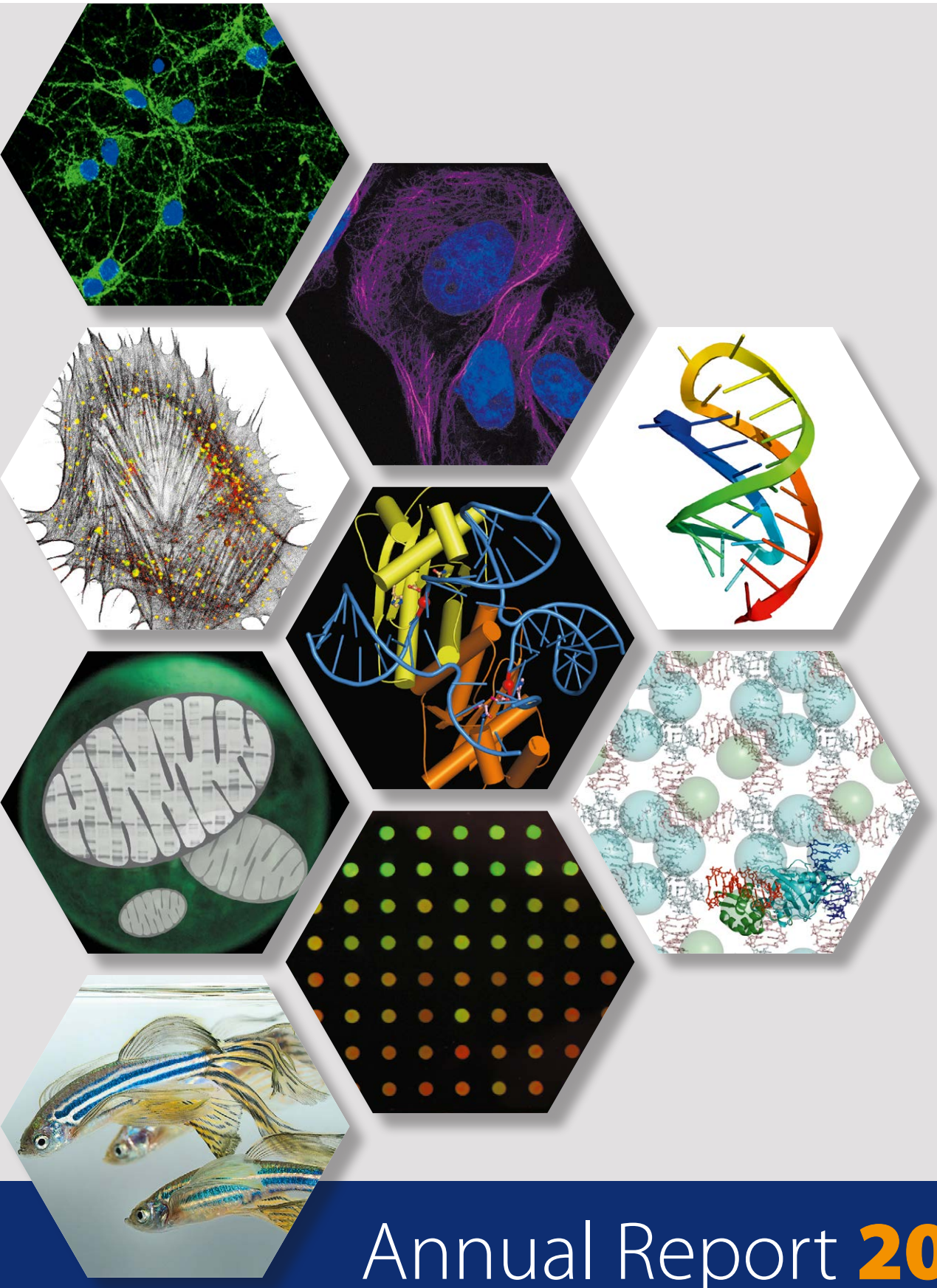
INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY



Annual Report 2013

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2010-2014 term

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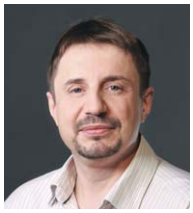
Deputy Scientific Director



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01.06.2011–31.05.2013

Marta Miączyńska
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01.06.2013–31.05.2014

Hanna Iwaniukowicz
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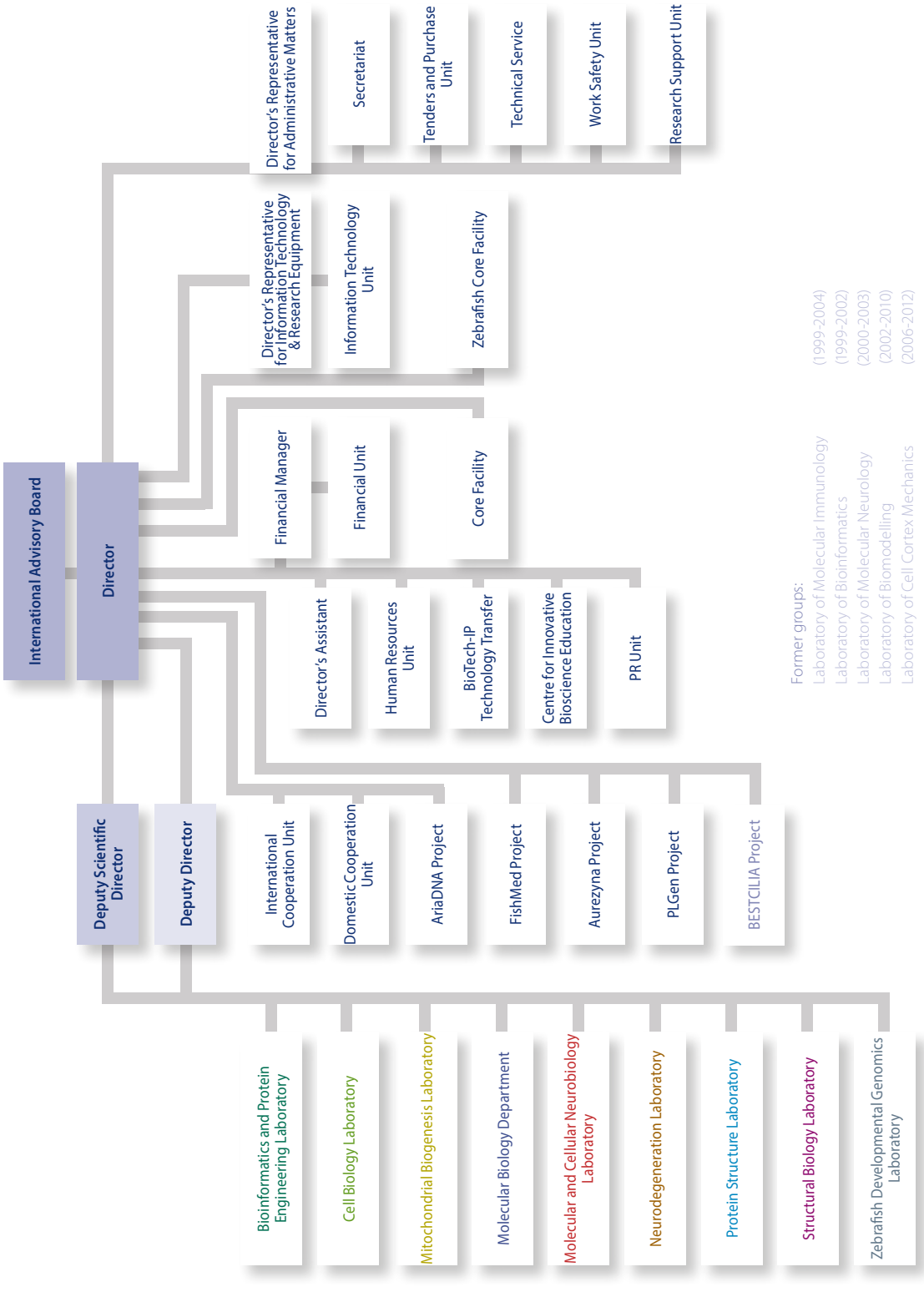
Alexander Wlodawer. National Cancer Institute, Frederick, USA

Permanent Advisor: **Angelo Azzi** Tufts University, Boston, USA

Structure of the International Institute of Molecular and Cell Biology



Introduction

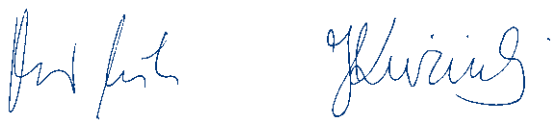
Directors' Note

We reached the end of 2013 totally involved in the procedures of yet another parametric evaluation of our Institute, scheduled, as prescribed by the Ministry of Science and Higher Education, to cover a period of four years. This technically and organizationally complex task, whose purposefulness and reliability will most likely continue to be a subject of debate in academic circles for a long time to come, ended in huge success for us: we were awarded the top A+ category in the field of Life Sciences (only 37 scientific institutions in Poland hold the A+ category across all fields, of which 9 are in the Life Sciences area). Not only does this mean prestige for us but it is also linked to the level of statutory financing over the next four years, undoubtedly offering us the possibility of more intensive development. This, certainly, is a reason for satisfaction.

Another reason for satisfaction is the finalization, after prolonged efforts, of the competition for the position of group leader for a research group operating under the FishMed Project. We are pleased to announce that the selected candidate is a researcher from Singapore, Dr. Cecilia Winata, who will carry out research into the transcriptional regulation of cardiac development in fish embryos in the laboratory of Zebrafish Developmental Genomics, IIMCB/Max Planck Research Group. This is an element which provides a considerable boost to the international stature of the Institute, while also strengthening our position in zebrafish model research. At the same time, it is also related to the development of our Zebrafish Core Facility, which provides the basis for a single biomedical research center using this model in Poland on such a scale. Further development of research into models of human diseases in zebrafish represents a priority in the future plans of the Institute. At the same time, it provides us with the foundation to strengthen our collaboration with the Max-Planck Society (MPG) and, specifically, with the MPG Institute of Heart and Lung Research in Bad Nauheim. It also points out that we need to be serious about our plans to enter the *Horizon 2020* program. The profiling of our basic research already towards certain interdisciplinary innovativeness and applicability makes us well suited to enter this European program, hopefully quite soon.

Bearing in mind the need for further expansion of the research base of our Institute, and being aware of the restrictions of available space in our building, which is already being used to capacity, we announced – under our consortia agreements – competitions for shared independent positions in the fields of systems biology (with the Intercollegiate Faculty of Biotechnology, University of Gdańsk-Medical University of Gdańsk) and genomics (with the Museum and Institute of Zoology PAN). It is worth mentioning here that our consistent and clear employment policy, based on international competitions, and selection based exclusively on the merits of the candidates, was behind the European Commission's decision to award us with the 'HR Excellence in Research' logo, which identifies particularly attractive work environments. The IIMCB is the third institution in Poland to receive this honourable distinction.

The regular day-to-day staff of the IIMCB has exceeded 200 people and we are already aware of the fact that we will not be able to develop further in the building presently occupied by our Institute. This is an issue with its own separate history that could become the subject of a separate text. The construction of a new Institute building is our most important task for the years to come.



Description of the Institute's Activities

Brief history and principles of activity

The International Institute of Molecular and Cell Biology in Warsaw (IIMCB) is one of the most modern country's research institutes in its field in Poland holding the A+ category resulting from the parametric evaluation of research entities in Poland by the Ministry of Science and Higher Education. Created with the support of the Polish Government, Polish Academy of Sciences (PAN) and UNESCO, the Institute started its activity on January 1, 1999, based on a separate parliamentary bill. Research topics at IIMCB cover the wide area of cancer biology, neurobiology, protein structural biology, intracellular communication, dendritic tree formation, bioinformatics/computer modeling, mitochondrial biogenesis, experimental embryology and developmental biology, etc.

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 are distinct and differ from other research institutes in the country: an important body of the Institute is the International Advisory Board, acting as the Scientific Council; the leader of each research team of the Institute is selected in an international competition; group leader's initial employment is limited to a five-year contract; three years after beginning the research work, progress of research is assessed by the International Advisory Board. There are no permanent scientific positions at the Institute, however a successful lab leader after seven years can be promoted to a senior position and fall into a rolling-tenure mechanism of employment. According to the IIMCB bylaws, the lab leader position constitutes an equivalent of a professorial position of other Polish research institutes.

The Institute is partly financed from the state budget (statutory subvention from Ministry of Science and Higher Education, budgetary subvention from Polish Academy of Science) and through numerous grants founded by Framework Programs of EU, Max Planck Society, Howard Hughes Medical Institute, European Molecular Biology Organisation, National Institutes of Health, Wellcome Trust, Polish-Norwegian Research Fund, Fogarty International Research Collaboration Center, Ministry of Science and Higher Education, National Science Centre, National Centre for Research and Development, Foundation for Polish Science, Structural Funds, etc.

More than 80% of funds arrive as competitive grant awards received by the group leaders. IIMCB is involved in various educational programs as well as popularization activities performed by the Centre for Innovative Bioscience Education. To explore commercialization opportunities IIMCB also develops cooperation with some industrial partners with BioTech IP as a special unit (page 67).

The current building, designed primarily as an office building and not as a laboratory facility, was made available to us in mid-1990s by the Polish Academy of Sciences (PAN). Numerous modifications have resulted in its present appearance and functionality and turned it into a fairly modern research institute. Today, our building offers in total about 4,000 m^2 of floor space on eight levels. It can accommodate up to nine research groups with full research equipment and core facilities including a zebrafish facility.

The space occupied by IIMCB is not sufficient for the proper functioning of the Institute in its present form, and it is a major limiting factor as it prevents the establishment of new research groups and largely halts the development of research equipment of the Institute. Consequently, it thwarts the innovative potential of activities undertaken. Due to the difficult housing conditions, a number of scientific instruments are located sub-optimally, which to some extent limits proper usage of equipment.

Relation of IIMCB to Polish Academy of Sciences

The International Institute of Molecular and Cell Biology is directly subordinate to the President of the Polish Academy of Sciences (PAN), who supervises the organization and activities of the Institute. The President of PAN nominates members of International Advisory Board (IAB) and the Institute's Directors. The IIMCB uses a building loaned to it by the Polish Academy of Sciences. PAN also played a crucial role as a party to the agreement with the Max Planck Society which made it possible to organize international laboratories on both sides.

The organization of research at IIMCB

Nine research groups comprise the structure of IIMCB: Bioinformatics and Protein Engineering Laboratory (Bujnicki), Cell Biology Laboratory (Miączyńska), Mitochondrial Biogenesis Laboratory (Chacińska), Molecular Biology Department (Żylicz), Molecular and Cellular Neurobiology Laboratory (Jaworski), Neurodegeneration Laboratory (Kuźnicki), Protein Structure Laboratory (Nowotny), Structural Biology Laboratory (Bochtler), and Zebrafish Developmental Genomics Laboratory (Winata) which will start activity in June 2014.

The research carried out at IIMCB is mainly focused on fundamental biomedical problems. The major research topics include the following:

1. Experimental and theoretical studies on structures of RNAs and proteins, and on protein-nucleic acid interactions, from the development of computer software, to comparative sequence analyses and molecular modeling, to biochemical analyses and protein engineering of enzymes that act on nucleic acids, to experimental structural biology (Bujnicki group).
2. Interdependence between endocytic transport, intracellular signal transduction and transcriptional regulation (Miączyńska group).
3. Biogenesis of mitochondrial proteins, cellular protein homeostasis, protein transport mechanisms, redox processes in mitochondria (Chacińska group).
4. Role of molecular chaperones in cell transformation, including analysis of interactions between human wildtype and mutant p53 with molecular chaperones and oncogenic activity of MDM2 (Żylicz group).
5. Molecular processes, including gene transcription, kinase dependent cell signaling, cytoskeleton dynamics, intracellular trafficking that underlie mTOR kinase-dependent neuronal development and plasticity, and central nervous

system pathologies (e.g., tuberous sclerosis, epilepsy, and neurodegenerative disorders) (Jaworski group).
6. Studies of calcium and β-catenin signaling in the brain and molecular mechanisms of neurodegeneration (Kuźnicki group).
7. Structural and biochemical studies of nucleic acid enzymes (Nowotny group).
8. Structural and biochemical studies of DNA methylation and hydroxymethylation (Bochtler group).
9. Complex transcriptional regulatory mechanism of embryonic cardiac development *in vivo*. (Winata group).

Awards and Honors

- **Marcin Nowotny** received the **Knight's Cross of the Order of Polonia Restituta** from the President of the Republic of Poland, for outstanding achievements in scientific research work and for the promotion of Polish scientific thought.
- **Marcin Nowotny** received an **Award of the Prime Minister** for outstanding achievements in research.
- **Jacek Kuźnicki** was awarded the **"Kryształowa Brukselka"** ("Crystal Brussels Sprout") prize of the Polish National Contact Point for Research Programmes of the European Union (KPK) for leadership in implementing and promoting European Projects.
- **Urszula Wojda**, **Marta Miączyńska** and **Jacek Jaworski** received **professorial titles** from the President of the Republic of Poland .
- **Janusz M. Bujnicki** shared the **award of the Polish Genetics Society** as a coauthor of the best Polish genetics-related publication in 2012
- **Marta B. Wiśniewska, Katarzyna Misztal, Andrzej Nagalski and Jacek Kuźnicki** received an **Award of the Division of Biological and Agricultural Sciences of the Polish Academy of Sciences** for a series of research papers focusing on β-catenin as a factor that influences the excitability of thalamic neurons by regulating gene expression.
- **Małgorzata Urbańska** from the Laboratory of Molecular and Cellular Neurobiology received the **L'Oreal Award for Women in Science** for women PhD students under 35 years of age conducting research in the fields of biology, biochemistry, biotechnology, agriculture, medicine, pharmacy and/or physiology.
- **Małgorzata Mossakowska,** Prof. Andrzej Więcek and Prof. Piotr Błędowski received the **Award of the Rector of the Warsaw School of Economics** for editing the monograph "Medical, psychological, sociological and economic aspects of ageing in Poland", published within the PolSenior project coordinated by IIMCB.
- **Katarzyna Kamińska** of the Laboratory of Bioinformatics and Protein Engineering and **Agnieszka Banrowska** of the Technology Transfer Unit (BioTech-IP) are laureates of the third edition of the **Top 500 Innovators - Science Management Commercialization Program,** funded by the Ministry of Science and Higher Education.
- **Janusz M. Bujnicki** is the winner in the Science category of the **"Poles with verve"** ("Polacy z werwą") national plebiscite initiated by PKN Orlen, which aims to promote Poland's most talented people and their achievements.

Cooperation with Other Institutions

Domestic Cooperation

Intercollegiate Faculty of Biotechnology

The partnership is based on a consortium agreement with the Intercollegiate Faculty of Biotechnology at the University of Gdańsk/ Medical University of Gdańsk (IFB UG-MUG), our strategic Polish Road Map Partner and one of the best academic biotechnology units in Poland. The partnership allows us to further increase our effectiveness in basic and translational research. IFB UG-MUG is ranked very high among scientific institutions (2nd place in biology in 2009, 3rd place in life sciences in 2013) and holds "excellence" status for educational achievements from the Ministry of Science and Higher Education. Thanks to this partnership, IIMCB is recognized as a strong academic unit with a superior ability to mentor top research work, run regular courses for students at higher levels of education, and admit PhD theses to be defended in a university environment. In bilateral cooperation, IIMCB performs activities that lead to improvements in PhD curricula through the Life Sciences and Mathematics Interdisciplinary Doctoral Studies (LiSMiDoS) PhD school and innovative research in the field of systems biology through the establishment of a new joint laboratory. This cooperation is also very promising in the field of medical biology and molecular diagnostics.

Museum and Institute of Zoology PAN (MIZ)

An agreement was reached to set up a joint research group between MIZ and IIMCB, centered on the sequencing capabilities (PacBio) acquired by MIZ where the laboratory will be located. The greatest overlap in the scientific interests of both institutes is seen in the field of modern sequencing, and members of IIMCB and MIZ expressed an interest in acquiring a group leader with expertise in this area. It was also agreed that the new group leader should have his or

her own scientific program, rather than just being a "high level" technician for genomic projects of interest to the two institutes. Possible areas of interest could be *de novo* sequencing and assembly, RNAseq, CHIPseq, and the sequencing of modified or ancient DNA. An international competition for a joint group leader is currently on-going.

Biocentrum Ochota (www.biocentrumochota.gov.pl)

In January 2008, the scientific activities of the Biocentrum Ochota Consortium of the Polish Academy of Sciences were launched at the initiative of six research institutes that operate at the Ochota Campus in Warsaw. The founders and members of the Consortium are the following:

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

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

The EU funds obtained by Biocentrum Ochota are used both for research projects and to expand the team of researchers. The scientists from the member institutions of Biocentrum Ochota are specialists who are recognized nationally and internationally as experts in their fields.

Cooperation with companies

IIMCB actively collaborates with several **pharmaceutical and biotechnology companies** (Adamed, Polfa-Tarchomin, Celon Pharma, Kucharczyk Biovectis, Fermentas) to develop new therapies in neurology and oncology. The Technology Transfer Unit supports scientists in their work on applicable R&D projects. IIMCB was instrumental in establishing a **spin-off company** Proteon Pharmaceuticals Ltd. Creation of the second company based on the ERC Proof of Concept grant currently is being negotiated.

IIMCB actively supports **social initiatives** serving groups of patients with particular diseases. It fostered two **patient support organizations**: Polish Association Supporting People with Inflammatory Bowel Disease "J-elita" (since 2005) and Polish Ciliary Dyskinesia Society "Cilium" (since 2011).

International Cooperation

Max Planck Society

First cooperation programme

The cooperation started in 2001 as an initiative of the Max Planck Society (MPG) and Polish Academy of Sciences (PAN).

According to the agreement, the Junior Research Group, with **Dr. Matthias Bochtler** as Lab Leader, was funded by MPG and hosted at IIMCB. The lab has been active in the structural biology of peptidases, proteases and protein degradation. The group has also been first to publish the structures of several new peptidase clans, and, in studies on the staphopain-staphostatin system, has discovered a novel cysteine peptidase inhibitor mechanism. Furthermore the group has focused on eukaryotic protein degradation system. Dr. Bochtler's laboratory got furnished with the modern protein crystallography equipment including a high brilliance rotating anode generator (RU-H3RHB from MSC), Max-Flux confocal optical mirrors, a MAR345 low noise Xray detector and a cryosystem. At that time this was the most advanced equipment of this kind in Poland (currently replaced with an even more advanced version). Due to its uniqueness, this equipment served and still serves other members of the scientific community interested in protein crystallography analysis. Dr. Bochtler's term at IIMCB under MPG funding lasted a full nine years and became a great scientific success. In period 2007-2011 he was a part-time Director of Structural Biology of Cardiff University (United Kingdom) and a Lab Leader in IIMCB. After this four years period he started a cross-appointment at IIMCB and the Institute of Biochemistry and Biophysics PAN in Warsaw as a full professor.

The Laboratory of Cell Cortex Mechanics MPG/PAN, headed by **Dr. Ewa Paluch** and a twin laboratory of Matthias Bochtler's MPG/PAN laboratory, was established in February 2006. The equipment and running costs of the laboratory, including personnel, were covered by Polish funds, but the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (MPI-CBG; a host for this laboratory) was responsible for local operational costs, maintenance, and administrative support. Dr. Ewa Paluch's group focused on the biochemical and physical mechanisms of cell shape and deformations. The research was funded mainly by the Polish Ministry of Science and Education and concentrated on movements of the actomyosin cortex and, in particular, the involvement of spontaneous cortical ruptures and flows in cell division. The group's most spectacular achievements to date include a paper published in *Nature* and a recently funded ERC grant. In 2013, Dr. Paluch relocated her research activities to University College London under an arrangement whereby she formally remained an IIMCB employee on a leave of absence for the duration of the ERC project and retained the use of part of our research equipment, which allowed her research at the new location to commence without undue delay.

Second cooperation programme

In March 2012, a new cooperation agreement was signed between IIMCB and MPG. The agreement concerned the establishment of two Max Planck/IIMCB Research Groups, one at IIMCB and the other at the Max-Planck Institute of Heart and

Lung Research (MPI-HLR) in Bad Nauheim. Each of the parties will finance a research group with its own budget.

The lab leader position at Bad Nauheim was filled by **Dr. Michael Potente** who started MaxPlanck/IIMCB Angiogenesis and Metabolism Laboratory, which constitutes the Independent Research Group at MPI-HLR. Dr. Potente was trained as a MD at the Universities of Frankfurt and Toronto with a major focus on cardiovascular physiology and medicine. I addition to building his scientific career, Dr. Potente has consistently continued with his clinical specialty training in cardiology. His research program is devoted to the molecular analysis of transcriptional regulatory circuits that govern the growth, maintenance and regression of blood vessels. He has focused on the analysis of Notch signaling and FOXO transcription factors, two pivotal transcriptional regulators of vascular growth and homeostasis, as well as their regulation by reversible acetylation. He explores specifically the function of sirtuins, which are NAD^+ – dependent deacetylases, for the dynamic regulation and adaptation of endothelial cell responses. Using conditional mouse mutants and *in vivo* models of vessels formation, combined with high-resolution imaging and state-of-the-art proteomics and genomics, his research aims to delineate novel regulatory pathways and mechanisms that control vascular growth and function in development, physiology and disease. Dr. Potente is a coauthor of many important papers e.g. in *Nature, Cell, J Clin Invest, PNAS, Dev Cell, J Biol Chem*.

A competition for the position in Warsaw has been recently completed, resulting in the appointment of **Dr. Cecilia Winata**, who will run the Zebrafish Developmental Genomics Laboratory, which is dedicated to the study of developmental processes of the heart by applying genomics methods in combination with experimental embryology and biochemistry. Winata's group will focus on transcriptional regulatory network of heart development and on epigenome profile of heart development. The group will base mainly on agenomics approach. This is supposed to be the first research laboratory in Poland which, together with an extensive experience of the Zebrafish Core Facility, is going to display top expertise in experimental studies on zebrafish model. The group is also affiliated with MPI-HLR in Bad Nauheim, our strategic partner for the creation and development of the FishMed Centre. The laboratory will thus have full access to MPI-HLR equipment, animal facilities, and genetic modification techniques for zebrafish and mice (for details see page 58-61).

Institute of Molecular Biology and Genetics, Kiev, Ukraine

For many years, IIMCB has collaborated with the Institute of Molecular Biology and Genetics (IMBG) in Kiev, Ukraine. Regular meetings of scientists from both institutions originated at the Polish-Ukrainian Parnas Conferences. This intense cooperation evolved into a common application for EU funding, resulting in shared participation in the COMBIOM project entitled, "Strengthening Cooperation in Molecular Biomedicine between EU and Ukraine" (01.12.2011-30.11.2014), supported by FP7 INCO, an ERA-WIDE activity. In addition to IMBG (coordinator) and IIMCB, COMBIOM involves a third partner, Institute Gustave-Roussy (IGR) from France. The role of IIMCB is to support IMBG with activities such as twinning with Ukrainian researchers in the development of the IMBG Biomed Research Strategy, soft skills workshops, and managerial training. All of this serves the overall COMBIOM aim of strengthening IMBG scientific and institutional potential and its future integration into the European Research Area.

Inspired by IIMCB experience, IMBG created an International Advisory Board (IAB) to support the development of the IMBG Strategy on Biomedicine for its implementation at the end of the project. IAB consists of internationally recognized scientists who are experienced in project and institutional management. The members from IIMCB are Prof. Jacek Kuznicki and Prof. Alicja Żylicz. Two annual IAB meetings resulted in a SWOT analysis and constructive recommendations on necessary improvements in spheres such as institutional organization and management, training for young scientists, and an internal support system for domestic and international grant competitions.

To date, IIMCB has organized two weekly practical workshops for IMBG young scientists (on Scientific Communication and Research Integrity and Responsible Conduct of Research) and two study visits of IMBG administrative staff.

A new initiative has been recently developed: Call for Young IMBG Scientists for the implementation of yearly research grants. The purpose was to provide young Ukrainian fellows the opportunity to develop their own ideas and experience research independence in cooperation with a European partner. To finance this initiative, IIMCB dedicated its own resources to cover the research portion of the winning grants, and the COMBIOM budget supported travel and networking activities. Approved by IAB Members, the call was announced, and two laureates were selected: Dr. Mykola Dergai and Dr. Aleksy Avdieiev.

‘HR Excellence in Research’ logo and Lab Leader Competitions

The International Institute of Molecular and Cell Biology in Warsaw received the prestigious ‘HR Excellence in Research’ logo for the implementation of the provisions of the European Charter for Researchers and the Code of Conduct for the Recruitment of Researchers. By implementing the Charter and Code, the IIMCB fosters international collaboration and contributes to the development of an open and attractive European labour market for researchers. Detailed scrutiny of European standards, analysis of Institute’s practices vis-a-vis the European ones, identification and elimination of shortcomings allow the IIMCB to upgrade and strengthen its recruitment practices and employment conditions.

The European Charter for Researchers and the Code of Conduct, adopted by the European Commission in 2005, specify the role, rights and duties of researchers, their employees and funding agencies. Until now, 155 research institutions from 23 European countries have been honored with the ‘HR Excellence in Research’ logo, which identifies particularly attractive work environments. The IIMCB is the third institution in Poland which has the right to use the ‘HR Excellence in Research’ logo. The two other institutions are the Foundation for Polish Science and the Nencki Institute of Experimental Biology.

In line with the above mentioned rules, international competitions for lab leaders’ positions are considered at IIMCB as an essential mechanism for ensuring proper intake of talented young researchers to the Institute. This procedure is mandatory, unquestionably leading to a continuous improvement in IIMCB scientific standards and enhancing the sense of integrity and democracy among employees.

As a rule, every Lab Leader competition is advertised in internationally visible media (NatureJobs, Euraxess, IIMCB web page). The applicants are initially screened formally at the Institute. Later, they get evaluated by the Selection Committee made up of several members of the International Advisory Board (IAB). Shortlisted candidates with the highest scores receive invitations to give a presentation in a symposium run publicly with the participation of IAB members. The final recommendation is made by IAB and passed to the Director, who comes with the binding decision. We believe that the strict selection criteria and an objective and completely fact-based recruitment process of lab leaders are key to the success of an institution such as IIMCB. This is the starting point for dynamic growth, the opening of new lines of research and introduction of modern technology at the Institute. The recruitment process makes it possible to hire the most talented researchers – and by providing them with appropriate conditions for development, IIMCB often becomes their first step to independent, international scientific careers.

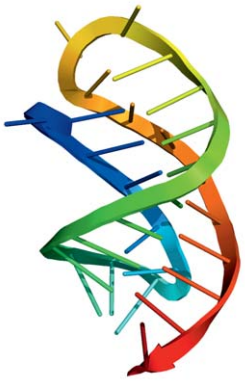
Competition	Year	Number of candidates	**Winners employed at IIMCB**
I	1998	6	**Jarosław Dastych**
II	1999	3	**Maciej Żylicz**
III	2000	6	**Michał Hetman**
IV[1)]	2000	7	**Matthias Bochtler, Leszek Rychlewski**
V	2002	9	**Janusz M. Bujnicki, Sławomir Filipek**
VI[2)]	2002	9	–
VII	2003	18	**Marta Miączyńska**
VIII[3)]	2004	26	–
IX	2005	26	**Jacek Jaworski**
X[1)]	2005	17	**Ewa Paluch**
XI	2006	25	**Marcin Nowotny**
XII[3)]	2007	16	–
XIII	2008	14	**Agnieszka Chacińska**
XIV[2)]	2010	20	–
XV[3,4)]	2012	18 & 15	–
XVI[4)]	2013	14	**Cecilia Lanny Winata**

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

Important Dates in the Institute's History

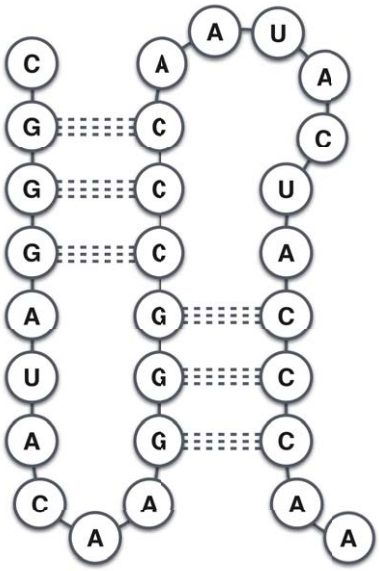
Sep. 1991	The proposal to create the Institute was published in the UNESCO Bulletin of MCBN
June 1994	State Committee for Scientific Research (KBN) accepts the activities aimed at establishing the Institute
Oct. 1994	Presidium of Polish Academy of Sciences (PAN) votes to support the Institute
May 1995	An agreement between Poland and UNESCO to establish the Institute
June 1996	The Molecular and Cell Biology Department is created by PAN with Prof. M.J. Nałęcz as the Head
June 1997	Polish Parliament passes a bill to found the Institute
May 1998	Prof. A. Azzi is nominated as the Director of IIMCB
Jan. 1999	The Institute commences its independent activities; Prof. J. Kuźnicki appointed as Acting Director
July 1999	Dr. J. Dastych is appointed as a first Lab Leader at IIMCB
Oct. 1999	Prof. M. Żylicz is appointed as Chair of the Department of Molecular Biology
April 2000	An agreement between the Max Planck Society (MPG) and the Polish Academy of Sciences (PAN) to launch a Joint MPG-PAN Junior Research Group
Jan. 2001	The MPG-PAN Junior Research Group commences its activities with Dr. M. Bochtler as a Lab Leader
June 2001	Prof. J. Kuźnicki is elected by the International Advisory Board as Director of the Institute for the term 2002-2006 (further nominations for terms: 2006-2010, 2010-2014, 2014-2018)
Jan. 2003	Status of the Centre of Excellence in Molecular Bio-Medicine is granted by the European Commission within 5th Framework Programme
Feb. 2006	Twin MPG-PAN laboratory established at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden Independent Junior Research Group with Dr. Ewa Paluch as a Lab Leader
Sep. 2010	IIMCB ranked first among 36 institutions in the section of Biological Sciences, and classified into category 1, according to the 2005-2009 parametric evaluation of Polish research institutions by Ministry of Science and Higher Education
March 2012	Agreement between Max Planck Society (MPG) and IIMCB on continuation of of MPG/IIMCB Research Groups
April 2013	Introduction of zebrafish as an animal model for research
Oct. 2013	Receipt of the A+ category by IIMCB within the parametric evaluation of Polish academic institutions

Research



Structure of a pseudoknotted RNA 28-mer designed in silico

Bioinformatics and Protein Engineering Laboratory



Postdoctoral Fellows and Research Associates (as of January 2014):
Piotr Bentkowski, PhD; Michał Boniecki, PhD; Grzegorz Chojnowski, PhD; Justyna Czarnecka, PhD; Stanisław Dunin-Horkawicz, PhD; Łukasz Kozłowski, PhD; Grzegorz Łach, PhD; Irina Tuszyńska, PhD; Martyna Nowacka, PhD; Elżbieta Purta, PhD; Krzysztof J. Skowronek, PhD (part-time); Agata Sulej, PhD

Junior Researchers (as of January 2014):
Astha, MSc; Magdalena Byszewska, MSc; Ilona Domagała, MSc; Dawid Głow, MSc; Elżbieta Jankowska, MSc; Magdalena Machnicka, MSc; Marcin Magnus, MSc; Dorota Matelska, MSc; Anna Olchowik, MSc; Paweł Piątkowski, MSc; Juliusz Stasiewicz, MSc; Krzysztof Szczepaniak, MSc

Associate researchers, working e.g. extramurally on their individual projects towards full independence (as of January 2014):
Joanna Kasprzak, PhD
Katarzyna Kamińska, MSc
Bogusław Kluge, PhD
Tomasz Waleń, PhD

Research Technicians:
Małgorzata Kurkowska, MSc
Sylwia Panek, MSc

Undergraduate Students:
Katarzyna Grudziąż, MSc

Office Manager:
Agnieszka Faliszewska, MSc

Computer Administrators:
Tomasz Jarzynka (part-time)
Jan Kogut, BSc (part-time)
Łukasz Munio

Lab Leader:
Janusz M. Bujnicki,
PhD, Professor

Degrees

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

Professional Experience

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

Selected professional affiliations

- Academy of Young Scientists, Polish Academy of Sciences, AMU-PAN (elected in 2011)
- Scientific Committee of the Innovative Medicines Initiative, member (selected in 2013)
- Science Europe, Life, Environmental and Geo Sciences (LEGS) Scientific Committee, member (selected in 2013)
- EC Advisory Group on European Research Infrastructures including e-Infrastructures, member (selected in 2013)
- Society of Bioinformatics in Northern Europe (SocBiN) (board member, 2004-Present)
- Member: Polish Bioinformatics Society, PTBI (founding member, Vice-President 2007-2010, President 2011-2013), International Society for Computational Biology, RNA Society,
- Executive Editor, *Nucleic Acids Research* (2013-Present)
- Series Editor, *Nucleic Acids and Molecular Biology* (Springer Verlag, 2009-Present)
- Deputy Section Editor, *BMC Bioinformatics* (2010--Present)
- Editorial Board, *Nucleic Acids Research* (2005-2012), *Journal of Applied Genetics* (2004-Present), *Database Journal* (2008-Present), *Biology Direct* (2013-Present)

Honors, Prizes and Awards

2013	Award in the Science category of the national plebiscite "Poles with Verve"
2013	Award of the Polish Genetics Society (coauthor in the best Polish genetics-related publication in 2012)
2013	Adam Mickiewicz University Rector Special Award for Top Performance in Publishing High Impact Research Articles in 2012
2012	Award for Outstanding Research Achievements, Ministry of Science and Higher Education
2011	Adam Mickiewicz University Rector Special Award for Top Performance in Publishing High Impact Research Articles in 2010
2010	ERC Starting Grant (2011-2015)
2009	Fellowship for Outstanding Young Scientists, Ministry of Science and Higher Education
2009	Award for Research Achievements, Ministry of Science and Higher Education (Individual work)
2008	Adam Mickiewicz University Rector Award for Research Achievements (Individual work)
2006	Award from Prime Minister for habilitation thesis
2006	Young Researcher Award in Structural and Evolutionary Biology, Visegrad Group Academies of Sciences
2003, 2004	Fellowship for Young Scientists, Foundation for Polish Science
2002	EMBO/Howard Hughes Medical Institute Young Investigator Program Award
2002	Award for best Polish genetics-related publication in 2001 (*Trends Biochem Sci* 2001, Jan, 26[1]:9-11), Polish Society of Genetics
2001	Award for best Polish publication on nucleic acid biochemistry in 2000 (*FASEB J* 2000, Nov, 14[14]:2365-2368), Polish Biochemical Society

Doctorates defended under Lab Leader's supervision

Żylicz-Stachula A, Chmiel A, Cymerman I, Czerwoniec A, Gajda M, Pawłowski M, Sasin-Kurowska J, Kosinski J, Obarska-Kosińska A, Pawlak S, Purta E, Tkaczuk K, Kosciński Ł, Rother M, Potrzebowski W, Korneta I, Puton T, Kasprzak J, Tuszyńska I, Kozłowski Ł, Werner M, Kamaszewska A, Philips A, Milanowska K, Piętal M

Selected awards of current group members

- START Fellowships (Foundation of Polish Science): Purta E (2009,2010); Chojnowski G (2011); Dunin-Horkawicz S (2012); Tuszyńska I (2012,2013);
- Fellowship for PhD Students (Marshall of the Masovia Province): Kozłowski Ł (2009); Tuszyńska I (2009, 2011);
- Fellowships for Outstanding Young Scientists (Polish Ministry of Science): Purta E (2011); Dunin-Horkawicz S (2013);
- Award of the Polish Biochemical Society and Sigma-Aldrich (the best PhD thesis in the field of biochemistry 2010); Purta E (2011)

