neuron transfected. Changes in surface staining for glutamate receptor subunits help an assessment of synapse activity by cell biology approach in our search for GSK3 and mTOR interplay in neuronal physiology and neurodegeneration (author: Jacek Jaworski).

Laboratory **of Molecular and Cellular Neurobiology**

Lab Leader:

Jacek Jaworski, PhD

Post-doctoral Fellows:

Magda Błażejczyk, PhD

Iwona Cymerman, PhD (starting January 2009)

Matylda Macias, PhD

Junior Researchers:

Anna Malik, MSc

Małgorzata Perycz, MSc

Łukasz Świech, MSc

Małgorzata Urbańska, MSc

Undergraduate Students:

Paweł Krawczyk

Kamil Parobczak

Patrycja Pietruszka

Anna Urbańska

Małgorzata Zarębska

Technician:

Monika Dudek

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 PAN, Prof. L. Kaczmarek, Warsaw, Poland; PhD student until 2001; postdoctoral associate until May 2002
1995-1996	Department of Genetics, Prof. P. Węgleński, Warsaw University, Poland, master degree

FELLOWSHIPS AND AWARDS

2005	Konorski Award of Polish Neuroscience Society and Polish Academy of Sciences for the best publication of year 2004 in the field of neuroscience (for publication by Kowalczyk et al, 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**, Kapitein LC, Montenegro Gouveia S, Dortland BR, Wulf PS, Grigoriev I, Camera P, Spangler SA, di Stefano P, Demmers J, Krugers H, Defilippi P, Akhmanova A, Hoogenraad CC. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity, Neuron, 2009; 61:85-100
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- ***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 2008

- **Jaworski J**, Kapitein LC, Montenegro Gouveia S, Dortland BR, Wulf PS, Grigoriev I, Camera P, Spangler SA, di Stefano P, Demmers J, Krugers H, Defilippi P, Akhmanova A, Hoogenraad CC. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity, Neuron, 2009; 61:85-100
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- Rylski M, Amborska A, Zybura K, Mioduszewska B, Michaluk P, **Jaworski J**, Kaczmarek L. Yin Yang 1 is a Critical Repressor of Matrix Metalloproteinase-9 Expression in Brain Neurons. J Biol Chem, 2008; 283:35140-53
- **Urbanska M, Blazejczyk M, Jaworski J**. Molecular basis of dendritic arborization. Acta Neurobiol Exp, 2008; 68:264–288
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- **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
- 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, 2008; 86:61-70.

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 neuronal cells in physiology and pathology. We mostly focus our research on two phenomena that are dependent on mTOR activity and are crucial for proper formation and functioning of the neuronal networks – dendritic arbor and synapse formation and stabilization. In this context, we attempt to understand the role of the 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 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 (Fig. 1). 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 for the first time that PI3K and its downstream kinase, Akt, regulate the complexity of dendritic branching in neurons by protein kinase mTOR (mammalian

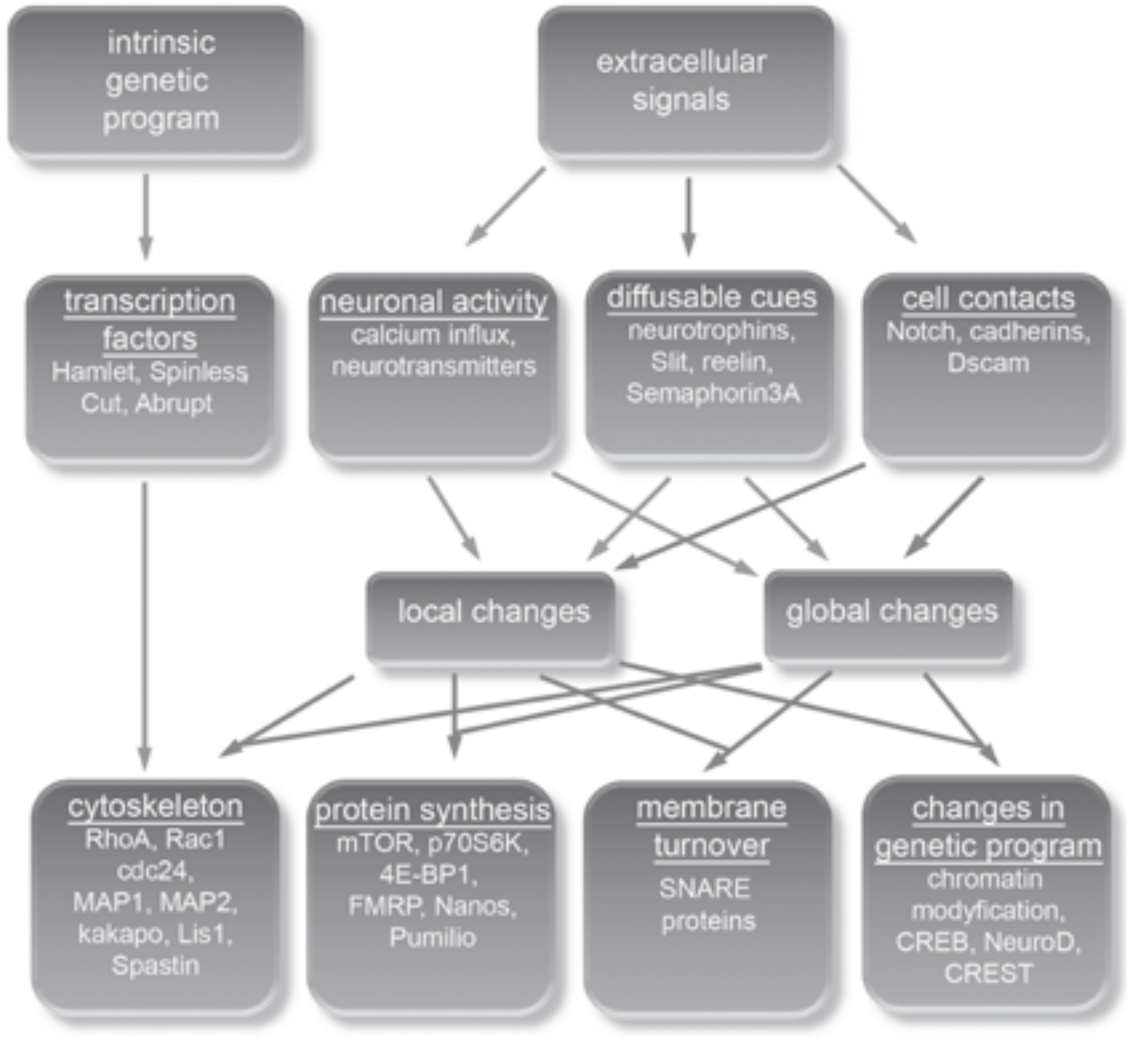


Fig. 1. Dendritogenesis is a process strictly controlled by the combination of an intrinsic genetic program and extracellular signals causing changes in the cytoskeleton, macromolecule synthesis and membrane turnover. Several changes occur either globally or only locally in dendrites. Adapted from Urbanska et al., 2008.