Selected Recent Publications

- **Smietanski M, Werner M, Purta E, Kaminska KH,** Stepinski J, Darzynkiewicz E, Nowotny M, **Bujnicki JM**. Structural analysis of human 2'-O-ribose methyltransferases involved in mRNA cap structure formation. ***Nature Commun***, 2014; 5:3004
- **Tuszynska I, Matelska D, Magnus M, Chojnowski G,** Kasprzak JM, **Kozlowski LP, Dunin-Horkawicz S, Bujnicki JM.** Computational modeling of protein-RNA complex structures. ***Methods***, 2013, Sep 29. pii: S1046-2023(13)00383-6
- Dzananovic E, Patel TR, **Chojnowski G, Boniecki MJ,** Deo S, McEleney K,Harding SE, **Bujnicki JM,** McKenna SA. Solution conformation of Adenovirus Virus Associated RNA-I and its interaction with PKR. ***J Struct Biol***, 2014; 185(1):48-57
- **Chojnowski G, Walen T, Bujnicki JM**. RNA Bricks - a database of RNA 3D motifs and their interactions. ***Nucleic Acids Res***, 2014; 42(1):D123-31
- **Tuszynska I, Matelska D, Magnus M, Chojnowski G,** Kasprzak JM, **Kozlowski L, Dunin-Horkawicz S, Bujnicki JM**. Computational modeling of protein-RNA complex structures. ***Methods***, 2013 Sep 29. doi:pii: S1046-2023(13)00383-6. 10.1016/j.ymeth.2013.09.014
- **Philips A, Milanowska K, Lach G, Bujnicki JM.** LigandRNA: computational predictor of RNA-ligand interactions. ***RNA***, 2013;19(12):1605-16
- **Matelska D, Purta E, Panek S, Boniecki MJ, Bujnicki JM, Dunin-Horkawicz S.** S6:S18 ribosomal protein complex interacts with a structural motif present in its own mRNA. ***RNA***, 2013;19(10):1341-8
- **Pawlowski M, Bogdanowicz A, Bujnicki JM.** QA-RecombineIt: a server for quality assessment and recombination of protein models. ***Nucleic Acids Res***, 2013; 41(Web Server issue):W389-97
- **Bujnicki JM**, Tiuryn J. Bioinformatics and Computational Biology in Poland. ***PLoS Comput Biol***, 2013; 9(5):e1003048
- **Puton T, Kozlowski LP, Rother KM, Bujnicki JM.** CompaRNA: a server for continuous benchmarking of automated methods for RNA secondary structure prediction. ***Nucleic Acids Res***, 2013; 41(7):4307-23
- Nowak E, **Potrzebowski W,** Konarev P, Rausch J, Bona M, Svergun D, **Bujnicki JM,** Le Grice S, Nowotny M. Structural analysis of monomeric retroviral reverse transcriptase in complex with an RNA/DNA hybrid. ***Nucleic Acids Res***, 2013; 41(6):3874-3887
- **Milanowska K**, Mikolajczak K, Lukasik A, Skorupski M, Balcer Z, **Machnicka MA, Nowacka M, Rother KM, Bujnicki JM**. RNApathwaysDB-a database of RNA maturation and decay pathways. ***Nucleic Acids Res***, 2013; 41(Database issue):D268-72
- **Machnicka MA, Milanowska K**, Osman Oglu O, **Purta E, Kurkowska M, Olchowik A, Januszewski W,** Kalinowski S, **Dunin-Horkawicz S, Rother KM,** Helm M, **Bujnicki JM**, Grosjean H. MODOMICS: a database of RNA modification pathways: 2012 update. ***Nucleic Acids Res***, 2013; 41(D1): D262-D267
- **Sulej A, Tuszynska I, Skowronek KJ,** Nowotny M, **Bujnicki JM** Sequence-specific cleavage of the RNA strand in DNA-RNA hybrids by the fusion of ribonuclease H with a zinc finger. ***Nucleic Acids Res***, 2012; 40(22):11563-70
- **Korneta I, Bujnicki JM** Intrinsic disorder in the human spliceosomal proteome. ***PLoS Comput Biol***, 2012; 8(8):e1002641
- **Skowronek KJ, Boniecki M, Kluge B, Bujnicki JM**. Rational engineering of sequence specificity in R.MwoI restriction endonuclease. ***Nucleic Acids Res***, 2012; 40(17):8579-92
- **Poleszak K, Kaminska KH, Dunin-Horkawicz S,** Lupas A, **Skowronek KJ, Bujnicki JM**. Delineation of structural domains and identification of functionally important residues in DNA repair enzyme Exonuclease VII. ***Nucleic Acids Res***, 2012; 40(16):8163-74
- **Siwek W,** Czapinska H, Bochtler M, **Bujnicki JM, Skowronek KJ**. Crystal structure and mechanism of action of the N6-methyladenine dependent type IIM restriction endonuclease R.DpnI. ***Nucleic Acids Res***, 2012; 40(15):7563-72
- **Korneta I, Magnus M, Bujnicki JM**. Structural bioinformatics of the human spliceosomal proteome. ***Nucleic Acids Res***, 2012; 40(15):7046-65
- **Chojnowski G, Bujnicki JM,** Bochtler M. RIBER/DIBER: a software suite for crystal content analysis in the studies of protein-nucleic acid complexes. ***Bioinformatics***, 2012; 28(6):880-1
- **Pietal M**, Szostak N, **Rother KM**, **Bujnicki JM**. RNAmap2D – calculation, visualization and analysis of contact and distance maps for RNA and protein-RNA complex structures. ***BMC Bioinformatics***, 2012; 13(1):333
- **Pawlowski M**, **Bujnicki JM**. The utility of comparative models and their local quality for protein crystal structure determination by Molecular Replacement. ***BMC Bioinformatics***, 2012; 13(1):289
- **Magnus M**, **Pawlowski M**, **Bujnicki JM**. MetaLocGramN: a meta-predictor of protein subcellular localization for Gram-negative bacteria. ***Biochim Biophys Acta***, 2012; 1824(12):1425-33
- **Kozlowski LP**, **Bujnicki JM**. MetaDisorder: a meta-server for the prediction of intrinsic disorder in proteins. ***BMC Bioinformatics***, 2012; 13(1):111
- Cruz JA, Blanchet MF, **Boniecki M**, **Bujnicki JM**, Chen SJ, Cao S, Das R, Ding F, Dokholyan NV, Flores SC, Huang L, Lavender CA, Lisi V, Major F, **Mikolajczak K**, Patel DJ, **Philips A**, Puton T, SantaLucia J, Sijenyi F, Hermann T, Rother K, Rother M, Serganov A, Skorupski M, **Soltysinski T**, Sripakdeevong P, **Tuszynska I**, Weeks KM, Waldsich C, Wildauer M, Leontis NB, Westhof E. RNA-Puzzles: A CASP-like evaluation of RNA three-dimensional structure prediction. ***RNA***, 2012; 18(4):610-625
- Fislage M, Roovers M, **Tuszynska I**, **Bujnicki JM**, Droogmans L, Versées W. Crystal structures of the tRNA:m2G6 methyltransferase Trm14/TrmN from two domains of life. ***Nucleic Acids Res***, 2012; 40(11):5149-61
- Kennaway CK, Taylor JE, Song CF, **Potrzebowski W**, Nicholson W, White JH, Swiderska A, **Obarska-Kosinska A**, Callow P, Cooper LP, Roberts GA, Artero JB, **Bujnicki JM**, Trinick J, Kneale GG, Dryden DTF. Structure and operation of the DNA-translocating Type I DNA restriction enzymes. ***Genes Dev***, 2012; 26(1):92-104
- **Philips A**, **Milanowska K**, **Lach G**, **Boniecki M**, Rother K, **Bujnicki JM**. MetalionRNA: computational predictor of metal-binding sites in RNA structures. ***Bioinformatics***, 2012; 28(2):198-205
- Puton T, **Kozlowski L**, **Tuszynska I**, Rother K, **Bujnicki JM**. Computational methods for prediction of protein-RNA interactions. ***J Struct Biol***, 2012; 179(3):261-268

Co-workers affiliated with IIMCB are given in bold

Description of Current Research

The Bioinformatics and Protein Engineering Laboratory is involved in theoretical and experimental research on sequence-structure-function relationships in proteins, nucleic acids, and macromolecular complexes. Theoretical research involves the development of computer software for the analysis of biological macromolecules. Currently, the focus is on the development of software for the structural prediction and modeling of RNA and RNA-protein complexes. Thus far, we have developed and made publicly available one of the first methods for the automated comparative modeling of RNA (ModeRNA; http://iimcb.genesilico.pl/moderna/), a method for the structure-based prediction of metal ion binding sites (MetalionRNA; http://metalionrna.genesilico.pl/), a method for the structure-based modeling of RNAligand complexes (LigandRNA; http://ligandrna.genesilico.pl/), a statistical potentials for predicting the structure of RNA-protein complexes (DARS-RNP and QUASI-RNP; http://iimcb.genesilico.pl/RNP/), a database of RNA 3D motifs and their interactions (RNA Bricks; http://iimcb.genesilico.pl/rnabricks/), and a server for continuous benchmarking of automated methods for RNA secondary structure prediction (CompaRNA; http://iimcb.genesilico.pl/comparna/).

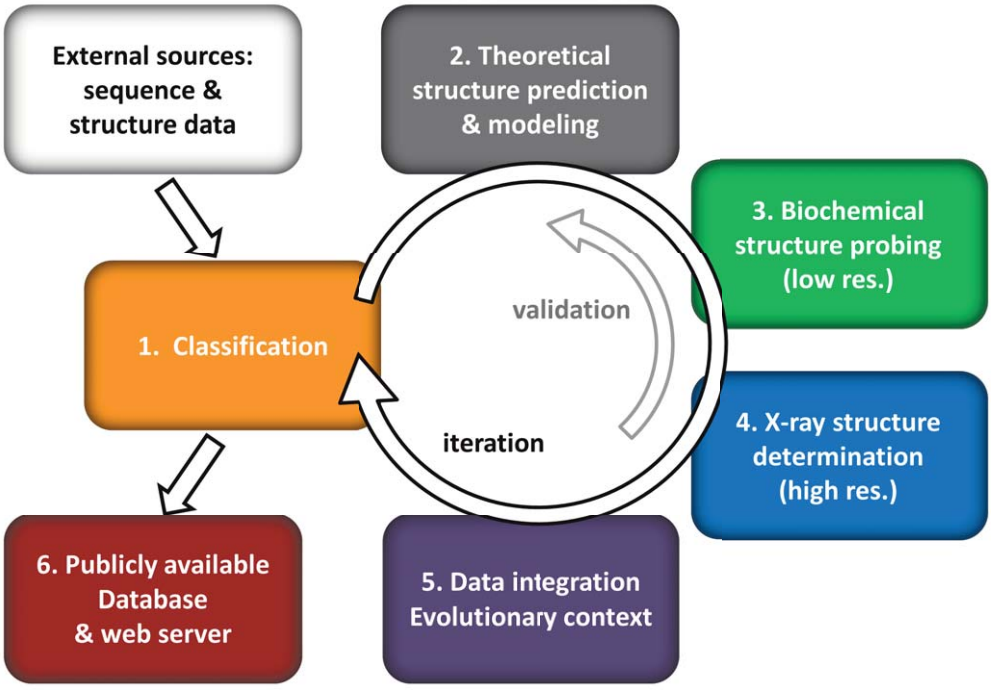
Our suite of programs for protein structure prediction and analysis includes the GeneSilico MetaServer for primary, secondary, and tertiary structure prediction (https://www.genesilico.pl/meta2/), the QA-RecombineIt server for quality assessment and recombination of protein models (http://iimcb.genesilico.pl/qarecombineit/), and a method for the discrimination of models according to their agreement with experimental data (FILTREST3D; http://filtrest3d.genesilico.pl/).

We also developed methods for the prediction of order/disorder in protein structures (http://iimcb.genesilico.pl/metadisorder/) and protein localization in Gram-negative bacterial cells (MetaLocGramN; http://genesilico.pl/MetaLocGramN/). A system of nucleic acid metabolism databases has also been developed. Published elements of this system include a database for the systems biology of RNA modification (MODOMICS; http://modomics.genesilico.pl/), a database for the systems biology of DNA repair (REPAIRtoire; http://repairtoire.genesilico.pl/), and a database of pathways of RNA maturation and decay (RNApathwaysDB; http://genesilico.pl/rnapathwaysdb/).

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

- Experimental testing of functional predictions by gene cloning or nucleic acid synthesis, protein or RNA expression, purification, development of *in vitro* and *in vivo* functional assays, and biochemical and cellular characterization.
- Experimental testing of protein or RNA structural predictions. We apply low-resolution structural probing methods, such as mutagenesis, chemical modification, crosslinking, mass spectrometry, and circular dichroism. We recently also started the crystallization of protein-nucleic acid complexes and structure determination using X-ray crystallography.
- Protein engineering to obtain enzymes with new, useful features, particularly altered substrate specificity (e.g., nucleases that recognize and cut new sequences in DNA or RNA).

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



A flowchart illustrating the main techniques and the direction of information and data flow in a research project focused on RNA structure prediction and determination, funded by MAESTRO grant ("Structural RNomics", 2012/04/A/NZ2/00455) by the National Science Center (NCN) to Prof. Bujnicki.

Recent Highlights

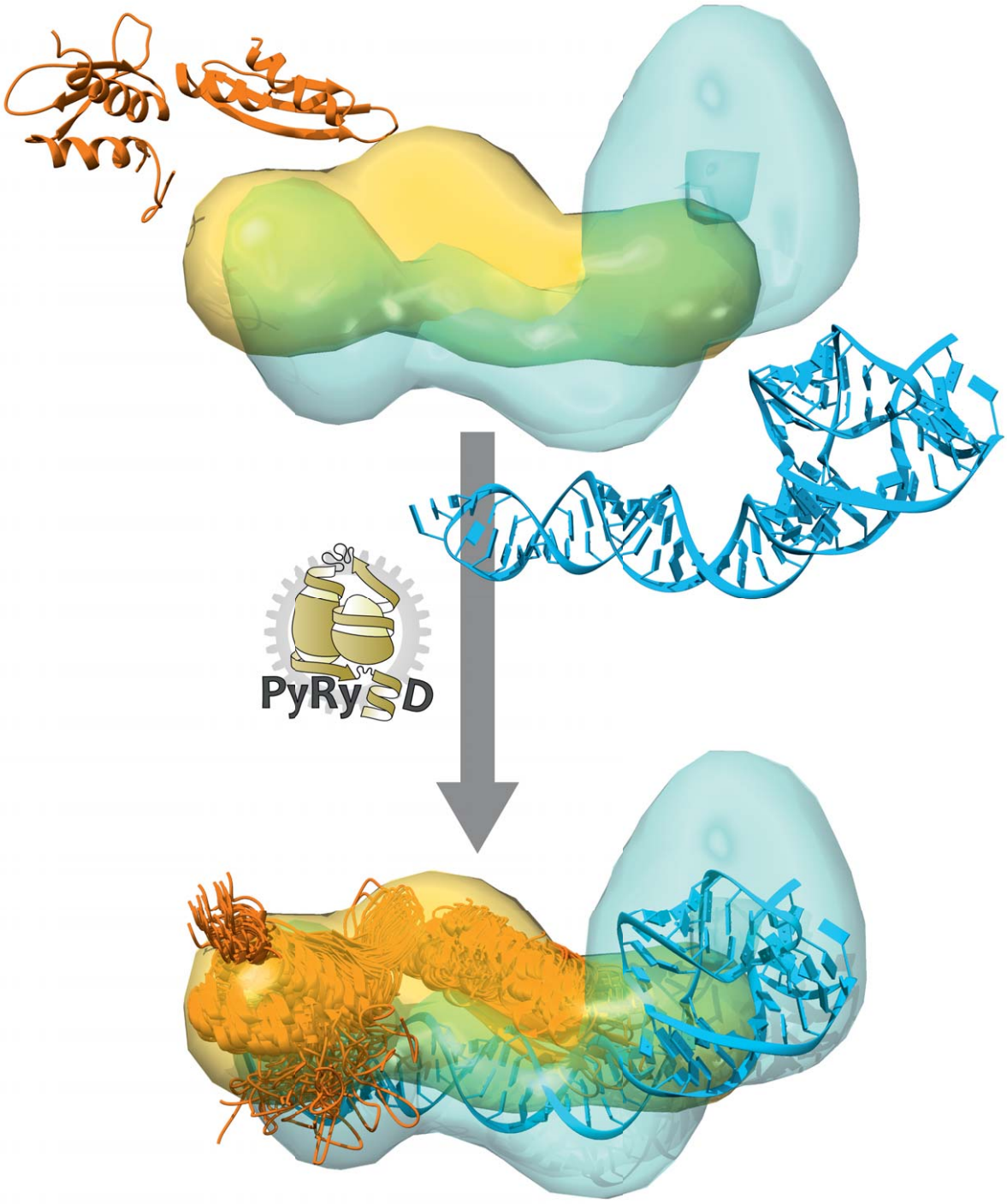
Determination of structure and mechanism of action of human methyltransferases that act on the cap structure in RNA

The 5′ cap of human messenger RNA contains 2′-*O*-methylation of the first and often second transcribed nucleotide that is important for its processing, translation, and stability. These modifications are also present in other RNAs, including snoRNA and snRNA. Human enzymes that methylate these nucleotides, termed cap methyltransferases 1 and 2 (CMTr1 and CMTr2, respectively), have recently been identified by the Bujnicki group (CMTr1, also independently identified by another group). However, the structures of these enzymes and their mechanisms of action remain unknown. Recently, the Bujnicki group, in collaboration with the Nowotny group at IIMCB and Darżynkiewicz group at University of Warsaw, determined the spatial structures and mechanism of action of CMTr1 and CMTr2. They first used X-ray crystallography to determine the atomic details of a complex formed by an enzymatically active catalytic domain of CMTr1 with a chemically synthetized analog of a "capped" RNA (comprising an N7-methylguanosine [m7G] linked via an inverted 5′-5′triphosphate bridge to the 5′-terminus of a tetranucleotide 5′-GAUC-3′]. They then used the crystallographically determined structure of the CMTr1-RNA complex as a template to computationally model an analogous complex formed by CMTr2 with an RNA that was a product of a reaction made by CMTr1. They also performed biochemical experiments on CMTr1 and CMTr2 to confirm the importance of individual amino acid residues predicted to be responsible for the recognition of RNA based on the crystallographic and theoretical models of molecular structures. Comparisons between the human and viral enzymes revealed that human and viral methyltransferases share extensive similarity in the active sites but differ completely in the way they recognize the m^7G moiety. The combination of different types of data explained why human cap methyltransferases are relatively insensitive to the presence of a methyl group at m^7G, which makes them different from viral enzymes. The new structures suggest how the cap analogs could be modified to make them block the viral enzymes but not the human enzymes, which may help in the development of new antiviral drugs.

Development of computational methods for structural analyses of RNA molecules and RNA-protein complexes

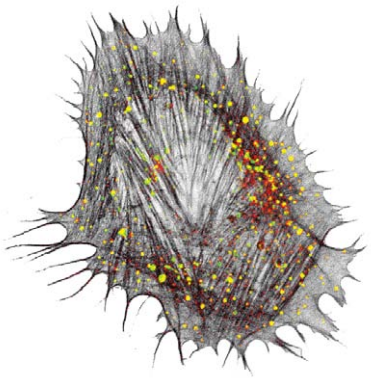
Importantly, the work on CMTr1 and CMTr2 methyltransferases performed by Bujnicki and coworkers would not have been possible without previous contributions made by the Bujnicki laboratory in the area of computational methods for modeling of RNA structure and the structure of protein-RNA complexes. They developed one of the first methods for comparative RNA modeling, ModeRNA, and an innovative method for modeling protein-RNA interactions and complexes. These methods have been used to predict the CMTr1 structure and subsequently model the structure of the CMTr2-RNA complex.

In the area of computational methods development, one of the most recent achievements of the Bujnicki group is the RNA Bricks database (http://iimcb.genesilico.pl/rnabricks). It stores information about recurrent RNA 3D motifs and their interactions that are found in experimentally determined RNA structures and RNA-protein complexes. In contrast to other similar tools (RNA 3D Motif Atlas, RNA Frabase, Rloom), RNA motifs (i.e. "RNA bricks") are presented in the molecular environment where they were determined, including RNA, protein, metal ions, water molecules, and ligands. All of the nucleotide residues in RNA bricks are annotated with structural quality scores that describe real-space correlation coefficients with the electron density data (if available), backbone geometry, and possible steric conflicts, which can be used to identify poorly modeled residues. The database is also equipped with an algorithm for 3D motif searches and comparisons. The algorithm compares the spatial positions of backbone atoms of the user-provided query structure and stored RNA motifs, without relying on sequence or secondary structure information. This enables the identification of local structural similarities among evolutionarily related and unrelated RNA molecules. Additionally, the search utility enables searching "RNA bricks" according to sequence similarity and allows the identification of motifs with modified ribonucleotide residues at specific positions.



Structural model of VAIΔTS RNA structure complexed with two RBD domains of the PKR protein, based on small angle X-ray scattering data, obtained with the PyRy3D program developed in prof. Bujnicki's group (Dzananovic et al., J Struct Biol. 2014 185(1):48-57., published electronically on 28 Nov 2013).

Cell Biology Laboratory



Human fibroblast that overexpresses constitutively active Cdc42 protein. Actin cytoskeleton and filopodia are visualized by staining with phalloidin (black). Colocalization between platelet-derived growth factor receptor (red) and early endosome antigen 1 (green) on endosomal vesicles is indicated in yellow. Author: Kamil Jastrzębski

Postdoctoral Fellows:
Magdalena Banach-Orłowska, PhD (part-time since April 2013; until Nov. 2013)
Anna Bartosik, PhD (since Feb. 2013), FishMed
Jarosław Cendrowski, PhD (since June 2013)
Beata Pyrzyńska, PhD (part-time since April 2013)
Ewelina Szymańska, PhD

Junior Researchers:
Kamil Jastrzębski, MSc
Agnieszka Mamińska, MSc
Sam D. Stephen, MSc
Anna Toruń, MSc

FishMed Technical Assistant:
Lidia Wolińska-Nizioł, MSc (joint with Molecular and Cellular Neurobiology Laboratory)

Volunteer:
Noga Budick-Harmelin, PhD (since Nov. 2013)

Undergraduate Student:
Rafał Sejdak, Eng

Grant Administrator and Lab Manager:
Izabela Zacharek, MSc, MSM (joint with Structural Biology Laboratory; until Feb. 2014)

Technicians:
Monika Matuszczyk
Alina Zielińska

Lab Leader:
Marta Miączyńska,
PhD, Professor

Degrees

2013	Professor of Biological Sciences, nomination by the President of the Republic of Poland
2008	DSc Habil in Cell Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1997	PhD in Genetics, University of Vienna, Austria
1993	MSc in Molecular Biology, Jagiellonian University, Cracow, Poland
1991	BSc in Biological Sciences, University of Wolverhampton, UK

Research Training

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

Fellowships and Awards

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

Selected Recent Publications

- **Miaczynska M.** Effects of membrane trafficking on signaling by receptor tyrosine kinases. (Review) Cold Spring Harb Perspect Biol, 2013; 5:a009035
- **Sadowski Ł, Jastrzębski K,** Kalaidzidis Y, Heldin CH, Hellberg C, **Miaczynska M.** Dynamin Inhibitors Impair Endocytosis and Mitogenic Signaling of PDGF. Traffic, 2013; 14:725-36
- **Pyrzynska B, Banach-Orlowska M, Teperek-Tkacz M**, Miekus K, Drabik G, Majka M, **Miaczynska M**. Multifunctional protein APPL2 contributes to survival of human glioma cells. Mol Oncol, 2013; 7:67-84
- Winiarska M, Nowis D, Bil J, Glodkowska-Mrowka E, Muchowicz A, Wanczyk M, Bojarczuk K, Dwojak M, Firczuk M, Wilczek E, Wachowska M, Roszczenko K, **Miaczynska M**, Chlebowska J, Basak GW, Golab J. Prenyltransferases Regulate CD20 Protein Levels and Influence Anti-CD20 Monoclonal Antibody-mediated Activation of Complement-dependent Cytotoxicity. J Biol Chem, 2012; 287:31983-93
- Zerrouqi A, **Pyrzynska B**, Febbraio M, Brat DJ, Van Meir EG. p14ARF inhibits human glioblastoma-induced angiogenesis by upregulating the expression of TIMP3. J Clin Invest, 2012; 122:1283-95
- **Hupalowska A, Pyrzynska B, Miaczynska M**. APPL1 regulates basal NF-κB activity by stabilizing NIK. J Cell Sci, 2012; 125: 4090-102
- **Hupalowska A**, **Miaczynska M**. The new faces of endocytosis in signaling. (Review) Traffic, 2012; 13:9-18
- **Urbanska A**, **Sadowski L**, Kalaidzidis Y, **Miaczynska M**. Biochemical Characterization of APPL Endosomes: The Role of Annexin A2 in APPL Membrane Recruitment. Traffic, 2011; 12:1227-41
- **Pilecka I**, **Sadowski L**, Kalaidzidis Y, **Miaczynska M**. Recruitment of APPL1 to ubiquitin-rich aggresomes in response to proteasomal impairment. Exp Cell Res, 2011; 317:1093-107
- **Miaczynska M**, Bar-Sagi D. Signaling endosomes: seeing is believing. (Review) Curr Opin Cell Biol, 2010; 22:535-540
- **Banach-Orlowska M, Pilecka I, Torun A, Pyrzynska B, Miaczynska M**. Functional characterization of the interactions between endosomal adaptor protein APPL1 and the NuRD co-repressor complex. Biochem J, 2009; 423:389–400
- **Pyrzynska B, Pilecka I, Miaczynska M**. Endocytic proteins in the regulation of nuclear signaling, transcription and tumorigenesis. (Review) Mol Oncol, 2009; 3: 321-338
- **Rashid S, Pilecka I, Torun A, Olchowik M, Bielinska B, Miaczynska M**. Endosomal Adaptor Proteins APPL1 and APPL2 Are Novel Activators of beta-Catenin/TCF-mediated Transcription. J Biol Chem, 2009; 284:18115-28
- **Sadowski L, Pilecka I, Miaczynska M**. Signaling from endosomes: Location makes a difference. (Review) Exp Cell Res, 2009; 315:1601-09
- *Ohya T, **Miaczynska M**, Coskun U, Lommer B, Runge A, Drechsel D, Kalaidzidis Y, Zerial M. Reconstitution of Rab and SNARE-dependent membrane fusion by synthetic endosomes. Nature, 2009; 459:1091-97
- **Miaczynska M**, Stenmark H. Mechanisms and functions of endocytosis. J Cell Biol, 2008; 80:7-11
- **Pilecka I, Banach-Orlowska M, Miaczynska M**. Nuclear functions of endocytic proteins. Eur J Cell Biol, 2007; 86:533-547
- *Mace G, **Miaczynska M**, Zerial M, Nebreda AR. Phosphorylation of EEA1 by p38 MAP kinase regulates μ opioid receptor endocytosis. EMBO J, 2005; 24:3235-46
- ***Miaczynska M**, Pelkmans L, Zerial M. Not just a sink: endosomes in control of signal transduction. (Review) Curr Opin Cell Biol, 2004; 16:400-406
- ***Miaczynska M**, Christoforidis S, Giner A, Shevchenko A, Uttenweiler-Joseph S, Habermann B, Wilm M, Parton RG, Zerial M. APPL proteins link Rab5 to signal transduction via an endosomal compartment. Cell, 2004; 116:445-56

* no IIMCB affiliation

Description of Current Research

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

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

II. Involvement of endocytic proteins in the regulation of intracellular signaling and transcription.

The intracellular compartmentalization of signal transduction processes may play an important role in modulating the overall cellular response. Endocytosis was first viewed simply as a mechanism of signal termination by the downregulation and degradation of surface receptors. However, more recent data strongly argue that endosomal compartments and their resident proteins play an important role in transmitting intracellular signals by transporting ligand-receptor complexes and affecting their activity inside the cell (Hupalowska and Miaczynska, *Traffic*, 2012; Miaczynska, *Cold Spring Harbor Perspectives in Biology*, 2013; Miaczynska and Bar-Sagi, *Current Opinion in Cell Biology*, 2010). The proposal that endosomes serve as signaling compartments, which was initially postulated in the mid-1990s, has gained increasing experimental support in recent years (Sadowski et al., *Experimental Cell Research*, 2009). Moreover, the relaying of signals from the plasma membrane via endosomes to the nucleus requires signal mediators to be transported between different cellular locations. Intriguingly, a growing number of clathrin adaptors and endosomal proteins are reported to undergo nucleocytoplasmic shuttling. Endocytic proteins can interact with nuclear molecules involved in transcription or chromatin remodeling, changing their localization or activity, and thus may directly modulate the levels or specificity of gene transcription. Certain endocytic proteins translocate to the nucleus in response to extracellular signals to exert a specific biological effect, thus serving as a vehicle for molecular communication between intracellular organelles. In most other cases, however, unclear is the extent to which endocytic and nuclear functions are related or represent disparate tasks (so-called moonlighting; Pilecka et al., *European Journal of Cell Biology*, 2007). Importantly, some such dual-function endocytic and nuclear proteins affect cell proliferation or act as tumor suppressors, or their expression is changed in human cancers (Pyrzynska et al., *Molecular Oncology*, 2009).

Our direct links to both lines of research include previous studies of adaptor proteins APPL1 and APPL2. These homologous proteins are localized to a particular subpopulation of endosomes but can also act as signal transducers capable of nuclear translocation. As such, they provide examples of both the involvement of endosomes in signaling and activity of endocytic proteins in the nucleus (Miaczynska et al., *Cell*, 2004). Our initial research efforts concentrated on APPL1 and APPL2 as exemplary proteins involved in endocytic trafficking and nuclear signaling, whereas more recently we have been extending our studies to exploring other dual-function endocytic proteins.

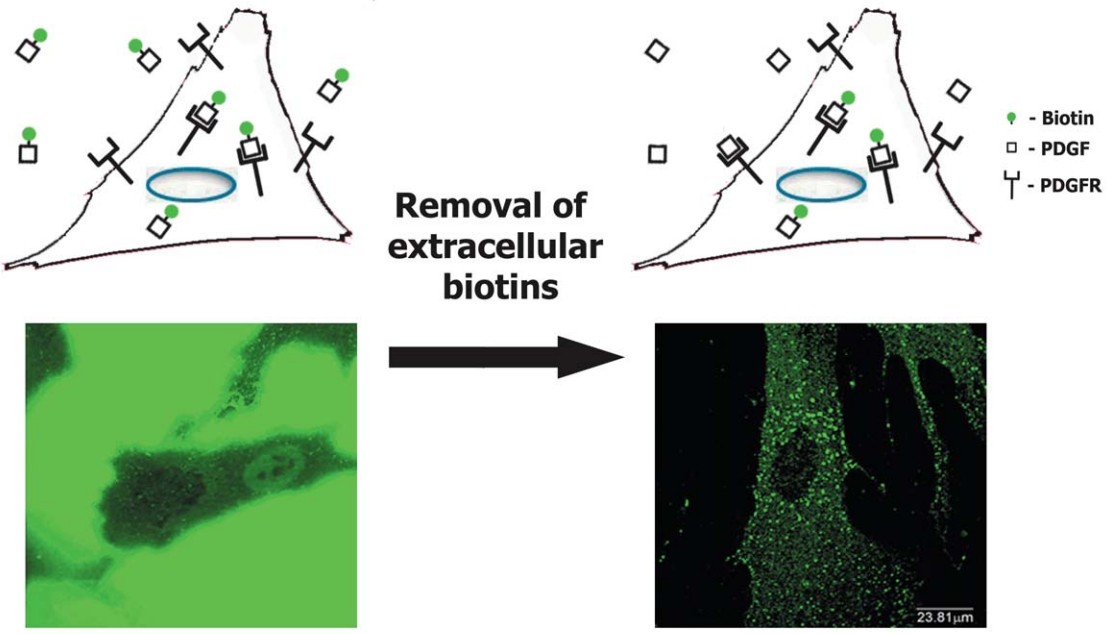


Fig. 1. Detection of PDGF endocytosis using a newly developed PDGF ligand labeled by reversible biotinylation. Cells are allowed to internalize the modified ligand. Subsequently, biotin molecules attached to extracellular PDGF are cleaved off by a reducing agent. This procedure removes the strong extracellular background of PDGF that adheres to the support (left image). The internalized biotinylated PDGF is left intact and can be detected by anti-biotin antibodies (right image). Author: Kamil Jastrzębski.

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

Within this general theme, in 2013 we completed a project that sought to characterize the endocytic trafficking of platelet-derived growth factor (PDGF) and evaluate the impact of endocytosis on PDGF-dependent signaling events (Sadowski et al., *Traffic*, 2013). This work was performed in collaboration with Dr. Carina Hellberg at University of Birmingham, Prof. Carl-Henrik Heldin at the Ludwig Institute for Cancer Research in Uppsala, and Dr. Yannis Kalaidzidis at Max Planck Institute in Dresden. This study provided novel mechanistic insights into the endocytosis and signaling of PDGF and the role of dynamin activity in these processes. In contrast to the well-studied epidermal growth factor (EGF), our knowledge of the mechanisms that link the endocytosis and signaling of other growth factors, including PDGF, is very limited, mainly because of a lack of appropriate tools. No commercial reagents (e.g., a labeled ligand) to visualize PDGF in cells are available. Moreover, tracking internalized PDGF using fluorescence microscopy has been challenging because of its pronounced adhesion to glass and plastic, which results in extreme extracellular background when using a directly labeled ligand, precluding any quantitative analysis. For this reason, most previous reports visualized PDGF receptors by indirect immunofluorescence to infer conclusions about ligand trafficking, although this approach cannot distinguish between ligand-bound and free receptors that traverse endocytic or biosynthetic pathways.

In our project, we employed reversible biotinylation to develop a tool to track internalized PDGF-BB using confocal microscopy in the absence of extracellular background (Fig. 1). We used this tool to quantitatively analyze the trafficking of PDGF-BB through subsequent endocytic compartments. By employing a panel of dynamin inhibitors, we further showed that PDGF was internalized by dynamin-dependent and -independent pathways, operating with different kinetics but ultimately leading to lysosomal degradation. Intriguingly, although these routes appeared to be functionally equivalent for the endocytic sorting of PDGF, they differed with respect to mitogenic signaling of PDGF. By testing the activation of individual signaling effectors upon cell stimulation with PDGF, we found that the inhibition of dynamin activity specifically decreased signaling via signal transducer and activator of transcription 3 (STAT3) but not via ERK1/2 MAPK or AKT kinase. This defect in STAT3 activation prevented the PDGF-induced expression of MYC and eventually reduced the mitogenic response of cells to PDGF. These data demonstrated that dynamin activity is required for PDGF-induced mitogenesis.

Our data support a general view that the components that govern endocytic trafficking, such as dynamin, may selectively regulate certain signaling effectors activated by a growth factor. This study broadens our knowledge of the cellular mechanisms of PDGF action and provides a basis for further research in the field of endocytosis and signaling, in which our newly developed and validated tool for tracking internalized PDGF may be potentially used in numerous experimental systems, or its principle of action may be applied to other growth factors. Currently, we are continuing our work to delineate which dynamin-independent pathways are exploited for PDGF internalization and how they affect PDGF signaling.

II. Involvement of endocytic proteins in the regulation of intracellular signaling and transcription.

To systematically study the possible mechanisms by which endocytic proteins may contribute to transcriptional regulation, we established and performed small-scale, targeted RNAi screens. We sought to identify the endocytic proteins that affect transcriptional responses in selected signaling pathways. Such proteins may act at various stages of these pathways, in the cytoplasm or in the nucleus, in a manner that results from their endocytic function or independently of it. We sought to identify important modulators of the investigated pathways that are able to provide fine-tuning and possible integration between different processes.

We initially chose signaling pathways that lead to the activation of TCF/LEF, AP-1, NF-κB, and STAT transcription factors. We selected these because of their physiological importance and different mechanisms of operation and because some endocytic proteins are already implicated in them (including our studies on APPL proteins). All of these pathways can be induced by extracellular ligands that bind appropriate plasma membrane receptors that undergo internalization, but how endocytosis affects the ultimate signaling responses remains poorly investigated or controversial.

For all of the screens, we preselected 80-160 candidate proteins that are known to function in endocytic internalization or endosomal sorting. These proteins were also implicated in nuclear signaling or oncogenic transformation. We used two complementary libraries of siRNA pools (esiRNA generated in the laboratory and commercial SMARTpool reagents from Dharmacon) that target the selected genes for screening in HEK293 cells under pathway-specific stimulation and, if detectable, also under basal conditions. Luciferase-based reporter tests were used as a primary screening assay to measure transcription that depends on the chosen factors upon knockdown of the genes that encode endocytic proteins.

The screens led to the identification of potential candidate regulators that act in a positive or negative manner in a given pathway. We performed validation of the identified primary hits to select proteins for further studies. Our aim is to delineate the molecular mechanisms of action of the newly identified regulators. To this end, we use two parallel experimental systems: cultured mammalian cells and zebrafish embryos (Fig. 2). In cultured cells, for each of the protein hits, we plan to identify (1) the target genes whose expression they regulate, (2) the stage of a signaling pathway at which they act, and (3) the relationship, if any, between their endocytic and transcriptional roles. In zebrafish embryos, we wish to (1) validate the evolutionary conservation of the observed phenotypes, (2) identify the set of target genes for each hit, and (3) describe the developmental phenotypes of knockdown or overexpression of each new regulator. This ongoing work is financed by a MAESTRO grant from the National Science Center and a project funded under the Polish-Swiss Research Programme.