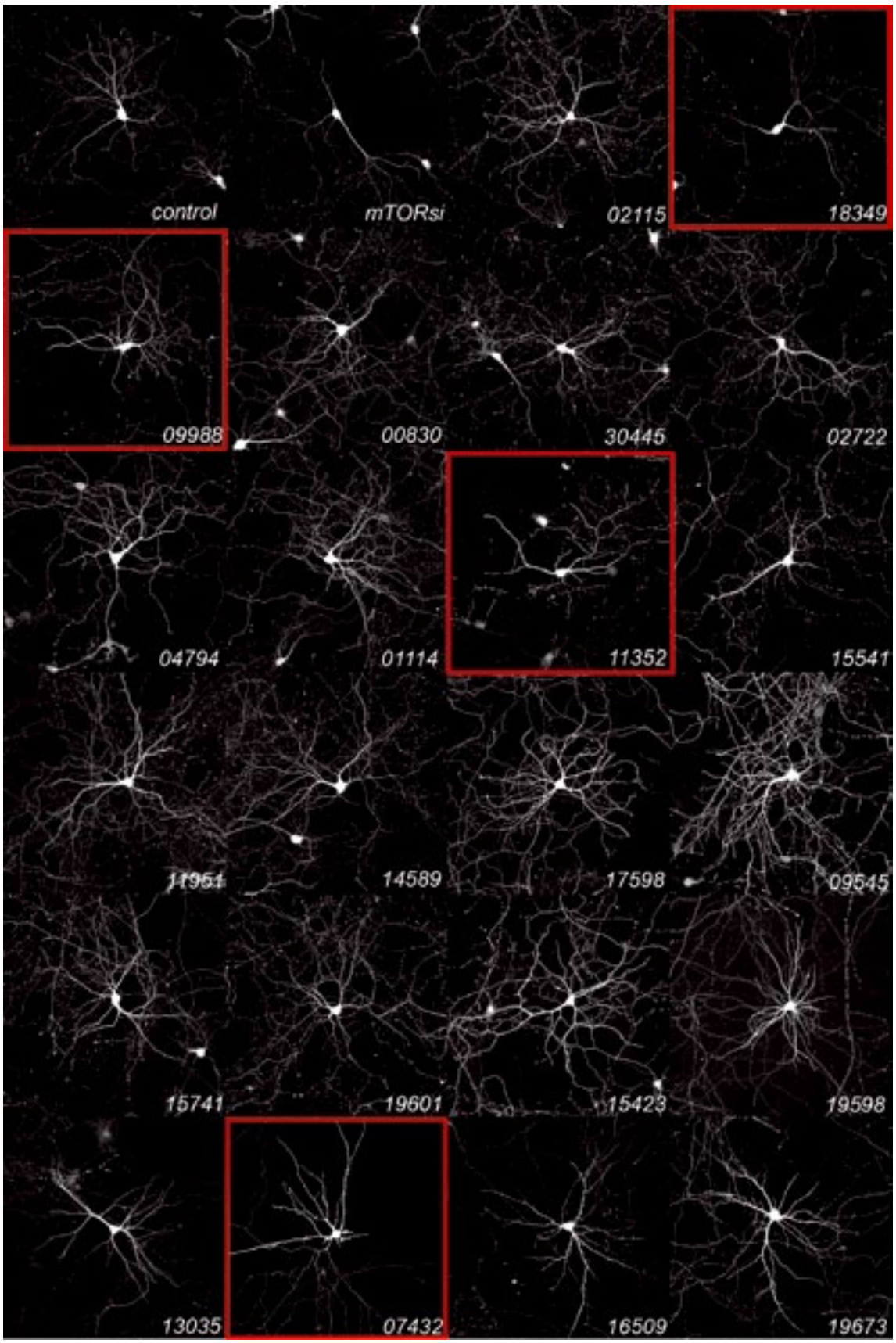


Fig. 2. Identification of mTOR regulated proteins involved in dendritic arborization of hippocampal neurons with use of siRNA library designed against mRNAs encoding proteins potentially regulated by this kinase. Exemplary images of neurons from the screen transfected with siRNA against 22 genes out of 150 selected for the library. Images in red frames highlight neurons with substantially changed dendritic morphology.

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. In neurons, mTOR 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. 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. 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's and Huntington's 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 synapse formation processes, 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 processes of dendritic branching and synapse formation and stabilization.
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. We selected 150 proteins potentially regulated by mTOR-Raptor complex, based on the bioinformatic approach, and have designed a library of siRNAs against all selected candidates. Next we prepared shRNA-pSUPER-plasmid based library, which consists of 450 plasmids encoding individual shRNAs against selected genes (3 hairpins per sequence). In 2008 we concentrated our research efforts on identification of genes crucial for dendritic arbor development and stability using this library (Fig. 2). So far we have identified few dozens of such genes that encode proteins involved in several cellular processes. Especially highly represented are genes encoding proteins regulating cellular membrane turnover. This finding is quite novel and intriguing, since mTOR is commonly known only for its involvement in protein synthesis regulation and membrane turnover is underinvestigated in the context of dendritic arbor development. As a next step, we are going to study in more detail molecular mechanisms underlying the role of selected genes in dendritogenesis as well as to ask questions for their potential role in neuropathology connected with dendritic arbor disturbances. Moreover, we plan to take a closer look for a connection between mTOR activity and cellular membrane turnover during dendritic arbor development.

As a supplementary approach to our bioinformatic search for mTOR regulated proteins and genes in 2008 we launched new project aiming to identify mTOR interacting partners specifically in neurons, that bind this kinase depending on mTOR activity status. With use of bio-IP techniques followed by mass spectrometry we identified so far 82 proteins, 72 of which co-IPed with mTOR differentially under conditions of mTOR inhibition with use of Rapamycin (Table 1). One of them was CLIP170, a protein that has already been thoroughly studied in our laboratory. CLIP170 belongs to a group of microtubule plus-end tracking proteins (+TIPs) and is believed to regulate microtubule dynamics at plus-end during polymerization by promoting the rescue phase. Our extensive research performed so far shows that mTOR and CLIP170 can interact in brain extracts and that inhibition of mTOR activity prevents full phosphorylation of CLIP170. Moreover, 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,

	50 kD	150 kD	200 kD
Total	**46**	**19**	**17**
Rapa-/+	**4**	**5**	**1**
Rapa-	**10**	**10**	**6**
Rapa+	**32**	**4**	**10**

Table 1. Numbers of proteins, identified by MS method, interacting with mTOR in neurons depending on mTOR activity

a decrease in the complexity of dendritic arbors and shrinkage of dendritic fields. Moreover, CLIP-170 knockdown exerts a strong effect on the shape of dendritic arbor even under conditions promoting dendritogenesis such as the overexpression of constitutively active forms of PI3K and Akt kinases, which are crucial upstream components of mTOR signaling pathway. Taken together, this data strongly suggests the role of CLIP170 in the development of dendritic arbor, which may be regulated in mTOR dependent manner. To support our hypothesis that mTOR is an important regulator of microtubule dynamics and CLIP-170 serves as a mediator, we performed microtubule regrowth assays under conditions of mTOR inhibition. As shown on Fig. 3 addition of rapamycin strongly impairs microtubule growth and attachment of CLIP170 to microtubules ends.

In 2008 we also have continued our research on potential involvement of rapamycin independent complex of mTOR, mTORC2 in dendritogenesis and spine formation. RNA interference mediated Rictor knockdown in developing rat hippocampal neurons in culture resulted in the significant reduction of the total dendritic length and complexity of dendritic arbor as well as in changes of number and morphology of dendritic spines. 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. Recently, we have also shown that effects of Rictor knockdown can be reversed by coexpresison of constitutively active Akt, another known target of mTORC2.

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

To study the role of local protein translation in dendritic arbor development, we have continued our studies on the effects of knockdown of proteins of mRNA dendritic transport machinery on dendritic arbor development. 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 Staufens 1 and 2 in hippocampal neurons. Indeed in all 3 cases knockdown led to simplification of dendritic arbor that in case of ZBP-1 was reversed by treatment with the actin polymerizing drug – jasplakinolide, pointing to actin mRNA transport and local translation being a major function of ZBP-1 during dendritogenesis. However, it is worth stressing that our bioinformatic screen performed in collaboration of Dr. Enrico Tongiorgii from Trieste, has identified additional 8 mRNAs encoded in rat genome that are potential targets for ZBP-1 and are expressed in neurons. Our current aim is to confirm these predictions experimentally and investigate role of those newly identified ZBP-1 targets during dendritogenesis and dendritic spine development.

Recently, it has been shown that ZBP-1 function is regulated by phosphorylation by Src kinase. That raised two important questions i) are other mRNA binding proteins involved in dendritic mRNA transport and translational silencing regulated by phosporylation, ii) which other kinases are involved in this process? To address these questions we tested 19 selected proteins of ribonucleoprotein complex (RNP) for existence of potential "generic" phosphorylation sites and for the probability of phosphorylation by selected panel of kinases using Netphos2.0 and NetphosK software (Blom et al., 1999, J. Mol. Biol., 294: 1351; Blom et al., 2004, Proteomics, 4: 1633), respectively. To avoid artifacts due to the usage of a single algorithm we repeated analysis of potential phosphorylation sites for ZBP1, Staufen1 and Staufen2 using Scansite 2.0 software (Obenauer et al., 2003, NAR, 31: 3635). Indeed, most of the obtained results were identical in both types of analysis. The performed analysis revealed few regularities. First, that phosphorylation of RNP proteins is a common event. Among analyzed kinases, PKC, PKA and tyrosine kinases ubiquitously phosphorylate proteins of RNPs. Other kinases are more selective, and p38MAPK phosphorylating only two substrates is the most spectacular example. Finally, we could distinguish proteins of RNPs potentially undergoing very heavy phosphorylation by several kinases (ZBP1, Satufen1, Pumilio) and those potentially very poorly regulated (Translin, hnRNPA2). Consequently, we performed experiments to confirm bioinformatic predictions regarding ZBP1 and Staufens. Indeed we were able to show phosphorylation of Staufen1 by Src kinase that has not been reported so far. Since neither NetphosK nor Scansite2.0 contain consensus phosphorylation motifs for our favorite, mTOR kinase in their libraries we used newly developed software, Group Based Position software (GPS2.0; Xue et al., 2008, Mol Cell Proteomics, 7: 1598) in order to test if RNP proteins can be phosphorylated by this kinase. Results of GPS analysis revealed that almost all analyzed proteins contained at least one highly probable phosphorylation site for mTOR. The only exceptions were Translin and hnRNPA2. FMRP contained medium probability consensus phosphorylation site. Similar results were obtained also for ERKs, another group of kinases capable of direct control of translation machinery. We next preliminarily confirmed our prediction that ZBP1 phosphorylation depends on mTOR activity using 2D protein electrophoresis. Results of both, bioinformatics and preliminary experiments suggest that mTOR and ERKs jointly control translational mRNA competence and translation itself and orchestrate local translational environment in neurons.

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

Our group is involved also in research projects aiming on understanding role of mTOR in neuropathology during development and aging. Together with several Polish groups (Commissioned Grant of the Ministry of Science and Higher Education), we 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 disease is upregulation of mTOR activity due to mutations in its inhibitors – hamartin and tuberin (TSC1/2 complex). 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. We used this observation as a readout for shRNA screen for

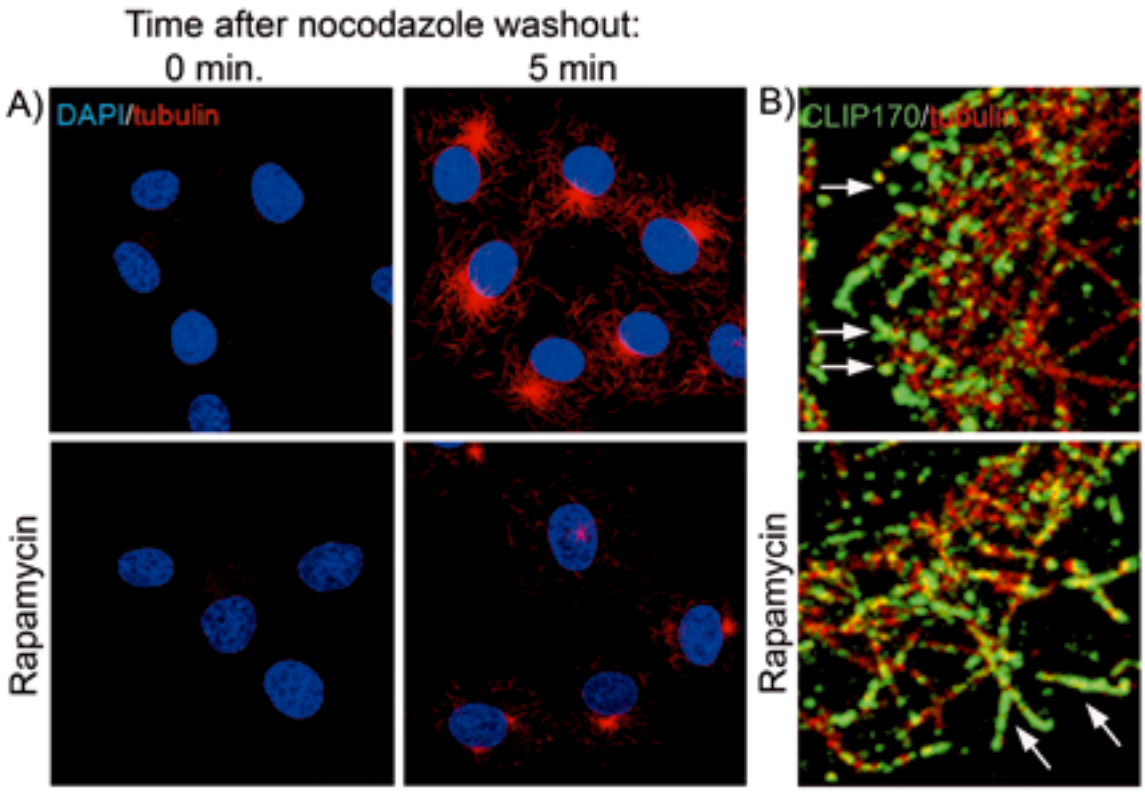


Fig. 3: mTOR inhibition impairs microtubule regrowth after depolimerization with nocodazole and increases CLIP170 binding to microtubules. mTOR inhibition impairs microtubule regrowth after depolimerization with nocodazole and increases CLIP170 binding to microtubules a) Rat-2 cells were treated with nocodazole or nocodazole together with rapamycin for 1 hour. After incubation cells were washed and put in a fresh medium or a medium with rapamycin, rescectively. Cells were fixed immediately after incubation or 5 minutes after washing and stained for α-tubulin. b) HeLa cells were treated with nocodazole and rapamycin as described above. Cells were fixed 5 minutes after washing out nocodazole and stained for α-tubulin and CLIP-170. Arrows indicate CLIP-170 staining localized on microtubules tips (author: Łukasz Świech).

mTOR regulated genes and proteins potentially involved in morphological changes occurring to cells of the nervous system during TSC development. We identified 20 genes, knockdown of which resulted in the cell soma size return to control level, involved in such cellular processes as: gene expression, translation, cytoskeleton dynamics, cell signaling and cellular membranes turnover.

In 2008 our group started another collaborative project within the 7FP EU focusing on understanding mechanisms underlying GSK3 kinase functions in neuronal plasticity in physiology and Alzheimer's disease. The major task of the Laboratory of Molecular and Cellular Neurobiology is to understand links between mTOR and GSK3 in neurons during various neuronal plasticity situations and check for disturbances of this interaction in AD animal models.

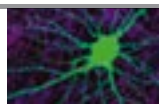
Collaborative projects

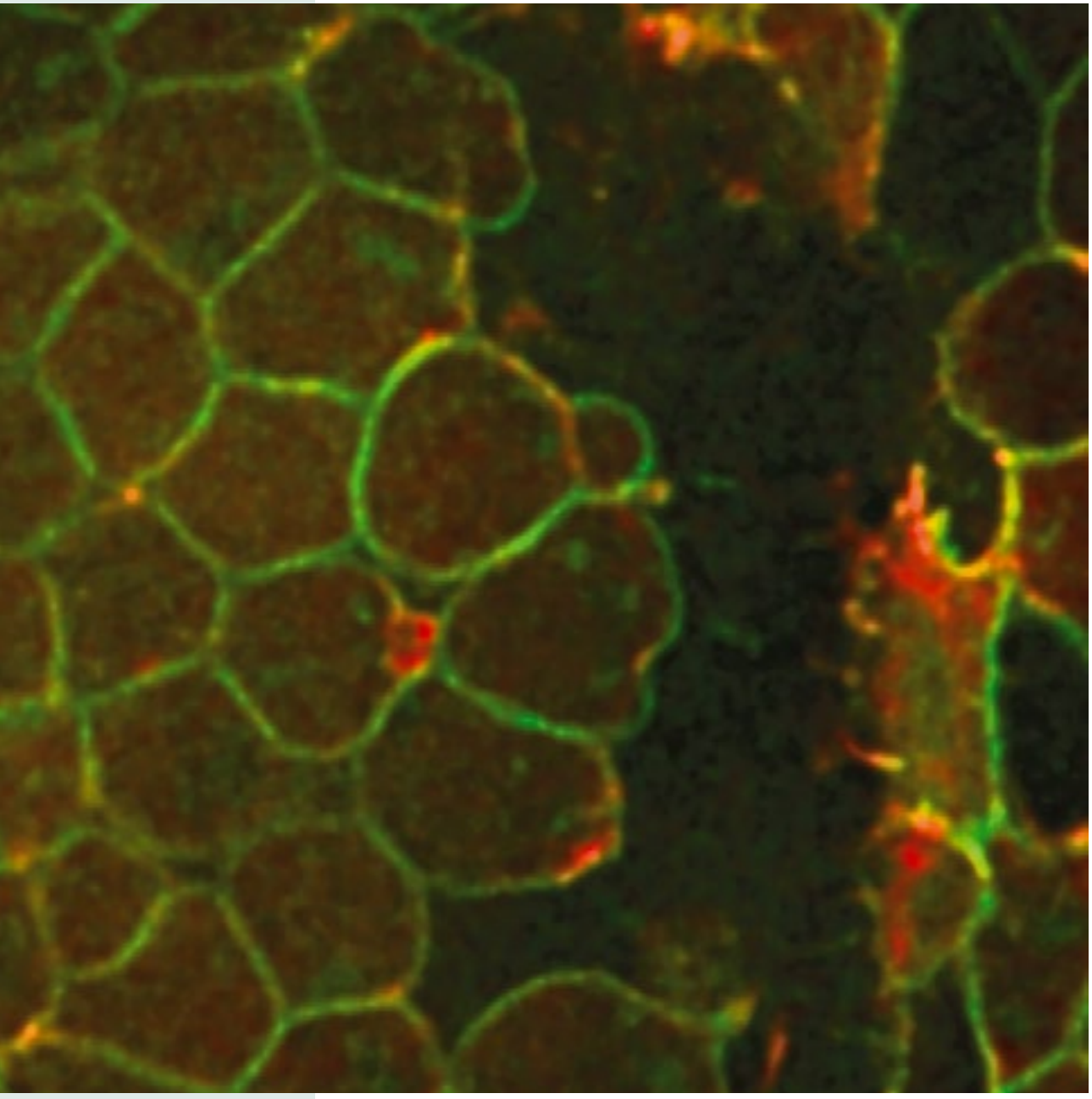
We also closely collaborated with the group of Dr. Hoogenraad (Erasmus MC, Rotterdam, Netherlands) in order to study the role of microtubule dynamics in dendritic spine development. Our research focused mostly on +TIP protein EB3. Together, we showed that growing microtubule plus-ends decorated by EB3 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. Further investigations showed that indeed EB3 exerts its effect through binding partner, p140CAP, a known regulator of actin. Additionally, cortactin was identified as binding partner for p140CAP, important for actin regulation involved in spine stabilization by invading dynamic microtubules. Importance of spine shape and number regulation by EB3 is supported by the fact that inhibition of microtubule dynamics by application of low doses of nocodazole, resulted in inhibition of long term synaptic plasticity (LTP). These observation has been recently published in Neuron (Jaworski et al., 2009). In the nearest future we hope for further close collaboration regarding role of mTOR in microtubule dynamics in frame of HEALTH_PROT Center of Excellence EU grant.