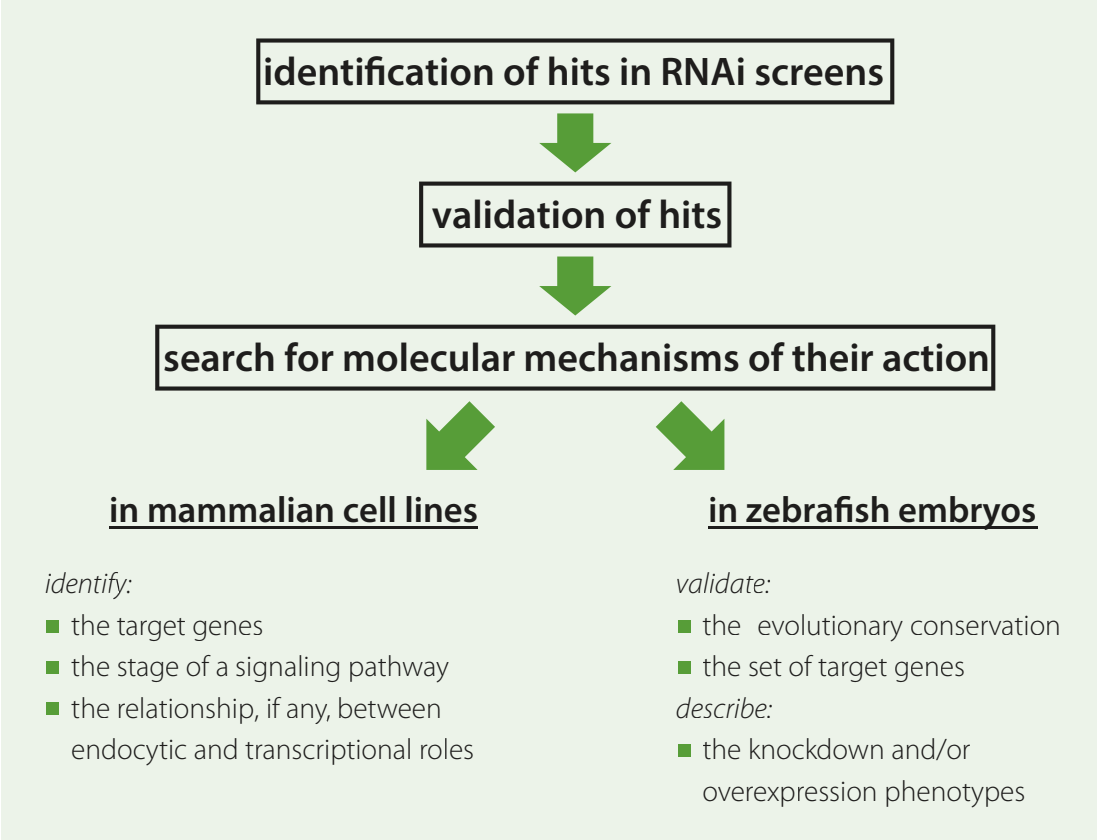
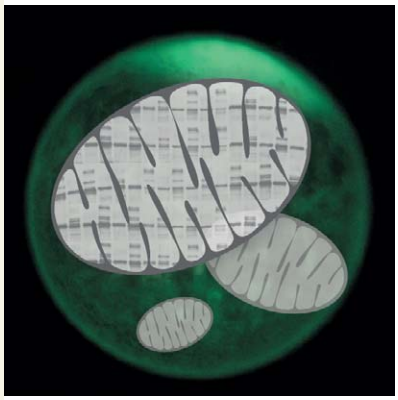


Fig. 2. The workflow of studies that seek to identify endocytic proteins that contribute to transcriptional regulation in selected signaling pathways. Author: Marta Miączyńska



Graphics by Agata Trojanowska

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Education and Degrees

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

Awards

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

Research experience and Appointments

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

Publications 2011-2013

- **Bragoszewski P, Gornicka A, Sztolsztener ME, Chacinska A**. The ubiquitin-proteasome system regulates mitochondrial intermembrane space proteins. Mol Cell Biol, 2013; 33:2136-48
- Qiu J, Wenz LS, Zerbes RM, Oeljeklaus S, Bohnert M, Stroud DA, Wirth C, Ellenrieder L, Thornton N, Kutik S, Wiese S, Schulze-Specking A, Zufall N, **Chacinska A**, Guiard B, Hunte C, Warscheid B, van der Laan M, Pfanner N, Wiedemann N, Becker T. Coupling of mitochondrial import and export translocases by receptor-mediated supercomplex formation. ***Cell***, 2013; 154:596-608
- **Varabyova A, Topf U, Kwiatkowska P, Wrobel L, Kaus-Drobek A, Chacinska A**. Mia40 and MINOS act in parallel with Ccs1 in the biogenesis of mitochondrial Sod1. ***FEBS J***, 2013; 280:4943-59
- **Wrobel L, Trojanowska A, Sztolsztener ME, Chacinska, A**. Mitochondrial protein import: Mia40 facilitates Tim22 translocation into the inner membrane of mitochondria. ***Mol Biol Cell***, 2013; 24:543-554
- **Varabyova A**, Stojanovski D, **Chacinska A**. Mitochondrial protein homeostasis. IUBMB Life, 2013; 65:191-201
- **Sztolsztener ME, Brewinska A**, Guiard B, **Chacinska A**. Disulfide bond formation: sulfhydryl oxidase ALR controls mitochondrial biogenesis of human MIA40. ***Traffic***, 2013; 14:309-320
- Bottinger L*, **Gornicka A***, **Czerwik T, Bragoszewski P, Loniewska-Lwowska A**, Schulze-Specking A, Truscott KN, Guiard B, Milenkovic D, **Chacinska A**. In vivo evidence for cooperation of Mia40 and Erv1 in the oxidation of mitochondrial proteins. ***Mol Biol Cell***, 2012; 23:3957-69 (*equal contribution)
- Stojanovski D, **Bragoszewski P, Chacinska A**. The MIA pathway: A tight bond between protein transport and oxidative folding in mitochondria. ***Biochim Biophys Acta***, 2012; 1823:1142-50
- Voegtle FN, Burkhart JM, Rao S, Gerbeth C, Hinrichs J, Martinou JC, **Chacinska A**, Sickmann A, Zahedi RP, Meisinger C. Intermembrane space proteome of yeast mitochondria. ***Mol Cell Proteomics***, 2012; 11:1840-52
- Bohnert M, Wenz LS, Zerbes RM, Horvath SE, Stroud DA, von der Malsburg K, Muller JM, Oeljeklaus S, Perschil I, Warscheid B, **Chacinska A**, Veenhuis M, van der Klei IJ, Daum G, Wiedemann N, Becker T, Pfanner N, van der Laan M. Role of MINOS in protein biogenesis of the mitochondrial outer membrane. ***Mol Biol Cell***, 2012; 23:3948-56
- von der Malsburg K, Muller JM, Bohnert M, Oeljeklaus S, **Kwiatkowska P**, Becker T, **Loniewska-Lwowska A**, Wiese S, Rao S, Milenkovic D, Hutu DP, Zerbes RM, Schulze-Specking A, Meyer HE, Martinou JC, Rospert S, Rehling P, Meisinger C, Veenhuis M, Warscheid B, van der Klei IJ, Pfanner N*, **Chacinska A***, van der Laan M. Dual Role of Mitofilin in mitochondrial membrane organization and protein biogenesis. ***Developmental Cell***, 2011; 21:694-707 (*co-corresponding authors)
- Schulz C, Lytovchenko O, Melin J, **Chacinska A**, Guiard B, Neumann P, Ficner R, Jahn O, Schmidt B, Rehling P. Tim50's presequence receptor domain is essential for signal driven transport across the TIM23 complex. ***J Cell Biol***, 2011; 195:643-656
- Becker T, Wenz LS, Kruger V, Lehmann W, Muller JM, Goroncy L, Zufall N, Lithgow T, Guiard B, **Chacinska A**, Wagner R, Meisinger C, Pfanner N. The mitochondrial import protein Mim1 promotes biogenesis of multispanning outer membrane proteins. ***J Cell Biol***, 2011; 194:387-395

Co-workers affiliated with IIMCB are given in bold

Other selected publications

- **Chacinska A**, Koehler CM, Milenkovic D, Lithgow T, Pfanner N. Importing mitochondrial proteins: machineries and mechanisms. ***Cell***, 2009; 138:628-644
- Milenkovic D, Ramming T, Muller JM, Wenz LS, Gebert N, Schulze-Specking A, Stojanovski D, Rospert S, **Chacinska A**. Identification of the signal directing Tim9 and Tim10 into the intermembrane space of mitochondria. ***Mol Biol Cell***, 2009; 20:2530-9
- **Chacinska A***, Guiard B*, Muller JM, Schulze-Specking A, Gabriel K, Kutik S, Pfanner N. Mitochondrial biogenesis: switching the sorting pathways of the intermembrane space receptor Mia40. ***J Biol Chem***, 2008; 283:29723-9 (*equal contribution)
- Stojanovski D, Milenkovic D, Muller JM, Gabriel K, Schulze-Specking A, Baker MJ, Ryan MT, Guiard B, Pfanner N, **Chacinska A**. Mitochondrial protein import: precursor oxidation in a ternary complex with disulfi de carrier and sulfhydryloxidase. ***J Cell Biol***, 2008; 183:195-202
- Muller JM, Milenkovic D, Guiard B, Pfanner N, **Chacinska A**. Precursor oxidation by Mia40 and Erv1 promotes vectorial transport of proteins into the mitochondrial intermembrane space. ***Mol Biol Cell***, 2008; 19:226-236
- Milenkovic D, Gabriel K, Guiard B, Schulze-Specking A, Pfanner N, **Chacinska A**. Biogenesis of the essential Tim9-Tim10 chaperone complex of mitochondria: site-specific recognition of cysteine residues by the intermemembrane space receptor Mia40. ***J Biol Chem***, 2007; 282:22472-80
- Meinecke M, Wagner R, Kovermann P, Guiard B, Mick DU, Hutu DP, Voos W, Truscott KN, **Chacinska A**, Pfanner N, Rehling P. Tim50 maintains the permeability barrier of the mitochondrial inner membrane. ***Science***, 2006; 312:1523-26
- Wiedemann N, Pfanner N, **Chacinska A**. Chaperoning through the mitochondrial intermembrane space. ***Mol Cell***, 2006; 21:145-148
- **Chacinska A***, Lind M*, Frazier AE, Dudek J, Meisinger C, Geissler A, Sickmann A, Meyer HE, Truscott KN, Guiard B, Pfanner N, Rehling P. Mitochondrial presequence translocase: switching between TOM tethering and motor recruitment involves Tim21 and Tim17. ***Cell***, 2005; 120:817-829 (*equal contribution)
- Rissler M, Wiedemann N, Pfannschmidt S, Gabriel K, Guiard B, Pfanner N, **Chacinska A**. The essential mitochondrial protein Erv1 cooperates with Mia40 in biogenesis of intermembrane space proteins. ***J Mol Biol***, 2005; 353:485-492
- **Chacinska A**, Pfannschmidt S, Wiedemann N, Kozjak V, Sanjuan Szklarz LK, Schulze-Specking A, Truscott KN, Guiard B, Meisinger C, Pfanner N. Essential role of Mia40 in import and assembly

of mitochondrial intermembrane space proteins. ***EMBO J***, 2004; 23:3735-46
- Meisinger C, Rissler M, **Chacinska A**, Sanjuan Szklarz LK, Milenkovic D, Kozjak V, Schonfisch B, Lohaus C, Meyer HE, Yaffe MP, Guiard B, Wiedemann N, Pfanner N. The mitochondrial morphology protein Mdm10 functions in assembly of the preprotein translocase of the outer membrane. ***Dev Cell***, 2004; 7:61-71
- Sickmann A, Reinders J, Wagner Y, Joppich C, Zahedi R, Meyer HE, Schonfisch B, Perschil I, **Chacinska A**, Guiard B, Rehling P, Pfanner N, Meisinger C. The proteome of *Saccharomyces cerevisiae mitochondria*. ***Proc Natl Acad Sci USA***, 2003; 100:13207-12
- Wiedemann N, Kozjak V, **Chacinska A**, Schonfisch B, Rospert S, Ryan MT, Pfanner N, Meisinger C. Machinery for protein sorting and assembly in the mitochondrial outer membrane. ***Nature***, 2003; 424:565-571
- **Chacinska A**, Rehling P, Guiard B, Frazier AE, Schulze-Specking A, Pfanner N, Voos W, Meisinger C. Mitochondrial translocation contact sites: separation of dynamic and stabilizing elements in formation of a TOM-TIM-preprotein supercomplex. ***EMBO J***, 2003; 22:5370-5381
- Geissler A*, **Chacinska A***, Truscott KN, Wiedemann N, Brandner K, Sickmann A, Meyer HE, Meisinger C, Pfanner N, Rehling P. The mitochondrial presequence translocase: an essential role of Tim50 in directing preproteins to the import channel. ***Cell***, 2002; 111:507-518 (*equal contribution).

Publications until 2009 have no IIMCB affiliation

Description of Current Research

Mitochondria play an important role in metabolism and regulatory processes in the cell. Thus, the formation of mitochondria is essential for cellular function in the entire eukaryotic kingdom, from unicellular organisms to mammals. Mitochondria comprise 1000-1500 cellular proteins that are synthesized outside the mitochondria in the cytosol and must be imported into mitochondria (Fig. 1). The biogenesis of mitochondria relies on the efficient import, sorting, and maturation of proteins governed by conserved protein translocases and other complex machineries. In the course of earlier work at the University of Freiburg, we made a surprising discovery that contradicted the dogma on the absence of disulfide bonds in reducing cellular compartments, such as mitochondria. We identified and characterized a novel mitochondrial intermembrane space assembly (MIA) pathway that utilizes the transfer of disulfide bonds and is dedicated to the import and biogenesis of intermembrane space proteins that lack a classic mitochondrial leader sequence (Fig. 2). Supported by a Welcome Grant from the Foundation for Polish Science, an EMBO Installation Grant, and grants from the Ministry of Science and Higher Education and National Science Centre, the group seeks to understand the complex and dynamic processes involved in the formation of functional mitochondria, the maintenance of mitochondrial protein homeostasis, and their failure that results in pathology. Our major interests are also directed to redox-dependent processes involved in mitochondrial protein biogenesis. We concentrate on the following issues (see also Fig. 1):

- Redox-related protein biogenesis events driven by MIA in yeast and higher eukaryotes.
- Impact of protein transport pathways on mitochondrial and cellular protein homeostasis.
- Biological consequences of oxidative protein biogenesis failure.

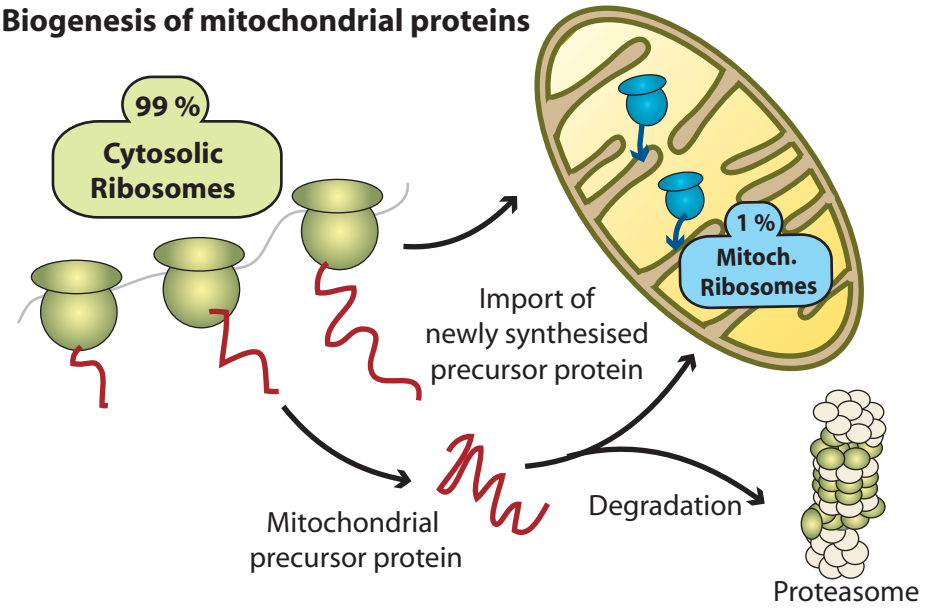
Redox-based protein biogenesis events in the intermembrane space of mitochondria

Our research aims to understand the biogenesis of proteins localized in the intermembrane space of mitochondria (Figure 1). To be entrapped in the intermembrane space of mitochondria, proteins utilize catalyzed thiol-disulfide exchange driven by mitochondrial MIA machinery. One interesting mechanistic aspect under debate is the mode of cooperation between Mia40 and Erv1, two major components of the MIA pathway. In contrast to the well-established view that Mia40 interacts with either substrate proteins to facilitate their oxidative folding or Erv1 for Mia40 reoxidation, we provided compelling evidence *in organelle* and *in vivo* that the oxidation of intermembrane space substrate proteins involves the simultaneous association of Mia40 and Erv1 to maintain the productivity of oxidative biogenesis (Bottinger et al., *Mol Biol Cell*, 2012). These findings led us to propose that the oxidative folding of intermembrane space proteins governed by MIA is a spatially and temporally coordinated chain of events (for review, see Stojanovski et al., *Biochim Biophys Acta*, 2012).

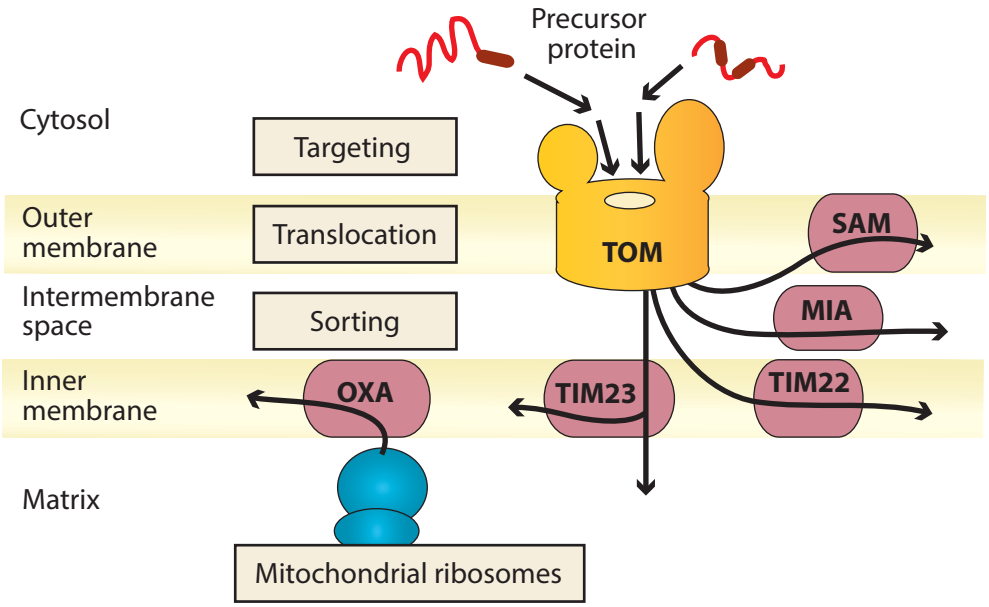
We previously demonstrated (Milenkovic et al., *Mol Biol Cell*, 2007; Milenkovic et al., *Mol Cell Biol*, 2009) that mitochondrial precursor proteins are specifically recognized by Mia40, the major component of the MIA pathway, after they pass a main entry gate into mitochondria formed by the TOM complex. In the search for factors that determine the localization of Mia40 in the vicinity of the TOM complex, we performed a comprehensive study of protein interactions. We identified a new interaction partner of Mia40, Fcj1 (Formation of Crista Junctions; Mic60; mitofilin in higher eukaryotes), and demonstrated that Mic60/Fcj1 interacts with the TOM complex. Thus, Mic60/Fcj1 is a regulatory factor that spatially organizes the biogenesis of mitochondria by positioning Mia40 in close proximity to the TOM complex (von der Malsburg et al., *Developmental Cell*, 2011). Moreover, consistent with this general function of Fcj1 in the spatial organization of mitochondria, a large complex formed by Mic60/Fcj1 was identified that was named MINOS/MICOS/MitOS for its critical role in mitochondrial inner membrane organization (von der Malsburg et al., *Developmental Cell*, 2011). We continue to study the relationship between the MIA pathway and Mic60/Fcj1 and its role in membrane organization.

In the search for the non-canonical functions of MIA, we investigated inner mitochondrial membrane proteins. Surprisingly, a multispanning membrane protein responsible for the transport of mitochondrial inner membrane proteins, Tim22, was found in the oxidized state in mitochondria. The oxidized state of Tim22 is necessary to properly assemble and form the functional TIM22 translocase complex. We demonstrated that Tim22 transiently interacts with Mia40. In conclusion, Mia40 serves not only as an oxidoreductase but also as a translocase that assists inner membrane proteins in their passage through the intermembrane space and membrane integration (Wrobel et al., *Mol Biol Cell*, 2013). This finding extends the function of the MIA pathway

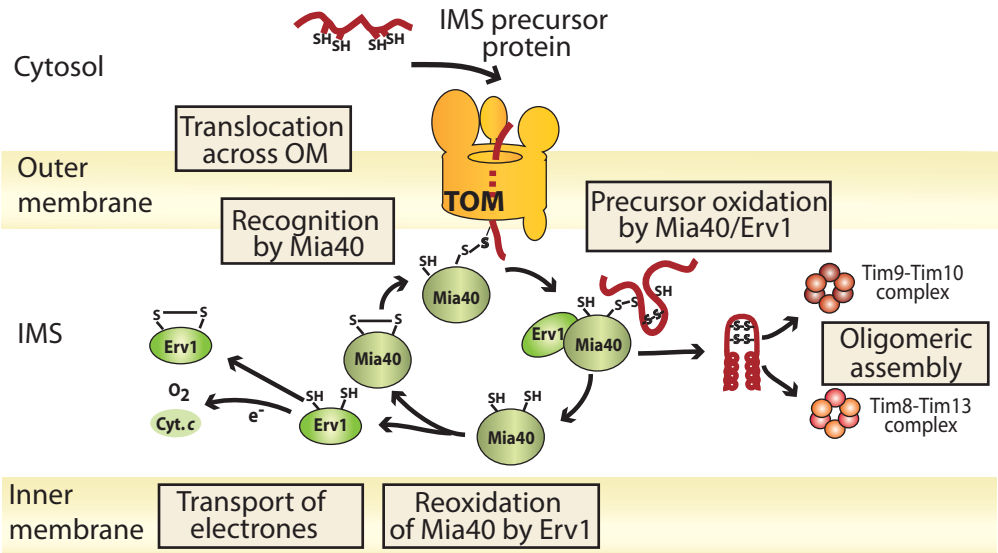
A. Biogenesis of mitochondrial proteins



B. Mitochondrial protein sorting



C. Mitochondrial intermembrane space import and assembly (MIA)



beyond the oxidative folding of intermembrane space proteins. The mechanism and precise role of cysteine residues in Tim22 and selected other membrane proteins in membrane insertion remain core subjects of our current research.

Impact of protein transport pathways on mitochondrial and cellular protein homeostasis

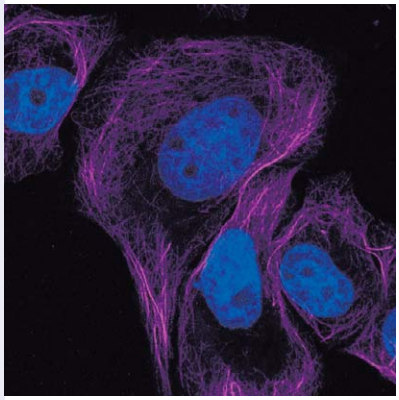
A fundamental and largely unanswered question in cell biology is how the cell protects itself against the accumulation of proteins that do not reach their proper destination. We have been interested in the fate of intermembrane space precursors in the cytosol under conditions of mitochondrial protein import limitations. We found that intermembrane space proteins are very efficiently degraded in the cytoplasm (Bragoszewski et al., *Mol Cell Biol*, 2013). We demonstrated that the process of degradation of the proteins destined to the intermembrane space of mitochondria occurs under normal conditions, in addition to conditions in which their presence in the cytosol is prolonged because of an import defect (MIA mutants). This process is executed by degradation machinery in the cytosol, the proteasome. Interestingly, the proteasome competes with mitochondrial protein import machinery. Our study systematically demonstrated the involvement of the proteasome in the biogenesis of mitochondrial proteins for the large class of mitochondrial proteins prior to their import into mitochondria (Bragoszewski et al., *Mol Cell Biol*, 2013). We also performed a global proteome analysis to identify changes that are caused by the defective import of proteins into mitochondria due to MIA dysfunction (in collaboration with Dr. Bettina Warscheid from University of Freiburg). Our quantitative proteomics analysis is currently completed and constitutes a rich source of information on changes in the abundance of cellular proteins upon mitochondrial protein import failure that drives our current and future research.

Biological consequences of oxidative protein biogenesis failure

We are interested in studies performed in higher eukaryotes. The components of the MIA pathway that are similar to other mitochondrial protein translocases are conserved, but they have remained only poorly understood. Our currently completed intermediate stage in reaching the goal of understanding redox-driven protein biogenesis in human cells involved reconstituting the human MIA pathway in yeast. We exchanged the essential yeast counterparts for the human proteins MIA40 and ALR (the sulfhydryl oxidase, yeast Erv1 homolog) and established the mechanistic principles of the human MIA pathway. Importantly, with the use of our "humanized" yeast, we addressed the exact molecular defect caused by the pathogenic mutant variant of ALR (Sztolsztener et al., *Mol Biosyst*., 2012).

We set up lines of research with cultured human cell lines. Our intensive efforts are now directed toward a better understanding of the role of the MIA pathway in human cell biology. Furthermore, to expand our scientific interests to multicellular organism, we have used zebrafish as an excellent model system thanks to a newly established zebrafish facility available for all groups in our institute. During the last year, we established basic expertise in zebrafish handling, morpholino technology, and an innovative TALEN technology (in collaboration with Dr. Didier Stainier from MPI in Bad Nauheim). We also made great progress in the visualization of zebrafish mitochondria (with the use of a stable zebrafish line, in which GFP is targeted specifically to mitochondria) and mitochondrial proteins (by intensively testing commercially available antibodies against conserved mitochondrial proteins).

Fig. 1. (previous page) (A) A large majority of mitochondrial proteins are synthesized on cytosolic ribosomes and enter mitochondria via a main gate formed by the TOM complex. The possibility exists that protein synthesis is coupled to protein transport. Non-imported mitochondrial proteins are cleared by the proteasome. (B) After crossing the TOM complex, mitochondrial precursor proteins are sorted inside mitochondria into their final destinations (i.e., one of two mitochondrial membranes, the matrix or intermembrane space) via specific translocase machineries. A small number of hydrophobic proteins are encoded by mitochondrial DNA and synthesized by mitochondrial ribosomes and enter the inner mitochondrial membrane in a cotranslational process. (C) After arriving on the trans side of the TOM complex, intermembrane space proteins enter the MIA pathway, which drives their import completion and maturation by catalyzing disulfide bond formation and electron transfer (adapted from Chacinska et al., *Cell*, 2009, 138:628).



Long, polymerized tubulin fibers (microtubules) in SK-BR-3 cells treated with taxol for 16 hours. Author: Milena Wiech

Molecular Biology Department

Postdoctoral Fellows:
Maciej Olszewski, PhD (since June 2013), FishMed

FishMed Research Assistant:
Marta Wawrzyniak, PhD (since June 2013, joint with Structural Biology Laboratory)

Junior Researchers:
Marcin Herok, MSc (since Oct. 2013)
Marta Małuszek, MSc
Magdalena Pruszko, MSc
Zuzanna Tracz-Gaszewska, MSc
Milena Wiech, MSc (till Nov. 2013)

Secretary:
Grażyna Orleańska, MSc

Technician:
Wanda Gocal

Lab Leader:
Maciej Żylicz,
PhD, Professor

Degrees

1992	Professor, nomination by the President of the Republic of Poland
1986	DSc Habil in Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
1980	PhD in Biochemistry, Medical University of Gdańsk, Poland
1977	MSc in Physics, University of Gdańsk, Poland (student of physics and biology)

Postdoctoral Training

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

Professional Employment

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

Other Professional Activities

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

Membership in Scientific Societies, Organizations, and Panels

- European Molecular Biology Organization (EMBO), Member
- Polish Academy of Sciences, Full Member
- German National Academy of Sciences Leopoldina, Member
- Polish Academy of Arts and Sciences, Member
- Academia Europaea, Member
- European Academy of Cancer Research, Member
- American Society of Biochemistry and Molecular Biology, Member
- Advisory Editorial Board, EMBO Journal, EMBO Reports (2004-2008), and IUBMB Life, Member
- EMBO Council (2004-2007), Member
- Selection Committee, EMBO Young Investigator Programme (2001-2003), Member
- European Molecular Biology Conference (2001-2004), Polish delegate
- European Science Foundation Life Science Committee (2003-2005), Polish Delegate
- Selection Committee, Special DFG Programmes (2001-2005), Member
- Max Planck Society, Member of Senate (2012-Present)
- State Committee for Scientific Research (1997-2004), Member

Honors, Prizes and Awards

2013	Doctor Honoris Causa, Jagiellonian University
2011	Doctor Honoris Causa, University of Gdańsk
2008	Officer's Cross of the Order of Polonia Restituta (awarded by the President of the Republic of Poland)
2007	Doctor Honoris Causa, University of Wrocław
2002	Prime Minister Award for Scientific Achievements
2001	Marchlewski Award, Committee of Biochemistry and Biophysics, Polish Academy of Sciences
1999	Award in biological/medical sciences, Foundation for Polish Science
1996, 2007, 2010	Awards for best biochemistry work performed in Polish laboratories, Polish Biochemical Society
1994	Award from Ministry of Education
1993	Heweliusz Prize for Scientific Achievements (awarded by President of Gdańsk)
1990	Award from Polish Academy of Sciences
1986	Individual Award for Scientific Achievements, Polish Academy of Sciences

Doctorates

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

Academic Habilitations

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

Professor Titles Received

Liberek K, Marszalek J, Konieczny I, Wawrzynów A

Publications

About 80 publications in primary scientific journals, including two papers published in *Cell*, six in *EMBO J*, six in *Proc Natl Acad Sci USA*, and more than 30 in *J Biol Chem*. These papers were cited more than 6000 times with a Hirsch index of H = 40.

Selected Publications

- **Wiech M, Olszewski M, Tracz-Gaszewska Z, Wawrzynow B, Zylicz M, Zylicz A**. Molecular Mechanism of Mutant p53 Stabilization: The Role of HSP70 and MDM2. ***PLoS One***, 2012; 7(12):e51426
- Hageman J, van Waarde MA, **Zylicz A**, **Walerych D**, Kampinga HH. The diverse members of the mammalian HSP70 machine show distinct chaperone-like activities. ***Biochem J***, 2011; 435:127-142
- **Walerych D, Gutkowska M, Klejman MP, Wawrzynow B, Tracz Z, Wiech M, Zylicz M, Zylicz A**. ATP binding to Hsp90 is sufficient for effective chaperoning of p53 protein. ***J Biol Chem***, 2010; 285:32020-8
- Zubrienė A, **Gutkowska M**, Matulienė J, Chaleckis R, Michailovienė V, Voroncova A, Venclovas C, **Zylicz A, Zylicz M**, Matulis D. Thermodynamics of radicicol binding to human Hsp90 alpha and beta isoforms. ***Biophys Chem***, 2010; 152:153-163
- **Zurawska A, Urbanski J**, Matulienė J, Baraniak J, **Klejman MP**, Filipek S, Matulis D, **Bieganowski P**. Mutations that increase both Hsp90 ATPase activity in vitro and Hsp90 drug resistance in vivo. ***BBA – Mol Cell Res***, 2010; 1803:575-583
- Kirkegaard T, Roth AG, Petersen NH, Mahalka AK, Olsen OD, Moilanen I, **Zylicz A**, Knudsen J, Sandhoff K, Arenz C, Kinnunen PK, Nylandsted J, Jäättelä M. Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated lysosomal pathology. ***Nature***, 2010; 463:549-553
- **Walerych D, Olszewski MB, Gutkowska M, Helwak A, Zylicz M, Zylicz A**. Hsp70 molecular chaperones are required to support p53 tumor suppressor activity under stress conditions. ***Oncogene***, 2009; 28:4284-94
- Narayan V, Eckert M, **Zylicz A, Zylicz M**, Ball KL. Cooperative regulation of the interferon regulatory factor-1 tumor suppressor protein by core components of the molecular chaperone machinery. ***J Biol Chem***, 2009; 284:25889-99
- Wawrzynow B, Pettersson S, **Zylicz A**, Bramham J, Worrall E, Hupp TR, Ball KL. A function for the RING finger domain in the allosteric control of MDM2 conformation and activity. ***J Biol Chem***, 2009; 284:11517-30
- **Szymanska Z, Zylicz M**. Mathematical modeling of heat shock protein synthesis in response to temperature change. ***J Theor Biol***, 2009; 259:562-569
- **Szymanska Z, Urbanski J**, Marciniak-Czochra A. Mathematical modelling of the influence of heat shock proteins on cancer invasion of tissue. ***J Math Biol***, 2009; 58:819-44
- **Zurawska A, Urbanski J, Bieganowski P**. Hsp90n - An accidental product of a fortuitous chromosomal translocation rather than a regular Hsp90 family member of human proteome. ***Biochim Biophys Acta***, 2008; 1784:1844-6
- Stevens C, Pettersson S, **Wawrzynow B**, Wallace M, Ball K, **Zylicz A**, Hupp TR. ATP stimulates MDM2-mediated inhibition of the DNA-binding function of E2F1. ***FEBS J***, 2008; 275:4875-86
- **Wawrzynow B, Zylicz A**, Wallace M, Hupp T, **Zylicz M**. MDM2 chaperones the p53 tumor suppressor. ***J Biol Chem***, 2007; 282:32603-12
- Issat T, Nowis D, Legat M, Makowski M, **Klejman MP, Urbanski J**, Skierski J, Koronkiewicz M, Stoklosa T, Brzezinska A, Bil J, Gietka J, Jakobisiak M, Golab J. Potentiated antitumor effects of the combination treatment with statins and pamidronate *in vitro* and *in vivo*. ***Int J Oncol***, 2007; 30:1413-25
- **Kudla G, Lipinski L, Caffin F, Helwak A, Zylicz M**. High guanine and cytosine content increases mRNA levels in mammalian cells. ***PLoS Biology***, 2006; 4:0933-42
- **Walerych D, Kudla G, Gutkowska M, Wawrzynow B**, Muller L, **King FW, Helwak A, Boros J, Zylicz A, Zylicz M**. Hsp90 chaperones wild-type p53 tumor suppressor protein. ***J Biol Chem***, 2004; 279: 48836-45
- Dworakowska D, Jassem E, Jassem J, Peters B, Dziadziuszko R, **Zylicz M**, Jakobkiewicz-Banecka J, Kobierska-Gulida G, Szymanowska A, Skokowski J, Roessner A, Schneider-Stock R. MDM2 gene amplification: a new independent factor of adverse prognosis in non-small cell lung cancer (NSCLC) ***Lung Cancer***, 2004; 43:285-295
- **Kudla G, Helwak A, Lipinski L**. Gene conversion and GCcontent evolution in mammalian Hsp70. ***Mol Biol Evol***, 2004; 21:1438-44
- **Zylicz M, King FW, Wawrzynow A**. Hsp70 interactions with the p53 tumour suppressor protein. ***EMBO J***, 2001; 20:4634-8
- **King FW, Wawrzynow A**, Hohfeld J, **Zylicz M**. Cochaperones Bag-1, Hop and Hsp40 regulate Hsc70 and Hsp90 interactions with wild-type or mutant p53. ***EMBO J***, 2001; 20:6297-305.

Description of Current Research

The research conducted in the of Molecular Biology Department mainly focuses on the activity of molecular chaperones in mammalian cells, including cell transformation. Using highly purified recombinant human proteins, we previously identified intermediate reactions that led to the assembly of molecular chaperone complexes with the wildtype or mutant p53 tumor suppressor protein. We also demonstrated that the heat shock protein 90 (HSP90) molecular chaperone was required for the binding of wildtype p53 to the promoter sequences under a physiological temperature of 37ºC and that chaperoning activity was adenosine triphosphate (ATP)-dependent. We provided *in vivo* evidence that under heat shock conditions, HSP90 and HSP70/HSPA chaperone machineries were required for the proper folding of wildtype p53, its specific binding to chromatin, and the transcription of p53-dependent genes (Walerych et al., *Oncogene*, 2009). The influence of chaperones on the binding of p53 to the *WAF1* promoter sequence was confirmed *in vitro* using highly purified proteins. HSP90 stabilized the binding of p53 to the promoter sequence at 37ºC, whereas the requirement for the HSP70-HSP40 system and its cooperation with HSP90 increased under heat shock conditions. (Walerych et al., *Oncogene*, 2009). We also elucidated the role of the adenine nucleotide in the HSP90 chaperone cycle by taking advantage of a unique *in vitro* assay that measures the HSP90-dependent binding of p53

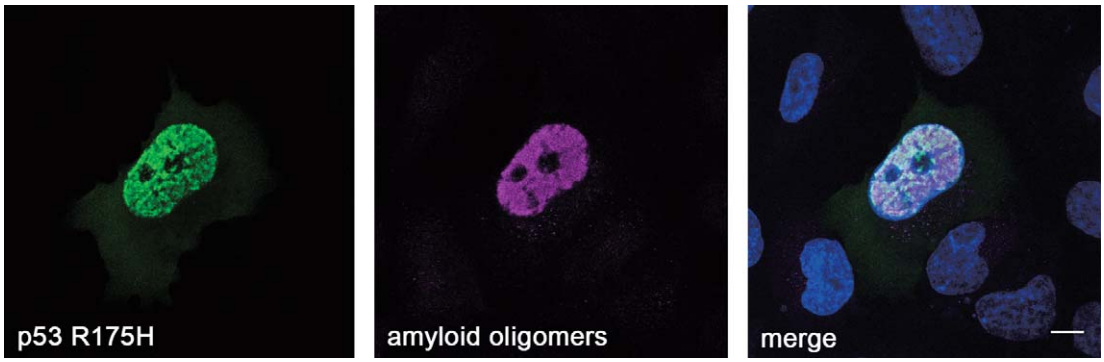


Fig. 1. In the presence of MDM2 excess, amyloid oligomer-specific antibodies bind to p53 R175H aggregates. H1299 cells were transfected with plasmids encoding p53 R175H and MDM2, treated with MG132 proteasome inhibitor for 16 hours prior to fixation and then labeled for p53 (DO-1 antibody, pseudocolour green) and amyloid oligomers (A11 antibody, pseudocolour magenta). Images were acquired using Zeiss LSM5 Exciter microscope. Nucleus was stained with DAPI (blue). Scale bar 10 μm. Author: Milena Wiech

to the promoter sequence (Walerych et al., *Journal of Biological Chemistry*, 2010). Using a trap version of the GroEL chaperonin, which irreversibly captures unfolded proteins, we showed that the action of the HSP90 chaperone on wildtype p53 resulted in a partial unfolding of the substrate. The ATP-dependent dissociation of the p53-HSP90 complex allowed further folding of the p53 protein to an active conformation that was able to bind to the promoter sequence. Altogether, our research indicated that the binding of ATP to HSP90β was a sufficient step for effective wildtype p53 client protein chaperoning (Walerych et al., *Journal of Biological Chemistry*, 2010).

Heat shock protein 70 (HSP70/HSPA1) is an evolutionarily highly conserved molecular chaperone that promotes the survival of stressed cells by inhibiting lysosomal membrane permeabilization, a hallmark of stress-induced cell death. Clues to its molecular mechanism of action may lie in the recently reported stress- and cancer-associated translocation of a small portion of HSP70 to the lysosomal compartment. Prof. Marja Jaattela's laboratory at the Denmark Cancer Institute, in collaboration with our department, showed that HSP70 stabilized lysosomes by binding to endolysosomal anionic phospholipid bis(monoacylglycero)phosphate (BMP), an essential cofactor for lysosomal sphingomyelin metabolism (Kirkegaard et al., *Nature*, 2010). Altogether, these data open exciting possibilities for the development of new treatments for lysosomal storage disorders and cancer with compounds that enter the lysosomal lumen through the endocytic delivery pathway (Kirkegaard et al., *Nature*, 2010).

We discovered that MDM2, in addition to its E3-ubiquitin ligase activity, exhibited molecular chaperone activity and demonstrated that a MDM2 mutant protein that is defective in ATP binding (K454A) lacked chaperone activity both *in vivo* and *in vitro*. Wildtype *MDM2* coexpressed with wildtype *p53* stimulated efficient p53 protein folding *in vivo*, and this effect was abrogated with an ATP binding-defective form of MDM2 (Wawrzynow et al., *Journal of Biological Chemistry*, 2007). In collaboration with Prof. Kathryn Ball at the University of Edinburgh, we showed that the binding affinity of MDM2's hydrophobic pocket could be regulated through the RING finger domain and that increases in pocket affinity were reflected by a gain in MDM2 transrepressor activity (Wawrzynow et al., *Journal of Biological Chemistry*, 2009). Thus, mutations within the RING domain that affect zinc coordination but not mutations that inhibit ATP binding produce MDM2 proteins that have a higher affinity for the BOX-I transactivation domain of p53 and a reduced affinity for p53 transrepression. An allosteric model of the regulation of the hydrophobic pocket was supported by differences in protein conformation and pocket accessibility between wildtype and RING domain mutant MDM2 proteins. Additionally, the data demonstrated that the complex relationship between different domains of MDM2 could impact the efficacy of anticancer drugs directed toward its hydrophobic pocket (Wawrzynow et al., *Journal of Biological Chemistry*, 2009).

Numerous p53 missense mutations possess gain-of-function activities. Studies in mouse models have demonstrated that the stabilization of p53 R172H (R175H in humans) mutant protein by currently unknown factors is a prerequisite for its oncogenic gain-of-function phenotype, such as tumor progression and metastasis. Recently, we showed that the MDM2-dependent ubiquitination and degradation of p53 R175H mutant protein in mouse embryonic fibroblasts was partially inhibited by increasing the concentration of HSP70/HSPA1-A. These phenomena correlated well with the appearance of HSP70-dependent folding intermediates in the form of dynamic amorphic aggregates that contain p53 R175H and several molecular chaperones. We propose that a transient but recurrent interaction with HSP70 may lead to an increase in mutant p53 protein half-life. In the presence of MDM2, these amorphic aggregates can form stable amyloid-like structures (Fig. 1). The refolding kinetics of p53 indicated that HSP70 caused transient exposure of the p53 aggregate-prone domains that apparently can interact with MDM2. Our results indicate that the HSP70 molecular chaperone binds and partially unfolds p53. Upon the ATP-dependent release of HSP70 from the complex with p53, part of the unfolded p53 protein, with the help of MDM2, is captured in the aggregation-prone conformation (Fig. 2). Moreover, the sequestration of p73 tumor suppressor protein by these nuclear aggregates may lead to gain-of-function phenotypes (Wiech et al., *PLoS One*, 2012).

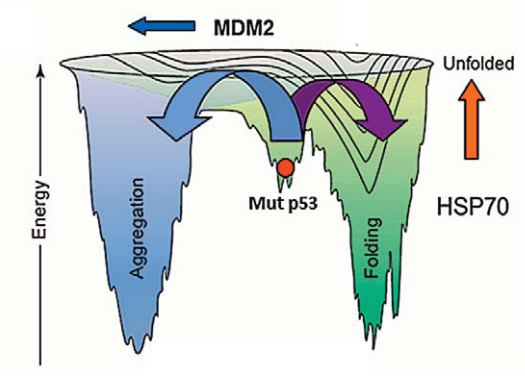
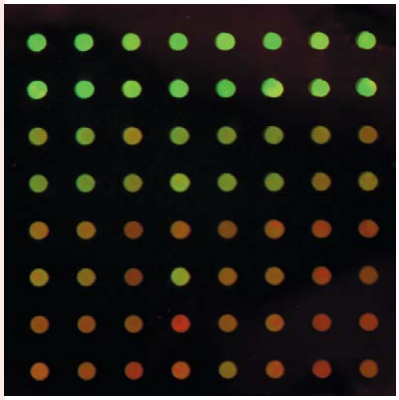


Fig. 2. HSP70 accelerates mutant p53 protein refolding reaction, but at the same time HSP70, by partial unfolding, transiently exposes the aggregation prone regions of p53 which can be trapped by MDM2.



Results of medium scale comparison of mTOR pathway activity in HEK cells upon transfection of various mTOR mutants described in human diseases. *Photo by Bartosz Tarkowski*

Molecular and Cellular Neurobiology Laboratory

Postdoctoral Fellows:
Magdalena Błażejczyk, PhD
Iwona Cymerman, PhD
Agata Goździ, PhD
Aleksandra Janusz, PhD (since Jan. 2014)
Justyna Jezierska, PhD, FishMed
Ewa Liszewska, PhD
Matylda Macias, PhD
Anna Malik, PhD
Bartosz Tarkowski, PhD

Junior Researchers:
Joanna Lipka, MSc (abroad as MPD student)
Agnieszka Kolka, MSc
Marcelina Pieprzyk, MSc
Aleksandra Piechnik, MSc
Agnieszka Skałecka, MSc
Katarzyna Świtoń, Msc
Anna Urbańska, MSc
Małgorzata Urbańska, MSc

FishMed Technical Assistant:
Lidia Wolińska, MSc (since Jan. 2013) joint with Cell Biology Laboratory

Technicians:
Monika Matuszczyk
Alina Zielińska

Lab Leader:
Jacek Jaworski,
PhD, Professor

Degrees

2014 Professor of Biological Sciences, nomination by the President of the Republic of Poland
2010 DSc Habil in Molecular Biology, Warsaw University, Poland
2001 PhD in Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1996 MSc in Biology, Department of Genetics, Warsaw University, Poland

Research Training

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

Fellowships and Awards

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

Membership in Scientific Societies, Organizations, and Panels

2011 Neurobiology Committee of the Polish Academy of Sciences, Member

Awards, Honors and Titles (Lab members - 2013)

2013 L'Oréal Poland for Women in Science 1-year PhD fellowship, M. Urbańska
2013 Mazovia 1-year PhD Scholarship, A. Urbańska
2013 Pomost from Foundation for Polish Science, A. Malik
2013 Etiuda from National Research Center, A. Skałecka

Awards, Honors and Titles (Lab members – 2011-2012)

2012 The Nencki Institute Scientific Council distinction for PhD thesis, M. Perycz
2012 PhD in Molecular Biology, Nencki Institute, M. Perycz
2012 The Nencki Institute Scientific Council distinction for PhD thesis, A. Malik
2012 PhD in Molecular Biology, Nencki Institute, A. Malik
2012 Mazovia 1-year PhD Scholarship A. Urbańska
2012 Homing Plus from Foundation for Polish Science, E. Liszewska
2012 Fuga from National Science Center, B. Tarkowski
2011 EMBO Long-Term Scholarship for postdoctoral training at Broad Institute, Ł. Świech
2011 Selection for "Top Innovator 500" Ministerial Program, I. Cymerman
2011 The Nencki Institute Scientific Council distinction for PhD thesis, Ł. Świech
2011 PhD in Molecular Biology, Nencki Institute, Ł. Świech
2011 Mazovia 1-year PhD Scholarship, M. Urbanska

Selected Publications

Publications in 2013

- **Malik AR, Urbanska M, Gozdz A, Swiech LJ**, Nagalski A, **Perycz M, Blazejczyk M, Jaworski J**. Cyr61, a matricellular protein, is needed for dendritic arborization of hippocampal neurons. J Biol Chem, 2013; 288(12):8544-59
- **Macias M, Blazejczyk M**, Kazmierska P, Caban B, **Skalecka A, Tarkowski B**, Rodo A, Konopacki J, **Jaworski J**. Spatiotemporal Characterization of mTOR Kinase Activity Following Kainic Acid Induced Status Epilepticus and Analysis of Rat Brain Response to Chronic Rapamycin Treatment. PLoS One, 2013; 8(5):e64455
- **Malik AR**, **Urbanska M**, **Macias M**, **Skalecka A**, **Jaworski J**. Beyond control of protein translation: What we have learned about the non-canonical regulation and function of mammalian target of rapamycin (mTOR). Biochim Biophys Acta – Proteins and Proteomics, 2013; 1834(7):1434-48
- Kuzniewska B, Rejmak E, **Malik AR, Jaworski J**, Kaczmarek L, Kalita K. Brain-derived neurotrophic factor induces matrix metalloproteinase 9 expression in neurons via the serum response factor/c-Fos pathway. Mol Cell Biol, 2013; 33(11):2149-62
- **Lipka J**, Kuijpers M**, Jaworski J**, Hoogenraad CC. Mutations in cytoplasmic dynein and its regulators cause malformations of cortical development and neurodegenerative diseases. Biochem Soc Trans, 2013; 41(6):1605-12
- van Spronsen M, Mikhaylova M, **Lipka J**, Schlager MA, van den Heuvel DJ, Kuijpers M, Wulf PS, Keijzer N, Demmers J, Kapitein LC, Jaarsma D, Gerritsen HC, Akhmanova A, Hoogenraad CC. TRAK/Milton Motor-Adaptor Proteins Steer Mitochondrial Trafficking to Axons and Dendrites. Neuron, 2013; 77(3):485-502
- Kapitein LC, van Bergeijk P, **Lipka J**, Keijzer N, Wulf PS, Katrukha EA, Akhmanova A, Hoogenraad CC. Myosin-V Opposes Microtubule-Based Cargo Transport and Drives Directional Motility on Cortical Actin. Curr Biol, 2013; 23(9):828-34
- Janusz A, Milek J, **Perycz M**, Pacini L, Bagni C, Kaczmarek L, Dziembowska M. The fragile x mental retardation protein regulates matrix metalloproteinase 9 mRNA at synapses. J Neurosci, 2013; 33(46):18234-41
- Bedzhov I, Alotaibi H, Basilicata MF, Ahlborn K, **Liszewska E**, Brabletz T, Stemmler MP. Adhesion, but not a specific cadherin code, is indispensable for ES cell and induced pluripotency. Stem Cell Res, 2013; 11(3):1250-1263

Other selected publications

- Knapska E#, **Macias M**, Mikosz M, Nowak A, Owczarek D, Wawrzyniak M, **Pieprzyk M**, **Cymerman IA**, Werka T, Sheng M, Maren S, **Jaworski J**#, Kaczmarek L#. Functional anatomy of neural circuits regulating fear and extinction. Proc Natl Acad Sci, 2012; 109(42):17093-8; # - corresponding authors
- **Urbanska M, Gozdz A, Swiech LJ, Jaworski J.** Mammalian target of rapamycin complex 1 (MTORC1) and 2 (MTORC2) control the dendritic arbor morphology of hippocampal neurons. J Biol Chem, 2012; 287(36):30240-56
- **Perycz M, Urbanska AS, Krawczyk PS, Parobczak K, Jaworski J.** Zipcode Binding Protein 1 Regulates the Development of Dendritic Arbors in Hippocampal Neurons. J Neurosci, 2011; 31(14):5271–85
- **Swiech L, Blazejczyk M, Urbanska M, Pietruszka P**, Dortland B. R, **Malik AR**, Wulf P. S, Hoogenraad C. C, **Jaworski J.** CLIP-170 and IQGAP1 Cooperatively Regulate Dendrite Morphology. J Neurosci, 2011; 31(12):4555-68
- **Jaworski J**, Kapitein LC, Montenegro Gouveia S, Dortland BR, Wulf PS, Grigoriev I, Camera P, Spangler SA, di Stefano P, Demmers J, Krugers H, Defi lippi P, Akhmanova A, Hoogenraad CC. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity, Neuron, 2009; 61:85-100
- **Swiech L, Perycz M, Malik A, Jaworski J.** Role of mTOR In physiology and pathology of the nervous system. Biochim Biophys Acta – Proteins and Proteomics, 2008; 1784:116-132
- *__Jaworski J__, Spangler S, Seeburg DP, Hoogenraad CC, Sheng M. Control of dendritic arborization by the PI3-kinase – Akt –mTOR pathway. J Neurosci, 2005; 25:11300-12
- *Dunah AW, Hueske E, Wyszynski M, Hoogenraad CC, **Jaworski J**, Pak DT, Simonetta A, Liu G, Sheng M. LAR receptor protein tyrosine phosphatases in the development and maintenance of excitatory synapses in hippocampal neurons. Nat Neurosci, 2005; 8:458-467
- *Chang CJ, **Jaworski J**, Nolan EM, Sheng M, Lippard SJ. A tautomeric zinc sensor for ratiometric fluorescence imaging: Application to nitric oxide-induced release of intracellular zinc. Proc Natl Acad Sci USA, 2004; 101:1129-34
- *__Jaworski J__, Mioduszewska B, Sanchez-Capelo A, Figiel I, Habas A, Gozdz A, Proszynki T, Hetman H, Mallet J, Kaczmarek L. Inducible cAMP Early Repressor (ICER), an endogenous antagonist of cAMP responsive element binding protein (CREB) evokes neuronal apoptosis *in vitro*. J Neurosci, 2003; 23:4519-26
- *__Jaworski J__, Biederman I, Lapinska J, Szklarczyk AW, Figiel I, Konopka D, Nowicka D, Filipkowski RK, Hetman M, Kowalczyk A, Kaczmarek L. Neuronal excitation driven and AP-1-dependent activation of timp1 gene expression in rodent hippocampus. J Biol Chem, 1999; 274: 28106-12

*no IIMCB affiliation
Co-workers affiliated with IIMCB are given in bold

Description of Current Research

Establishing proper neuronal morphology is necessary for correct brain function. Dendrites are the main site of information input into neurons, and different neurons have distinctive and characteristic dendrite branching patterns. Dendritic arbor development is a multi-step process that is controlled by both external signals and intrinsic genetic programs. Among the proteins that transduce extracellular or cell-surface signals into changes in dendritic arbor and dendritic spine shape is mammalian/mechanistic target of rapamycin (mTOR). mTOR is a serine-threonine kinase involved in almost every aspect of mammalian cell function. It forms two protein complexes, initially identified as translation-regulating (mTORC1) or influencing the actin cytoskeleton (mTORC2). In fact, my postdoctoral work showed that mTOR-dependent translation regulation contributes to dendritogenesis (Jaworski et al., 2005). However, the list of cellular processes that involve both mTORCs has expanded, and new ways of regulating mTORC activity, novel mTOR partners, and mTOR effectors have been discovered. Nonetheless, their contribution to the neuronal functions of mTOR and neuropathology is poorly understood. Therefore, since the inception of our laboratory, we have sought to identify mTOR partners and regulated proteins involved in the processes of dendritic branching and synapse formation and stabilization and characterization of mTOR dysfunction in neuropathology. To reach our scientific objectives, we have been using a well-established, relatively simple, and robust model of dendritogenesis of neurons cultured *in vitro*. Using this approach, we previously performed both proof-of-principle experiments and unbiased screens that clearly showed that mTOR functions during neuronal development beyond the canonical control of translation. This research showed that (*i*) CLIP-170, a non-canonical substrate of mTORC1 (Swiech et al., 2011), and (*ii*) mTORC2 are needed for proper dendritogenesis (Urbanska et al., 2012). We also identified a matricellular protein, Cyr61, as a potential downstream effector of mTOR that is regulated at the transcriptional level (Malik et al., 2013). Collectively, these findings suggest that we are still far from revealing the full complexity of the mTOR signaling network, both in neurons and in non-neuronal cells. However, the results of our shRNA screens combined with the results of proteomic analysis of mTOR interactions at the subcellular level resulted in narrowing our research toward identifying the cellular compartment-specific regulation and functions of mTOR in neurons with a special focus on membrane trafficking events. To raise our research efforts on these topics to the highest level, we have begun establishing adequate animal models to confirm the *in vivo* relevance of our *in vitro* culture findings (e.g., *in vivo*

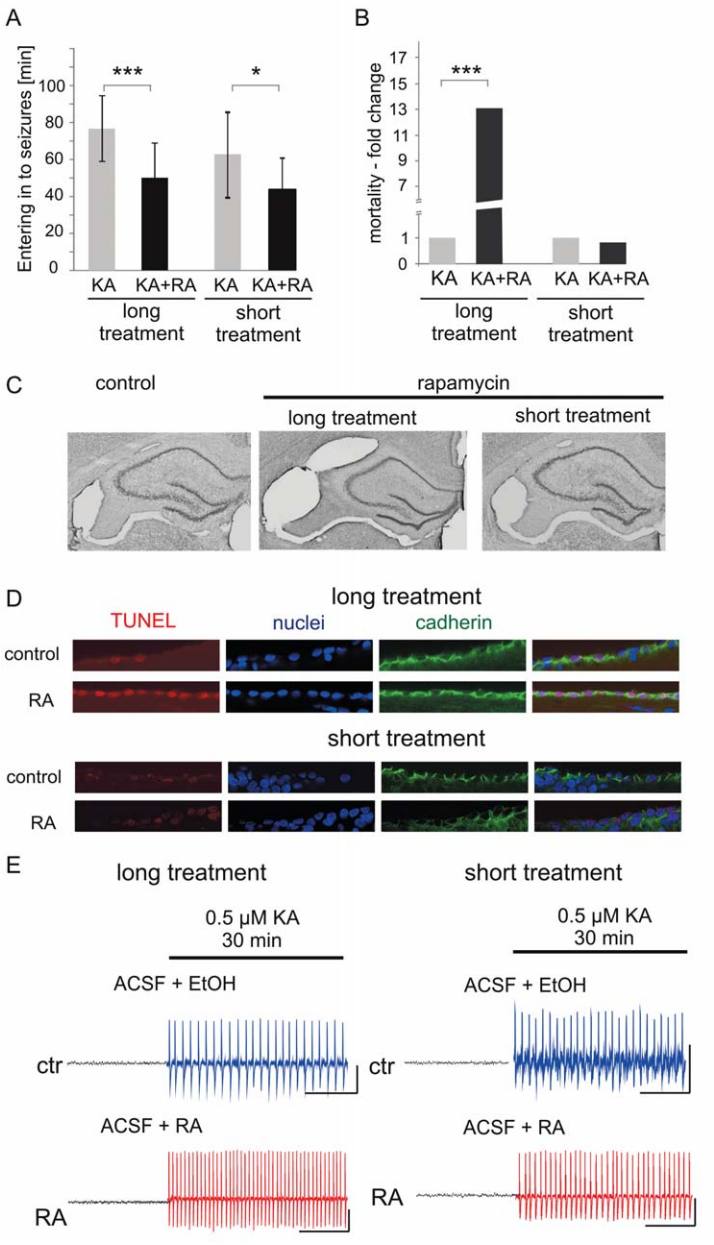


Fig. 1. Effects of chronic rapamycin treatment on brain morphology and physiology (A, B) Analysis of mean latency from kainic acid (KA) injection to stage 3 seizures (A) and mortality (B), induced by KA in rats treated for either one week (short treatment) or four weeks (long treatment) with vehicle or rapamycin (RA). Error bars represent the standard deviation. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; Mann Whitney U-test. **(C)** Representative images of Nissl-stained hippocampal sections in animals after either short or long treatment with RA. **(D)** Representative confocal images of ependymal cells surrounding lateral ventricle, stained for presence of apoptotic cells (TUNEL staining, red) and counterstained with cadherin antibody to visualize ependymal cells (green) and nuclear dye Hoechst 33258 (blue), obtained from animals after after either short or long treatment with RA. **(E)** Analogue examples of epileptiform activity recorded in 30 min after KA administration (0.5 µM) in CA3c region of hippocampal slices delivered from animals treated for either 1 or 4 weeks with vehicle (ACSF+EtOH, marked blue) or RA (ACSF+RA, marked red). *Photo Matylda Macias and Agnieszka Skałecka. Recordings performed in Konopacki Lab, University of Łódź, Poland. More details can be found in Masias et al. (2013).*

electroporation of a brain, mutant zebrafish). In parallel, we have been intensively working with clinically relevant models to study the neuronal dysfunction of mTOR, namely in animal models of seizures, subependymal giant astrocytomas (SEGAs), and induced pluripotent stem cells (iPSC) from patients with tuberous sclerosis (TSC), a disease most likely caused by mTOR hyperactivation. In 2013, in addition to developing a new research line that concerns the cell compartment-specific role of mTOR in neurons, significant efforts have been made in investigating these clinically relevant topics.

Epilepsy is a chronic neurological disorder with a complex pathogenesis. Triggers of epileptogenesis still remain largely unexplored, but this process is accompanied by reactive gliosis, neuronal loss, and neuronal circuitry rearrangements. Genetic disorders characterized by mTOR hyperactivity (e.g., TSC) are often associated with a high probability of epilepsy. Several groups have recently reported that seizures increase mTOR activity, and such an increase in activity in genetic animal models can contribute to spontaneous seizures. However, the consequences of insufficient mTOR activity on seizure induction (status epilepticus) have been thus far poorly understood. Therefore, we systematically investigated this issue over the last few years. In 2013, we extended our observations of the timeline of adverse effects of pharmacologically inhibiting mTOR with rapamycin treatment on brain morphology and function. Our studies revealed that chronic rapamycin treatment for 4-6 weeks led to increased mortality upon the induction of status epilepticus with kainic acid (KA; Macias et al., 2013). We also observed hydrocephaly, the apoptosis of brain ventricle ependymal cells, and a more rapid response to KA in animals chronically treated with rapamycin. This diversity of rapamycin's side effects obscured a clear understanding of why rats with lower mTOR activity exhibit an increase in KA sensitivity. To find a primary cause, in collaboration with the Konopacki laboratory from Łódź, we monitored the behavioral, anatomical, and electrophysiological responses to rapamycin for 1 week after drug application. This approach revealed that the shorter treatment duration, similarly to chronic treatment, was capable of accelerating status epilepticus in response to KA and decreased the threshold of electrophysiological responses to KA (Fig. 1). We did not observe any anatomical changes, cell death, or KA-induced mortality (Fig. 1). As rapamycin treatment was extended, the responses to KA became stronger at both the behavioral and electrophysiological levels, and changes in brain anatomy became evident (Macias et al. 2013; Fig. 1). These observations clearly suggest that the acceleration of KA-induced status epilepticus in rapamycin-treated mice reflects changes in synaptic transmission rather than anatomical abnormalities. Higher mortality can stem from both neuronal overexcitation and brain damage. We believe that our observations contribute to the very vivid scientific discussion regarding the potential role of mTOR in the epileptic brain and therapeutic potential of mTOR inhibitors. Our observation of very pronounced effects of rapamycin on the brain should also be of interest to neurobiologists who work in the fields of brain plasticity, feeding behavior, aging, and neurodegenerative disorders, in which mTOR has recently gained much attention.

Tuberous sclerosis is a multiorgan disease caused by mutations in the mTORC1 inhibitors hamartin and tuberin (TSC1 and TSC2, respectively) and is characterized by epilepsy, autism, and the formation of benign tumors in the brain, among other symptoms. Improper proliferation, migration, differentiation, and synaptic transmission are believed to be the major mechanisms that underlie the brain manifestations of TSC. Still unknown, however, is how mTOR contributes to the development of the disease. Additionally, much evidence from research on cells and animals experimentally devoid of either the *Tsc1* or *Tsc2* gene suggests that *TSC* mutations can alter translation, cause major transcriptomic and metabolic changes, and possibly affect cellular transport. These observations should be thoroughly studied in clinically relevant models (e.g., SEGAs, iPSCs) that can also be used to screen drugs that are complementary

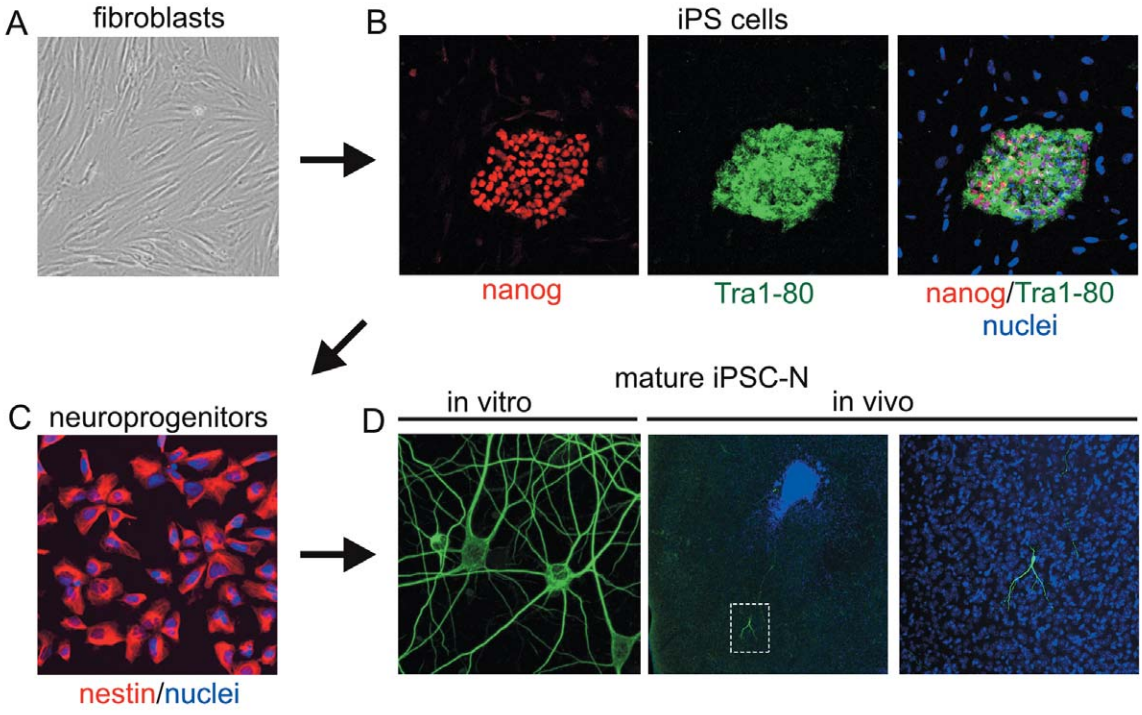


Fig. 2. Representative micrographs of cells at different stages of reprogramming of fibroblasts to neurons. (A) human primary fibroblasts from skin biopsy cultured *in vitro*. **(B)** confocal image of iPS cells colony cultured in vitro immunostained for pluripotency markers – Nanog and Tra1-80. **(C)** confocal image of iPS cells-derived neuroprogenitors in culture *in vitro* immunostained for nestin. **(D)** confocal images of iPS-cells derived neurons cultured in vitro (left panel) and upon transplantation to the mouse brain. *Photo by Ewa Liszewska*

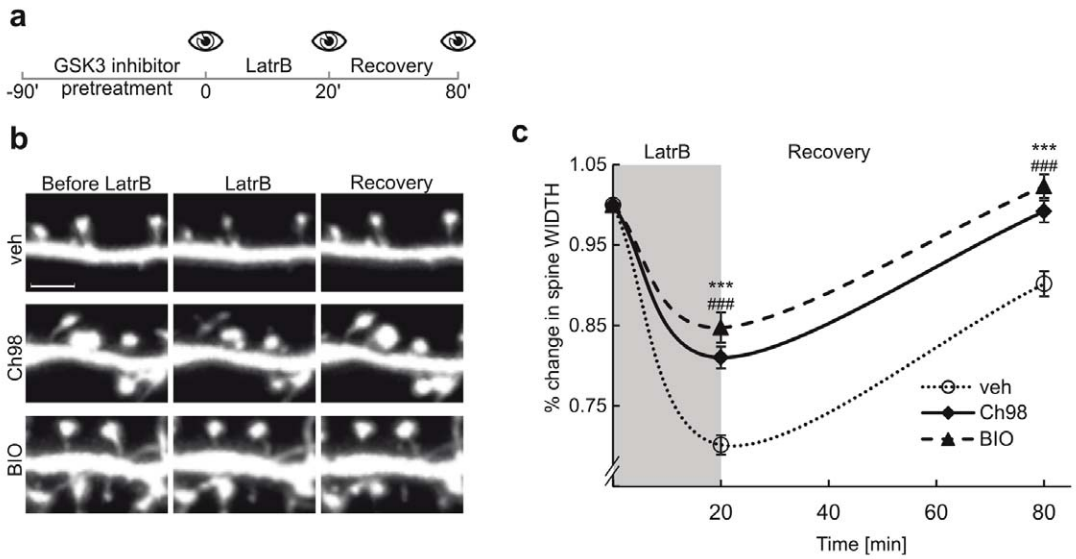
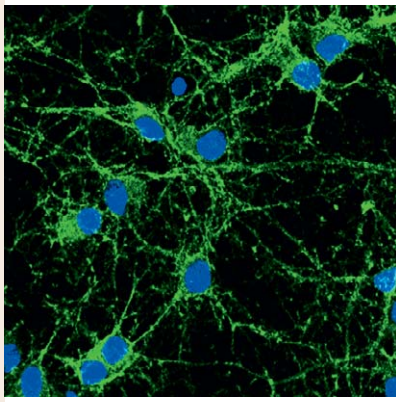


Fig. 3. GSK3 pharmacological inhibition hinders dendritic spine structural plasticity. (A) Schematic representation of experiment setup. **(B)** Representative images of cultured murine hippocampal 18 DIV neurons illustrate the differences in spine shape changes induced by LatrunculinB treatment and spine shape recovery after LatrunculinB removal under basal conditions and after prior GSK3 inhibition. (**C**) Timecourse of spine shape changes, expressed as % change relative to the initial values. * and # indicate statistical significance for measurements of spines upon GSK3 inhibition with Ch98 and BIO respectively when compared to the control values at the corresponding time points; p<0.05. *Photo by Iwona Cymerman and Agata Góźdź*

to rapamycin. Thus, one of our substantial achievements in modeling this mTOR-related disease was establishing protocols for iPSC work *in vitro* and *in vivo*. In 2013, Dr. Ewa Liszewska optimized the production of iPSC cells from skin biopsies and their differentiation *in vitro* to an expandable population of neuroprogenitors (Fig. 2). These iPSC-derived neuroprogenitors were then grafted to the subventricular zone (SVZ), striatum, cortex, and hippocampi in mice of different ages. The most promising results were obtained after cell transplantation to the SVZ at early postnatal stages. Under such conditions, transplanted iPSC-derived neuroprogenitors populated the neurogenic niche of the SVZ, migrated to several brain areas, and developed further into mature neurons (Fig. 2). Using those optimized protocols, Dr. Liszewska was also able to obtain iPSCs from three patients who carried mutations in the *TSC1* gene, which is responsible for TSC development and mTOR hyperactivation. These neuroprecursors are now being characterized both *in vitro* and *in vivo* for defects typical of TSC. Notably, our laboratory was among the first in Poland to successfully obtain human iPSCs from patients.

The EPISTOP project consortium was also initiated in 2013 (http://www.epistop.eu), in which our laboratory participates. The main aim of EPISTOP is to identify the molecular predictors of epileptogenesis progression in TSC patients. Our team, together with others, will search for potential markers of epileptogenesis identified primarily by RNAseq and proteomics of blood samples and cortical tubers of TSC patients. This important project should result in new approaches to TSC treatment. It will also generate vast amounts of data on transcriptomic and proteomic changes in TSC that can serve as a starting point to dissect the mTOR-dependent and -independent aspects of TSC, epilepsy, and autism spectrum disorders.

In 2013, we also finalized an important project that stemmed from our interest in cross-talk between mTOR and GSK3 kinases in the context of both brain physiology and pathology (e.g. Alzheimer's disease). This particular line of research led to the important discovery of a unique function of GSK3α in the structural plasticity of dendritic spines. Although GSK3 kinases have been known to affect long-term synaptic plasticity, no data were available on their role in structural plasticity (e.g., plasticity-induced changes in the shape of dendritic spines). This is an intriguing inconsistency because the major postulate of Ramon y Cajal and his adherents is that any significant changes in synaptic plasticity should be followed by structural adjustments (which is currently dogma in modern neurobiology). For example, the long-term depression (LTD) of a given synapse is reflected by shrinkage of a corresponding dendritic spine. We hypothesized that this inconsistency regarding the participation of GSK3 in synaptic and structural plasticity reflects an effect of the experimental approaches utilized to date (i.e., investigations of steady-state situations under basal conditions) rather than the sole participation of GSK3 in synaptic plasticity. Using a combination of pharmacological and genetic approaches with time-lapse spine imaging, Dr. Cymerman and Dr. Góźdź demonstrated that GSK3, particularly its mysterious isoform GSK3α, is key for the induction of structural changes upon chemical LTD, and most likely the actin cytoskeleton is an important substrate downstream of this kinase. Our study also discovered a particular biological process that differentially requires both GSK3 isoforms. Intriguingly, the sole involvement of GSK3α was specific for chemical LTD-induced dendritic spine changes. When spine changes were induced with the addition of latruncullin, an actin depolymerization inducer, spine shape recovery required the presence of both GSK3α and GSK3β (Fig. 3).



Rat cortical neurons at 7 div in culture. Blue (nuclei), green (b-catenin). Author: Katarzyna Misztal.

Neurodegeneration Laboratory

Vice Head:
Urszula Wojda, PhD, Professor (until June 2013)

Senior Scientists:
Tomasz Węgierski, PhD
Marta Wiśniewska, PhD, DSc Habil (until August 2013)

Senior Postdoctoral Fellow:
Joanna Gruszczyńska-Biegała, PhD (maternal leave)

Postdoctoral Fellows:
Magda Czeredys, PhD
Łukasz Majewski, PhD
Smijin Karthully Soman, PhD (since March 2013), FishMed

FishMed Technical Assistant:
Michał Bazała, MSc, joint with Mitochondrial Biogenesis Laboratory

Junior Researchers:
Kinga Gazda, MSc in Engineering
Anna Jaworska, MSc (maternal leave)
Andrzej Nagalski, MSc (until October 2013)
Łukasz Szewczyk, MSc
Aleksandra Szybińska, MSc

MSc Students:
Aleksandra Kurek (since June 2013)

Technician:
Elżbieta Grzelak

Current affiliations of some former PhD students and coworkers

- Wojciech Michowski, postdoctoral research fellow, Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA
- Katarzyna Misztal, postdoctoral research fellow, Laboratory of Structural Biology, IIMCB
- Andrzej Nagalski, PhD student, Laboratory of Molecular Neurobiology, CeNT, University of Warsaw
- Adam Sobczak, postdoctoral research fellow, Institute of Biochemistry and Biophysics PAN, and Bio&Technology Innovations Platform of BioCentrum Ochota (BioTech-IP)
- Marta B. Wiśniewska, research group leader, Laboratory of Molecular Neurobiology, CeNT, Uni of Warsaw
- Urszula Wojda, research group leader, Laboratory of Advanced Preclinical Studies, Neurobiology Centre at the Nencki Institute of Experimental Biology

Lab leader:
Jacek Kuźnicki,
PhD, Professor

Degrees:

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

Postdoctoral Training

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

Professional Employment:

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

Membership in Scientific Societies, Organizations, and Panels:

Jul 1, 2013 –	Dec 31, 2013 President, Biocentrum Ochota Consortium (rotating presidency)
2012-2014	Expert, National Science Centre
Jul 1, 2012 –	Dec 31, 2012 President of the Science Policy Committee at the Ministry of Science and Higher Education (rotating presidency); member since 2011
2012-Present	Board Member of Marceli Nencki's Foundation to Support the Biological Sciences
2011-Present	Member, International Expert Council of the Research and Education Center, State Key Laboratory of Molecular and Cellular Biology (SKL) in Ukraine
Oct-Nov 2011	Chairman of the Commission for the Assessment of Property and Legal and Organizational Joined PAN Scientific Units (units operating under the name of the Department of Antarctic Biology Polish Academy of Sciences and Institute of Biochemistry and Biophysics)
2011-Present	Member, BIO-IMAGINE Steering Committee, 7th Framework Program at the Nencki Institute of Experimental Biology
2011-Present	Member, Science Policy Committee, Ministry of Science and Higher Education
Jul 1, 2010 –	Dec 31, 2010 President, Consortium Biocentrum Ochota (rotating presidency)
2010-Present	Member, Society for Neuroscience
2008-2010	Head, Scientific and Organizing Committees, 11th Meeting of the European Calcium Society
2009-Present	Member, Polish Alzheimer's Society
2008-Present	Board Member, European Calcium Society
2006-Present	Member, Health Research Advisory Group, 7th Framework Program European Commission
2004-Present	Member, Polish Academy of Sciences
2003-Present	Member, American Society for Biochemistry and Molecular Biology
2002-Present	Head, Advisory Board, Centre for Innovative Bioscience Education
1991-Present	Member, Polish Neuroscience Society
1991-2009	Member, Polish Society for the Advancement of Science and Arts
1996-1999, 2000-2002	Vice-President, Polish Biotechnology Committee
1990-2002	Member, Polish Biotechnology Committee
1989-1992	Co-Editor, *Advances in Biochemistry* (published in Polish)
1989-1991	General Secretary, Polish Biochemical Society
1977-Present	Member, Polish Biochemical Society

Honors, Prizes, and Awards:

2013	Award of the 2nd Division of Biological and Agricultural Sciences of the Polish Academy of Sciences for Marta B. Wiśniewska, Katarzyna Misztal, Andrzej Nagalski and Jacek Kuźnicki for a series of research papers entitled *β- catenin as a factor that influences the excitability of thalamic neurons by regulating gene expression*
2013	Crystal Brussels Prize for outstanding achievements in 7th Framework Programme of the European Union for Research and Development
2011	Konorski Award for the best Polish research work in neurobiology (awarded by the Polish Neuroscience Society and Committee on Neurobiology of PAN)
2008	Officer's Cross of the Order of Polonia Restituta (awarded by the President of the Republic of Poland)
2004-2008	Professorial Subsidy Program Award, Foundation for Polish Science
2003	Prime Minister Award for Scientific Achievement
2001	Award from the Division of Biological Sciences, Polish Academy of Sciences (for work on calcium binding proteins)
1998	Knight's Cross of the Order of Polonia Restituta (awarded by the President of the Republic of Poland)
1987	Polish Anatomical Society Award for article on calcium binding proteins (*Advances in Cell Biology*)
1986	Skarżyński Award, Polish Biochemical Society (for best review article in *Advances in Biochemistry*)
1977	Parnas Award, Polish Biochemical Society (for publishing the best paper in biochemical research)
1977	Mozołowski Award, Polish Biochemical Society (for outstanding young Polish biochemists)
1976	MSc, Magna cum laude, University of Warsaw, Poland

Selected Publications

- **Czeredys M**, **Gruszczynska-Biegala J**, Schacht T, Methner A, **Kuznicki J**. Expression of genes encoding the calcium signalosome in cellular and transgenic models of Huntington's disease. ***Front Mol Neurosci***, 2013; 6:42
- Honarnejad K, Daschner A, Giese A, Zall A, Schmidt B, **Szybinska A**, **Kuznicki J**, Herms J. Development and implementation of a high-throughput compound screening assay for targeting disrupted ER calcium homeostasis in Alzheimer's disease. ***PLoS One***, 2013; 8(11):e80645
- Honarnejad K, Kirsch AK, Daschner A, **Szybinska A**, **Kuznicki J**, Herms J. FRET-based calcium imaging: a tool for high-throughput/content phenotypic drug screening in Alzheimer disease. ***J Biomol Screen***, 2013; 18(10):1309-20
- **Gruszczynska-Biegala J**, **Kuznicki J**. Native STIM2 and ORAI1 proteins form a calcium-sensitive and thapsigargin-insensitive complex in cortical neurons. ***J Neurochem***, 2013; 126(6):727-38
- **Jaworska A**, Dzbek J, Styczynska M, **Kuznicki J**. Analysis of calcium homeostasis in fresh lymphocytes from patients with sporadic Alzheimer's disease or mild cognitive impairment. ***BBA - Mol Cell Res***, 2013; 1833(7):1692-9
- **Wojda U**, **Kuznicki J**. Alzheimer's disease modeling: ups, downs, and perspectives for human induced pluripotent stem cells. ***J Alzheimers Dis***, 2013; 34(3):563-88. Review
- Esteras N, Alquézar C, Bermejo-Pareja F, **Bialopiotrowicz E**, **Wojda U**, Martín-Requero A. Downregulation of extracellular signal-regulated kinase 1/2 activity by calmodulin KII modulates p21Cip1 levels and survival of immortalized lymphocytes from Alzheimer's disease patients. ***Neurobiol Aging***, 2013; 34(4):1090-100
- Golanska E, Sieruta M, Corder E, Gresner SM, Pfeffer A, Chodakowska-Zebrowska M, Sobow TM, Klich I, Mossakowska M, **Szybinska A**, Barcikowska M, Liberski PP. The prion protein M129V polymorphism: longevity and cognitive impairment among Polish centenarians. ***Prion***, 2013; 7(3):244-247
- Golanska E, Sieruta M, Gresner SM, Pfeffer A, Chodakowska-Zebrowska M, Sobow TM, Klich I, **Szybinska A**, Barcikowska M, Liberski PP. APBB2 genetic polymorphisms are associated with severe cognitive impairment in centenarians. ***Exp Gerontol***, 2013; 48(4):391-394
- **Wisniewska MB**. Physiological role of β-catenin/TCF signaling in neurons of the adult brain. ***Neurochem Res***, 2013; 38(6):1144-55 Review
- **Nagalski A**, Irimia M, **Szewczyk L**, Ferran JL, **Misztal K**, **Kuznicki J**, **Wisniewska MB**. Postnatal isoform switch and protein localization of LEF1 and TCF7L2 transcription factors in cortical, thalamic, and mesencephalic regions of the adult mouse brain. ***Brain Struct Funct***, 2013; 218(6):1531-49
- Malik AR, Urbanska M, Gozdz A, Swiech LJ, **Nagalski A**, Perycz M, Blazejczyk M, Jaworski J. Cyr61, a matricellular protein, is needed for dendritic arborization of hippocampal neurons. ***J Biol Chem***, 2013; 288(12):8544-59
- **Węgierski T**. Mikroskopowe metody rejestracji wewnątrzkomórkowych odpowiedzi wapniowych. ***Kosmos***, 2013, 62, 2(299):193-203 (in Polish)
- **Wisniewska MB, Nagalski A**, **Dabrowski M, Misztal K, Kuznicki J**. Novel β-catenin target genes identified in thalamic neurons encode modulators of neuronal excitability. ***BMC Genomics***, 2012; 13:635
- **Bialopiotrowicz E, Szybinska A**, Kuzniewska B, Buizza L, Uberti D, **Kuznicki J, Wojda U**. Highly pathogenic Alzheimer's disease presenilin 1 P117R mutation causes a specific increase in p53 and p21 protein levels and cell cycle dysregulation in human lymphocytes. ***J Alzheimers Dis***, 2012; 32(2):397-415
- **Bialopiotrowicz E**, **Kuzniewska B**, **Kachamakova-Trojanowska N**, Barcikowska M, **Kuznicki J**, **Wojda U**. Cell cycle regulation distinguishes lymphocytes from sporadic and familial AD patients. ***Neurobiol Aging***, 2011; 32(12):2319.e13-26
- **Gruszczynska-Biegala J**, Pomorski P, **Wisniewska MB**, **Kuznicki J**. Differential Roles for STIM1 and STIM2 in Store-Operated Calcium Entry in Rat Neurons. ***PLoS One***, 2011 Apr 26;6(4):e19285
- **Misztal K**, **Wisniewska MB**, **Ambrozkiewicz M**, **Nagalski A**, **Kuznicki J**. Wnt-independent constitutive nuclear localization of b-catenin and its low degradation rate in thalamic neurons. ***J Biol Chem***, 2011, 286(36):31781-8
- **Sobczak A**, **Debowska K**, **Blazejczyk M**, Kreutz MR, **Kuznicki J**, **Wojda U**. Calmyrin1 binds to SCG10 protein (stathmin2) to modulate neurite outgrowth. ***BBA - Mol Cell Res***, 2011; 1813(5):1025- 37
- Steinbeck JA, Henke N, Opatz J, **Gruszczynska-Biegala J**, Schneider L, Theiss S, Hamacher N, Steinfarz B, Golz S, Brustle O, **Kuznicki J**, Methner A. Store-operated calcium entry modulates neuronal network activity in a model of chronic epilepsy. ***Exp Neurol***, 2011; 232(2):185-194
- **Wisniewska M**, **Misztal K**, **Michowski W**, Szczot M, Purta E, Lesniak W, **Klejman M**, Dąbrowski M, Filipkowski R, **Nagalski A**, Mozrzymas J, **Kuznicki J**. LEF1/beta-catenin complex regulates transcription of the Cav3.1 calcium channel gene (Cacna1g) in thalamic neurons of the adult brain. ***J Neurosci***, 2010; 30:4957-69
- **Michowski W**, Ferretti R, **Wisniewska MB**, **Ambrozkiewicz M**, Beresewicz M, Fusella F, **Skibinska- Kijek A**, Zablocka B, Brancaccio M, Tarone G, **Kuznicki J**. Morgana/CHP-1 is a novel chaperone able to protect cells from stress. ***BBA - Mol Cell Res***, 2010; 1803:1043-9
- **Blazejczyk M**, **Sobczak A**, **Debowska K**, **Wisniewska MB**, Kirilenko A, Pikula S, Jaworski J, **Kuznicki J**, **Wojda U**. Biochemical characterization and expression analysis of a novel EF hand Ca^{2+} binding protein calmyrin2 (Cib2) in brain indicates its function in NMDA receptor mediated Ca^{2+} signaling. ***Arch Biochem Biophys***, 2009; 487:66-78
- **Bojarski L**, Pomorski P, **Szybinska A**, **Drab M**, **Skibinska-Kijek A**, **Gruszczynska-Biegala J**, **Kuznicki J**. Presenilindependent expression of STIM proteins and dysregulation of capacitative Ca^{2+} entry in familial Alzheimer's disease. ***BBA - Mol Cell Res***, 2009; 1793:1050-7
- **Klejman M**, **Gruszczynska-Biegala J**, **Skibinska-Kijek A**, **Wisniewska MB**, **Misztal K**, **Blazejczyk M**, **Bojarski L**, **Kuznicki J**. Expression of STIM1 in brain and puncta-like colocalization of STIM1 and ORAI1 upon depletion of Ca^{2+} store in neurons. ***Neurochem Int***, 2009; 54:49-55
- Puzianowska-Kuznicka M, **Kuznicki J**. The ER and ageing II: calcium homeostasis. ***Ageing Res Rev***, 2009; 8:160-72. Review
- ***Peng H, Lewandrowski U, Muller B, Sickmann A, Walz G, **Wegierski T**. Identification of a Protein Kinase C-dependent phosphorylation site involved in sensitization of TRPV4 channel. ***Biochem Biophys Res Commun***, 2010; 391:1721-5
- ***Gao H, Wang Y, **Wegierski T**, Skouloudaki K, Putz M, Fu X, Engel C, Boehlke C, Peng H, Kuehn EW, Kim E, Kramer-Zucker A, Walz G. PRKCSH/80K-H, the protein mutated in polycystic liver

disease, protects polycystin-2/TRPP2 against HERP-mediated degradation. ***Hum Mol Genet***, 2010; 19:16-24

- ***Ganner A, Lienkamp S, Schafer T, Romaker D, **Wegierski T**, Park TJ, Spreitzer S, Simons M, Gloy J, Kim E, Wallingford JB, Walz G. Regulation of ciliary polarity by the APC/C. ***Proc Natl Acad Sci USA***, 2009; 106:17799-804
- ***Wegierski T**, Steffl D, Kopp C, Tauber R, Buchholz B, Nitschke R, Kuehn EW, Walz G, Kottgen M. TRPP2 channels regulate apoptosis through the Ca^{2+} concentration in the endoplasmic reticulum. ***EMBO J***, 2009; 28:490-499
- ***Wegierski T**, Lewandrowski U, Muller B, Sickmann A, Walz G. Tyrosine phosphorylation modulates the activity of TRPV4 in response to defined stimuli. ***J Biol Chem***, 2009; 284: 2923-33

***** no IIMCB affiliation
Co-workers affiliated with IIMCB are given in bold

Description of Current Research

We are interested in the molecular mechanisms involved in neurodegeneration and memory formation, with a special emphasis on the role of β-catenin transcription regulation, calcium homeostasis, and signaling. These processes are being studied at the genomic, proteomic, and cellular levels and using zebrafish and mice as model organisms. Our major projects focus on the following:

1. Dysregulation of calcium homeostasis in neurodegenerative diseases.
2. Role of STIM proteins in store-operated calcium entry in neurons.
3. Role and regulation of β-catenin and transcription factors LEF1/TCF in mature neurons.