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

Our research plans for 2009 include:

- further research on genes identified in shRNA screens in context of dendritogenesis and TSC development
- further investigation of the role of phosphorylation of mRNA binding proteins: Staufen1, Staufen2 and hnRNPA for their biological functions
- conducting the kick off proteomic screens for mTOR interacting partners in neurons under pathological (epilepsy) conditions
- investigate reciprocal regulations loops between mTOR and GSK3 under physiological and AD mimicking conditions.





Animal view of the prechordal plate of a zebrafish embryo at 80% epiboly. Micrograph is a 8 um maximum intensity projection of a spinning disk stack. Green: membrane (HRAS-GFP), red: Lifeact-RFP (Actin-F marker). Blebs are clearly visible at the leading edge (author: Alb Diz Munoz).

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

Junior Researchers:

Jakub Sędzinski, MSc

Maté Biro, MSc

Alba Diz Muñoz, MSc

Andrew G. Clark, BSc

MSc Student:

Sonja Kroschwald, BSc

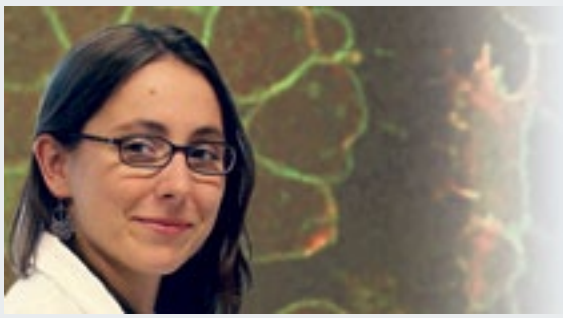
Technician:

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).



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
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

- Charras G, **Paluch E**. Blebs lead the way: how to migrate without lamellipodia (review). Nat Rev Mol Cell Biol, 2008; 9:730-736
- **Paluch E**, Van der Gucht J, Sykes C. Cracking up: symmetry breaking in cellular systems. J Cell Biol, 2006; 175:687-692
- ***Paluch E**, van der Gucht J, Joanny J-F, Sykes C. Deformations in actin comets from rocketing beads. Biophys J, 2006; 91:3113-22
- ***Paluch E**, Sykes C, Prost J, Bornens M. Dynamic modes of the cortical actomyosin gel during cell locomotion and division. Trends Cell Biol, 2006; 16:5-10
- ***Paluch E**, Piel M, Prost J, Bornens M, Sykes C. Cortical actomyosin breakage triggers shape oscillation in cells and cell fragments, Biophys J, 2005; 89:724-33
- *Gucht J, **Paluch E**, Plastino J, Sykes C. Stress release drives symmetry breaking for actin-based movement, Proc Natl Acad Sci USA, 2005; 102:7847-52.

*Papers marked with an asterisk do not have the IIMCB affiliation of the authors

Publications (other than scientific articles)

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

Grants

- 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; 2006-2009
- Human Frontier Science Program Young Investigators' Grant, (RGY67/2008), 112 500 $/annualy for 3 years, 2008-2011

Research

The main goal of the group's research is to understand how the mechanical properties of the cell are regulated at the protein-level in order to achieve controlled cellular deformations. We particularly focus on the cell cortex, a network of actin, myosin and associated proteins that lies beneath the plasma membrane and determines the shape of the cell body. The cortex enables the cell to resist externally applied forces and 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 patho-physiology of diseases such as cancer where cortical contractility is often upregulated. Despite its importance, very little is known about how the cortex is assembled and regulated.

The biological function of the cortex relies on its ability to contract and to exert forces. As such, the cortex is an intrinsically mechanical structure and its biological properties cannot be understood in isolation from its mechanics. Our main focus is to investigate how cortical mechanical properties are determined by the molecular components of the cortex and how these properties are regulated, locally and globally, to allow the cell to undergo deformations during cell division and migration. We particularly focus on blebs, spherical membrane protrusions driven by contractions of the actomyosin cortex, which commonly occur during apoptosis, cell spreading, cytokinesis and migration.

The staff composed of biologists and physicists combine biophysical and molecular approaches. Our main lines of research are:

1. Regulation of the mechanical properties of the cortex

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.

We have measured cortical tension in various cell lines and have shown that it has a well-defined quantity for a given cell line but that it can also considerably vary between lines. This suggests the existence of feedback loops allowing the cell to adjust its own tension. We are currently investigating how such mechanosensing feedbacks are achieved. Moreover, we have started assessing the influence of various cortical proteins on cortical tension. We have shown that tension depends not only on the activity of myosin motors, but also on the level of proteins involved in actin turnover (Fig. 1). We are currently extending this analysis to a larger set of cortical components. Concomitantly, we will check the effect of depletion of cortical proteins on cortex dynamics. To that aim, we are analyzing cortical flows during cortical oscillations, which can be triggered by depolymerization of microtubules (Paluch et al., Biophys. J. 2005). In an alternative approach, we plan to monitor cortical flows during cleavage furrow establishment during cytokinesis and analyze the effect of target protein depletion on the dynamics of these flows.

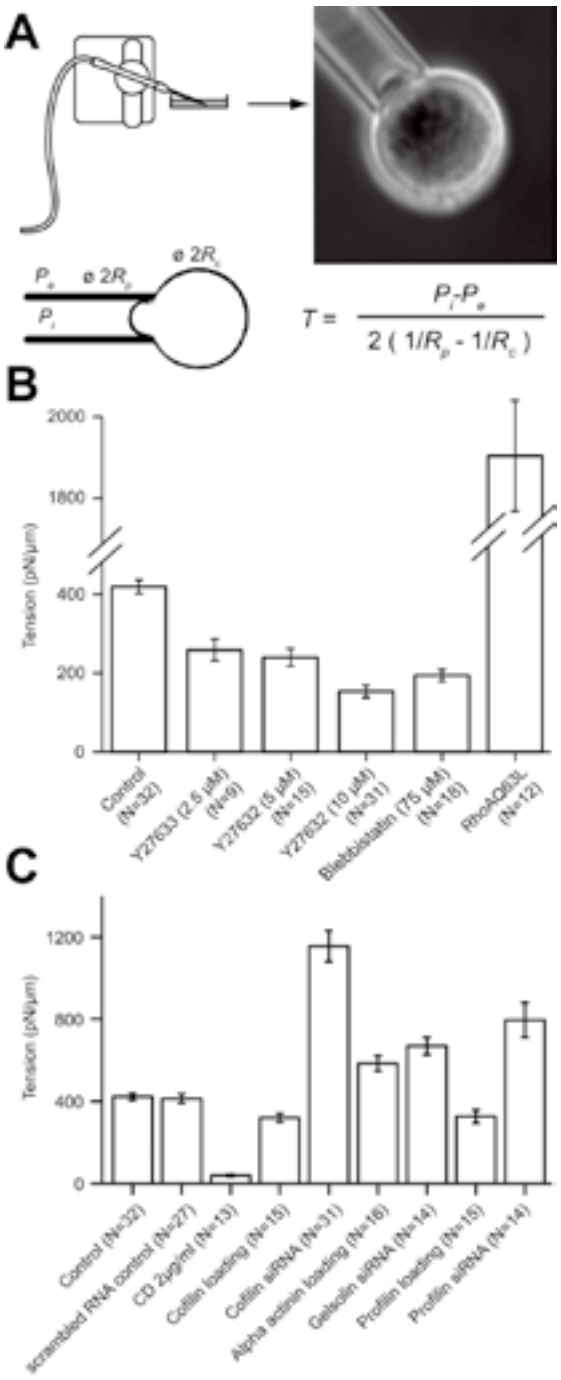


Fig. 1: Cortex tension depends on the level of myosin activity and on actin turnover. **A.** Schematic of the aspiration setup: a cell is gradually aspirated into a micropipette until it forms a hemispherical bulge inside the pipette. At this critical pressure, cortical tension is given by the Laplace law (formula indicated). The image displays a L929 detached fibroblast aspirated into a micropipette close to the critical pressure. **B.** Cortex tension after various treatments affecting myosin activity. Y27632: ROCK inhibitor. Blebbistatin: myosin II inhibitor. RhoAQ63L: constitutively active RhoA. **C.** Cortex tension after various treatments affecting actin and actin binding proteins. CD: Cytochalasin D (authors: Jean-Yves Tineves and Ulrike Schulze).