1. Dysregulation of calcium homeostasis in neurodegenerative diseases.

Calcium dyshomeostasis is an early event in the pathogenesis of neurodegenerative diseases. Therefore, in one project we focused on the expression of calcium-related genes in transgenic mouse models of Alzheimer's disease (AD) and Huntington's disease (HD). We hypothesized that mutated proteins related to AD and HD might affect the expression of components of calcium homeostasis and signaling pathways, thereby initiating or propagating the neurodegenerative processes of AD and HD. To test this hypothesis, we analyzed mRNA levels in the brains of transgenic AD and HD mice using custom-made TaqMan Low Density Microarrays. Some genes whose expression was changed compared with control brains were further analyzed by RT-PCR and Western blot. The overexpression of HAP1, CacyBP/SIP, and Calb2 has been confirmed in HD transgenic mice (*Frontiers in Molecular Neuroscience*, 2013). Moreover, the increased expression of a few other genes was observed, and one of them (*Aph1b*) met the criterion of Bonferroni correction ($p < 0.00052$).

Many studies have shown that disturbed cellular calcium homeostasis is one of the key features of AD. Calcium changes can be observed not only in neurons but also in peripheral cells, such as skin fibroblasts and lymphocytes. Lymphocytes, in contrast to other cell types, can be easily obtained and therefore have great diagnostic potential. Disturbed calcium

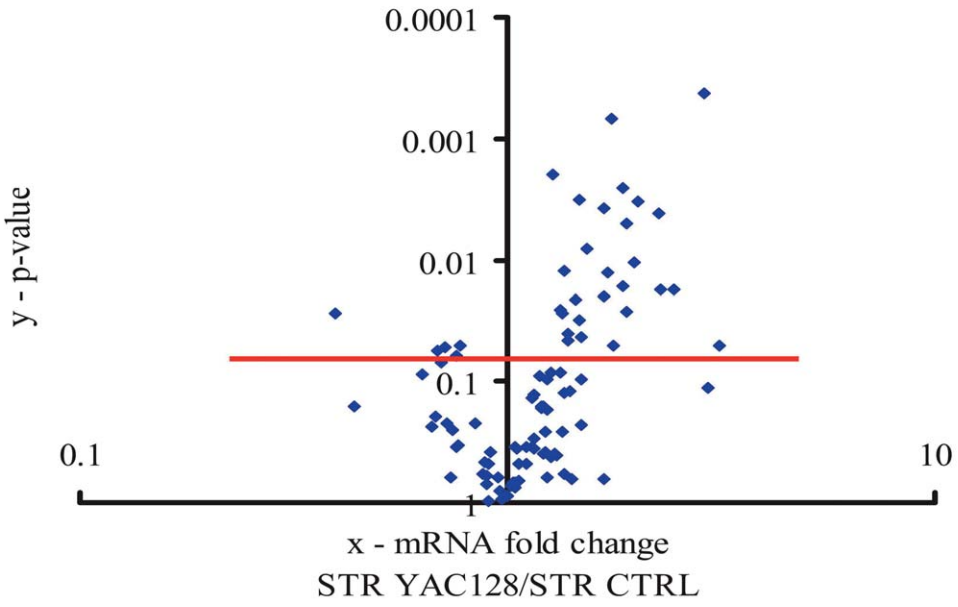


Fig. 1. Expression of calcium signaling and homeostasis genes in the striatum in HD mice. The volcano plot arranges genes along the dimensions of (*x*) mean expression fold difference between the striatum (STR) in YAC128 mice and control mice (CTRL), and (*y*) *p* value (Student's *t*-test). A logarithmic scale is used. Points located above the red lines represent genes whose expression was significantly changed ($p < 0.05$). The results represent data based on three independent mRNA preparations from the striatum in 3-month-old mice.

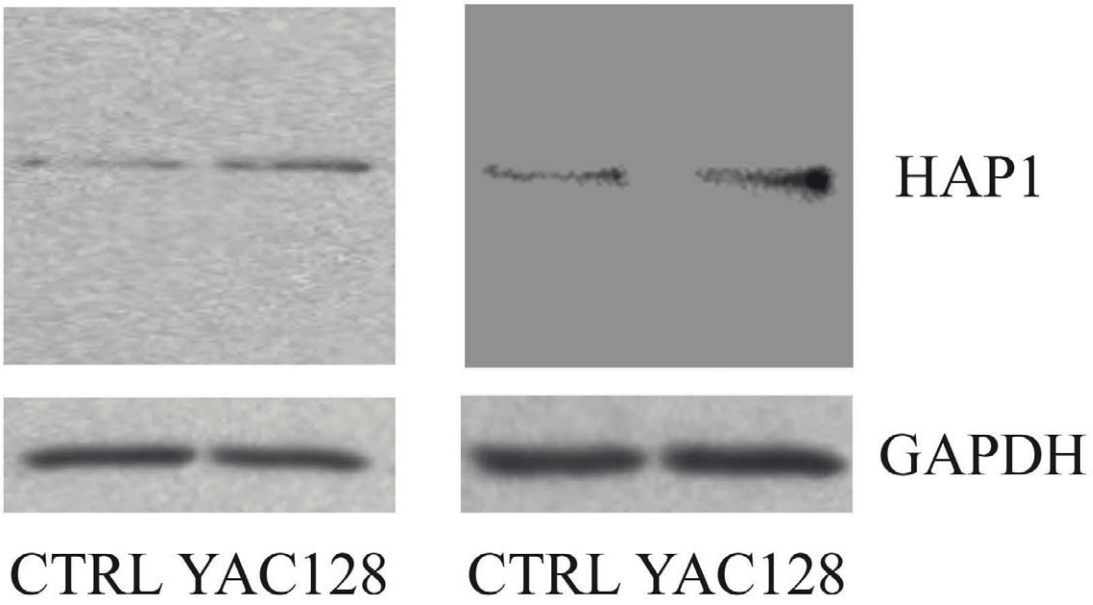


Fig. 2. HAP1 protein expression increases in the striatum in YAC128 mice. Immunoblots of HAP1 in the striatum in two YAC128 mice and age-matched control mice (CTRL) show a 1.9× increase in HAP1 level. HAP1 densitometry was performed using the intensity of GAPDH.

handling was found by many research groups in immortalized human B-lymphocytes derived from patients with an inherited form of AD (i.e., familial AD), but observations of similar changes observed in cells derived from patients with the sporadic form of AD (SAD) are very limited. Mild cognitive impairment (MCI) is found to be a transitional stage between normal aging and dementia. It is often observed in individuals who develop AD later in life and therefore may be considered a risk factor for AD. To explore calcium homeostasis during the early stages of SAD and MCI, we investigated SOCE and inositol triphosphate receptor (IP3R)-mediated calcium release into the cytoplasm in unmodified B-lymphocytes from MCI subjects and SAD patients and compared them with non-demented subjects (NDS). Calcium levels in the endoplasmic reticulum (ER) were similar in all three groups. However, we found that SAD and MCI cells were more prone to IP3R activation than NDS cells. Mild cognitive impairment cells exhibited an enhanced magnitude of calcium influx during SOCE, and MCI cells but not SAD cells were characterized by higher basal cellular calcium levels than NDS cells. In summary, perturbed calcium homeostasis was observed in peripheral cells from MCI and SAD patients, supporting the hypothesis that SAD is a systemic disease, and MCI is a risk factor for AD. Thus, lymphocytes obtained from MCI subjects may be promising in the early diagnosis of individuals who will eventually develop SAD (*BBA Molecular Cell Research*, 2013).

Calcium dyshomeostasis is an early event in the pathogenesis of AD that precedes other disease symptoms and can affect many cellular processes. Drugs with the ability to restore calcium homeostasis to values observed in healthy control cells could be applied as therapeutics in AD. In collaboration with Prof. Jochen Herms at Ludwig Maximilian University, Munich, Germany we screened approximately 20,000 chemical compounds to determine their ability to influence intracellular calcium concentrations. The screen revealed over 300 compounds that decreased calcium levels. To address their putative mechanism of action, almost 160 of the best compounds were chosen for an enzyme-linked immunosorbent assay (ELISA) for γ-secretase activity, whose gain of function is believed to be a major factor in familial AD pathology. Using ELISA, we measured β-amyloid 1-42 levels in HEK293 cells that overexpressed the wildtype or mutated presenilin 1 gene. Only a few compounds decreased β-amyloid 1-42 to control levels; thus, the majority of the compounds that influenced calcium signaling did not affect γ-secretase activity (*PLoS One*, 2013; *Journal of Biomolecular Screening*, 2013).

The vast majority of available animal models of AD are based on the β-amyloid/tau hypothesis. These mice overexpress one or more mutated proteins known to be responsible for early-onset FAD. The FAD models, representing less than 5% of all human cases, appear to have little value for understanding the mechanisms of SAD. In one project, we generated and began to characterize transgenic mouse models that might have dysregulated calcium homeostasis. Transgenic mice will be a suitable model for verifying the hypothesis that sustained increases in basal calcium levels might be one of the early changes that lead to neurodegeneration (*work in progress*).

The genetic manipulation of proteins linked to AD results in disturbances in cellular calcium homeostasis. Specifically, alterations in the receptor-induced release of calcium from the ER and SOCE have been described. These observations support the calcium hypothesis of the development of AD, but the precise mechanisms that underlie the dysregulation of calcium homeostasis in AD models are unclear. We aim to elucidate these mechanisms, particularly whether AD proteins exert a direct regulatory effect on key players of calcium homeostasis, such as the SOCE complex. Using a split-ubiquitin system (i.e., a yeast genetic system) to search for interacting partners, we found a physical interaction between SOCE machinery and proteins crucially involved in the development of neurodegeneration. The interaction was confirmed using independent methodology, such as co-immunoprecipitation and co-immunolocalization assays. The functional relevance of this finding is being studied using overexpression systems and the ablation of gene expression by RNA interference in various cell lines. We are analyzing the regulation of SOCE complexes using PLAs and calcium measurements with the aid of the calcium indicator Fura-2 (*work in progress*).

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

The calcium sensors STIM1 and STIM2, located in the ER, and calcium channel-forming protein ORAI1 are involved in SOCE. The process relies on extracellular calcium influx through plasma membrane channels. In non-excitable cells, the STIM interaction with ORAI1 is a crucial element of SOCE, but its mechanism in neurons remains unclear. We previously showed that STIM1 is likely involved in thapsigargin-induced SOCE, whereas STIM2 is mostly active after the ethylene glycol tetraacetic acid (EGTA)-driven depletion of extracellular calcium (*PLoS One*, 2011). The depletion of calcium from the ER by thapsigargin increased the number of puncta-like colocalization of YFP-STIM1 and ORAI1 but not YFP-STIM2 and ORAI1. In contrast, a reduction of extracellular calcium levels triggered puncta formation for both YFP-STIM1/ORAI1 and YFP-STIM2/ORAI1. As a next step, we focused on detecting complexes that contain endogenous STIM2 and ORAI1. Using a proximity ligation assay (PLA), we were able to visualize fluorescent dots that represent the site where two antibodies are bound: one against ORAI1 and another against STIM2. These dots identified the complexes between STIM2 and ORAI1 (*Journal of Neurochemistry*, 2013). To confirm that the observed PLA dots represented authentic STIM2-ORAI1 complexes, we used different pairs of anti-STIM2 and anti-ORAI1 antibodies. The number of these complexes increased when intracellular and subsequently ER calcium concentrations decreased under the influence of BAPTA-AM or a medium without calcium ions. These results were confirmed by the co-immunoprecipitation of endogenous STIM2 and ORAI1 proteins. We also showed a strong correlation between the number of endogenous STIM2-ORAI1 complexes and calcium responses studied in the same neuronal cell. Our results indicated that STIM2 responds to changes in intracellular calcium levels and the small decrease in calcium levels in the ER in rat cortical neurons by interacting with ORAI1. We are now attempting to confirm these observations in animal models.

3. Role and regulation of β-catenin and transcription factors LEF1/TCF in mature neurons.

β-catenin is a gene expression regulator in the canonical Wnt pathway that is involved in early brain patterning and neurogenesis. Growing evidence also implicates Wnt/β-catenin signaling in the proper functioning of the adult central nervous system. The aberrant regulation of β-catenin has been associated with psychotic and affective disorders (e.g., major depression, bipolar disorder, and schizophrenia) and neurodegenerative diseases (e.g., AD, HD, and Parkinson's disease). However, the physiological role of Wnt/β-catenin in the adult brain has remained elusive. Pioneering research by our group demonstrated that β-catenin is constitutively and specifically present in the nuclei of thalamic neurons, independent of Wnt signaling activation, and associated with low levels of the proteins involved in β-catenin degradation (*Journal of Biological Chemistry*, 2011). Moreover, we demonstrated that β-catenin, together with LEF/TCF transcription factors, regulated the transcription of the *Cacna1g* gene that encodes Cav3.1 voltage-gated calcium channels, contributing to electrical signal propagation in thalamic neurons (*Journal of Neuroscience*, 2010). Recently, we identified new β-catenin target genes in thalamic neurons by combining bioinformatics and experimental approaches: *Gabra3* for the GABA receptor, *Calb2* for the calcium-binding protein calretinin, and *Kcna6* for the voltage-gated potassium channel; *BMC Genomics*, 2012). Two other genes, *Cacna2d2* and *Kcnh8*, appeared to be regulated by β-catenin, but the binding of β-catenin to the regulatory sequences of these genes could not be confirmed. We conclude that β-catenin in the thalamus regulates the expression of a novel group of genes that encode proteins involved in neuronal excitation. This implies that the transcriptional activity of β-catenin is necessary for the proper excitability of thalamic neurons, may influence activity in the thalamocortical circuit, and may contribute to thalamic pathologies. Additionally, we provided a comprehensive analysis of LEF1/TCF protein localization and the expression profile of their isoforms in cortical, thalamic, and midbrain regions in mice. The analysis of alternative splicing and promoter usage identified brain-specific TCF7L2 isoforms and revealed a developmentally coordinated transition in the composition of LEF1 and TCF7L2, suggesting that the role of these proteins in the adult brain might be different from their role in the embryonic brain. One of the projects focuses on involvement of the transcription factors LEF1 and TCF7L2 in β-catenin transport to nuclei of thalamic neurons in the adult brain (*work in progress*; figure below from Dr. Katarzyna Misztal).

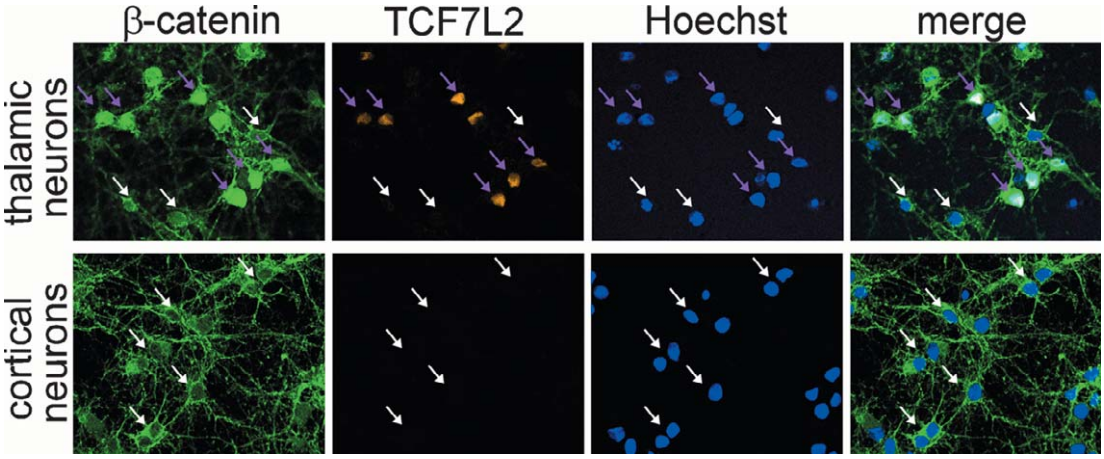


Fig. 3. Colocalization of β-catenin with TCF7L2 in nuclei of thalamic neurons but not cortical neurons: β-catenin (green), TCF7L2 (orange), nuclei (blue). Pink arrows indicate neurons with colocalization of β-catenin with TCF7L2.

Protein Structure Laboratory

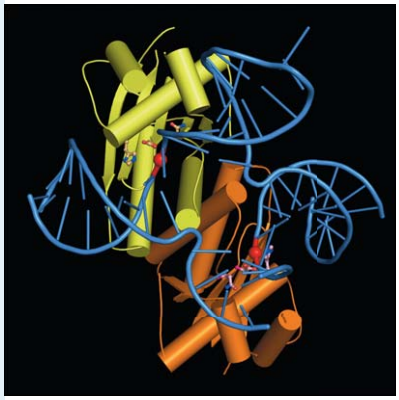


Fig. 1. Crystal structure of RuvC Holliday junction resolvase in complex with DNA substrate (blue). The two subunits of the protein dimer are shown in yellow and orange. The scissile phosphate (site of the cleavage) is shown as red sphere. Active site residues are shown and ball-and-stick.

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Lab Leader:
Marcin Nowotny,
PhD, DSc Habil

Degrees

2013	DSc Habil in Molecular Biology, Institute of Biochemistry and Biophysics, Warsaw, Poland
2002	PhD *magna cum laude* in Biochemistry, under the supervision of Prof. dr hab. Jacek Kuźnicki, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1998	MSc in Organic Chemistry and Biochemistry, Department of Chemistry, Warsaw University, Poland

Postdoctoral Training

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

Professional Employment

2008-Present	Head, Protein Structure Laboratory, IIMCB

Honors, Prizes, Awards

2013	Academia Europea Burgen Scholar
2013	Knight's Cross Polonia Restituta from the President of the Republic of Poland
2012	Polish Prime Minister's Award for scientific achievement
2012	„Ideas For Poland" Award, Foundation for Polish Science
2012	Jan Karol Parnas Award for the best Polish biochemical publication
2012	Wellcome Trust Senior Research Fellowship (renewal)
2012	HHMI Early Career Scientist Award
2011	ERC Starting Grant
2007	EMBO Installation Grant
2007	Wellcome Trust Senior Research Fellowship
2003	Prime Minister's Award for PhD thesis
2001, 2002	Annual Stipend for Young Scientists, Foundation for Polish Science

Selected Publications

- **Nowak E**, Miller JT, Bona MK, **Studnicka J**, Szczepanowski RH, **Jurkowski J**, Le Grice SFJ†, **Nowotny M**†. Ty3 reverse transcriptase complexed with an RNA-DNA hybrid shows structural and functional asymmetry. ***Nat Struct Mol Biol***, 2014; doi:10.1038/nsmb.2785; †*corresponding authors*
- **Smietanski M,** Werner M, Purta E, Kaminska KH, Stepinski J, Darzynkiewicz E, **Nowotny M**†**,** Bujnicki JM† Structural analysis of human 2'-O-ribose methyltransferases involved in mRNA cap structure formation. ***Nat Commun***. 2014 Jan 9;5:3004; †*corresponding authors*
- **Górecka KM, Komorowska W, Nowotny M**. Crystal structure of RuvC resolvase in complex with Holliday junction substrate. ***Nucleic Acids Res***. 2013; 41(21):9945-55
- Chon H, Sparks JL, **Rychlik M, Nowotny M**, Burgers PM, Crouch RJ, Cerritelli SM. RNase H2 roles in genome integrity revealed by unlinking its activities. ***Nucleic Acids Res***. 2013; 41(5):3130-43
- **Nowak E**, Potrzebowski W, Konarev P, Rausch J, Bona M, Svergun D, Bujnicki JM, Le Grice S, **Nowotny M.** Structural analysis of monomeric retroviral reverse transcriptase in complex with an RNA/DNA hybrid. ***Nucleic Acids Res***, 2013; 41(6):3874-87
- Rosta E, **Nowotny M,** Yang W, Hummer G. Catalytic mechanism of RNA backbone cleavage by ribonuclease h from quantum mechanics/molecular mechanics simulations. ***J Am Chem Soc***, 2011; 133(23):8934-41
- **Figiel M**, Chon H, Cerritelli SM, **Cybulska M**, Crouch RJ, **Nowotny M**. The structural and biochemical characterization of human RNase H2 complex reveals the molecular basis for substrate recognition and Aicardi-Goutieres syndrome defects. ***J Biol Chem***, 2011; 286:10540-50
- **Jaciuk M**, **Nowak E**, Skowronek K, **Tanska A**, **Nowotny M**. Structure of UvrA nucleotide excision repair protein in complex with modifi ed DNA. ***Nat Struct Mol Biol***, 2011; 18:191-197
- **Rychlik MP**, Chon H, Cerritelli SM, **Klimek P**, Crouch RJ, **Nowotny M.** Crystal Structures of RNase H2 in Complex with Nucleic Acid Reveal the Mechanism of RNA-DNA Junction Recognition and Cleavage. ***Mol Cell***, 2010; 40:658-670
- **Nowotny M**. Retroviral integrase superfamily: the structural perspective (review). ***EMBO Rep***, 2009; 10:144-51
- **Nowotny M**, Yang W. Structural and functional modules in RNA interference. ***Curr Opin Struct Biol***. 2009;19:286-293. Review
- *Nowotny M, Cerritelli SM, Ghirlando R, Gaidamakov SA, Crouch RJ, Yang W. Specific recognition of RNA/DNA hybrid and enhancement of human RNase H1 activity by HBD. ***EMBO J***, 2008; 27:1172-81
- *Nowotny M, Gaidamakov SA, Ghirlando R, Cerritelli SM, Crouch RJ, Yang W. Structure of human RNase H1 complexed with an RNA/DNA hybrid: Insight into HIV Reverse Transcription. ***Mol Cell***, 2007; 28:264-276
- *Nowotny M, Yang W. Stepwise analyses of metal Ions in RNase H catalysis: From substrate destabilization to product release. ***EMBO J***, 2006; 25:1924-33
- *Nowotny M, Gaidamakov SA, Crouch RJ, Yang W. Crystal structures of RNase H bound to an RNA/DNA hybrid: substrate specifi city and metal-dependent catalysis. ***Cell***, 2005; 121:1005-16

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Co-workers affiliated with IIMCB are given in bold

Description of Current Research

Our laboratory focuses on structural and biochemical studies of nucleic acid enzymes using protein crystallography as a primary method. The key results obtained recently in our laboratory concern RuvC Holliday junction (HJ) resolvase and reverse transcriptases.

RuvC is a small nuclease that cleaves HJs—four-way DNA structures that arise when two DNA duplexes are joined through the exchange of strands. Holliday junctions are intermediates in homologous recombination, a process used for the repair of dangerous DNA damage, such as double-stranded breaks. In eukaryotes, it also occurs in meiosis and is used to reshuffle genes to generate genetic diversity. Holliday junctions are mutagenic and have to be removed. One of the pathways to achieve this is nucleolytic cleavage by specialized enzymes called resolvases. In bacteria, the canonical resolvase and one of the best characterized enzymes in its class is RuvC. It is a dimeric protein whose structure was first reported in 1995. However, a crystal structure of its complex with DNA substrate, which would explain the details of substrate recognition, remained elusive.

We solved the first crystal structure of the RuvC-substrate complex. It was determined at relatively low resolution (3.75 Å). Therefore, we complemented our structural data with sophisticated cross-linking experiments that involved the introduction of a thiol group to either the bases of the DNA or its backbone and engineering of cysteine residues into the protein in the vicinity of the DNA modification. The formation of the disulfide bridge-linked complexes was used to verify the correctness of our structural model.

In the structure, the HJ adopts a tetrahedral conformation that has not been observed previously. The site of DNA cleavage is located one nucleotide from the exchange point. Two important helices form the dimer interface of the protein and also stabilize the opening of the HJ. The mode of substrate binding by RuvC is strikingly different from the other two resolvases of phage origin for which substrate complex structures are known (T4 Endo VII and T7 Endo I). This implies that multiple modes of HJ recognition evolved by taking advantage of its highly mobile nature.

Reverse transcription involves the conversion of single-stranded RNA to double-stranded DNA and is essential for the proliferation of retrotransposons and retroviruses, such as human immunodeficiency virus (HIV-1). Reverse transcription is an intricate multi-step process that is catalyzed by very versatile enzymes called reverse transcriptases (RTs). These enzymes possess two activities: (1) DNA polymerase synthesizes the new DNA, and (2) RNase H degrades the RNA strand in the RNA/DNA intermediates of the reaction, thus removing the original genetic information. Retroviral RTs can be divided into two groups based on their architecture. Dimeric enzymes, such as HIV-1 RT, are very well characterized structurally and biochemically, but the mechanism of monomeric RTs is less well understood. To characterize the mechanism, we solved the crystal structure of monomeric RT from xenotropic murine leukemia virus-related virus (XMRV) in complex with an RNA/DNA substrate. The structure comprised the polymerase domain, but the RNase H was disordered and hence not visible. The structure revealed that the active site and substrate contacts around it were well conserved between monomeric and dimeric RTs. Further toward the RNase H domain, however, substrate binding was mediated by a different set of residues. Our structure also revealed the role of a "pin" structure that guided the trajectory of the template strand and was important for DNA polymerase processivity. We also explained the ability of XMRV RT to perform so-called strand-displacement DNA synthesis, during which a nucleic acid that is hybridized with the template ahead of the polymerase active site is removed concurrently with polymerization. In collaboration with Janusz Bujnicki at IIMCB and Dmitri Svergun at EMBL in Hamburg, we used small-angle X-ray diffraction data and combined them with the crystal structures to model the full-length enzyme. These studies revealed that the RNase H domain was very mobile in the absence of nucleic acid and became organized on the substrate when it was present. Transient and infrequent interactions between the RNase H domain and substrate appear to be a universal feature of RTs. It likely allows the enzyme to regulate RNase H activity, which has to perform very precise cuts at several stages of reverse transcription. However, the mechanism of the regulation of the RNase H interaction with the substrate is very different for the HIV-1 enzyme. Unlike in monomeric XMRV RT, the RNase H domain in dimeric RTs is rigidly placed on the non-catalytic subunit, and the substrate has to be deformed to reach its active site. Reverse transcriptases, therefore, exhibit an intriguing variety of mechanisms to perform their function. The XMRV RT studies were performed in collaboration with Dr. Stuart Le Grice at the National Institutes of Health in the United States.

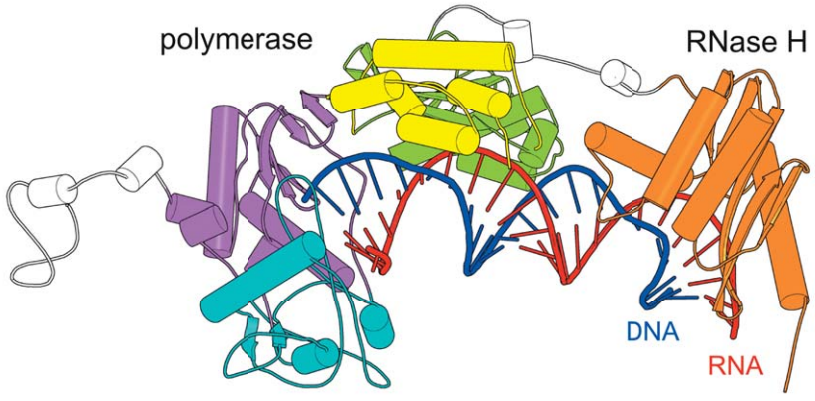
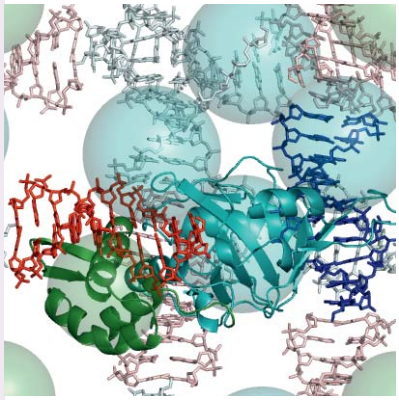


Fig. 2. **Model of the full-length XMRV reverse transcriptase based on crystal structures and SAXS data.** The polymerase domain is in cyan (fingers subdomain), pink (palm), yellow (thumb), and green (connection). The RNase H domain is in orange and the RNA/DNA hybrid substrate in red and blue.



Crystal packing of R.DpnI-DNA complex.
The DNA duplexes bound to either catalytic (cyan/blue) or winged helix (green/red) domains of R.DpnI endonuclease alternate to form long DNA "rods" that run throughout the whole crystal.

Structural Biology Laboratory

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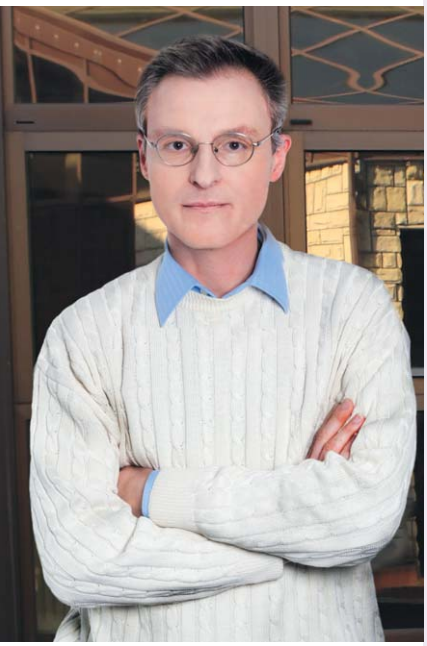


Lab Leader:
Matthias Bochtler,
PhD, Professor

Degrees

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

Research Training

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

Professional Employment

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

Honors, Prizes, Awards

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

Selected Recent Publications

Protein-nucleic acid interactions

- Kazrani AA, Kowalska M, Czapinska H, **Bochtler M**. Crystal structure of the 5hmC specific endonuclease PvuRts1I. ***Nucleic Acids Res***. 2014 Mar 14. [Epub ahead of print]
- **Wojciechowski M, Czapinska H, Bochtler M**. CpG Underrepresentation and the Bacterial CpG Specific DNA Methyltransferase M.MpeI. ***Proc Natl Acad Sci USA***, 2013; 110(1):105-110
- **Bochtler M**. Structural basis of the TAL effector-DNA interaction. Biol Chem, 2012; 393(10):1055-66
- **Siwek W, Czapinska H, Bochtler M, Bujnicki JM, Skowronek K**. Crystal structure and mechanism of action of the N6-methyladenine dependent type IIM restriction endonuclease. ***Nucleic Acids Res***, 2012; 40(15):7563-72
- **Chojnowski G,** Bujnicki JM, **Bochtler M**. RIBER/DIBER: a software suite for crystal content analysis in the studies of protein-nucleic acid complexes. ***Bioinformatics***, 2012; 28(6):880-881
- **Chojnowski G, Bochtler M**. DIBER: protein, DNA or both? Acta ***Crystallogr D***, 2010; 66:643-653
- Antonczak AK, Simova Z, Yonemoto IT, **Bochtler M**, Piasecka A, **Czapinska H**, Brancale A, Tippmann EM. Importance of single molecular determinants in the fidelity of expanded genetic codes. ***Proc Natl Acad Sci USA***, 2011; 108:1320-5
- Braun S, Humphreys C, Fraser E, Brancale A, **Bochtler M**, Dale TC. Amyloid-Associated Nucleic Acid Hybridisation. ***PLoS One***, 2011; 6:e19125
- **Sokolowska M, Czapinska H, Bochtler M.** Hpy188I-DNA pre- and post-cleavage complexes - snapshots of the GIYYIG nuclease mediated catalysis. ***Nucleic Acid Res***, 2011; 39:1554-64
- **Firczuk M, Wojciechowski M, Czapinska H, Bochtler M**. DNA intercalation without flipping in the specific ThaIDNA complex. ***Nucleic Acid Res***, 2011 39:744-754
- **Sokolowska M, Czapinska H, Bochtler M**. Crystal structure of the ββα-Me type II restriction endonuclease Hpy99I with target DNA. ***Nucleic Acid Res***, 2009; 37:3799-810
- **Szczepanowski RH**, Carpenter MA, **Czapinska H**, Zaremba M, Tamulaitis G, Siksnys V, Bhagwat AS, **Bochtler M**. Central base pair flipping and discrimination by PspGI. ***Nucleic Acids Res***, 2008; 36:6109-17
- Tamulaitis G, Zaremba M, **Szczepanowski RH**, **Bochtler M**, Siksnys V. Central base pair flipping and discrimination by PspGI. How PspGI, catalytic domain of EcoRII and EcI18kI acquire specificities for different DNA targets. ***Nucleic Acids Res***, 2008; 36:6101-8
- Sukackaite R, Grazulis S, **Bochtler M**, Siksnys V. The recognition domain of the BpuJI restriction endonuclease in complex with cognate DNA at 1.3-A resolution. ***J Mol Biol***, 2008; 378:1084-93
- Tamulaitis G, Zaremba M, **Szczepanowski RH**, **Bochtler M**, Siksnys V. Nucleotide flipping by restriction enzymes analyzed by 2-aminopurine steady-state fluorescence. ***Nucleic Acids Res***, 2007; 35:4792-9
- **Sokolowska M, Kaus-Drobek M, Czapinska H**, Tamulaitis G, **Szczepanowski RH**, Urbanke C, Siksnys V, **Bochtler M**. Monomeric restriction endonuclease BcnI in the apo form and in an asymmetric complex with target DNA. ***J Mol Biol***, 2007; 369:722-34
- **Kaus-Drobek M, Czapinska H, Sokolowska M**, Tamulaitis G, **Szczepanowski RH**, Urbanke C, Siksnys V, **Bochtler M**. Restriction endonuclease MvaI is a monomer that recognizes its target sequence asymmetrically. ***Nucleic Acids Res***, 2007; 35:2035-46
- **Bochtler M, Szczepanowski RH**, Tamulaitis G, Grazulis S, **Czapinska H**, Manakova E, Siksnys V. Nucleotide flips determine the specificity of the EcI18kI restriction endonuclease. ***EMBO J***, 2006; 25:2219-29
- Grazulis S, Manakova E, Rössle M, **Bochtler M**, Tamulaitiene G, Huber R, Siksnys V. Structure of the metal-independent restriction enzyme BfiI reveals fusion of a specific DNAbinding domain with a nonspecific nuclease. ***Proc Natl Acad Sci USA***, 2005; 102:15797-802

Other

- **Haniewicz P**, De Sanctis D, Büchel C, Schröder WP, Loi MC, Kieselbach T, **Bochtler M, Piano D**. Isolation of monomeric photosystem II that retains the subunit PsbS. ***Photosynth Res***, 2013; 118(3):199-207.
- Jaremko M, Jaremko L, Nowakowski M, **Wojciechowski M, Szczepanowski RH, Panecka R**, Zhukov I, **Bochtler M**, Ejchart A. NMR structural studies of the first catalytic half-domain of ubiquitin activating enzyme. ***J Struct Biol***, 2013: S1047-8477(13):00299-2
- **Sabala I**, Jonsson IM, Tarkowski A, **Bochtler M**. Anti-staphylococcal activities of lysostaphin and LytM catalytic domain. ***BMC Microbiol***, 2012; 12:97
- Gentsch M, **Kaczmarczyk A**, van Leeuwen K, de Boer M, **Kaus-Drobek M**, Dagher MC, Kaiser P, Arkwright PD, Gahr M, Rösen-Wolff A, **Bochtler M**, Secord E, Britto-Williams P, Saifi GM, Maddalena A, Dbaibo G, Bustamante J, Casanova JL, Roos D, Roesler J. Alu-repeat-induced deletions within the NCF2 gene causing p67-phoxdeficient chronic granulomatous disease (CGD). ***Hum Mutat***, 2010; 31:151-158
- **Chojnowski G**, Breer K, Narczyk M, Wielgus-Kutrowska B, **Czapinska H**, Hashimoto M, Hikishima S, Yokomatsu T, **Bochtler M**, Girstun A, Staron K, Bzowska A. 1.45 A resolution crystal structure of recombinant PNP in complex with a pM multisubstrate analogue inhibitor bearing one feature of the postulated transition state. ***Biochem Biophys Res Commun***, 2010; 391:703-708
- **Piano D, El Alaoui S, Korza HJ, Filipek R, Sabala I, Haniewicz P**, Buechel C, De Sanctis D, **Bochtler M**. Crystallization of the photosystem II core complex and its chlorophyll binding subunit CP43 from transplastomic plants of Nicotiana tabacum. ***Photosyn. Res***, 2010; 106:221-226

Description of Current Research

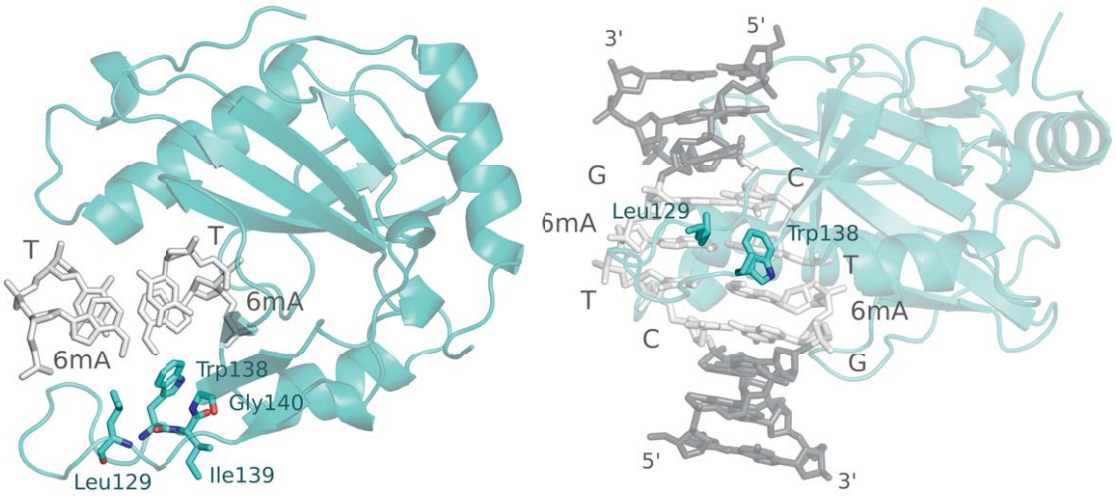
The group wants to understand the mechanistic aspects of DNA methylation and hydroxymethylation and role of these modifications in (relatively) simple model organisms.

To better understand sequence specificity, we looked for more bacterial methyltransferase model proteins with the same sequence specificity as the eukaryotic Dnmt proteins. We investigated the long-term impact of DNA methylation on genomes. In both eukaryotes and prokaryotes, cytosine methylation drives cytosine deamination, and sequences that are methylated are therefore underrepresented in genomes. Using CpG underrepresentation as a guide, we discovered that many Mycoplasm species harbor active CpG methyltransferases. We used these as model systems for CpG-specific DNA methyltransferases in eukaryotes and found features of CpG recognition (i.e., unstacking of the "weak CpG" sequence) that were independently reported for the eukaryotic Dnmt1-DNA complex by Prof. Dinshaw Patel's group. The role of CpG methyltransferases in Mycoplasms is still unclear, but we suspect that they help pathogens evade the host-specific and -nonspecific immune response against non-CpG methylated DNA. This work was published in *Proceedings of the National Academy of Sciences USA* in 2013 (Wojciechowski et al., selected publications, second reference).

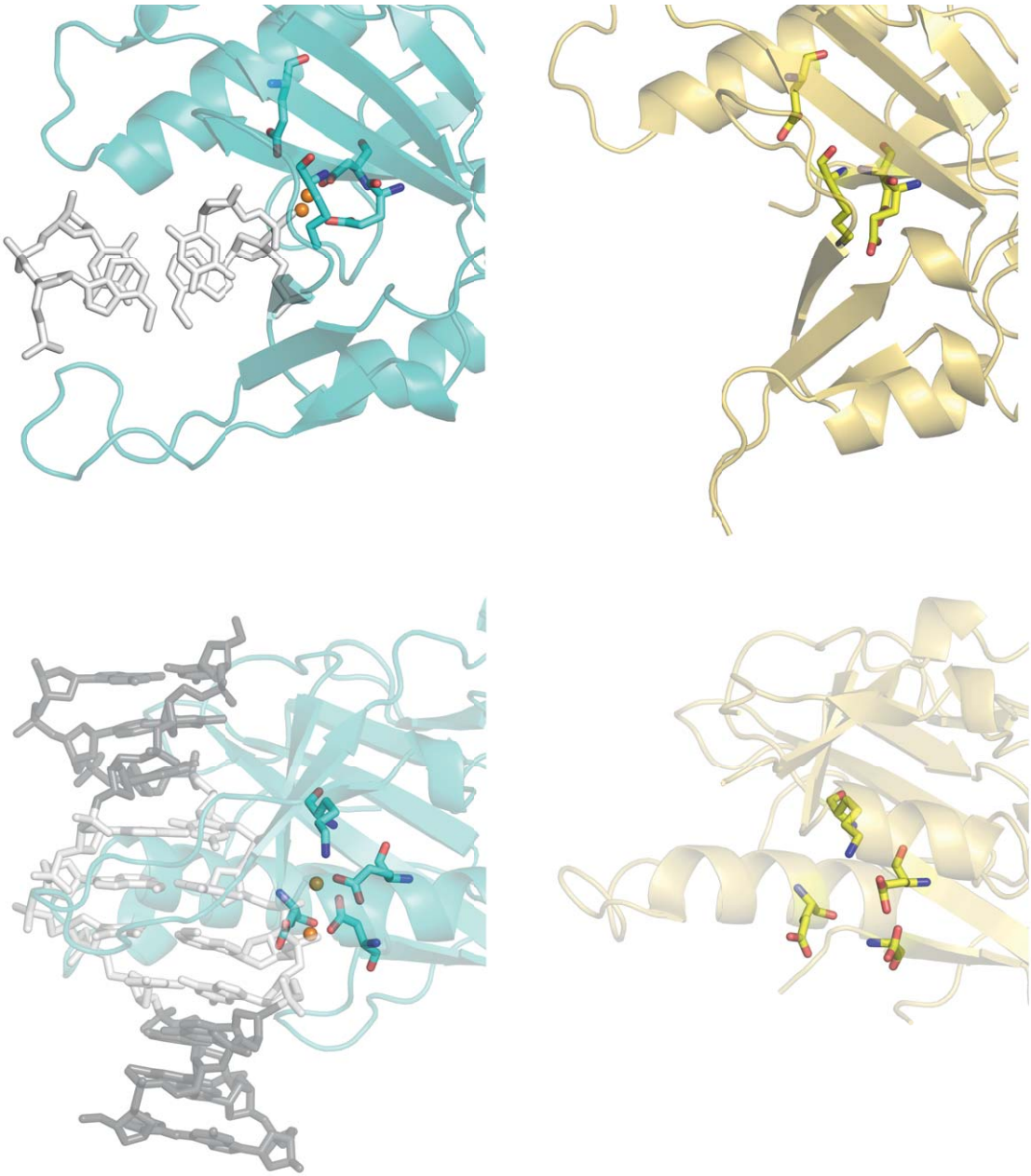
In parallel with the work on DNA methyltransferases, the group has also focused on the readout of DNA methylation. As a model protein, we used the 6-methyladenine-dependent restriction endonuclease R.DpnI, which cleaves only methylated DNA and not unmethylated DNA with high stringency. In 2012, we published a first structure of R.DpnI, which showed DNA bound to the winged helix but not the catalytic domain of the enzyme. This work suggested that methylation specificity is enhanced by multiple readouts, but it did not answer the more fundamental question how the individual domains become methylation-specific. Based on a detailed structural analysis of readouts of both adenine and cytosine methylation, we now believe that the mechanisms of specificity could be surprisingly different. From the outset, repulsive van der Waals interactions are clearly strong enough to prevent protein-DNA binding, whereas attractive van der Waals forces are too weak to explain how just one or two methyl groups can be required for the binding to occur. In the case of cytosine methylation, desolvation effects (which are more favorable for methylated than unmethylated DNA bases) have been hypothesized to be the main driving force. Based on structural data and molecular modeling, we propose that methyl group proximity in the case of Dam GATC adenine methylation imposes conformational restrictions on DNA. This effect lowers the entropic penalty for binding to the protein which "fixes" the DNA, regardless of prior constraints on its conformational freedom. Our analysis predicts that the entropic mechanism for methylation detection is not applicable for cytosine and works for adenine methylation only if methylated adenines are staggered as in the Dam GATC target (incidentally explaining the "evolutionary success" of the Dam methyltransferase system and its adoption for many different biological purposes). This work is currently under review at *Nucleic Acids Research* (Mierzejewska et al).

Much structural information is already available on the readout of 5-methylcytosine, and we have focused on the readout of 5-hydroxymethylcytosine. As a model protein, we have used the 5-hydroxymethylcytosine-specific DNA endonuclease PvuRts1I. Using recombinantly produced protein, we were able to obtain crystals and solved an apo-structure of the protein. Based on this structure, predicting the DNA binding mode and performing biochemical experiments to test the model were possible. The work has been summarized in a manuscript that has just been accepted in *Nucleic Acids Research* (Kazrani et al).

In vertebrates, 5-hydroxymethylcytosine is generated by the oxidation of 5-methylcytosine by oxoglutarate-dependent TET dioxygenases. In collaboration with Prof. Ryszard Maleszka at



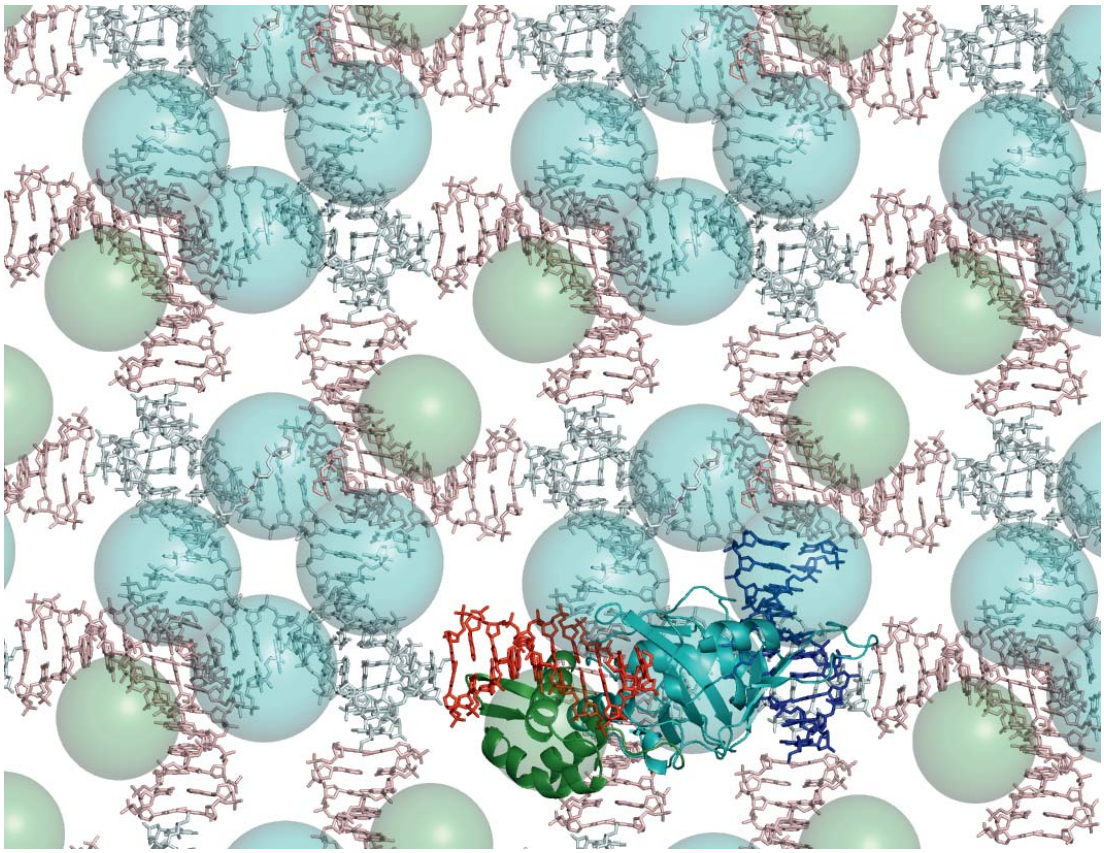
The recognition of the Dam methylated GATC sequence by the catalytic domain of R.DpnI restriction endonuclease.
The side chains of Trp138 and Leu129 residues form the methyl binding pocket of the enzyme. Gly140 stabilizes the conformation of Trp138 that simultaneously stacks against the two methyl groups of the 6-methyladenines. The left panel presents only the central two 6mA:T base pairs of the R.DpnI target sequence. The right panel is 90° rotated and presents the whole DNA fragment with the target sequence marked in white.



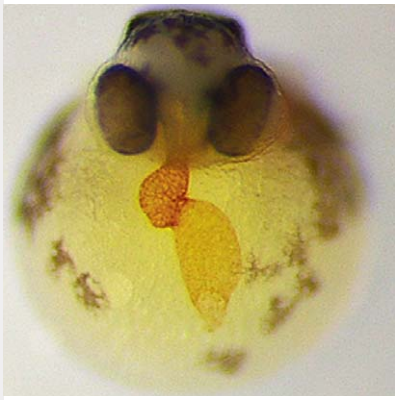
Ordering of the R.DpnI catalytic domain upon DNA binding.
The presence of DNA and divalent metal cations leads to the ordering of the PD-(D/E)XK active site of R.DpnI endonuclease. Moreover, the long loop that could not be reliably traced in the absence of the substrate (yellow, right), in its presence wedges into the DNA major groove to recognize the target bases (blue, left).

Australian National University, we discovered a homologue of vertebrate TETs in the honeybee *A. mellifera*. We demonstrated that the protein is active both in a standard HEK293 cell assay and in the native host. The presence of 5hmC in *A. mellifera* was confirmed independently by thin-layer chromatography, dot-blot analysis, and a glucosyl transfer assay. The data consistently showed that the endogenous 5hmC bases occur at a frequency of 5-25 per million bases. Hence, absolute 5hmC levels are approximately 1000-fold lower in *A. mellifera* than in 5hmC-rich mammalian tissues. However, because of the much lower levels of methylation in *A. mellifera* compared with mammals, the relative levels of 5hmC and 5mC bases are comparable. A manuscript that summarizes this work is currently under review (Wojciechowski et al).

According to several high-profile papers (published before the discovery of the oxidative demethylation pathway), demethylation in zebrafish is supposed to proceed through a deaminative route and is claimed to involve a thymine intermediate. The mechanism is suspicious because such an intermediate in a genome-wide demethylation pathway should be highly mutagenic. Moreover, we found that the TET genes in zebrafish are bona fide orthologues of their mammalian counterparts. Indeed, using commercially available antibodies, we demonstrated 5hmC in early zebrafish embryos (before zygotic genome activation), which was much earlier than reported previously. However, the functional role of 5hmC in zebrafish is not yet clear. Also unknown is whether the base is involved in DNA demethylation, acts as an epigenetic signal, or might have both roles. To address this issue, we are using genetic methods and small molecules to inactivate TET proteins. This work is part of the FishMed project and still at a relatively early stage.



Crystal packing of R.DpnI-DNA complex.
The DNA duplexes bound to either catalytic (cyan/blue) or winged helix (green/red) domains of R.DpnI endonuclease alternate to form long DNA "rods" that run throughout the whole crystal.



Zebrafish heart stained with the MF20 antibody (for cardiac myosin) Photo by Dr. Igor Kondrychyn.

Zebrafish Developmental Genomics Laboratory

(under organization)

Postdoctoral Fellow:
Katarzyna Nieścierowicz, PhD, FishMed (since April 2014)

Technician:
Monika Rychlik, FishMed (since April 2014)

Lab Leader:
Cecilia Lanny Winata,
PhD

Degrees

2009	PhD in Biology, Department of Biological Sciences, National University of Singapore
2004	BSc (Hons.) in Biology, Department of Biological Sciences, National University of Singapore

Research experience

2014	Head, Zebrafish Developmental Genomics Laboratory, IIMCB, Warsaw, Poland
2013-2014	Research Associate, Genome Institute of Singapore.
2013	Research visit, laboratory of Prof. Peter Aleström, Norwegian School of Veterinary Sciences, Oslo, Norway.
2009-2013	Postdoctoral Fellow with Dr. Sinnakaruppan Mathavan, Genome Institute of Singapore.
2004-2009	Doctoral research with Profs. Gong Zhiyuan and Vladimir Korzh, Department of Biological Sciences, National University of Singapore.

Honors and Awards

2000-2004	ASEAN Undergraduate Scholarship.
2003	Science Faculty Dean's List, National University of Singapore.

Selected Recent Publications

- Aanes H., **Winata C.L.**, Moen L.F., Østrup O., Mathavan S., Collas P., Rognes, T., Aleström P. (2014) Normalization of RNA-sequencing data from samples with varying mRNA levels. ***PLoS One*** (accepted).
- **Winata C.L.**, Kondrychyn I., Kumar V., Srinivasan K.G., Orlov Y., Ravishankar A., Prabhakar S., Stanton L.W., Korzh V., Mathavan S. (2013) Genome-wide analysis reveals Zic3 interaction with distal regulatory elements to regulate zebrafish developmental genes. ***PLoS Genetics*** 9(10): e1003852.
- Aanes H.*, **Winata C.L.***, Lin C.H., Chen J.P., Srinivasan K.G., Lee S.G., Lim A.Y., Hajan H.S., Collas P., Bourque G., Gong Z., Korzh V., Aleström P., Mathavan S. (2011) Zebrafish mRNA sequencing deciphers novelties in transcriptome dynamics during maternal to zygotic transition. ***Genome Research*** 21(8): 1328-1338. (* equal contribution).
- Lindeman L.C., Andersen I.S., Reiner A.H., Li N., Aanes H., Østrup O., **Winata C.**, Mathavan S., Müller F., Aleström P., Collas P. (2011) Prepatterning of developmental gene expression by modified histones before zygotic genome activation. ***Developmental Cell*** 21(6):993-1004.
- Lindeman L.C., **Winata C.L.**, Aanes H., Mathavan S., Alestrom P., Collas P. (2010) Chromatin states of developmentally-regulated genes revealed by DNA and histone methylation patterns in zebrafish embryos. ***International Journal of Developmental Biology*** 54(5):803-13.
- Korzh S., **Winata C.L.**, Zheng W., Yang S., Yin A., Ingham P., Korzh V., Gong Z. (2011) The interaction of epithelial Ihha and mesenchymal Fgf10 in zebrafish esophageal and swimbladder development. ***Developmental Biology*** 359(2): 262-76.
- Yin A., Korzh S., **Winata C.L.**, Korzh V., Gong Z. (2011) Wnt signaling is required for early development of zebrafish swimbladder. ***PLoS One*** 6(3): e18431. IF (5-year): 4.244; times cited: 4 (status on the 3rd December 2013).
- **Winata C.L.**, Korzh S., Kondrychyn I., Korzh V., Gong Z. (2010) The role of vasculature and blood circulation in zebrafish swimbladder development. ***BMC Developmental Biology*** 10:3.
- Yin A., **Winata C.L.**, Korzh S., Korzh V., Gong Z. (2010) Expression of components of Wnt and Hedgehog pathways in different tissue layers during lung development in ***Xenopus laevis***. ***Gene Expression Patterns*** 10(7-8):338-44.
- Ung C.Y., Lam S.H., Hlaing M.M., **Winata C.L.**, Korzh S., Mathavan S., Gong Z. (2010) Mercury-induced hepatotoxicity in zebrafish: in vivo mechanistic insights from transcriptome analysis, phenotype anchoring and targeted gene expression validation. ***BMC Genomics*** 11:212.
- **Winata C. L.**, Korzh S., Kondrychyn I., Zheng W., Korzh V., Gong Z. (2009) Development of the zebrafish swimbladder: the requirement of Hedgehog signaling. ***Developmental Biology*** 331(2):222-36.
- Korzh S., Pan X., Garcia-Lecea M., **Winata C.L.**, Pan X., Wohland T., Korzh V., Gong Z. (2008) Requirement of vasculogenesis and blood circulation in late stages of liver growth in zebrafish. BMC Developmental Biology. ***BMC Developmental Biology*** 8:84.
- Lam S.H.*, **Winata C.L.***, Tong Y., Korzh S., Lim W.S., Korzh V., Spitsbergen J., Mathavan S., Miller L.D., Liu E.T., Gong Z. (2006) Transcriptome kinetics of arsenic-induced adaptive response in zebrafish liver. ***Physiol. Genomics*** 27(3):351-61.(* equal contribution)
- Lam S.H., Mathavan S., Tong Y., Hu J., **Winata C.L.**, Lee S., Miller L.D., Liu E.T., and Gong Z. (2004) Preliminary microarray analyses of gene expression in zebrafish treated with xenobiotic and bioactive compounds. ***Marine Biotechnology*** 6: S468-S474.

Publications without IIMCB affiliation

Description of Current Research

The Zebrafish Developmental Genomics Laboratory is dedicated to the study of developmental processes by applying genomics methods in combination with experimental embryology, genetics, and biochemistry. The aim is to understand the complex transcriptional regulatory mechanism of embryonic development *in vivo*. Currently our research focuses on elucidating the downstream regulatory mechanism of heart development by cardiac transcription factors (TFs) and characterization of epigenetic profiles during heart development. A comprehensive understanding of the molecular regulatory networks governing heart development will be a crucial step to a better understanding of the mechanism of congenital heart diseases.

The study of heart development poses a unique challenge due to the importance of the organ for survival. Disruption to factors regulating the early steps of heart formation, cause early embryonic lethality. The zebrafish (*Danio rerio*) alleviates this problem by allowing access to developing embryos right after fertilization and its ability to survive without a functioning heart up to a comparatively late stage of development. Taking advantage of this model organism, many genes regulating heart development have been identified. However, despite these advances, considerable challenges to understand the mechanism of heart development still exist. Firstly, there is still a lack of knowledge of molecular mechanism and downstream targets of cardiac TFs. Furthermore, the interconnectivity of their mechanisms and functions render it difficult, and possibly of little meaning, to make isolated assessments of individual factors in the characterization of a particular phenotypic outcome. Secondly, the transcription of genes are modulated by cis regulatory elements located in non-coding regions of the genome, which also serve as binding sites for TFs. Thus, mutations in these regulatory elements equally affect developmental outcome as mutations in coding regions. However, there is still a lack of systematic resource for these elements and understanding of their roles in heart development. Thirdly, an additional layer of regulation exists in the form of epigenetics. Cardiac TFs have been shown to interact with chromatin modifying factors, and loss of function of several histone modifying enzymes have been found to affect various aspects of cardiac development.

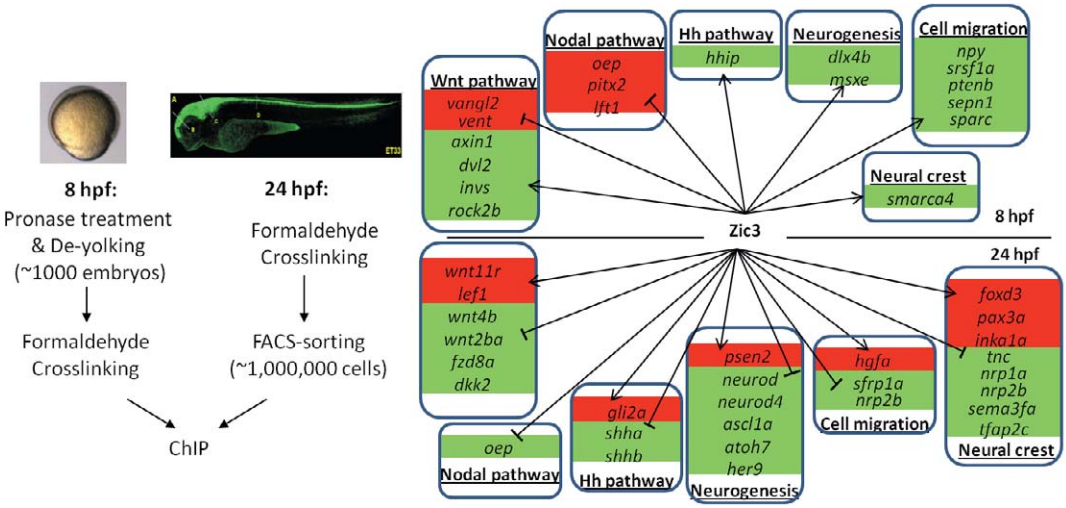
The high degree of complexity in developmental regulation *in vivo* necessitates an approach which takes into account both

genetic and epigenetic factors. Using a genomics approach and capitalizing on the advantages of zebrafish, we want to uncover genetic and epigenetic factors contributing to the process of heart development and elucidate their regulatory mechanism.

1. Transcriptional regulatory network of heart development

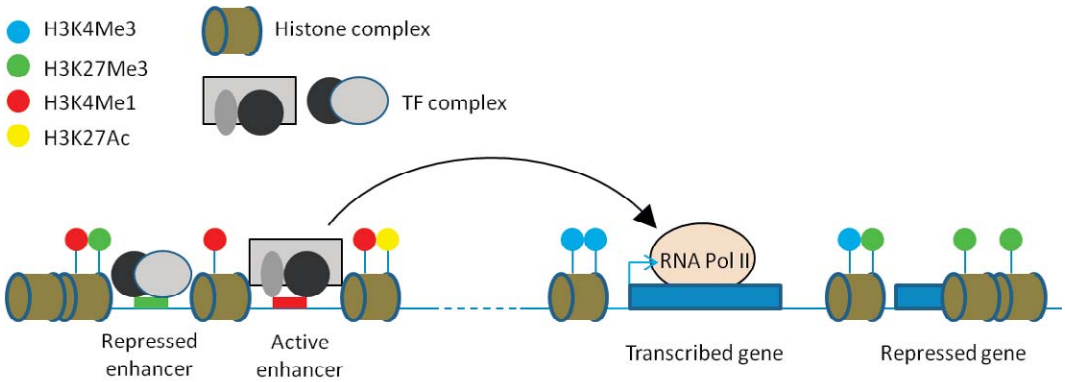
The vertebrate heart undergoes three key stages of morphogenesis: specification and migration of cardiac progenitors, formation of the beating linear heart tube, and looping to form a multi-chambered organ. In each of these stages, TFs play a crucial role in initiating transcription of cardiac genes, leading to a cascade of genetic regulation. At the core of this regulation machinery is the interaction between cardiac TFs Nkx2.5, Gata5, Tbx5, and Hand2 which is necessary for the establishment of cardiac identity in cells of the embryonic mesoderm, their subsequent diversification into atrial and ventricular progenitors, and their migration to the midline to form the linear heart tube.

In our previous work, we have used ChIP-seq on zebrafish whole embryos and FACS-sorted cells to study Zic3, a TF implicated in left-right patterning and neural development. We identified novel target genes of Zic3 and uncovered links to several developmental pathways during gastrulation and neural patterning. Building upon the experience and knowledge gained from this study, we want to characterize the downstream regulatory network of cardiac TFs during key phases of heart development.



2. Epigenome profile of heart development

Epigenetic marks in the form of modified histones have been commonly used to identify chromatin states, indicating the transcriptional status or activity of particular genetic elements, such as enhancers and promoter. A systematic catalogue of these marks, combined with the information on TF binding sites in the genome, would provide a comprehensive and unbiased view of transcriptional regulatory landscape during heart development *in vivo*. Together with functional analysis in zebrafish mutants, we aim to identify genome-wide elements associated with heart defects, and to characterize epigenetic contributions to heart development.



Core Facilities

Core Facility

X-ray generator. Photo by Roman Szczepanowski

Head: **Alicja Żylicz**, PhD, Professor

Senior Staff Scientists:
Krzysztof Skowronek, PhD
Roman Szczepanowski, PhD
Tomasz Węgierski, PhD

Radiation Safety Officer:
Piotr Brągoszewski, PhD

Confocal microscope (Zeiss LSM5 Exciter).

Crystallographic Robot (Art Robbins Crystal Phoenix).

The Core Facility Laboratory was created in October 2011 with the goal of supporting innovative research at IIMCB, giving investigators access to a broad spectrum of cutting-edge research technology platforms that are extensively used in such diverse areas as structural biology, bioinformatics, molecular and cell biology. It is being run by experienced scientists who devote their time to maintaining the most sophisticated equipment and are top authorities in research applications for such equipment. More than 100 equipment items are grouped according to biophysical, biochemical and visualization applications for protein and nucleic acid structures and functional determination.

1. The Macromolecule Crystallography Unit is one of the most advanced in Poland. Proteins are purified with several chromatographic FPLC systems and subjected to crystallization using ~1,000 crystallization conditions (buffers). Crystallization progress is tracked by a CCD camera, and the crystals are analyzed using an X-ray generator (Proteum Bruker) equipped with a CCD detector (Platinum 135) and cryosystem (Cryostream series 700). This facility allows the collection of a complete set of diffraction data within a few hours.
2. The Biochemical and Biophysical Analysis of Proteins and Nucleic Acids Unit is equipped with analytical instruments for in-depth analyses of proteins and nucleic acids using different methods. Interactions between molecules are studied using several complementary techniques (microcalorimetry, analytical ultracentrifugation, and surface plasmon resonance). The size of the macromolecular complexes is measured by SEC-MALS (size exclusion chromatography with multiangle light-scattering detector) and analytical ultracentrifugation.
3. The Mass Spectrometry of Proteins and Nucleic Acids Unit has two mass spectrometers: MALDI-TOF-TOF (ultrafleXtreem, Bruker) and LC-ESI-Ion Trap (amaZone, Bruker). They provide analyses of macromolecules other than standard proteomics, particularly analyses of RNA samples. The use of MS analysis for RNA targets makes this facility a unique entity nationwide.
4. The Microscopy Bioimaging Unit equipment includes a Zeiss LSM710 NLO dual confocal/multiphoton microscope for live imaging of cells and tissues, Leica TCS SP2 confocal microscope for live cell imaging and FRAP experiments, Zeiss LSM5 Exciter for routine confocal scanning of fixed samples, Olympus CellR/ScanR imaging station for intracellular calcium measurements with Fura-2 and semi-high throughput quantitative analysis of fluorescence signals, and Nikon 80i Eclipse microscope with a scanning stage for mosaic imaging of histochemically or fluorescently stained tissue sections. A Zeiss Lightsheet Z.1 single-plane illumination microscope (SPIM) was installed at IIMCB in October 2013. This is the first installation of this innovative technology in Poland and likely the second in Europe. At the end of 2013, a spinning-disk confocal microscope for very fast (real-time) imaging of live cells was delivered to the Institute.
5. The Bioinformatics and Computational Biology Facility provides free services to the research community worldwide, based on unique computer software developed at IIMCB (mainly in the Bujnicki group) and an in-house computer cluster that consists of >2,000 nodes. The genesilico.pl server is currently the most widely used Polish web portal for bioinformatics tools developed in Poland. The software provided includes web servers for the prediction of protein and RNA structures (e.g., GeneSilico metaserver, ModeRNA), the prediction of interactions (e.g., LigandRNA), and monitoring the quality of predictions (e.g., CompaRNA). The facility also provides databases that focus on nucleic acids (e.g., the MODOMICS database of RNA modification pathways, recently selected for the F1000Prime list by Faculty 1000). The planned development of the facility is focused on the computational analysis of imaging data, particularly from the SPIM microscope.

The Laboratory provides sufficient assistance with methodological principles, experimental design, initial training, the procedures needed for an experiment, data analysis, and final interpretation. The use of the equipment is free of charge to all faculty members and students. The Core Facility is also available to scientists from other institutes.

IIMCB emphasizes cooperation with rapidly developing biotech companies in Poland. Within the framework of bilateral agreements, IIMCB laboratories collaborated with biotech companies, such as Adamed, Celon Pharma, Fermentas, BIOVECTIS, and Polfa.

Zebrafish Core Facility

Zebrafish (*Danio renio*). Photo by Michał Bazała

Head: **Małgorzata Wiweger**, PhD, FishMed

FishMed Technicians:
Piotr Korzeniowski, DVM,
Maciej Mańk, MSc
Krzysztof Surga, MSc,
Monika Turniak, MSc

The Zebrafish Core Facility (ZCF) is a licensed breeding and research facility that was established in 2012 as a base for the FishMed project (for more information, see p. 75) and other biomedical studies. ZCF is registered as a closed facility that is entitled to keep genetically modified organisms and use them for FishMed and other projects. All of the research activities are performed in compliance with fundamental ethical principles, and all of the procedures are implemented in compliance with relevant European and international guidelines on animal welfare (FELASA) and relevant Polish regulations.

This state-of-the-art facility includes a water plant, stand-alone quarantine unit, and main system consisting of 326 tanks. The system can hold approximately 6,000 adult fish, and further expansion is scheduled. Presently, 32 different lines of zebrafish are being housed. In addition to the aquarium, which is a restricted area, our Zebrafish Core Facility has a laboratory space equipped with a needle puller and beveller that is suited for the production of micro needles that are suitable for the injection of cells, zebrafish, Drosophila, and other organisms. This laboratory is open to internal and external users.

Where possible, ZCF provides a sufficient number of eggs/adult fish for all individuals who use the facility, with priority given to FishMed researchers and other IIMCB employees. The facility endeavors to provide various lines. If the line is not in stock, then ZCF will try to source the line from another facility or stock center. Presently, ZCF does not offer any fish hotels for external users. Personnel are available if users would like to discuss zebrafish biology, husbandry, and research or seek help in solving technical issues. The cost for the fish and access to ZCF is free for academic users, but the cost for chemicals and the equipment needed for a specific project is charged to individual users.

ZCF holds a formal permission to house and breed *Danio rerio*, granted by the District Veterinary Inspectorate (license number 14656251). ZCF is registered as a closed facility, entitled to keep genetically modified organisms (GMO: 01-101/2012 and 01-105/2013) and use them for FishMed and other projects. All research activities are carried out in compliance with fundamental ethical principles on the basis of the resolutions received from the 1st Local Ethical Commission for animal experimentation in Warsaw. Research procedures are implemented in compliance with the relevant European guidelines on animal welfare (Directive 2010/63/EU on the protection of animals used for scientific purposes), the guidelines and recommendations of the Federation of European Laboratory Animal Science Associations (FELASA), and the relevant Polish regulations.

Zebrafish are freshwater, small (3-5 cm) tropical fish with a life-cycle of approximately 3-4 months. External fertilization, a transparent body, large mutant/transgenic collection, and the availability of various genetic tools make zebrafish an excellent organism to study multiple aspects of human diseases. Furthermore, the use of this lower vertebrate animal model opens new possibilities to introduce "3R" (reduction, replacement, and refinement) ethical guidance at the Ochota Campus.

Zebrafish lines

	name	afected gene	mutation type
wild type	AB		wild type
	ABTL		wild type
	Kakadu		wild type
	TL		wild type
	TU		wild type
	WIK		wild type
	Wet-Aqua WT		wild type
mutants	*albino*	*slc45a2*	*unknown*
	casper	*(roy x nacre)*	*unknown*
	dackel	*ext2*	*to273b*
	hi307	*b3gat3*	*hi307Tg*
	hi954	*uxs1*	*hi954Tg*
	hi1002	*csnk1a1*	*hi1002Tg*
	knypek	*glypican 4*	*u34.8*
	nacre	*mitfa*	*unknown*
	pink-1	*pink-1*	
	pinscher	*slc35b2*	*to216z*
	roy	*unknown*	*unknown*
	siberblick	*wnt11*	*tx226*
	trilobite	*vangl2*	*m209*
	tsc2	*tsc2*	*vu242*
	zTOR	*ztor*	*xu015*
transgenic lines	*Tg(cmlc2:mRPF)*		
	Tg(fli:GFP)		
	Tg(HaC;GCaMP3)		
	Tg(kop:EGFP-UTRnanos3)er1		
	Tg(mnx1:TagRFP)		
	Tg(vasa:GFP)		
	Tg(Xla.Eef1a1:mlsEGFP)		
	Wet-Aqua pink		


1


2


3

Zebrafish lines (from left): 1) Wet-Aqua pink x *necre*, 2) mutant roy, 3) mutant casper. Photo by Michał Bazała

Bio&Technology Innovations Platform (BioTech-IP)

From left: Leszek Lipiński (Specialist), Adam Sobczak (Project Manager), Magdalena Powierża (Manager), Hubert Ludwiczak (Assistant), Piotr Potepa (Assistant).

The Bio&Technology Innovations Platform (BioTech-IP) Technology Transfer Office at IIMCB was established in 2010 to support the commercialization of inventions and technologies developed by scientists in six public research institutes affiliated with the Ochota Biocentre Consortium in Warsaw in areas such as biotechnology, biomedicine, bioinformatics, bioengineering, material technologies, and bionanotechnology (www.biotech-ip.pl).

Main tasks of the BioTech-IP

- to encourage a creative and entrepreneurial attitude in the academic environment by supporting creative activities and promoting the commercial exploitation of research results;
- to raise awareness among academics with regard to intellectual property rights through a series of lectures and workshops;
- to search for and verify research projects with high commercial potential and commercialize them through the formation of spin-off companies or licensing of technologies to industrial partners;
- to initiate science-business networking activities and get in touch with business angels, venture funds, and business supporting institutions;
- to promote research services offered by Ochota Biocentre institutes.

2013 events and achievements

ETTBio project meeting in Warsaw

IIMCB/BioTech-IP was a host of the Effective Technology Transfer in Biotechnology (ETTBio) project meeting in September 2013. The meeting was attended by project partners from seven European regions. Among the speakers were Vice President of the City of Warsaw Michał Olszewski and FNP President Prof. Maciej Żylicz. The event had wide media coverage: http://www.youtube.com/watch?v=FchJn2s4m_Q. The project seeks to identify, exchange, and transmit best practices to improve local and regional policies. The project is supported by funds from the INTERREG IVC program, which helps European regions share knowledge and transfer experience to improve regional policies. The ETTBio budget is more than €2 million for the 2012-2014 period.

PhD scholarships

Thirteen PhD students from six Ochota Biocentre institutes were granted scholarships for their research projects. Awarded scholarships are funded by the project *Support for bio-tech-med scientists in technology transfer through scholarships, training courses, and internships*. The grants are sponsored by the Operational Programme - Human Capital co-funded by the European Union under the European Social Fund.

Internship program

Two scientific researchers were supported by internships that took place in Dr. Irena Eris and Synapsis Foundation and were sponsored by the Operational Programme - Human Capital co-funded by the European Union under the European Social Fund within the project *Support for bio-tech-med scientists in technology transfer through scholarships, training courses, and internships*.

Workshops and lectures

BioTech-IP organized a series of lectures and workshops for PhD students and scientists around such topics as soft skills development, management, commercialization strategies, and project management attended by a total of 176 participants.

Science-to-business brunches

BioTech-IP organized three science-to-business brunches, during which Ochota Biocentre scientists presented their research outcomes to invited entrepreneurs and investors. The brunches were attended by a total of 70 people.

Purpose Vehicle Company – SpinTech by NCRD

In October 2013, IIMCB signed an agreement with NCRD that sought to set up a Purpose Vehicle Company (PVC). The first stage involves the preparation of regulatory documents to found the future company.

FishMed project

BioTech-IP started activities within the FishMed project, with the goal of boosting BioTech-IP's development to lead to the successful commercialization of R&D results. The budget in this project is more than €225,000.

Funds secured for patent protection

The BioTech-IP team secured approximately €1.5 million to support the Patent Cooperation Treaty (PCT) patent procedures of 11 technologies of the Ochota Biocentre, supported by the Operational Programme – Innovative Economy 1.3.2 or PatentPlus program. Two of the technologies are owned by IIMCB:

1. "A method of peptide hydrolysis, peptidase, the composition for use as a bacteriostatic and bactericidal agent, a kit and the uses of the active form of LytM from S. aureus or derivatives thereof"
2. "Sequence-specific engineered ribonuclease H and the method for determining the sequence preference of DNA-RNA hybrid binding proteins"

In 2013, four new technologies from Ochota Biocentre were subject to international patent procedures.

Joint science-to-business projects

BioTech-IP assisted in the preparation of six Ochota Biocentre proposals to the Applied Research Programme of the National Centre for Research and Development. One of these projects is performed at IIMCB ("Biotechnological applications of bacteriolytic protein"), in which a Cooperation Research and Development Agreement was signed with one biotech company for the further improvement of LytM enzyme for commercial purposes.

Management of IP and commercialization of R&D results

BioTech-IP coordinated activities that sought to commercialize a joint invention by IIMCB and Proteon Pharmaceuticals Ltd. (http://proteonpharma.com), a spin-off company based on the invention, "Cells and methods useful in characterizing the immunotoxic activity of xenobiotic substances." This invention was granted a US patent in 2013 and is subject to a pending PCT patent procedure.

Two new patent applications were prepared in cooperation with scientific institutes in Poland and filed with the Polish Patent Office.

One patent application, "Method for selection of hDIS3 PIN domain inhibitors and use of hDIS3 PIN domain inhibitors for cancer treatment," was the result of a collaboration between the Institute of Biochemistry and Biophysics PAN and OncoArendi company.

The initial negotiations started with investors to establish a spin-off company for commercialization of the invention "Sequence-specific RNA nucleases."

IT Unit

From left: Jakub Skaruz (IT Specialist), Michał Romiszewski (System Administrator), Roman Szczepanowski (Director's Representative for Information Technology & Research Equipment).

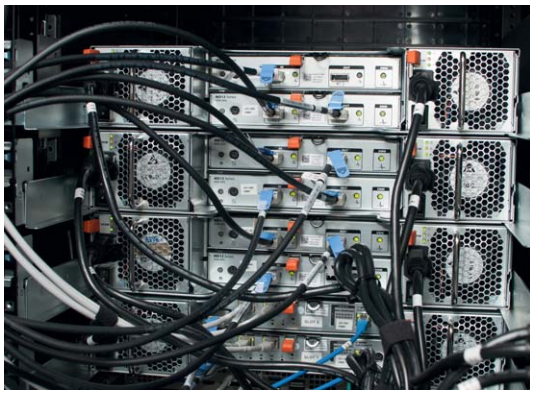
The tasks of the IT Unit focus on supporting various scientific activities of the Institute and on aiding the administrative staff in their core responsibilities. These objectives embrace many diverse, and highly technical fields, as listed below:

- Maintenance and administration of the computer network
- Administration of the e-mail system, DNS, DHCP and Proxy servers
- Helpdesk – providing user support, assistance with regard to the installation of hardware and software
- Ensuring the security of computer and e-mail data
- Maintaining and updating the anti-spam filter
- Administration of the Institute's web servers
- Maintenance of the Intranet service
- Providing remote external user access to computing resources of the Institute over the VPN protocol
- Creation and administration of diary information, e.g. task diaries containing information about the availability and use of scientific equipment
- Administration and continuous updating of financial and accounting software
- Providing back-ups to strategic computer servers
- Purchasing and managing computer software and making sure it is legally licensed
- Providing IT support for the seminars and conferences organized by IIMCB
- Hardware purchase coordination; consulting, preparation of tender specifications
- Keeping and updating the multimedia information service
- Setting up dedicated websites designated for conferences organized by the Institute.