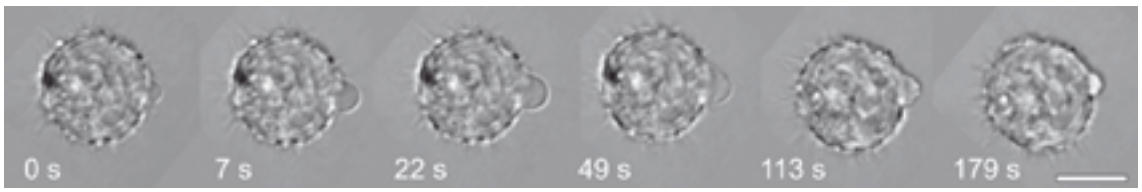


Fig. 2: Bleb induced by laser ablation in a detached L929 fibroblast. 0s: offset of ablation. Bar, 10µm (author: Julia Roensch).

In the long term, this approach will help defining the minimal ingredients necessary for cortex contractility, paving the way for a biomimetic system of the cell cortex.

2. Mechanisms of formation of blebs

The growth of blebs depends on myosin activity and is commonly believed to directly result from intracellular pressure, however this hypothesis has not been tested and the mechanisms of bleb growth remain elusive.

We have shown that laser ablation of the cell cortex leads to the formation of a bleb, supporting the view that bleb expansion is a direct, mechanical, result of intracellular pressure (Fig. 2). Moreover, multiple ablations of the same cell at different locations indicate that the growth of a bleb considerably reduces pressure. We have then induced blebs on cells with different tensions and shown that the size of a bleb directly depends on tension. This dependence can be fitted with a theoretical model of the actomyosin cortex and allows us to estimate elastic parameters of the cortex and of the cytoplasm, and to accurately predict bleb shape (collaboration with the group of J.F. Joanny, Institut Curie, Paris). We now plan to further analyze the dynamics of bleb expansion in cells with different tensions. Coupled to theoretical modeling, this will allow us to elucidate which dissipation source is the major limiting factor for bleb growth mechanics. This is particularly important because the type of protrusion formed by a cell is likely to modify its migration pattern (cf point 4) – bellow. Modifying the magnitude of dissipation linked to bleb growth can be used by cells to favor or reduce the formation of blebs versus other protrusion types such as lamellipodia.

3. Role of cortex tension and blebs during cytokinesis

We have discovered that ablation of the actin cortex during cytokinesis leads to oscillations of the cleavage furrow and results in division failure (Fig. 3). Similar furrow oscillations can be observed after depletion of different actin binding proteins. Strikingly, small oscillations of the furrow can also sometimes be observed in control divisions, although their amplitude remains limited, allowing for division to proceed. Based on our observations, we have proposed that the cortex controls its own contractility during furrow ingression and prevents the built-up of an imbalance in contractile forces by self-disassembling and forming blebs above a threshold tension. When this control is removed, like for example after laser ablation, the cleavage becomes unstable and cytokinesis fails. We are currently testing this interpretation using both biophysical and molecular techniques.

4. Protrusion formation during migration in 3D-environments

In 3D-environments, bleb-based migration is a widespread alternative to lamellipodial migration, and is commonly used by cancer cells and during development. It is not known why cells form one or the other type of protrusion, and how the cells can switch between protrusion types is poorly understood. Strikingly, certain cell types, e.g. mesendodermal cells in Danio rerio (zebrafish) embryos, are able to form both lamellipodia and blebs at the same time. We have initiated a study of the mechanisms of formation of these protrusions and of their respective contributions to cell migration in the zebrafish embryo (collaboration with the lab of C.P. Heisenberg, MPI-CBG). We have characterized wild type migration and have shown that the protrusions formed by mesendodermal progenitors consist of blebs (50%), lamellipodia (35%) and filopodia (15%). We have also shown that the expression of dominant negative (resp. constitutively active) ezrin (a protein linking the actin cortex to the membrane) shifts this distribution and leads to the formation of more (resp. fewer) blebs. We are currently investigating the effects of these treatments on migration. The long term perpective is to better understand the mechanisms of protrusion formation and the role of blebs during migration in vivo and in 3D environments.

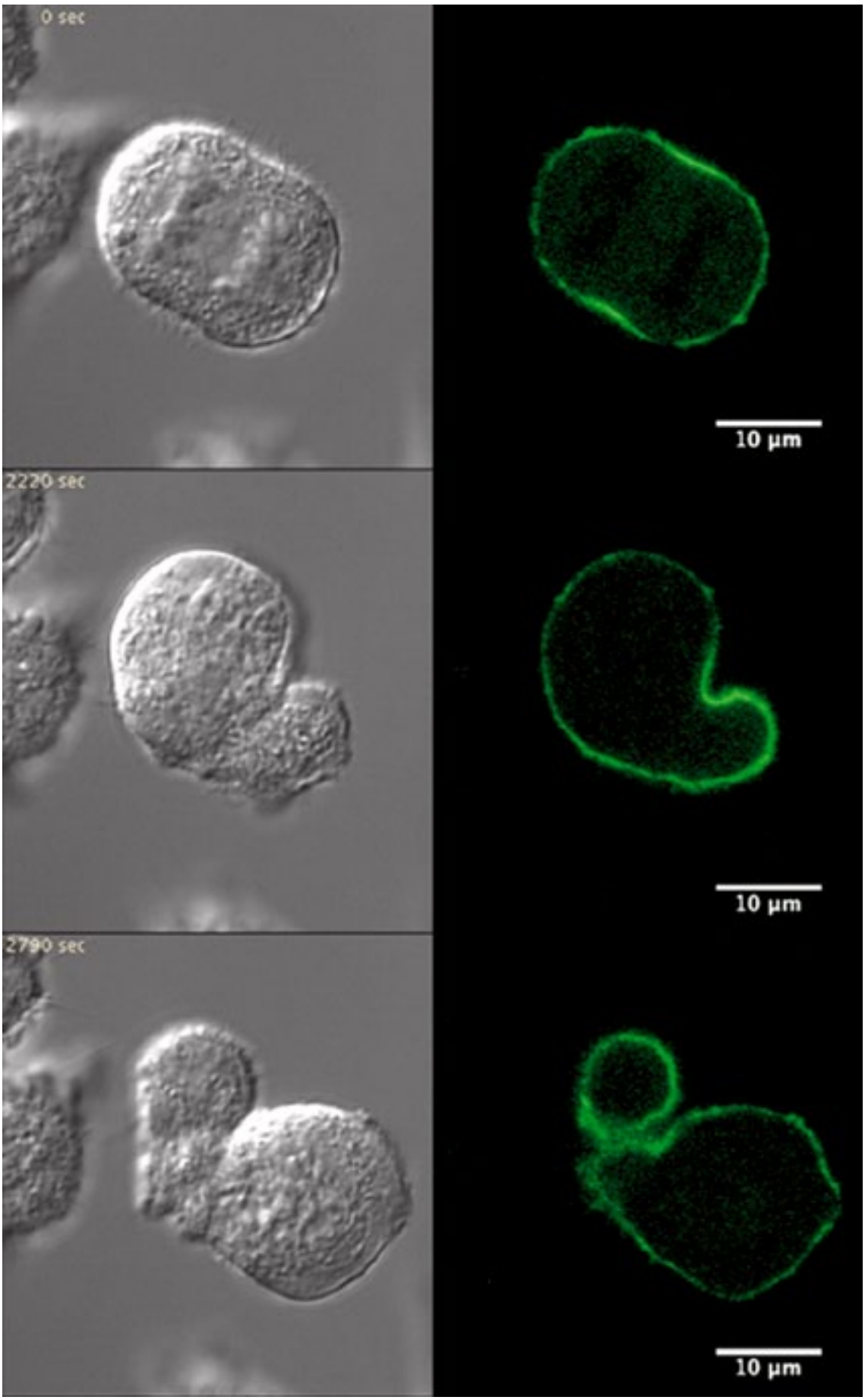
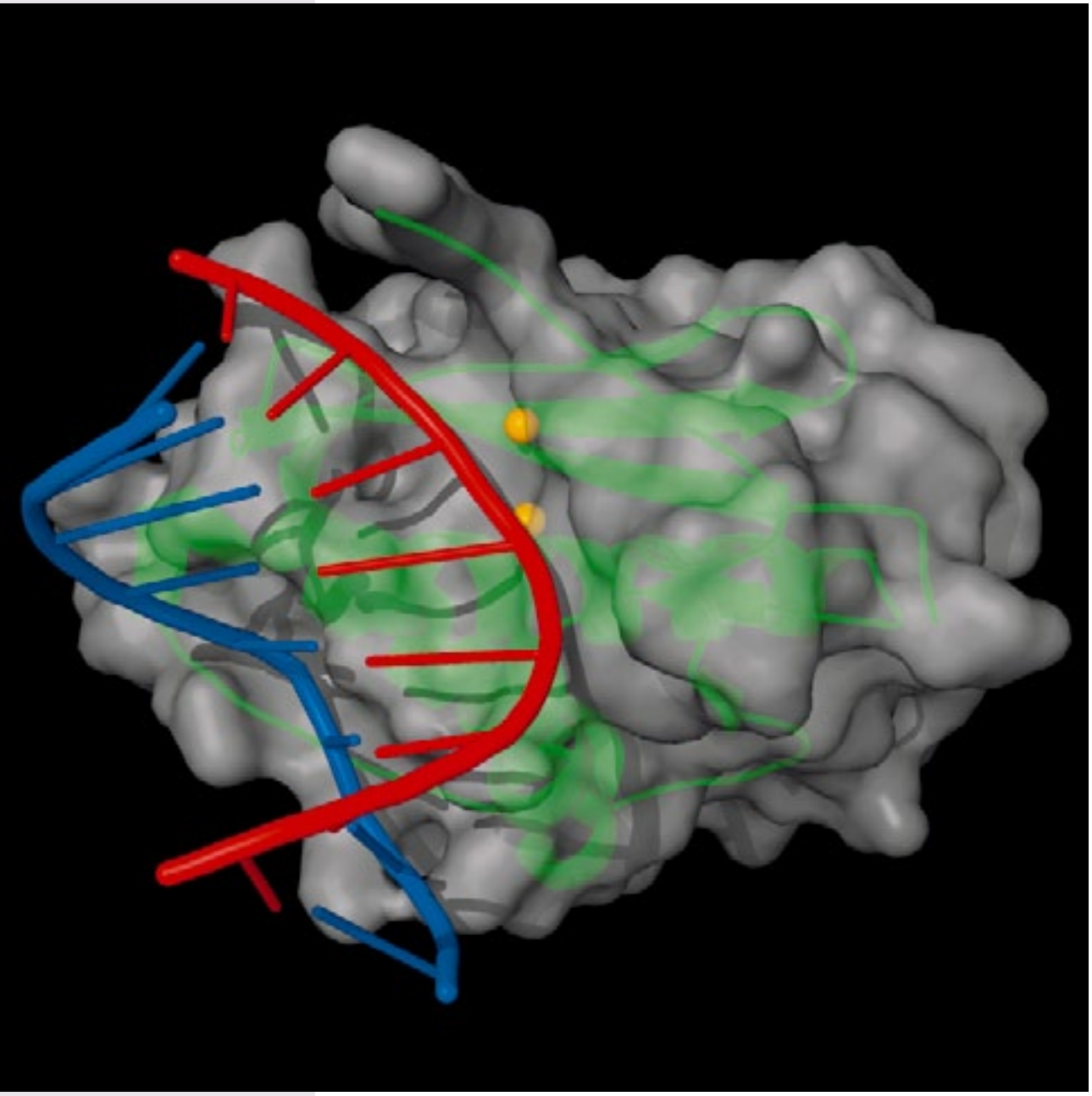


Fig. 3: Oscillations induced by laser ablation of the cortex in L929 fibroblast during cytokinesis. Left: DIC. Right: Myosin regulatory light chain-GFP (author: Jakub Sedziński).



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 (author: Marcin Nowotny).

Laboratory **of Protein Structure**

Lab Leader:

Marcin Nowotny, PhD

Post-doctoral Fellow:

Karolina Górecka, PhD

Junior Researchers:

Małgorzata Figiel, MSc

Marcin Jaciuk, MSc

Jakub Jurkowski, MSc

Monika Rychlik, MSc

Technician:

Jadwiga Dyttus

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**. Retroviral integrase superfamily: the structural perspective (review). EMBO Rep, 2009; 10:144-51
- ***Nowotny M,** Cerritelli SM, Ghirlando R, Gaidamakov SA, Crouch RJ, Yang W. Specific recognition of RNA/DNA hybrid and enhancement of human RNase H1 activity by HBD. EMBO J, 2008; 27:1172-81
- ***Nowotny M**, Gaidamakov SA, Ghirlando R, Cerritelli SM, Crouch RJ, Yang W. Structure of human RNase H1 complexed with an RNA/DNA hybrid: Insight into HIV Reverse Transcription. Mol Cell, 2007; 28:264-276
- ***Nowotny M,** Yang W. Stepwise analyses of metal Ions in RNase H catalysis: From substrate destabilization to product release. EMBO J, 2006; 25:1924-33
- *Yang W, Lee JY, **Nowotny M**. Making and Breaking Nucleic Acids: Two-Mg^{2+}-ion Catalysis and Substrate Specificity, (review). Mol Cell, 2006; 22:5-13
- ***Nowotny M**, Gaidamakov SA, Crouch RJ, Yang W. Crystal structures of RNase H bound to an RNA/DNA hybrid: substrate specificity and metal-dependent catalysis. Cell, 2005; 121:1005-16
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- ***Nowotny M**, Spiechowicz M, Jastrzebska B, Filipek A, Kitagawa K, **Kuznicki J**. Calcium-regulated interaction of Sgt1 with S100A6 (calcyclin) and other S100 proteins. J Biol Chem, 2003; 278:26923-8
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- *Filipek A, Jastrzebska B, **Nowotny M,** Kwiatkowska K, Hetman M, Surmacz L, Wyroba E, **Kuznicki J**. Ca^{2+}-dependent translocation of the calcyclin-binding protein in neurons and neuroblastoma NB-2a cells. J Biol Chem, 2002; 277:21103-9
- ***Nowotny M**, Bhattacharya S, Filipek A, Krezel AM, Chazin W, **Kuznicki J**. Characterization of the interaction of calcyclin (S100A6) and calcyclin-binding protein. J Biol Chem, 2000; 275:31178-82.

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

Description of Current Research

Our laboratory focuses on structural and biochemical studies of nucleic acid enzymes. Our primary method is 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, repairing 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. Substrate complex structures of type 1 RNases H revealed the mechanism of RNA/DNA recognition and demonstrated that the catalysis relies on two metal ions (Nowotny et al. Cell 2005, Nowotny et al. Mol Cell 2007). 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-Goutiéres 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 have 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 structures of complexes of HIV RT with 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 M. 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.

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 apoUvrA 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. Cocrystallization 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.

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. Bućko-Justyna (M. Żylicz lab, IIMCB) in 2005, K. Starowicz (R. Przewłocki lab, Institute of Pharmacology PAN, Kraków) 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, Poznań: Genetic analysis of primary ciliary dyskinesia/ Kartagener Syndrome [PCD/KS]), M. Łukowiak (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 Urbański (M. Żylicz 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, Poznań, Szczecin, Gdańsk, 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 postgraduate PhD program in the field of molecular medicine, which is addressed to medical, biology and pharmacology postgraduate students in Poland. Since 2002, SMM has been 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. 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 1998-2008, including seven 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. 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 were supported so far by subsidies from the Polish Ministry of Health, the Ministry of Science and Higher Education, the Kronenberg Foundation, UNESCO-ROSTE, the European Commission and The National Center for Scientific Research (CNRS), France. Additional financial support came 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 2008, the following courses were organized:

- SMM Spring School lecture course "From gene to protein, from structure to function and dysfunction", 14-18.04.2008, Warsaw. This annual course, obligatory for first-year students, was organized by Prof. Liliana Konarska. The lectures were given by twenty-eight outstanding scientists and academic teachers from the top clinical and research institutions in Poland.
- Practical course "Scientific communication", 12-16.05.2008, Warsaw, organized by SMM and IIMCB for first-year students. The course was ran by Prof. Edward Potworowski from the Armand-Frappier Institute of Montreal, Canada. This course 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 23-27.06.2008, Poznań, "Progress in Molecular Biology" - for first-year students.
- X Annual Inaugural and Research Report SMM Session, 20-21.11.2008, Warsaw, organized by SMM office and SMM students. Inaugural lecture was given by Prof. Sławomir Majewski from Medical University of Warsaw. During the session, 23 SMM students presented their research results obtained during the academic year 2007/2008. The presentations were divided into four subsessions: protein engineering and biomodelling, molecular diagnosis of cancer, molecular diagnosis of human and miscellaneous.

In 2008 SMM experienced a very sad and severe loss: Prof. Liliana Konarska, Director of SMM, died in August. Her successor became Prof. Bożena Kamińska-Kaczmarek. Currently SMM is in the process of general changes in its organizational status (new agreements between founding institutions). Final new arrangements will be completed at the beginning of the 2009 year.

Centre for Innovative Bioscience Education (CIBE)

(Formerly: Science Festival School)

The aim of the Center for Innovative Bioscience Education (CIBE) is to reduce the gap between science and society in Poland by conducting educational activities popularizing biology: workshops for students and all interested participants as well as courses for biology teachers and various science communication events. All activities are focused on improving biology education and awareness of biology in society. The co-founders of CIBE are as follows: International Institute of Molecular and Cell Biology (IIMCB), Nencki Institute of Experimental Biology PAN (NIEB), Institute of Biochemistry and Biophysics PAN (IBB), Warsaw University of Life Sciences (SGGW), BioEducation Foundation and Warsaw Science Festival. IIMCB hosts the CIBE laboratory, office and administration. Additionally, CIBE leads another laboratory at Warsaw University of Life Sciences. In 2008, over 1,600 young participants visited laboratory workshops. At the same time, over 200 biology teachers attended laboratory workshops and courses, approximately 350 children attended hands-on practice experiments.