The Institute has a modern computer network (1Gb/s), consisting of 7 nodes connected by optic fiber and structured cabling. The network is composed of 150 computers, both personal computers and dedicated units, supporting research equipment. The local network is connected to the Internet by optic fiber cables with a capacity of 1 Gb/s. Thanks to a subsidy from the Ministry of Science and Higher Education and the Institute's own resources, the IIMCB was able to build a state-of-the-art server room. In accordance with the latest trends in the construction of such facilities, the server room has an independent power supply system, consisting of two power lines, a battery backup system (UPS), a modern cooling system consisting of four networked air-conditioning cabinets, a raised technical floor, and a system designed for the monitoring of all environmental parameters, including the monitoring of water damage. Fire safety is ensured by a neutral gas extinguishing system.

The facility, described above, includes both the main servers of the Institute as well as the servers belonging to individual research groups. Particularly noteworthy are the resources of the Bioinformatics and Protein Engineering Laboratory: they include a computer cluster consisting of more than 2000 cores, with a file system built on the basis of SSD storage, a 100 TB backup memory and 14 multi-processor computing and application servers. These resources are used to develop, test, host and share publically original software such as PROTMAP2D, MetaServer, FRankenstein3D, ModeRNA server etc. and to develop specialized databases, such as MODOMICS, REPAIRtoire, SpliProt3D, RNA Bricks, etc.

The crystallographic servers used by the Protein Structure Laboratory and the Structural Biology Laboratory are also located in the server room. This is where the databases of the PolSenior centenarians' project can be accessed.



Thanks to a modern network infrastructure that provides very fast data transfer, the server room is also a place to collect and store all kinds of scientific image data (high-quality pictures and videos) produced by new microscopes such as the state-of- the-art fluorescence microscope Lightsheet.Z1.

In 2013 the activity of the IT Unit focused primarily on the virtualization of the main network services of the Institute. The consolidation of physical equipment was one of the main advantages of server virtualization. By reducing the number of old physical servers to two server hosts, we have reduced the costs of the maintenance and renovation of the server farm and in addition expanded the average utilization of equipment.

Currently our virtualization platform consists of:

- Two Citrix XenServer hosts – 2 x Dell PowerEdge R510 (each host has 2 processors with 4 cores and 64GB RAM)
- Shared Storage Repository – Dell PowerVault MD3600i with three expansion enclosures Dell PowerVault MD1200
- SAN Switch – 2 x Dell PowerConnect 8132 which support iSCSI data traffic.

With XenServer, applications and platforms are not tied down to server hardware. If there is particular demand for an application, the virtual machine can be rapidly moved to a server that is able to provide significantly higher processing capacity. This flexibility allows for the infrastructure to respond according to business demands and to eliminate slow/staggering performance of the system. Abstracting the platform from the physical infrastructure enables the optimization of resources through shared use. Allowing multiple users to share resources results in higher utilization thereof and thus a more efficient and effective use of the infrastructure.

The total size of the surfaced virtual disk offered by a set of disk arrays with expansion enclosures is now 56 TB. This performance potential is divided into a space for the virtual machine, for user data and a space for scientific equipment data.

After moving to a virtual platform the backup system has been improved and simplified. PHD Virtual Backup has been selected as a solution for backing up virtual machines from XenServer. Data backups are replicated on a dedicated physical backup server and ultimately will be replicated offsite. The implementation of new software has reduced backup creation time and the time needed to recover data.

Selected Projects

Fishing for medicines and their targets using Zebrafish models of human diseases

Coordination and Support Actions Project financed by the 7th Framework Programme of the European Union within the Research Potential scheme
fishmed.iimcb.gov.pl

IIMCB's strategic objective is to achieve the quality of research and innovative activities of leading research entities in the world. To achieve this level of excellence and increase our innovative potential, we introduced a new research model: zebrafish. The FishMed Centre, supported by the European Union and Ministry of Science and Higher Education, is composed of a Zebrafish Core Facility and research groups that use zebrafish in innovative projects that study the molecular mechanisms of disease. The EU and national funding will be used to finance the employment of 20 scientists, technicians, and managers, purchase state-of-the-art equipment, finance exchange visits between IIMCB researchers and their European partners, and participate in and organize various events, including those related to innovation and technology transfer.

Management

The FishMed project is coordinated by **Prof. Jacek Kuźnicki**, the author of the concept and application to set up the FishMed Centre at IIMCB. He is advised by the International Advisory Board and FishMed Committee. The project management team consists of a project manager, **Dr. Urszula Białek-Wyrzykowska**, and leaders of workpackages: Prof. Jacek Kuźnicki (WP1), Prof. Michał Witt (WP2), Prof. Alicja Żylicz (WP3), Magdalena Powierża (WP4), Dorota Libiszowska (WP5), and Dr. Urszula Białek-Wyrzykowska (WP6 and WP7).

Objectives

- Twinning of seven IIMCB groups with excellent European zebrafish centers to develop innovative potential using fish models.
- Development of a Zebrafish Core Facility and establishment of a new research group headed by a leader selected through an open international competition.
- Acquisition and upgrading of research equipment for a Zebrafish Core Facility and new zebrafish research laboratory.
- Reinforcement of IIMCB innovation potential with the Bio&Technology Innovations Platform (BioTech-IP).
- Construction of an interactive visibility platform to popularize the FishMed Centre and research with zebrafish models among scientific and non-scientific communities, including promotion of the project's innovative results.

Twinning and research

The FishMed Centre is a consortium of eight groups from IIMCB and six European institutions, including the Max Planck Institute for Heart and Lung Research (MPI-HLR) as a strategic partner. The twinning partners were chosen based on their expertise in research using zebrafish models, excellent publication records, and compatibility with the scientific interests of the FishMed Centre groups at IIMCB. European partners share with us their zebrafish models and expertise related to fish research. Twinning allows smooth passage through the initial phase of accommodating a new experimental model and quickly focusing on cutting-edge research that is likely to lead to innovations.

Twinning partners and research projects

- **Matthias Bochtler**, Laboratory of Structural Biology, IIMCB, and **Carl-Philipp Heisenberg**, Austrian Institute of Science and Technology (IST), Klosterneuburg, Austria.

Project: DNA methylation and demethylation in zebrafish.
Postdoctoral fellow: Agnieszka Kolano, PhD
Research assistant: Marta Wawrzyniak, PhD

The aim of this project is to decipher the role of TET proteins and 5-hydroxymethylcytosine (5hmC) in early zebrafish embryos. If the roles are different from those in mammals, as suggested by the first publications on 5hmC in zebrafish and preliminary data, then the project will also shed light on the evolution of a major system of epigenetic reprogramming in vertebrates.

In the case of DNA, reprogramming is associated with DNA demethylation. The pathways of DNA demethylation are still under debate. Currently, both passive and active DNA demethylation mechanisms are discussed. Active DNA demethylation requires the conversion of 5-methylcytosines (5mCs) back to cytosines in the absence of DNA replication. The best experimental support is for an oxidative DNA demethylation pathway that relies on the oxidation of 5mC to 5hmC, mediated by Ten-Eleven-Translocation (TET) dioxygenases.

In mouse zygotes, the TET3 enzyme strictly controls active DNA demethylation in the paternal pronucleus. However, recent data from non-mammalian vertebrates suggest that the biological functions of 5hmC and TETs might differ. In *Xenopus*, the morpholino-mediated knockdown of TET3 causes only a relatively "late" phenotype in ocular and neural development. More importantly, some studies report the absence of global

DNA demethylation in early *Danio rerio* embryos, whereas others support the existence of such demethylation in later stages.

Our results show that all TET enzymes (TET1-TET3) in the mouse have homologues in zebrafish. The expression pattern of TETs as analyzed by quantitative PCR corresponds to what could be expected based of the available data for mouse—higher TET3 levels before zygotic genome activation (2 h post-fertilization [2hpf] embryos) and higher TET1 and TET2 levels after zygotic genome activation (5 days post-fertilization [dpf] embryos). Moreover, the enzymatic product of Tet-mediated oxidation, 5hmC, was detected by performing dot-blot assays on genomic DNA isolated from 3.3 hpf and 12 hpf and 1-5 dpf embryos. To estimate the global 5-hmC content of genomic DNAs at the time points of zebrafish development mentioned above, a 5hmC glucosylation assay was used, and the level of 5hmC was at the level of UDP-[^{3}H] glucose incorporation by β-glucosyltransferase. For the earlier stages from zygotes to 1,000-cell stage (3 hpf), an immunofluorescence staining protocol was set up. A two-photon microscope that is available at IIMCB was utilized to show the localization of 5hmC in early zebrafish embryos.

To study the function of TET proteins in zebrafish embryogenesis and development experiments, we plan to perform TALEN- and CRISPR-mediated knockout experiments. To date, five TALENs that target TET proteins (deposited at Addgene) and five sgRNAs designed by our group have been tested. Primary analysis shows that one TALEN and one sgRNA are working. The focus will now be on raising the next generation of zebrafish lines with knockout of TET enzymes.

Localization of 5-methylcytosine (green) and 5-hydroxymethylcytosine (red) in 64-cell stage zebrafish embryo. Photo by Agnieszka Kolano.

- **Janusz M. Bujnicki**, Laboratory of Bioinformatics and Protein Engineering, IIMCB, and **Thomas Braun**, Max-Planck-Institute for Heart and Lung Research, Bad Nauheim, Germany.

Project: The development and application of bioinformatics software for the prediction of the pathogenic effects of mutations in protein- and RNA-coding loci.

Postdoctoral fellow: Wayne Dawson, PhD (since February 2014)

Research assistant: Michal Dyzma, MSc (since February 2014)

The planned activities of the Bujnicki group have been delayed because of the lack of appropriate candidates for the postdoctoral position, despite wide advertisement. In 2013, two postdoctoral candidates were recruited, who will work on complementary aspects of the project to make up for the delay. The work of the Bujnicki group that is related to the FishMed project has thus far mainly involved the work of the group leader himself, who coordinated the work of two MSc students (funded by sources outside of FishMed). This work primarily involved setting up and benchmarking third-party methods for predicting the effect of mutations on protein or RNA structure stability. To date, several methods for predicting the effects of mutations on protein stability (Dmutation, MuPro, I-Mutant, FoldX, CombMutate, and ERIS) have been evaluated, and a meta-predictor called MetaMisTher (based on the results of these methods) has been developed. Analogous analyses for RNA stability prediction are currently underway. The results obtained to date provide a solid basis for evaluating the predictive ability of methods based on statistical potentials to be analyzed by postdocs employed by FishMed in the Bujnicki group.

- **Agnieszka Chacińska**, Laboratory of Mitochondrial Biogenesis, IIMCB, and **Didier Stainier**, Max-Planck-Institute for Heart and Lung Research, Bad Nauheim, Germany.

Project: The role of protein import pathways in zebrafish development.

Postdoctoral fellow: Anna Sokół, PhD

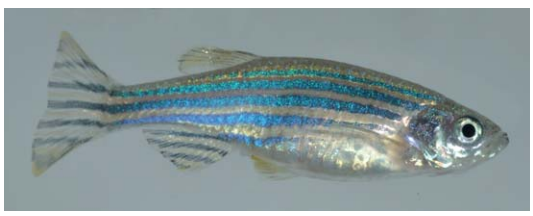
Research assistant: Michał Bazała, MSc

Mitochondria are important organelles involved in cellular metabolism and many regulatory processes. The focus of our group is to study the pathways that contribute to mitochondrial homeostasis and include the transport and turnover of mitochondrial proteins and consequences of their malfunction. We seek to understand how mutations in key players of mitochondrial protein import pathways influence the embryonic development of *Danio rerio*.

To address our scientific questions, we developed and optimized a protocol that allows the isolation of proteins from zebrafish embryos and larvae and study their levels and diversity at various stages of zebrafish development. Additionally and specifically to our research interests, we succeeded in enriching mitochondria protein extracts by isolating zebrafish mitochondria. Conceptually, we first focused our study on the importance of mitochondrial biogenesis in the MIA pathway, which plays a crucial role in protein import into the intermembrane space of mitochondria. We used well-established antisense techniques that exploit Morpholinos to achieve protein knock-down. Our goal was to knock-down Mia40, a key player of the MIA pathway and its substrate Timm9. The optimization and initial analyses of phenotypes, including inspections by a newly acquired Zeiss Lightsheet.Z1 confocal microscope system, are currently underway.

Additionally, we initiated our study by knocking out target proteins in zebrafish using an innovative technique that involves TALEN methodology. To achieve this, we collaborated with our FishMed research partner Dr. Didier Stainier in the Max Planck Institute in Bad Nauheim, Germany. During the past year, Dr. Anna Sokół has visited the laboratory of Prof. Stainier twice and stayed for a total period of 4 months. During the first visit, the TALEN technique was acquired and has already been successfully used to generate the first mutations in the zebrafish genome. The second visit to the Dr. Stainier laboratory was focused on learning and executing initial progeny screens. The visits to the laboratory of Dr. Stainier provided the opportunity to learn from and collaborate with scientists with top expertise in the *Danio rerio* model system, which greatly enhanced our know-how and conceptual expertise.

During the past year, scientists who were involved in the project, including research assistant Michal Bazała and postdoctoral fellow Dr. Anna Sokół, participated in specific training to improve their skills with the zebrafish model system. This includes training in microinjections, microdissections, genotyping, and microscopy. Additionally, Dr. Sokół participated in the "Working with Zebrafish Genome Resources" workshop organized by the Sanger Institute during the 8th European Zebrafish Meeting in Barcelona.



Zebrafish (*Danio rerio*). Photo by Michał Bazała.

• **Jacek Jaworski**, Laboratory of Molecular and Cellular Neurobiology, IIMCB, and **William Harris**, University of Cambridge, United Kingdom.

Project: Development of the zebrafish visual system as an *in vivo* model to study zTOR function and dysfunction in neurons.

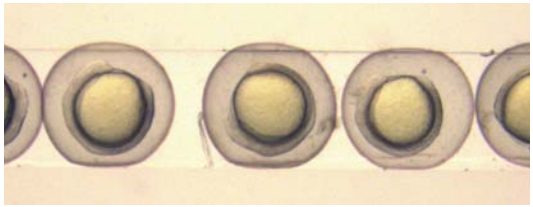
Postdoctoral fellow: Justyna Jezierska, PhD

Research assistant: Lidia Wolińska-Nizioł, MSc

The major aim of LMCN work within the FishMed grant is to establish zebrafish as a model to study the role of TOR kinase in neuronal development at the molecular and cellular levels in intact, living animals. More specifically, the project focuses on understanding the role of TOR kinase in the development of different classes of neurons in zebrafish retina. During the reporting period, the major efforts of LMCN were focused on the following:

– establishing optimal conditions for DNA transfer to fish embryos for the mosaic visualization of selected types of fish retina neurons (e.g., retinal ganglion neurons)
– optimizing conditions for confocal microscopy and SPIM microscopy based on the visualization of dendritic arbors of neurons within fish retina
– optimizing the conditions for the immunofluorescent staining of whole-mount and retinal sections for classical protein markers of different types of retinal neurons
– optimizing the conditions for the immunofluorescent staining of whole-mount and retinal sections for basic components of the TOR pathway.
– importing mutants of TSC2 and zTOR to the IIMCB fish facility
– developing a system for the visualization of zTORC1 activity in fish retinal neurons

To date, we have been able to optimize the conditions for efficient DNA injections and the visualization of retinal ganglion neurons based on an Ath5 promoter fluorescent reporter. Whole-mount immunofluorescence conditions were also optimized for retinal markers. Finally, both mutant fish lines (*tsc2* and *ztor*) were successfully introduced to IIMCB, genotyped, bred, and transferred to the main tank system. During the next reporting period, we plan to use those lines to understand the role of zTOR in retinal neuron development. A visit to the Harris lab is also planned to perform embryo transplantation experiments.



Zebrafish embryos. Photo by Lidia Wolińska.

• **Jacek Kuźnicki**, Laboratory of Neurodegeneration, IIMCB, and **Oliver Bandmann**, MRC at the University of Sheffield, United Kingdom.

Project: The mechanism of calcium perturbation in *pink-1* mutant of zebrafish, a model of Parkinson's disease.

Postdoctoral fellow: Smijin Soman, PhD

Research assistant: Michal Bazała, MSc

Research activities: Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects dopaminergic neurons in the substantia nigra, leading to a severe motor deficit. It affects more than 2% of the ageing population, and no permanent cure for the disease has yet been discovered.

The major objective of this project is to study alterations in calcium homeostasis in PD. We used a *pink-1* mutant (*pink*$^{-/-}$) zebrafish line with a premature stop mutation (Y431*) in the PINK1 kinase domain to identify the molecular mechanisms involved in altered calcium homeostasis. To understand changes in calcium homeostasis after PINK1 knockdown, studying calcium influx, efflux, and basal levels is important. Therefore, the estimation of calcium using fluorescent imaging was performed in the early phase of the project. Accordingly, zebrafish neuronal cultures were developed, but the viability of the neurons was significantly lower after 24 h *in vitro*. As a result, we moved to an *in vivo* quantification approach. Calcium Green was used as a calcium indicator, which exhibits an increase in fluorescence upon binding to Ca^{2+}. It was injected into single cell stage zebrafish eggs, and calcium imaging was performed in 1 day post-fertilization (dpf) eggs to 3 dpf fish. This procedure was standardized by considering various factors like egg viability and Calcium Green concentration, toxicity, and robustness.

As part of the twinning activities, Dr. Smijin Soman visited the University of Sheffield for 3 months to learn new techniques and widen the scope of the project. It is widely accepted that there is an enhanced state of mitochondrial calcium in PD and especially in PINK1 deficiency. The objective during his visit was to study the role of mitochondrial calcium channels, especially the mitochondrial calcium uniporter (MCU) and voltage-dependent anion channel (VDAC). He received extensive training in techniques like morpholino-based gene knockdown in zebrafish and *in situ* hybridization. He used these techniques in the experiments and obtained preliminary results regarding knocking down calcium channels in a PINK1 mutant zebrafish model. He also gained experience in genome engineering technologies, such as Targeting Induced Local Lesions in Genomes (TILLING). Such expertise in TILLING will enable the design of stable zebrafish mutants, which is considerably important for future research.

Zebrafish line Wet-Aqua pink x *casper*. Photo by Michał Bazała.

• **Marta Miączyńska**, Laboratory of Cell Biology, IIMCB, and **Marcos Gonzalez-Gaitan**, Department of Biochemistry, University of Geneva, Switzerland.

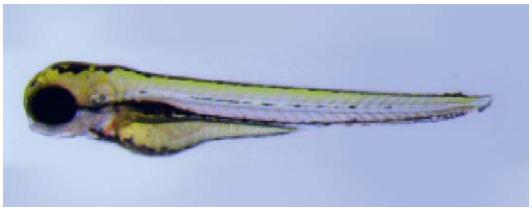
Project: The role of endocytic proteins in signaling and transcriptional regulation in zebrafish.

Postdoctoral fellow: Anna Bartosik, PhD

Research assistant: Lidia Wolińska-Nizioł, MSc

The Laboratory of Cell Biology collaborates with Prof. Marcos Gonzalez-Gaitan to investigate the role of endocytic proteins in signaling and transcriptional regulation in zebrafish. An emerging view is that endocytosis and other vesicular trafficking processes can profoundly affect gene expression patterns, and aberrant endocytosis is now strongly linked to carcinogenesis and neurodegenerative diseases. The common project between the two twinning groups seeks to identify the mechanisms and molecular targets that link endocytosis and transcriptional control. In unbiased RNAi screens performed in cultured mammalian cells, the Laboratory of Cell Biology identified candidate endocytic proteins that are involved in transcriptional regulation in several signaling cascades, including the Wnt and NF-κB pathways. These proteins are now being investigated with regard to their roles in zebrafish development. We first wish to determine whether the novel roles of such proteins,

identified in mammalian cells, are evolutionary conserved. To this end, in the first year of the project, we established procedures to downregulate or overexpress the proteins of interest in fish embryos. Moreover, we set up methods to evaluate the activity of the Wnt and NF-κB pathways in early zebrafish development. This included morphological measurements of the developing embryos, *in situ* hybridization to assess the expression of pathway markers, and real-time PCR to quantify the expression of pathway target genes. This allowed us to create a methodological toolbox to study the selected proteins in zebrafish development. Our initial results indicated that at least some of the effects caused by the depletion of endocytic proteins in cultured mammalian cell lines may be conserved in fish. This opens up several possibilities for continuation of the project to precisely characterize the observed developmental phenotypes and describe their underlying mechanisms.



Zebrafish larvae. Photo by Lidia Wolińska.

- **Maciej Żylicz**, Department of Molecular Biology, IIMCB, and **Ewa Snaar-Jagalska**, Department of Molecular Cell Biology, Institute of Biology, Leiden University, The Netherlands.

Project: The Heat Shock Protein network and p53 response in zebrafish.

Postdoctoral fellow: Maciej Olszewski, PhD

Research assistant: Marta Wawrzyniak, PhD

The incidence of cancer is ever-increasing. The diversity of processes that lead to cancerogenesis makes the detection and treatment of cancers very difficult. However, more than 50% of cancers have a mutation in the gene that encodes the tumor suppressor protein p53. Such mutations either render the p53 protein inactive or let it acquire new, oncogenic functions. The disruption of p53 has been shown to lead to uncontrolled cell division. Increasing evidence also indicates that mutated forms of p53 are involved in the stimulation of angiogenesis and regulation of cell migration, which are both required for the cancer to metastasize. Wildtype p53 negatively regulates VEGF bioactivity, but mutated p53 can stimulate angiogenesis by upregulating VEGF bioactivity. Similarly, several p53 mutants have been reported to contribute to the epithelial-mesenchymal transition that is a hallmark of migrating tumor cells.

The major objective of this project is to study the molecular mechanisms that link the mutation status of p53 and tumor cell migration or angiogenesis stimulation. We employ two biological models: *in vitro* cultures of cancer cell lines and zebrafish (*Danio rerio*) as a host for the xenotransplantation of cancer cells.

Our cell-based model consists of a panel of cancer cell lines, in which their endogenous p53 with various mutations is replaced by wildtype protein or a mutant of our choice. This is a significant improvement over current models because it removes the variability of the genetic background that makes deciphering the effect of particular p53 mutations difficult. Transplants of CM-DiI-labeled cancer cells into Casper fli GFP zebrafish with fluorescent endothelium were performed to monitor metastasis and early angiogenesis. As an alternative, we are also testing genetic labeling of the implanted cells with the novel fluorescent protein tdTomato. We are concurrently working on the details of the molecular mechanisms that regulate VEGF bioactivity in response to the change in p53 status.

Zebrafish Core Facility

Leader: Małgorzata Wiweger, PhD

Technician: Monika Turniak, ing. of zootechnics

Technician: Maciej Mańk, MSc in biology

Technician: Krzysztof Surga, MSc in biology

Veterinarian: Piotr Korzeniowski, MVD

The state-of-the-art facility includes a water plant, stand-alone quarantine unit, and main system manufactured by Tecniplast. The system can hold approximately 6,000 adult fish, and further expansion is ongoing. Two new rooms have recently been adapted, and the installation of new aquatic systems should begin soon. Currently, 32 different lines of zebrafish are being housed in 302 tanks, and more lines will be introduced shortly. In addition to the aquarium room, which is a restricted area, our Zebrafish Core Facility (ZCF) has a new laboratory space that is fully equipped for standard fish work. Alongside a needle puller and beveller, suited for the production of capillary needles for microinjection in zebrafish that were purchased before from other sources, ZCF purchased three incubators, five microscopes, four injectors with micromanipulators, one micromanipulator (CellTram) for transplantation, a PCR machine, and two systems for imaging and data storage that accompany the microscopes.

Since 2013, ZCF has been registered in the Zebrafish Model Organism Database (ZFIN), the main international zebrafish database, and European Society for Fish Models in Biology and Medicine (EuFishBioMed), a European network that is devoted to fostering the exchange of information, techniques, materials, and expertise within and beyond the fish community.

In 2013, we secured five wildtype lines, five transgenic lines, and five mutant lines that were requested by FishMed groups.

New research group

Developmental Genomics, IIMCB /Max PlanckResearch Group, is headed by **Dr. Cecilia L. Winata**. Dr. Winata was selected during an open international competition conducted by IIMCB and the Max Planck Society. The group is also affiliated with MPI-HLR in Bad Nauheim, our strategic partner for the creation and development of the FishMed Centre. The laboratory will thus have full access to MPI-HLR equipment, animal facilities, and genetic modification techniques for zebrafish and mice.

The expertise of the new group leader and her staff will further help us achieve a critical mass for the development of competitive and innovative zebrafish projects.

Research equipment

IIMCB laboratories are equipped with state-of-the-art scientific equipment. Several large pieces of equipment have been purchased for zebrafish-related research. The best example is Lightsheet.Z1, a fluorescence microscope manufactured by Zeiss. It works on the principle of single-plane illumination microscopy (SPIM), in which the illuminating planar sheet of

Fluorescence microscope – Lightsheet Z1 and Zebrafish head, 2 days after fertilization. Visualization in 3D of GFP localized in mitochondria. Photo by Roman Szczepanowski & Michał Bazała.

light is perpendicular to the detection axis. This allows for the much more efficient usage of light illumination, thus reducing the unwanted effects of photobleaching and phototoxicity. The sample, typically embedded in agarose rods, can be moved in all directions and rotated 360° during imaging. Lightsheet. Z1 is particularly well suited for the long-term live imaging of medium-sized objects, such as zebrafish larvae.

Bio&Technology Innovations Platform

In response to the growing potential of IIMCB, a separate unit was established in March 2010 to deal with applied technology generated at IIMCB, referred to as the Bio&Technology Innovations Platform (BioTech-IP). Presently, Biotech-IP's aim is to find, protect, and commercialize projects that display market potential.

BioTech-IP started cooperation with two technology transfer experts from the USA and UK. One of them assisted with the commercialization of a technology developed at IIMCB and later deployed by a spin-off company, Proteon Pharmaceuticals. The other one was involved in negotiations with investors to establish a spin-off on the basis of a new technology developed in Prof. Bujnicki's laboratory. In 2013, BioTech-IP organized three brunches, one of which was under the auspices of the FishMed project. It was a satellite meeting to the FishMed kick-off that took place on April 12th. The brunch featured five presentations and was attended by seven entrepreneurs and 15 scientists. On October 9-10, 2013, a representative of BioTech-IP participated in the largest European biotechnology trade fair, Biotechnica 2013, held in Hanover, Germany. This was an excellent opportunity to showcase innovative products, exchange scientific experience, establish and maintain business contacts, network, and present technology offers at the biopartnering session. The purpose of the delegation was to present BioTech-IP's technology offers and establish new business contacts.

For more information, please refer to the BioTech-IP website: http://www.biotech-ip.pl/en.

FishMed visibility

The FishMed project gave IIMCB an unprecedented opportunity to develop widespread, professional PR activities at the Institute. For the first time these can be focused on both the research community – by promoting zebrafish model and disseminating scientific results, and on wider society – by inspiring it to take interest in research and develop a dialogue with the scientific community.

The PR activities began in the early 2013 with the creation of the PR Unit and execution of the social communication audit among the Institute's employees. The outcomes of the latter laid the ground for the development of a PR strategy comprising the plan for activities which are currently being implemented.

FishMed communication tool-kit – a friendly package

The FishMed logo, a roll-up and a brochure promoting the project were developed as a part of IIMCB corporate identity system to be fully completed in 2014. The project website was launched at fishmed.iimcb.gov.pl. Constantly updated, the website popularizes FishMed and zebrafish research among scientific and non-scientific communities and it is also used for commercialization purposes.

Initial FishMed event in the limelight

The FishMed kick-off meeting took place in April 2013. The press conference attracted a wide media coverage, including numerous press articles, TV spots, interviews and radio programs (full list at fishmed.iimcb.gov.pl). The meeting was described in a movie published in internet (IIMCB and FishMed web pages, YouTube, etc.)

The open part of the kick-off meeting hosted around 150 people, including representatives of the Ministry of Science and Higher Education, National Contact Point for Research Programs of the EU, embassies, SMEs, media and scientists. The event was opened by Prof. Jacek Guliński, the Under-Secretary of State in the Ministry of Science and Higher Education. Grzegorz Ambroziewicz, the Project Officer at the European Commission, highlighted HORIZON2020, a new EU funding program. Prof. Jacek Kuźnicki presented FishMed, and Prof. Snaar-Jagalska gave a closing talk explaining why and how zebrafish is used in research.

During the seminar part of the meeting presentations were given by:

- Thomas Braun, Max-Planck Institute for Heart and Lung Research, Germany
- Didier Stainier, Max-Planck Institute for Heart and Lung Research, Germany
- Carl-Philipp Heisenberg, the Austrian Inst. of Science and Technology, Austria
- William Harris, University of Cambridge, United Kingdom
- Oliver Bandmann, University of Sheffield, United Kingdom
- Marcos Gonzalez-Gaitan, University of Geneva, Switzerland
- Ewa Snaar-Jagalska, Leiden University, The Netherlands.

Discussion platform on zebrafish usage

Discussion forum for the Polish scientific community on the zebrafish usage was initiated based on the open seminars provided at IIMCB by renowned experts in the field:

- Robert Huber (Max-Planck Institute of Biochemistry Martinsried, Germany)
- Branko Latinkić (Cardiff University, UK)
- Jarema Malicki (University of Sheffield, UK)
- Maximilian Fürthauer (Nice Sophia Antipolis Univ. France)
- Daniela Panakova (Max-Delbrück-Center for Molecular Medicine, Germany)
- Cecilia Lanny Winata (National University of Singapore)

Dissemination of scientific results

Active presence of FishMed researchers on the international research scene facilitated spreading knowledge on the project and its first scientific results:

- 8th European Zebrafish Meeting (Barcelona Spain, July 2013) – Jacek Jaworski, Jacek Kuźnicki, Marta Miączyńska, Anna Sokół, Lidia Wolińska, Małgorzata Wiweger
- Jacques Monod Conference "DNA Methylation and Demethylation" (Roscoff, France, September 2013) – Agnieszka Kolano, poster presentation
- National Veterinary Institute training and reporting meeting for fish pathologists (Puławy, October 2013) – Piotr Korzeniowski, oral presentation.

Activating and initiating a dialogue with wider society

As a new quality to dissemination activities, the FishMed allowed to introduce a visibility strategy targeted at circles outside academia. Actions towards the general public were initiated by an internal PR action – an art competition for the children of IIMCB employees.

Further FishMed popularization activities involved:

- organization of a „Genetic Zoo" program within the XVII Festival of Science (Biotech Café, September 2013)
- a promotional stand at the WIRE 2013, Cork, Ireland (A. Nałęcz-Tolak, September 2013)
- talk at the conference EU HORIZON 2020 Program and Teaming for Excellence in the European Research Area in Prague, Czech Republic (J. Kuźnicki, October 2013)
- organization at IIMCB of an info day for talented youth (IIMCB and the Polish Children's Fund, March 2014)

Study on Ageing and Longevity

From left: Małgorzata Mossakowska, Przemysław Ślusarczyk, Katarzyna Wodzyńska, Aleksandra Szybalska.

Study on ageing and longevity

In 1999, at the time when the pilot research of the Polish Centenarians Programme (Polish acronym: ***PolStu99***) was launched, there was very scarce information on problems related to longevity in Poland and there were no studies carried out on the group of people aged 100+. Research on Polish centenarians was proposed by Prof. Ewa Sikora from the Nencki Institute of Experimental Biology PAN who had collaborated for a number of years with Prof. Claudio Franceschi from the University of Bologna. The *PolStu99* project, developed and managed by Prof. Jacek Kuźnicki, was a pioneering venture modeled after Italian experience of many years. Research tools to be included in the Comprehensive Geriatric Assessment were selected in collaboration with Polish experts in geriatrics and neurology, and the scope of the interview, physical examination and laboratory tests was determined. An integral part of the project was the development of a data digitization tool which, further into the project, made it possible to archive the results and enabled effective processing and statistical analysis of data. The resulting database and its categories have been, with some modifications, in use to this day, offering a convenient tool for data collection and analysis.

The *PolStu99* project, with over ninety participants aged 100+ residing in four regions of Poland, was a multi-center study and it involved a questionnaire survey, a geriatric medical examination, the collection of biological samples (venous blood) and the banking of DNA isolated from peripheral blood leukocytes. The project represented an innovative development in Polish gerontology – because of the subject and scope of the research. At the same time it presented an organizational challenge, due to the need to address various methodological and recruitment-related issues, to conduct a structured interview and collect biological material at the respondents' homes, and to transport the blood to regional laboratories and the central laboratory. This part of the project yielded biological samples collected from over 80 centenarians, which enabled the researchers to create a unique collection of biological material for further research.

The experience and results of the *PolStu99* project formed a basis for the development of a research project commissioned by the Committee for Scientific Research (KBN) named *Genetic and environmental factors of longevity of Polish centenarians* (***PolStu2001***). On the part of the IIMCB, this nationwide and multidisciplinary project was co-authored by **Prof. Jacek Kuźnicki** (Project Leader) and **Dr. Małgorzata Mossakowska** (Project Coordinator). The results of the study enabled the assessment of the health status and the physical and cognitive performance of a representative group of about 350 Polish centenarians (Mossakowska et al., 2008). The results of the study documented the mismatch between the Polish health and care services system and the needs of the oldest residents across the entire country. The level of disability affecting people aged 100+ has an impact on the scope of their needs for care which, in the Polish reality, is met primarily by the family. It was demonstrated that the main predictor of survival of people in such an advanced age was their cognitive performance and functional status (Mossakowska et al., 2014).

A continuation of the research on ageing was another centrally commissioned project, known under the acronym ***PolSenior*** (full title: *Medical, psychological, sociological and economic aspects of ageing in Poland*). IIMCB was appointed as the leader of the research consortium, with **Prof. Piotr Błędowski** (Warsaw School of Economics) as Project Leader and **Dr. Małgorzata Mossakowska** (IIMCB) as Project Coordinator.

The value of the *PolSenior* project lay in the interdisciplinary nature of the research covering not only medical, but also social, economic and psychological aspects with impact on the ageing population. A new quality was infused into the project thanks to the cooperation between experts from various academic fields reaching beyond biological sciences, the partnership with private contractors experienced in population-based research, and the involvement of NGOs in the project. By making it possible to estimate the prevalence of certain health conditions on the basis of data going beyond the records of the Central Statistical Office (GUS) and National Health Fund (NFZ), the project contributed also to a better understanding of problems related to public health policies. This was possible because the project was based not only on a questionnaire survey but also on objective measurements, geriatric scales, and biological material testing.

The *PolSenior* project, carried out in 2007-2012, was the largest gerontology research project in Poland and one of the largest in Europe. Among the project's contributing participants were

over 30 academic centres, about 170 researchers and almost 500 nurses taking part in fieldwork; the research sample included 5,695 respondents (Błędowski et al., 2011). Despite problems related to recruitment of respondents, the response rate for the entire *PolSenior* study reached 43%.

The results of *PolSenior* served as the basis for recommendations developed with regard to public health and social policies targeting the elderly population, both on a national and local scale. It should be emphasized that a comprehensive approach to the problems of an ageing population is in line with the assumptions of policies aimed at senior citizens and provides a solid academic basis as the foundation for pursuing these policies. The results of *PolSenior* were published in 20 scientific publications. A comprehensive summary of the key aspects of this research project can be found in the monograph *Medical, psychological, sociological and economic aspects of ageing in Poland* (Mossakowska et al., 2012, eds; http://polsenior.iimcb.gov.pl/monografia).

Currently, the group led by Dr. Mossakowska is involved in a 3-year project named *Polish Reference Genome for Genomic Diagnostics and Personalized Medicine (**PLGen**)*, financed by NCBR. The project aims to determinate the reference sequence and complete the genomic databases of Polish subpopulations for commercial diagnostic applications and research in the field of personalized medicine. The project will be carried out with the use of the biological material and clinical data yielded by the *PolStu* and *PolSenior* projects. In 2013 the databases of the *PolStu* and *PolSenior* projects were searched for a selection of healthy long-living individuals (aged 95 years or more). To enhance the previous study, a group of about 150 centenarians and nonagenarians from Warsaw took part in the *PLGen* project till March 2014.

Selected publications:

- **Mossakowska M**, Broczek K, Wieczorowska-Tobis K, Klich-Rączka A, Jonas M, Pawlik-Pachucka E, Safranow K, **Kuznicki J**, Puzianowska-Kuznicka M. *Cognitive performance and functional status are the major factors predicting survival of centenarians in Poland*. J Gerontol A Biol Sci Med Sci. 2014. doi:10.1093/gerona/glu003
- Iakoubov L, **Mossakowska M**, Szwed M, Duan Z, Sesti F, Puzianowska-Kuznicka M. *A Common Copy Number Variation (CNV) Polymorphism in the CNTNAP4 Gene: Association with Aging in Females*. PLoS One, 2013; 8(11), e79790
- Nadrowski P, Chudek J, Grodzicki T, **Mossakowska M**, Skrzypek M, Wiecek A, Zdrojewski T, Kozakiewicz K. *Plasma level of N-terminal pro brain natriuretic peptide (NT-proBNP) in elderly population in Poland - The PolSenior Study*. Exp Gerontol. 2013; 48(9):852-857
- Bik W, Baranowska-Bik A, Wolinska-Witort E, Kalisz M, Broczek K, **Mossakowska M**, Baranowska B. *Assessment of adiponectin and its isoforms in Polish centenarians*. Exp Gerontol. 2013; 48(4):401-407
- Golanska E, Sieruta M, Gresner SM, Pfeffer A, Chodakowska-Zebrowska M, Sobow TM, Klich I, **Mossakowska M**, **Szybinska A**, Barcikowska M, Liberski PP. *APBB2 genetic polymorphisms are associated with severe cognitive impairment in centenarians*. Exp Gerontol. 2013; 48(4):391-394
- Skalska A, Wizner B, Piotrowicz K, Klich-Rączka A, Klimek E, **Mossakowska M**, Rowiński R, Kozak-Szkopek E, Jóźwiak A, Gąsowski J, Grodzicki T. *The prevalence of falls and their relation to visual and hearing impairments among a nation-wide cohort of older Poles.* Exp Gerontol. 2013; 48(2):140-146
- Bledowski P, **Mossakowska M**, Chudek J, Grodzicki T, Milewicz A, **Szybalska A**, Wieczorowska-Tobis K, Wiecek A, Bartoszek A, Dabrowski A, Zdrojewski T. *Medical, psychological and socioeconomic aspects of aging in Poland*
Assumptions and objectives of the PolSenior project. Exp Gerontol. 2011; 46(12):1003-9
- Polosak J, Roszkowska-Gancarz M, Kurylowicz A, Owczarz M, Dobosz P, **Mossakowska M**, **Szybinska A**, Puzianowska-Kuznicka M. *Decreased expression and the Lys751Gln polymorphism of the XPD gene are associated with extreme longevity*. Biogerontol. 2010; 11(3):287-297
- **Mossakowska M**, Barcikowska M, Broczek K, Grodzicki T, Klich-Raczka A, Kupisz-Urbanska M, Podsiadly-Marczykowska T, Sikora E, **Szybinska A**, Wieczorowska-Tobis K, Zyczkowska J, **Kuznicki J**. *Polish Centenarians Programme. Multidisciplinary studies of successful ageing: aims, methods, and preliminary results*. Exp Gerontol. 2008; 4(3):238-244
- **Mossakowska M**, Broczek K, **Witt M**. [eds.]: *Skazani na długowieczność. W poszukiwaniu czynników pomyślnego starzenia*. Poznań: Ośrodek Wydawnictw Naukowych, 2007; http://cent.iimcb.gov.pl/publications/PolStu-monograph.pdf

Selected Projects

Interdisciplinary Innovative Projects

RNA+P=123D, ERC Starting Grant, FP7

The project, "Breaking the code of RNA sequence-structure-function relationships: New strategies and tools for modelling and engineering DNA and RNA-protein complexes," was awarded to **Prof. Janusz M. Bujnicki**, the first laureate of this prestigious EU grant at IIMCB. The aim of the 5-year project is to use bioinformatics and experimental methods to develop tools for predicting structures of RNA and RNA protein complexes and design RNA molecules with new structures.