12th Science Picnic (14 June 2008)

Like in previous years, The BioEducation Foundation and CIBE organized an exhibition and science show during the 12th Science Picnic in Warsaw. The year 2008's motto was "The language of science".

- Can you see DNA? – DNA isolation from onion
- How scientific investigation works? – hands-on practice experiments
- Let's become a researcher! – children could make their own investigations on Picnic's visitors
- The magic of colors – how the pH indication works.

Science Festival

The goal of the XII Warsaw Science Festival (19-28 September 2008) was 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 every year by scientists, not by the government. About 140 scientific institutes and about 1000 scientists are involved every year in the organization of this event. In 2008 CIBE organized laboratory workshops for open public and for school groups such as:

- "Explore your own DNA"
- "Do you know, what you eat?"
- "Yeast – the leaving micro-factory"
- "Investigate evolution signs in your DNA"

"Explore the biological world by yourself" was the title of our presentations during XII Science Festival in Jabłonna Palace, 28 September 2008. The same presentations were repeated during X Science and Art Festival in Siedlce in October 2008.

Laboratory workshops

Guests of our biological laboratory may take part in real life experiments and learn how to use laboratory techniques and equipment. The practical experiments are always supported by lectures presenting the theoretical basis of molecular biology, genetics and its techniques. A total number of 1,606 students visited CIBE laboratories last year. The workshops for secondary school students take one day. We offered the following themes:

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

Courses for biology teachers

During our workshops for biology teachers we try to build a connection between them and scientists so they can feel they are a part of the scientific 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, equipment and reagents that can be used in a school environment. A total number of 202 biology teachers participated in our laboratory workshops and scientific meetings last year.

In 2008 following events were organized by CIBE as a part of teachers' excellence projects:

- "Molecular biology from the kitchen", 7-8 March 2008, Oświęcim, Poland
- "Introducing new approaches to teaching evolution in secondary schools" 12-14 March 2008, EMBL Heidelberg, Germany[http://www.embl.de/training/ells/learninglab/2008/llmar2008/index.html]

- "Practical experiments at school" 3 April 2008, Siedlce, Poland
- "Volvox – let's teach to experiment!" 6-7 June, 21-22 November, 9-10 December 2008, CIBE laboratory, Warsaw, Poland
- "Let's teach to experiment – biotechnology every day" 24-26 October 2008, part of a bigger project for 30 secondary school teachers from small cities and villages in Poland, this workshop was run at CIBE laboratory in Warsaw. Participants were also given valuable sets of school laboratory equipment
- "The VIIth scientific conference for biology teachers" 6 December 2008, NIEB, Warsaw

The final year of the European "VOLVOX" project

"Volvox – Innovative network for bioscience education" (www.eurovolvox.org) was a European project founded by European Commission in 6. Framework Programme; IIMCB represented a Polish partner in this project. It aimed to help to enliven school biology teaching, so that more young Europeans would continue to study biological science, follow scientific carriers and, as engaged citizens, help to shape Europe's scientific culture and economy. To achieve its aims, Volvox has:

- established an international network for European bioscience education
- implemented mechanisms to help teachers, scientists and others develop, exchange, translate and adapt resources for biology teaching
- identified barriers that prevent the exchange of new and novel ideas between those with a professional interest in bioscience education
- investigated practical means of enhancing the uptake of new and novel ideas by European biology teachers
- investigated ways in which such innovation networks can be expanded to create a 'critical mass' and so become sustainable.

The current reporting period covers the fourth and final year of the Volvox project. During this year we have focused on translating and adapting the educational resources and setting up web sites so that the materials can be made available to teachers. An on-line evaluation system, linked to the web sites, has also been implemented. Several events have been held to promote the project and its resources across Poland and the European Union. Although the project has now officially ended, the project partners will continue to exchange their educational experiences and materials to publish them for the foreseeable future.

Four meetings of Volvox partners were held during this reporting period - a meeting of Volvox Management Committee in Reading in January, a small meeting to become more adept in the use of the In Design software in Reading in February, two Volvox Consortium meetings in Reading in April and in October in Luxemburg were focused on production of resources for publication and involving an official press launch and public promotion of the Volvox project.

Volvox web sites were mentioned as a recommendable source of educational materials in the May issue of Nature [Nature, vol 453; 1 May 2008].

The Polish Volvox web site (http://www.sfn.edu.pl/volvox) now serves 21 practical protocols, Polish version of 'The Cell World' model animation, two text based activities, one presentation, one educational board game and a set of 200 educational images.

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

CIBE is a Polish distributor of "Science in School", free-available journal for European science teachers. The Journal 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. A printed English version of the journal is freely available as well as an on-line version, where articles are published in several European languages. CIBE translated into Polish some of the biology-based articles which are published on the web site http://www.scienceinschool.org/polish.

Staff and co-workers

Persons who coordinate and administrate CIBE are: Agnieszka Chołuj, Joanna Lilpop, Marta Badurek, and Marcin Wiśniewski as a coordinator at SGGW.

Animators and co-workers: Anna Fogtman, Justyna Rudzka, Kamil Koper, Aleksandra Kwiatkowska, Maciej Kotliński, MajaCieplak, Kamila Ornoch, Damian Graczyk, Takao Ishikawa, Anna Karnkowska-Ishikawa, Maciej Węsierski, Krzysztof Brewczyński, Grzegorz Olszewski, Monika Ostaszewska, Anna Łach, Izabela Szczupakowska, Wojciech Siwek, Monika Hejnowicz and students from Biotechnology Students' Association at SGGW.

Staff at IIMCB (as of 31 March 2009)

Administration		Funding
Jacek Kuźnicki	Director	IIMCB
Michał Witt	Deputy Scientific Director	IIMCB(1/2)
Jarosław Filiński	Deputy Administrative Director	IIMCB
Zbigniew Przygoda	Director's Advisor	IIMCB(1/2)
Hanna Iwaniukowicz	Financial Manager	IIMCB
Monika Nowicka	Payroll Specialist	IIMCB
Renata Knyziak	Accounting Specialist	IIMCB
Krystyna Domańska	Human Resources Specialist	IIMCB
Beata Tkacz	Human Resources Specialist	IIMCB
Urszula Białek-Wyrzykowska	International Cooperation Manager	IIMCB(1/2)
Dorota Wasiak-Libiszowska	Foreign Grants Specialist	IIMCB
Magdalena Powierża	International Cooperation Specialist	IIMCB
Marcin Ogonowski	International Cooperation Specialist	IIMCB
Agnieszka Wagner-Ziemka	Planning and Reporting Specialist	IIMCB
Agnieszka Karbowska	Tender Specialist	IIMCB
Dominika Dubicka	Director's Assistant	IIMCB
Anna Brzezińska	Secretary	IIMCB
Marcin Biedacha	IT Manager	IIMCB
Jakub Skaruz	IT Specialist	IIMCB
Robert Banasiak	Maintenance Specialist	IIMCB

Department of Molecular Biology		
Maciej Żylicz	Head	IIMCB
Alicja Żylicz	Vice Head	IIMCB
Marcin Klejman	Research Assistant	IIMCB
Dawid Walerych	Research Assistant	IIMCB/Ministerial grant
Paweł Burdiak	Junior Researcher	Ministerial grant
Marta Frankowska	Junior Researcher	IBB Fellowship/Ministerial grant
Milena Ostrysz	Junior Researcher	Nencki Fellowship/Ministerial grant
Jakub Urbański	Junior Researcher	Utrecht University Fellowship
Anna Żurawska	Junior Researcher	IBB Fellowship/Ministerial grant (till Sep. 2008)
Natalia Sikorska	Volunteer	
Grażyna Orleańska	Secretary	IIMCB(1/2)

Laboratory of Structural Biology MPG/PAN		
Matthias Bochtler	Head	Max Planck
Izabela Sabala	Post-doctoral Fellow	EU grant/Ministerial funds*
Honorata Czapińska	Post-doctoral Fellow	Ministerial grant
Renata Filipek	Post-doctoral Fellow	EU grant/Ministerial funds*
Grzegorz Chojnowski	Junior Researcher	Ministerial grant
Henryk Korza	Junior Researcher	EU grant/Ministerial funds*
Patrycja Kubajek	Junior Researcher	Ministerial funds*
Monika Sokołowska	Junior Researcher	Ministerial grant/Max Planck
Roman Szczepanowski	Junior Researcher	Max Planck
Magdalena Kaus-Drobek	Junior Researcher	Nencki PhD School
Marek Wojciechowski	Junior Researcher	HHMI
Dario Piano	EU visiting expert	EU grant
Sabah El Alaoui	EU visiting expert	EU grant

Laboratory of Biomodelling		
Sławomir Filipek	Head	IIMCB
Michał Koliński	Junior Researcher	IIMCB
Aleksander Dębiński	Junior Researcher	IIMCB/Warsaw Univ. Fellowship
Wojciech Puławski	Junior Researcher	IIMCB
Krzysztof Młynarczyk	MSc Student	Volunteer

* – Ministerial matching funds to EU grant

Laboratory of Protein Structure		
Marcin Nowotny	Head	Wellcome Trust/EMBO
Karolina Górecka	Post-doctoral Fellow	Wellcome Trust
Małgorzata Figiel	Junior Researcher	IIMCB
Marcin Jaciuk	Junior Researcher	Ministerial grant/EMBO
Jakub Jurkowski	Junior Researcher	Ministerial grant/EMBO
Monika Rychlik	Junior Researcher	IIMCB
Jadwiga Dyttus	Technician	IIMCB

Laboratory of Bioinformatics and Protein Engineering		
Janusz M. Bujnicki	Head	IIMCB
Krzysztof Skowronek	Research Coordinator	IIMCB
Kristian Rother	Post-doctoral Fellow	EU grant
Michał Boniecki	Post-doctoral Fellow	Ministerial grant
Ewa Wywiał	Post-doctoral Fellow	Ministerial funds*
Małgorzata Durawa	Junior Researcher	Ministerial grant
Agata Kamaszewska	Junior Researcher	Ministerial grant
Katarzyna H. Kamińska	Junior Researcher	Ministerial funds*
Iga Korneta	Junior Researcher	Ministerial funds*
Jan Kosiński	Junior Researcher	Ministerial grant
Łukasz Kozłowski	Junior Researcher	Ministerial grant
Agnieszka Obarska-Kosińska	Junior Researcher	Ministerial funds*
Jerzy Orłowski	Junior Researcher	Ministerial funds*
Marcin Pawłowski	Junior Researcher	Ministerial grant
Dariusz Pianka	Junior Researcher	Ministerial grant
Michał Piętal	Junior Researcher	NIH grant
Katarzyna Poleszak	Junior Researcher	Ministerial grant
Wojciech Potrzebowski	Junior Researcher	Ministerial grant
Elżbieta Purta	Junior Researcher	Ministerial grant
Wojciech Siwek	Junior Researcher	Ministerial grant
Karolina Tkaczuk	Junior Researcher	Ministerial grant
Irina Truszyńska	Junior Researcher	Ministerial grant
Maria Werner	Junior Researcher	Ministerial grant
Natalia Borkowska	Undergraduate Student	Volunteer
Tomasz Kościółek	Undergraduate Student	Volunteer
Katarzyna Kolczyńska	Undergraduate Student	Volunteer
Justyna Lesiak	Undergraduate Student	Volunteer
Paweł Łukasz	Undergraduate Student	Volunteer
Magdalena Mika	Undergraduate Student	Volunteer
Ewelina Osińska	Undergraduate Student	Volunteer
Tomasz Stępniewski	Undergraduate Student	Volunteer
Konrad Tomala	Undergraduate Student	Volunteer
Agnieszka Faliszewska	Office Manager	Ministerial funds*
Jan Kogut	Computer Administrator	Scientific Network
Tomasz Jarzynka	Computer Administrator	NIH grant
Łukasz Munio	Computer Administrator	Ministerial grant

Laboratory of Neurodegeneration		
Jacek Kuźnicki	Head	IIMCB
Urszula Wojda	Associate Professor	IIMCB
Joanna Gruszczyńska-Biegała	Post-doctoral Fellow	Polish-German grant
Anna Skibińska - Kijek	Post-doctoral Fellow	EU grant
Marta Wiśniewska	Post-doctoral Fellow	EU grant
Adam Sobczak	Post-doctoral Fellow	IIMCB
Łukasz Bojarski	Post-doctoral Fellow	Polish-German grant
Emilia Białopiotrowicz	Junior researcher	Ministerial grant
Katarzyna Dębowska	Junior researcher	Ministerial grant
Bożena Kuźniewska	Junior researcher	Ministerial grant
Wojciech Michowski	Junior researcher	Nencki PhD School
Katarzyna Misztal	Junior researcher	IIMCB
Andrzej Nagalski	Junior researcher	Scientific Network
Aleksandra Szybińska	Junior researcher	IIMCB

* – Ministerial matching funds to EU grant

Laboratory of Molecular and Cell Neurobiology		
Jacek Jaworski	Head	IIMCB/Polish-Norwegian Research Found
Magda Błażejczyk	Post-doctoral Fellow	EU grant/Nencki Institute
Iwona Cymerman	Post-doctoral Fellow	EU grant
Matylda Macias	Post-doctoral Fellow	EU grant/Nencki Institute
Anna Malik	Junior researcher	Ministerial grant/Nencki PhD School
Małgorzata Perycz	Junior researcher	Nencki PhD School
Łukasz Świech	Junior researcher	Nencki PhD School
Małgorzata Urbańska	Junior researcher	Ministerial funds*/Nencki PhD School
Paweł Krawczyk	MSc Student	Volunteer
Kamil Parobczak	MSc Student	Volunteer
Patrycja Pietruszka	MSc Student	Volunteer
Anna Urbańska	MSc Student	Volunteer
Małgorzata Zarębska	MSc Student	Volunteer

Laboratory of Cell Biology		
Marta Miączyńska	Head	Wellcome Trust
Magdalena Banach-Orłowska	Research Assistant	Wellcome Trust
Iwona Pilecka	Post-doctoral Fellow	Wellcome Trust
Beata Pyrzyńska	Post-doctoral Fellow	HHMI
Sajid Rashid	Post-doctoral Fellow	EU
Anna Hupałowska	Junior Researcher	EU/Nencki PhD School
Marta Olchowik	Junior Researcher	HHMI/Nencki PhD School
Łukasz Sadowski	Junior Researcher	EU/Nencki PhD School
Anna Toruń	Junior Researcher	IIMCB/Nencki PhD School
Anna Urbańska	Junior Researcher	IIMCB/Nencki PhD School
Michał Mlącki	MSc Student	Volunteer

Laboratory of Cell Cortex Mechanics MPG/ PAN		
Ewa Paluch	Head	Ministerial grant
Jakub Sędzinski	Jounior Researcher	Ministerial grant
Maté Biro	Jounior Researcher	HFSP grant
Alba Diz Munoz	Jounior Researcher	Ministerial grant
Andrew G. Clark	Jounior Researcher	HFSP grant
Sonja Kroschwald	MSc Student	Volunteer
Julia Roensch	Technician	Ministerial grant

Molecular Haematology Project		
Michał Witt	Project Director	IIMCB
Małgorzata Mossakowska	Coordinator	IIMCB
Przemysław Ślusarczyk	IT Specjalist	Ministerial grant
Maria Wojnowska	Technician	Ministerial grant

PolSenior Project		
Małgorzata Mossakowska	Coordinator	IIMCB
Aleksandra Szybalska	Project Assistant	Ministerial grant
Magdalena Owczarz	Assistant	Ministerial grant
Przemysław Ślusarczyk	IT Specjalist	Ministerial grant
Marta Świech	Technician	Ministerial grant
Ewa Tondys	Technician	Ministerial grant

Research Equipment Laboratory		
Wanda Gocal	Technician	IIMCB
Monika Dudek	Technician	IIMCB
Leszek Lipinski	Technician	IIMCB(1/4)
Adam Sobczak	Technician	IIMCB(1/4)

Centre for Innovative Bioscience Education		
Joanna Lilpop	Coordinator	IIMCB/Nencki/IBB
Agnieszka Chołuj	Coordinator	Volunteer
Marta Badurek	Coordinator	Volunteer
Marcin Wiśniewski	Coordinator	SGGW
Anna Fogtman	Teacher	Volunteer
Justyna Rudzka	Teacher	Volunteer
Kamil Koper	Teacher	Volunteer
Aleksandra Kwiatkowska	Teacher	Volunteer
Maciej Kotliński	Teacher	Volunteer
Maja Cieplak	Teacher	Volunteer
Kamila Ornoch	Teacher	Volunteer
Damian Graczyk	Teacher	Volunteer
Takao Ishikawa	Teacher	Volunteer
Anna Karnkowska-Ishikawa	Teacher	Volunteer
Maciej Węsierski	Teacher	Volunteer
Krzysztof Brewczyński	Teacher	Volunteer
Grzegorz Olszewski	Teacher	Volunteer
Monika Ostaszewska	Teacher	Volunteer
Anna Łach	Teacher	Volunteer
Izabela Szczupakowska	Teacher	Volunteer
Wojciech Siwek	Teacher	Volunteer
Monika Hejnowicz	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

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

International Institute of Molecular and Cell Biology

4 Ks. Trojdena Street, 02-109 Warsaw, Poland
www.iimcb.gov.pl