NERCOMP, ERC Starting Grant, FP7

The laureate of the ERC StG project, "Structural studies of nucleotide excision repair complexes," is **Dr. Marcin Nowotny**. The overall objective of NERCOMP is to expand knowledge about DNA repair mechanisms. Specifically, Dr. Nowotny focuses on the structural and biochemical characterization of protein complexes involved in NER pathways in bacteria and eukaryotes. This is a key process for a basic understanding of genome stability and because a disturbance in these mechanisms in humans can entail tumorigenesis.

International Early Career Award (IECS), HHMI

With support from HHMI for the project, "Structural and mechanistic studies of nucleic acid processing," **Dr. Marcin Nowotny** investigates enzymes that act on RNA and DNA. He takes a special interest in deadenylases, enzymes that kick-start RNA degradation, an essential cellular process. By revealing the crystal structures of deadenylases, Dr. Nowotny hopes to gain insights into the mechanisms of their activity.

International Senior Research Fellowship (ISRF), Wellcome Trust

The project, "Structural and biochemical studies of Holliday junction resolution," is an extension and completion of the first ISRF grant awarded to **Dr. Marcin Nowotny**. Its aim is to determine the structural basis of the recognition of four-way DNA structures by proteins. This is important for such processes as DNA repair.

WELCOME Programme, FNP

The Welcome grant of the Foundation for Polish Science was awarded to **Dr. Agnieszka Chacińska** after her relocation from the Freiburg University to IIMCB to support the research project „Biogenesis and turnover of mitochondrial intermembrane space proteins". The aim of this project is to discover dynamic reactions that contribute to building and maintaining of the proteome of cellular power plants - mitochondria. In-depth understanding of these processes is an important step towards understanding pathologies caused by malfunction of mitochondria and proteotoxicity.

MAESTRO grant, NCN. New functions of endocytic proteins in transcriptional regulation

The objective of the project led by **Prof. Marta Miączyńska** is to characterize the molecular mechanisms by which endocytic proteins may participate in transcriptional regulation controlled by intracellular signaling pathways. Selected endocytic proteins were first identified in RNAi-based screens as novel regulators of transcription. For each of these proteins, the researchers plan to characterize its target genes, the relationship between its endocytic and transcriptional roles, its domains, activities, or interaction partners required for transcriptional regulation, and the signaling pathway stage at which it acts.

MAESTRO grant, NCN. Transgenic mice with elevated basal level of calcium ions in neurons as a model of aged-induced neurodegeneration of sporadic Alzheimer's disease

The project led by **Prof. Jacek Kuźnicki** seeks to generate and characterize transgenic mice that exhibit dysregulated Ca^{2+} homeostasis by overexpressing STIM proteins involved in store-operated calcium entry (SOCE). The dysregulation of neuronal Ca^{2+} homeostasis in the proposed model is expected to have consequences for neurons that are similar to those that occur during ageing or produced by large increases in Ca^{2+} during excitotoxicity that will create conditions that predispose neurons to the pathological changes observed in human sporadic Alzheimer's disease (SAD).

MAESTRO grant, NCN. Structural RNomics

The scientific goal of this project headed by **Prof. Janusz M. Bujnicki** is to characterize the relationships between sequence, structure, and function for all RNAs using combined bioinformatics, experimental biochemistry, and structural biology tools. This will be accomplished by classifying ncRNA molecules, predicting their secondary and tertiary structures, validating the structural predictions, determining high-resolution structures, interpreting the results in an evolutionary context, and constructing a publicly available database that contains the results of this study.

MAESTRO grant, NCN. Molecular mechanisms of pro-survival processes in breast cancer

The goal of the project led by **Prof. Maciej Żylicz** is to demonstrate a new role for MDM2 protein as the main oncogenic driver in breast cancer survival processes that function independently of p53 mutational status. The outcomes of this research may provide new ways to develop novel cancer therapies, in which tumor growth and resistance to standard therapies can be reversed by specific MDM2 inhibitors. The approach is unique because previous strategies sought to discover inhibitors that interfere with interactions between MDM2 and p53.

Application-oriented Projects

EPISTOP, Collaborative project, FP7

The aim of the EPISTOP project is to better understand the mechanisms of epilepsy in patients with tuberous sclerosis complex (TSC). The title of the project is, "Long-term, prospective study evaluating clinical and molecular biomarkers of epileptogenesis in a genetic model of epilepsy – tuberous sclerosis complex." This is a multicenter study, involving 14 hospitals and laboratories from Europe and the United States, at IIMCB coordinated by **Prof. Jacek Jaworski**. The project seeks to identify the risk factors and biomarkers of epileptogenesis, including the development of clinical seizures and course of a disease. Another important goal of the project is to identify means and targets that enable epilepsy prevention and disease development modifications. In the long term, we plan to prepare new directions and guidelines regarding the treatment of patients with TSC and epilepsy that could improve patients' quality of life.

BESTCILIA, Collaborative project, FP7

Prof. Michał Witt is a partner in the research consortium, "Better Experimental Screening and Treatment for Primary Ciliary Dyskinesia." Coordinated by Prof. Heymut Omran from the University of Munster, this multi-partner project concentrates on observational studies to characterize the clinical course and improve the diagnosis and treatment of primary ciliary dyskinesia (PCD). Prof. Witt's responsibilities in BESTICILIA are to supervise observational studies performed by a third party, the Institute of Tuberculosis and Lung Diseases in Rabka-Zdrój, and lead the project's training and dissemination activities.

eRNAses, ERC Proof of Concept, FP7

Awarded to **Prof. Janusz M. Bujnicki**, the eRNAses project is the only ERC Proof-of-Concept grant that has been conducted in Poland. This project lasted 12 months and was completed in 2013. It continued research on RNases that are capable of sequence-specific RNA cleavage. The commercialization process of the invention has been initiated. The results have significantly contributed to the practical value of the invention made in Prof. Bujnicki's laboratory and now serve as a basis for further steps in the commercialization process.

Aurezyna, project within Applied Research Program, NCBR

The group headed by **Dr. Izabela Sabała** works on the project, "Biotechnological applications of bacteriolytic protein," awarded to a consortium established by IIMCB (project leader) and A&A Biotechnology (commercial partner). While working on the structural and biochemical characterization of an autolysin from *Staphylococcus aureus*, very unusual and commercially valuable features of the enzyme were discovered, including the very efficient lysis of staphylococcal cells under unique environmental conditions of low temperature and exceptionally low ionic strength. The aim of the project is to explore commercial applications of the enzyme, including staphylococcal cell lysis that allows the isolation of cellular components, diagnostic tests, and a wide range of bacteriostatic and bacteriolytic applications (e.g., the elimination of staphylococci from food and hospital environments). Further basic research will also be performed to expand environmental tolerance of the enzyme and modify its specificity.

New drugs for targeted therapy of multiple myelomas, NCBR

A consortium headed by Dr. Andrzej Dziembowski (IBB PAN) works on developing new inhibitors of cellular targets that are essential for the survival of multiple myelomas. **Dr. Marcin Nowotny** is responsible for the structural biology part of the project, including solving the crystal structures of complexes between protein targets and inhibitors to aid structure-activity relationship analyses. The ultimate goal is to develop potent inhibitors that specifically block the targets.

The study of genetic variation at the cellular level – new opportunities for forensic identification (AriaDNA 2010), NCBR

The aim of this project coordinated by **Prof. Michał Witt**/**Prof. Jacek Kuźnicki**, was to develop modern and innovative methods of biological sample identification based on complex genetic analysis combined with advanced separation of cells using laser dissection and FACS. Sets of transcriptional and methylation markers and SNPs were defined and successfully tested to identify single cells in mixed samples. The methods that were developed formed the core of three patent applications and are currently being tested in forensic laboratories. The participants on the project include groups of IIMCB (led by **Dr. Izabela Sabała**) the Maria Sklodowska-Curie Memorial Cancer Center, Institute of Oncology-Gliwice Branch(led by **Prof. Barbara Jarząb**), Institute of Human Genetics of the Polish Academy of Sciences (led by **Prof. Ewa Ziętkiewicz**), Internal Security Agency (led by **Dr. Rafał Wierzchosławski**), and Precoptic Co (led by **Michał Wojciechowski**).

& Facts Figures

Grants

7th Framework Programme

ERC Grants

- **eRNAses** "Engineered Sequence-Specific RNases: New reagents for RNA biotechnology" ERC Proof of Concept; (324624); 149,970 EUR; 2013; **J.M. Bujnicki**
- **NERCOMP** "Structural studies of Nucleotide Excision Repair complexes" ERC, (281500); 1,498,000 EUR; 2012-2016; **M. Nowotny**
- **RNA+P=123D** "Breaking the code of RNA sequence-structure-function relationships: New strategies and tools for modelling and engineering of RNA and RNA-protein complexes" ERC, (261351); 1,500,000 EUR; 2011-2015; **J.M. Bujnicki**

Other

- **EPISTOP** "Long-term, prospective study evaluating clinical and molecular biomarkers of epileptogenesis in a genetic model of epilepsy – tuberous sclerosis complex"; (602391); 774,818 EUR; matching funds 829,113 PLN; 2013-2018; **J. Jaworski**
- **FishMed** "Fishing for Medicines and their targets using Zebrafish models of human diseases"; (316125); 3,574,100 EUR; matching funds 1,393,769 PLN; 2012-2016; **J. Kuźnicki**
- **BIOMARKAPD** "Biomarkers for Alzheimer's disease and Parkinson's disease"; (2/BIOMARKAPD/JPND/2012); 240,804.27 PLN; 2012-2015; **J. Kuźnicki**
- **BESTCILIA** "Better Experimental Screening and Treatment for Primary Ciliary Dyskinesia"; (305404); 321,720 EUR; matching funds 201,397 PLN; 2012-2015; **M. Witt**
- **TargetSOCE** "Pathways of Store-Operated Calcium Entry (SOCE) as a novel therapeutic target in neurodegenerative diseases"; (NCBR/ERA NET RUS/03/2012); 545,623.47 PLN; 2012-2014; **J. Kuźnicki**
- **COMBIOM** "Strengthening Cooperation in Molecular Biomedicine between EU and Ukraine" ERA-WIDE, (294932); 80,036 EUR; matching funds 32,718 PLN; 2011-2014; **J. Kuźnicki**
- **NeuConnect** "Novel strategies for the treatment of schizophrenia based on genetic variation of the neural cell adhesion molecule NCAM and enzymes involved in its posttranslational modifications" (ERA-NET-NEURON/01/2011); 973,080 PLN; 2011-2014; **J. Kuźnicki/ M. Wiśniewska**
- **AMPREPACELL** "Development of new experimental models for mental retardation and autism by iPS technology: generation of human affected and animal model neurons by reprogramming skin fibroblasts and testing gene correction using *in vitro* and *in vivo* models" (ERA-NET-NEURON/03/2011); 1,419,075 PLN; 2011-2014; **J. Jaworski**
- **EXGENOMES** "Exgenome Molecular Enzymes" Research for SME (286556); 156,000 EUR; 2011-2013; **J.M. Bujnicki**
- **TRANSPOL** "Transport and signalling mechanism in polarized cells" ITN, (264399); 225,523 EUR; matching funds 475,200 PLN; 2010-2014; **M. Miączyńska**
- **ImageNinND** "Imaging Neurogenesis in Neurodegenerative Disease: *In vivo* imaging of dopaminergic adult-born neurons in the olfactory bulb of animal models of Parkinson's disease" (ERANET-NEURON/03/2010); 1,085,875 PLN; 2010-2013; **J. Jaworski**

Other International Funds

- **Wellcome Trust International Senior Research Fellowship** "Structural and Biochemical studies of Holliday junction resolution" (0988022); 3,369,854 PLN; 2013-2018; **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; 2007-2013; **M. Nowotny**
- **Howard Hughes Medical Institute, International Early Career Award** "Structural and Mechanistic Studies of Nucleic Acid Processing"; 715,000 USD; 2012-2017; **M. Nowotny**
- **Polish Swiss Research Programme** "The role of tumor susceptibility gene 101 (Tsg101) in transcriptional regulation in health and disease" (PSPB-094/2010); 5,365,200 PLN; 2012-2016; **M. Miączyńska**
- **International Centre for Genetic Engineering and Biotechnology**, "mTOR-driven phosphorylation of ZBP1 and Ago2 in neuronal development" (CRP/12/010); 48,000 EUR; 2012-2015; **J. Jaworski**
- **INTERREG IV C, ETTBio** "Effective Technology Transfer in Biotechnology"; (1210R4); 128,070 EUR; 2012-2014; **M. Powierża**
- **EMBO Installation Grant** "Protein biogenesis and redox homeostasis in mitochondria" (1966); 250,000 EUR; 2010-2014, **A. Chacińska**
- **DFG Program** Sensory and Regulatory RNAs in Prokaryotes "Single-molecule fluorescence analysis of the temperature dependent structure and dynamics of an RNA thermometer: consequences for its molecular function" (SE 1195/12-2); 90,450 EUR; 2010-2013; **J.M. Bujnicki**
- **PAN/CNRS** "Ca^{2+} toolkit coded proteins as drug targets in animal and plant cells", 2010-2013, **J. Kuźnicki**

Structural Funds

FNP Programmes

- IE OP 1.1.2. **WELCOME** "Biogenesis and turnover of mitochondria intermembrane space proteins" (WELCOME/2009/1); 5,940,670 PLN; 2009-2014; **A. Chacińska**
- IE OP 1.1.2. **TEAM** "Structural biology of methylation and hydroxymethylation" (TEAM/2010-6/1); 2,023,940 PLN; 2011-2015; **M. Bochtler**
- IE OP 1.1.2. **TEAM** "Modeling of RNA and protein-RNA complexes: from sequence to structure to function" (TEAM/2009-4/2); 2,200,000 PLN; 2010-2014; **J.M. Bujnicki**
- IE OP 1.1.2. **MPD** "PhD Programme in Molecular Biology: Studies of nucleic acids and proteins – from basic to applied research" (MPD/2009-3/2); 2,265,421 PLN; 2010-2015; **M. Witt** (7 PhD fellowships for all group leaders, see page 87)
- IE OP 1.2. **VENTURES** "The acquisition of chemotherapy resistance in non-small cell lung cancer – role of the p53 family proteins" (VENTURES/2010-6/8) 231,000 PLN; 2011-2014; **Z. Tracz**
- IE OP 1.2. FNP Programme **HOMING PLUS** "Structural and functional characterization of photosystem II from *Nicotiana tabacum*" (HOMING PLUS/2012-6/10); 326,000 PLN; 2013-2015; **D. Piano**
- IE OP 1.2. **HOMING PLUS** "Modeling tuberous sclerosis with induced pluripotent steam cells" (HOMING PLUS/2012-5/6); 302,000 PLN; 2013-2014; **E. Liszewska**
- IE OP 1.2. **POMOST** "Huntingtin-associated Protein 1 Induces Store-Operated Calcium Entry by Activating IP3" (POMOST/2013-8/4); 268,333 PLN; 2014-2015; **M. Czeredys**
- IE OP 1.2. **POMOST** "The role of the TET proteins in zebrafish" (POMOST/2013-7/4); 280,000 PLN; 2013-2015; **A. Kolano**
- IE OP 1.2. **POMOST** "Role of S6-kinase interaction with μ-adaptin in clathrin-mediated endocytosis and its implications for pathology of tuberous sclerosis" (POMOST/2013-7/10); 210,000 PLN; 2013-2015; **A. Malik**
- IE OP 1.2. FNP Programme **POMOST** "Characterization of selected endocytic proteins as novel regulators of AP-1 mediated transcription" (POMOST/2011-3/11); 430,000 PLN; 2012-2014; **E. Szymańska**
- IE OP 1.2. FNP Programme **POMOST** „Functional characterization of the interactions between endosomal adaptor proteins APPL and Dvl proteins in the Wnt signaling pathway" (POMOST/2010-1/1); 420,000 PLN; 2010-2013; **M. Banach-Orłowska**

Other

- IE OP 2.2.2 NCBR "Centre of Pre-clinical Research and Technology" (**CePT**); (POIG.02.02.00-14-024/08-00); 14,625,545 PLN; 2008–2014; **J. Kuźnicki**
- IE OP 1.3.2 OPI "Support for patent procedure of the invention: Tools and methods useful in characterising the immunotoxic activity of xenobiotic substances" (UDA-POIG.01.03.02-00-063/10-00); 230,315 PLN; 2011-2015; **M. Powierża**
- HC OP 8.2.1 MJWPU "Support for bio tech med. Scientists in technology transfer" (UDA-POKL.08.02.01-14-041/09-00); 2,586,221 PLN; 2010-2013; **M. Powierża**
- IE OP 2.2.3 NCBR "Biocentrum Ochota – IT infrastructure for development of strategic directions of the biology and medicine", (POIG.02.03.00-00-003/09); 4,834,300 PLN; 2009-2013; **J.M. Bujnicki and S. Filipek**

NCBR Research Grants

- **Applied Research Programme (PBS)** "Biotechnological applications of bacteriolytic protein" (**AUREZYNA**); (177126); 2,059,000 PLN (total grant budget: 2,443,260 PLN); 2013-2015; Coordinator **I. Sabała**
- **Applied Research Programme (PBS)** "New drugs for targeted therapy of multiple myelomas" (176911); 368,880 PLN (total grant budget: 5,327,452 PLN); 2012-2015; **M. Nowotny** (partner); Coordinator: A. Dziembowski, IBB PAN
- **INNOTECH** "Polish reference genome for genomic diagnostics and personalized medicine" (181852); 732,347 PLN (total grant budget: 4,648,937 PLN); 2013-2016; **M. Mossakowska** (partner); Coordinator: Genomed S.A.
- **Programmes and Projects – Defence, Security** "The study of genetic variation at the cellular level – new opportunities for forensic identification" (**AriaDNA 2010**); (OR00002712); 2,613,152 PLN (total grant budget: 9,904,670 PLN); 2010-2013; **J. Kuźnicki**
- **Innovativeness creator** "Innovation Creator (Kreator Innowacyjności) – to encourage entrepreneurship among scientists" (31/PMKI/U/30-06.09/2010); 422,990 PLN; 2010-2013; **M. Powierża**

NCN Research Grants

Regular Programmes

- **MAESTRO** "Molecular mechanisms of pro-survival processes in breast cancer" (2012/06/A/NZ1/00089); 3,000,000 PLN; 2013-2017; **M. Żylicz**
- **MAESTRO** "Structural RNomics" (2012/04/A/NZ2/00455); 3,000,000 PLN; 2012-2017; **J.M. Bujnicki**
- **MAESTRO** "Transgenic mice with elevated basal level of calcium ions in neurons as a model of aged-induced neurodegeneration of sporadic Alzheimer's disease" (2011/02/A/NZ3/00144); 2,989,800 PLN; 2012-2017; **J. Kuźnicki**
- **MAESTRO** "New functions of endocytic proteins in transcriptional regulation" 2,875,000 PLN; 2012-2017; **M. Miączyńska**
- **SONATA BIS** "Role of Rap proteins in regulation of mTOR function" (2012/07/E/NZ3/00503); 1,500,000 PLN; 2013-2018; **J. Jaworski**
- **SONATA BIS** "Architecture and evolution of protein-RNA networks and their relevance in the process of virulence regulation" (2011/03/D/NZ8/03011); 720,000 PLN; 2012-2016; **S. Dunin-Horkawicz**

- **SONATA BIS** "The contribution of STIM proteins and the role of Store Operated Calcium Entry (SOCE) in calcium homeostasis of neurons" (2011/01/D/NZ3/02051); 684,000 PLN; 2011-2016; **J. Gruszczyńska-Biegała**
- **OPUS** "Interplay between MIA pathway and reactive oxygen species in mitochondrial homeostasis" (2012/05/B/NZ3/00781); 663,500 PLN; 2013-2016; **M. Wasilewski**
- **OPUS** "Nuclear functions of mTOR in neurons" (2012/05/B/NZ3/00429); 750,000 PLN; 2013-2015; **J. Jaworski**
- **OPUS** "Oxidation landscape of mitochondrial proteins upon ROS production and in ageing" (2011/02/B/NZ2/01402); 997,500 PLN; 2012-2015; **A. Chacińska**
- **OPUS** "The canonical Wnt signaling pathway in the development of the thalamus" (2011/03/B/NZ3/04480); 842,500 PLN; 2012-2015; **M. Wiśniewska**
- **OPUS** "Regulation of clathrin-dependent endocytosis by mTOR kinase in neuronal development" (2011/03/B/NZ3/01970); 813,125 PLN; 2012-2015; **J. Jaworski**
- **OPUS** "The role of Amyloid Precursor Protein in the regulation of Store-Operated Calcium Entry" (2011/03/B/NZ3/01760); 504,000 PLN; 2012-2015; **T. Węgierski**
- **OPUS** "Investigation of arc protein role in synaptic plasticity of hippocampal and cortical neurons and mechanisms regulating arc expression with the special focus on GSK3-dependent degradation" (2011/01/B/NZ3/05397); 450,000 PLN; 2011-2014; **A. Goździ**
- **OPUS** "Sequence specifi city and its determinants in dsRNA endoribonucleases" (2011/01/B/NZ1/00209); 350,000 PLN; 2011-2014; **K. Skowronek**
- **HARMONIA** "The relationship between GSK3α and GSK3β activities and neuronal plasticity in chronic stress" (2011/01/M/NZ3/05413); 499,964 PLN; 2011-2014; **I. Cymerman**
- **SONATA** "Identification of post-transcriptional modifications in RNA sequences through mass spectrometry" (2012/05/D/ST/6/0382); 493,125 PLN; 2013-2016; **B. Kluge**
- **SONATA** "Determination of composition structure and substrate specificity of the mRNA_m6A methyltransferase protein complex" (2011/03/D/NZ1/03247); 750,000 PLN; 2012-2015; **E. Purta**
- **SONATA** "Structural and functional characterization of novel non-coding RNAs from Helicobacter pylori" (2011/01/D/NZ1/00212); 550,000 PLN; 2011-2014; **G. Chojnowski**
- **PRELUDIUM** "Genome wide high throughput analysis of 5-hydroxymethyl cytosine in *Danio rerio*" (2012/05/N/NZ2/02233); 150,000 PLN; 2013-2016; **K. Shanmuganandam**
- **PRELUDIUM** "Regulation of mTOR kinase signaling pathway by ESCRT-II complex in dendritogenesis" (2012/07/N/NZ3/01661); 140,000 PLN; 2013-2016; **M. Pieprzyk**
- **PRELUDIUM** "Bioinformatic analysis of GmrSD, a Type IV Modification-Dependent Restriction Systems" (2012/07/N/NZ2/01562); 100,000 PLN; 2013-2015; **M. Machnicka**
- **PRELUDIUM** "Structural basis of the recognition of postreplicative DNA modifications" (2012/05/N/NZ1/01912); 100,000 PLN; 2013-2015; **W. Siwek**
- **PRELUDIUM** "Analysis role of the PsbS subunit from photosystem II in the non-photochemical quenching" (2012/05/N/NZ1/01922); 99,200 PLN; 2013-2015; **P. Haniewicz**
- **PRELUDIUM** "Modeling of charge transport in RNA structural motifs" (2012/05/N/NZ1/02970); 75,000 PLN; 2013-2014; **J. Stasiewicz**
- **PRELUDIUM** "The interplay between the processes of inner membrane formation and protein transport in mitochondria" (2011/03/N/NZ3/01614); 318,750 PLN; 2012-2015; **P. Sakowska**
- **PRELUDIUM** "Development of a new scoring function for models of protein-small molecule complexes and its use for studying the mechanism of protein-ligand recognition" (2011/03/N/NZ2/03241); 230,000 PLN; 2012-2015;, **I. Tuszyńska**
- **PRELUDIUM** "Characterization of mTOR-dependent phosphorylation of ZBP1 and CLIP-170 and description of its contribution to dendritic arbor development" (2011/01/N/NZ3/05405); 180,000 PLN; 2011-2014; **A. Urbańska**
- **PRELUDIUM** "The role of HSP70 in the stabilization of p53 mutants in cancer cells" (2011/01/N/NZ1/00202); 192,000 PLN; 2011-2013; **M. Wiech**
- **PRELUDIUM** "Defining the mechanism of GSK3 dependent regulation of mTOR kinase activity in neurons in physiology and pathology" (2011/01/N/NZ3/05409); 150,000 PLN; 2011-2013; **M. Urbańska**
- **PRELUDIUM** "Generation of knockouts of HMTR1 and HMTR2 genes in human somatic cells and functional analysis of cap1 and cap2 methyltransferases encoded by these genes" (2011/01/N/NZ1/00211); 100,000 PLN; 2011-2013; **M. Werner**
- **PRELUDIUM** "Role of transcription factor TCF7L2 in establishment of thalamocortical connectivity and identity of thalamic neurons" (2011/01/N/NZ3/05345); 96,000 PLN; 2011-2013; **A. Nagalski**
- **FUGA** "A code for RNA recognition in RNA–RRM interactions" (2012/04/S/NZ1/00729); 612,000 PLN; 2012-2015; **M. Nowacka**
- **FUGA** "Does the hyperactivation of mTOR kinase interfere with cell differentiation into neurons?" (2012/04/S/NZ3/00264); 608,100 PLN; 2012-2015; **B. Tarkowski**

Other

- "Changes in cell cycle and apoptosis as a basis for diagnosis and potential therapeutic targets in Alzheimer's disease" (NN401596840); 408,000 PLN; 2011-2014; **U. Wojda**
- "Is there a "universal" RNA-guided DNA endonuclease?" (NN302654640); 400,000 PLN; 2011-2014; **M. Bochtler**
- „The Elmo1 function of regulated by mTOR kinase activity in neuronal plasticity" (NN301779040); 250,000 PLN; 2011-2015; **M. Błażejczyk**
- "Biochemical and structural studies of lentiviral reverse transcriptases" (NN301439738); 599,800 PLN; 2010-2014; **M. Nowotny**
- "Function of STIM proteins in capacitative calcium entry to the ER of healthy neurons and of cells with calcium dyshomeostasis in Alzheimer's disease" (NN301190039); 480,000 PLN; 2010-2014; **J. Kuźnicki**
- "Mechanism of oncogenic activities of mutated TP53" (NN302621838); 600,000 PLN; 2010-2013; **A. Żylicz**
- "Experimental characterization of hMTcap1 and hMTcap2 – last missing enzymes taking part in biosynthesis of the cap structure of human mRNA" (NN301425338); 500,000 PLN; 2010-2013; **J.M. Bujnicki**
- "Identification of the genetic program activated by Lef1/β-catenin complex in mature neurons" (NN301424538); 372,000 PLN; 2010-2013; **M. Wiśniewska**
- „The role of multifunctional adaptor proteins APPL1 and APPL2 in the regulation of cell growth and tumorigenic potential" (NN301189839); 336,000 PLN; 2010-2013; **B. Pyrzyńska**
- "Towards a new drug against influenza: Identification and characterization of compounds which abolish the activity of the influenza virus mRNA polymerase by the inhibition of virus endonuclease" (NN401585738); 150,000 PLN; 2010-2013; **K.H. Kamińska**
- "Identification and characteristics of endocytic proteins involved in regulation of gene transcription" (NN301296437); 340,740 PLN; 2009-2013; **I. Pilecka/M. Miączyńska**

Ministerial Research Grants

Iuventus Plus Initiative

- "mTOR compex 2 role in the regulation of actin cytoskeleton and neuronal dendritogenesis" (IP2012037872); 288,750 PLN; 2013-2015; **M. Urbańska**
- "Zinc finger Com-RNA complex as an example of specific protein-RNA interaction" (IP2012049072); 200,000 PLN; 2013-2015; **M. Nowacka**
- "Molecular determinants of sequence-specific DNA-RNA hybrid recognition and cleavage" (IP2012065672); 180,000 PLN; 2013-2015; **A. Sulej**
- "Bioinformatics search and analysis of protelomerase and its DNA recognition sites" (IP2012030172); 152,000 PLN; 2013-2014; **Ł. Kozłowski**
- "Coordinating proteasome subunit expression: structural biology of the master regulator Rpn4" (IP2011050971); 400,000 PLN; 2012-2014; **M. Kowalska**
- "Development and application of new methods for protein-RNA and protein-DNA complexes modeling" (IP2011057071); 175,000 PLN; 2012-2014; **I. Tuszyńska**
- "Structural analysis of the RNA-RNA and RNA-protein interactions" (IP2011006671); 145,000 PLN; 2012-2014; **G. Chojnowski**
- "Practical algorithms for graph isomorphism testing in the computational biology" (IP2011058671); 160,000 PLN; 2012-2014; **T. Waleń**
- "Structural biology of anti-cancer DNA methyltransferase inhibitors" (IP2011060971); 200,000 PLN; 2012-2014; **M. Wojciechowski**
- "Analysis of the relationship between sequence and structure in coiled-coil protein domains" (IP2011011071); 178,000 PLN; 2012-2014; **S. Dunin-Horkawicz**
- "Structural analysis of RNase H3 in complex with a substrate - the mechanism of action and substrate specificity in the context of an enzyme family" (IP2011060971); 150,000 PLN; 2012-2013; **M. Figiel**
- "Structural studies of mechanism of action of UvrC protein from bacterial DNA repair system called nucleotide excision repair system" (IP2011018671); 150,000 PLN; 2012-2013, **M. Jaciuk**
- "Casimir-Polder effect in scattering of atoms on liquid surfaces" (IP2011030771); 150,000 PLN; 2012-2013; **G. Łach**
- "Bioinformatics analysis of sequence-structure-function relationships in the GIY-YIG nuclease superfamily" (IP2011021871); 100,000 PLN; 2012-2013; **K. Kamińska**

Scientific Meetings and Lectures

- **TargetSOCE** project kick-off meeting, 15.02.2013, IIMCB, Warsaw
- The **NeuConnect** Consortium meeting, 24.02.2013, IIMCB, Warsaw
- **FishMed** kick-off meeting, 12.04.2013, IIMCB, Warsaw
- **IIMCB Annual Report** Session, 07.06.2013, Mierki
- 11th International **Congress of the Polish Neuroscience Society**, 15-17.09.2013, Poznań, co-organized by IIMCB
- **ETTBio** Project Meeting, 12-13.09.2013, IIMCB, Warsaw
- **COMBIOM** Course on Research Integrity and Responsible Conduct of Research, 30.09-3.10.2013, IIMCB, Warsaw

Seminars of invited speakers

• Special Lecture Series: Frontiers of Polish Biosciences*

Jan Potempa (Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Laureate of FNP Prize 2011) "A scientific reason for not looking a gift horse in the mouth: An association of periodontitis with systemic diseases", 31.01.2013

Leslie P. Kozak (Institute of Animal Reproduction and Food Resarch PAS, Olsztyn) "The influence of the early post-natal environment on the development of brown and white adipocytes", 21.02.2013

Joanna Cichy (Department of Immunology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków) "Plasmacytoid dendritic cell and neutrophil crosstalk in autoimmunity", 07.03.2013

Dorota Włoga (Nencki Institute of Experimental Biology PAS, Warsaw) "Identification of proteins implicated in cilia assembly and function", 16.05.2013

• Lab Leader Competition seminars

Cecilia Lanny Winata (Genome Institute of Singapore, Singapore) "Studying early developmental mechanisms using genomics in zebrafish", 26.09.2013

• Regular IIMCB seminars

Olaf Selchow (Carl Zeiss Jena) "Light sheet microscopy for multiview imaging of large specimens", 18.01.2013

Artur Jarmołowski (Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland) "Introns of plant pri-miRNAs are required for proper biogenesis and function of miRNAs", 24.01.2013

Tytus Bernaś (Laboratory of Imaging Tissue Structure and Function, Nencki Institute of Experimental Biology PAS, Warsaw, Poland) "Multi - dimensional optical microscopy", 07.02.2013

Elena Kaznacheyeva, Alexey Shalygin and Maria Ryazantseva (Laboratory of Ionic Channels of Cell Mambranes, Institute of Cytology of the Russian Academy of Sciences, Saint-Petersburg, Russia) "Store-operated channels – activation mechanisms and implications in neurodegeneration", 15.02.2013

Hannelore Ehrenreich (Max Planck Institute of Experimental Medicine Clinical Neurosciences Göttingen, Germany) "Shifting paradigms in neuropsychiatry", 25.02.2013

Katarzyna Piwocka (Nencki Institute of Experimental Biology PAS, Warsaw, Poland) "Unfolded protein response in leukemia implications in translation, progression and resistance", 14.03.2013

Mikołaj Olejniczak (Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland) "Bacterial regulatory RNAs in competition for chaperone protein Hfq",21.03.2013

Gosia Trynka (Harvard Medical School and Broad Institute of Harvard and MIT, Cambridge, USA) "Learning the biology of complex diseases, integrating SNP associations with histone modifications to identify disease critical cell types and functional variants", 25.03.2013

Witold Filipowicz (Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland) "Mechanisms and regulation of miRNA repression and metabolism in mammalian cells", 04.04.2013

Robert Huber (Nobel Laureate in Chemistry 1988, Max-Planck Institute of Biochemistry Martinsried, Germany) "Protein crystallography at the interface of physics, chemistry and biology", 19.04.2013

Krzysztof Giannopoulos (Experimental Hematooncology Department, Medical University of Lublin, Poland) "The Holy Grail of cancer immunotherapy: induction of specific immune response and inhibition of immunotolerance?", 23.05.2013

Piotr Podlasz (Laboratory of Genomics and Transcriptomics Department of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland) "Galanin: How zebrafish allows us to discover the full potential of this peptide", 03.06.2013

Wojciech Wiszniewski (Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, USA) "Personal genome sequencing and genomic medicine", 06.06.2013

Branko Latinkć (Cardiff University, Cardiff, United Kingdom) "Functional anatomy of cardiogenic transcription factor GATA4", 13.06.2013

Jan de Sonneville (From Life Science Methods BV, Leiden, The Netherlands) "Automated microinjection of cell-polymer suspensions in 3D ECM scaffolds for high-throughput quantitative cancer invasion screens" and "Automating microinjection in animal models", 18.06.2013

Anna Ajduk (Department of Embryology, Institute of Zoology Faculty of Biology, University of Warsaw, Poland) "Cytoplasmic

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

quality determines developmental potential of mammalian oocytes", 20.06.2013

Bennett Van Houten (University of Pittsburgh, Pittsburgh, USA) "Watching DNA repair: one molecule at a time", 21.06.2013

Andrei Lupas (Department of Protein Evolution, Max-Planck-Institute for Developmental Biology, Tuebingen, Germany) "On the origin of folded proteins", 25.06.2013

Irinka Castanon (Department of Biochemistry, University of Geneva, Switzerland) "Orientation of cell divisions: the physiological role of Anthrax Toxin Receptors", 27.06.2013

Ryszard Maleszka (Research School of Biology the Australian National University Canberra, Australia) "The rise of the epigenomes: bullet-train biology or a step forward in bridging the gap between genotype and phenotype?", 18.07.2013

Paul Hurd (The School of Biological and Chemical Sciences Queen Mary University of London, United Kingdom) "ChIPing away at the epigenome",18.07.2013

Maga Rowicka (Department of Biochemistry & Molecular Biology, Institute for Translational Sciences, University of Texas Medical Branch, USA) "The high-resolution genomic landscape of DNA double-strand breaks in human cells revealed by next-generation sequencing", 18.07.2013

Aleksei Sazanow (Chair of Natural Sciences and Geography, Leningrad State University named after A.S. Pushkin, St Petersburg, Russia) "Evolutionary and functional genomics of animals", 29.07.2013

Christian Koenig (Pacific Biosciences) "Single-Molecule, Real-Time (SMRT™) DNA Sequencing: Technology Overview and Recent Applications", 29.07.2013

M. Isabel Dominguez (Boston University School of Medicine Boston, USA) "Role of CK2 in Wnt/beta-catenin signaling", 18.09.2013

Nils Axelsen (Ombudsman for Research Integrity at Statens Serum Institut in Copenhagen) "Scientific Integrity and Responsible Conduct of Research - hot topics in the turbulent global scientific world", 30.09.2013

Jarema Malicki (Biomedical Genetics Department of Biomedical Science, the University of Sheffield, United Kingdom) "Ciliogenesis and cell polarity in the vertebrate embryo", 10.10.2013

Katarzyna Radwanska (Nencki Institute of Experimental Biology PAS, Warsaw, Poland) "The mechanisms for long-term memory formation when synaptic strengthening is impaired", 07.11.2013

Charles R. Cantor (Scripps Research Institute, La Jolla, and Sequenome Inc., USA) "Towards Predictive Medicine", 12.11.2013

Kamil R. Kranc (University of Edinburgh, UK) "The quest for regulatory pathways of haematopoietic stem cell fate decisions", 21.11.2013

Wieslaw I. Gruszecki (Department of Biophysics, Institute of Physics, Maria Curie-Sklodowska University, Lublin, Poland) "Do plants measure light intensity?", 05.12.2013

Piotr Szwedziak (Medical Research Council, Laboratory of Molecular Biology, Cambridge, United Kingdom) "Architecture of the FtsZ-ring in vivo and in vitro suggests a sliding filament constriction mechanism during bacterial cell division", 19.12.2013

IIMCB Researchers' Seminars

Lidia Wróbel (Laboratory of Mitochondrial Biogenesis) "Mitochondrial protein import: Mia40 facilitates Tim22 translocation into the inner membrane of mitochondria", 10.01.2013

Agnieszka Skałecka (Laboratory of Molecular and Cellular Neurobiology) "*In vivo* model to study neuron development", 28.03.2013

Aksana Varabyova (Laboratory of Mitochondrial Biogenesis) "Mia40 and MINOS act in parallel with Ccs1 in the biogenesis of mitochondrial Sod1", 11.04.2013

Michał Boniecki (Laboratory of Bioinformatics and Protein Engineering) "SimRNA: a coarse-grained method for RNA 3D folding simulations and structure prediction", 25.04.2013

Michał Witt (IIMCB and Institute of Human Genetics PAN) "Genetic testing - a source of ethical and legal dilemmas", 03.10.2013

Małgorzata Figiel (Laboratory of Protein Structure) "Crystal structure of RNase H3 substrate complex - the complete family picture of RNases H", 17.10.2013

Marcin Nowotny (Laboratory of Protein Structure) "Reverse transcriptases - structure and mechanism", 28.11.2013

IIMCB Annual Report Session, 15.06.2012, Mierki, Poland

Michael Potente (Max Planck/IIMCB Research Group located in Max-Planck-Institute for Heart and Lung Research, Angiogenesis & Metabolism, Germany) "Metabolic regulation of angiogenic blood vessel growth" – key note lecture

Michał Wasilewski (Laboratory of Mitochondrial Biogenesis) "MIA40 and mitochondrial biogenesis in human cells"

Łukasz Szewczyk (Laboratory of Neurodegeneration) "Polysialylation of Neural Cell Adhesion Molecule (NCAM) and myelination in the Central Nervous System (CNS)"

Karolina Mierzejewska (Laboratory of Structural Biology) "DpnI-DNA complex: How DNA methylation licences rather than prevents DNA cleavage"

Bartosz Wawrzynów (Department of Molecular Biology) "MDM2 oncoprotein modulates DNA damage response in human cancer"

Kamil Jastrzębski (Laboratory of Cell Biology) "The hitchhiker's guide to the PDGF endocytosis"

Dorota Matelska (Laboratory of Bioinformatics and Protein Engineering) "Comparative genomics of RNA regulatory systems"

Iwona Cymerman (Laboratory of Molecular and Cellular Neurobiology) "GSK3 kinase activity steers structural spine plasticity"

Michał Miętus (Laboratory of Protein Structure) "Structural studies of Rad2 DNA repair nuclease"

Agnieszka Kolano (Laboratory of Structural Biology) "5hmC in zebrafish – the role of TET enzymes"

Jacek Kuźnicki „Institute's annual report"

Publications in 2013

No	Publication	5-Year Impact Factor	Journal Category	Journal Rank in Category / Total Journals in Category
1	Bednarczyk P, Wieckowski MR, Broszkiewicz M, **Skowronek K**, Siemen D, Szewczyk A. Putative Structural and Functional Coupling of the Mitochondrial BKCa Channel to the Respiratory Chain. ***PLoS One***. 2013; 8(6):e68125.	4,244	Multidisciplinary Sciences	7/56
2	Bedzhov I, Alotaibi H, Basilicata MF, Ahlborn K, **Liszewska E**, Brabletz T, Stemmler MP. Adhesion, but not a specific cadherin code, is indispensable for ES cell and induced pluripotency. ***Stem Cell Res***. 2013; 11(3):1250-63.	4,76	Biotechnology & Applied Microbiology	20/160
3	Bik W, Baranowska-Bik A, Wolinska-Witort E, Kalisz M, Broczek K, **Mossakowska M**, Baranowska B. Assessment of adiponectin and its isoforms in Polish centenarians. ***Exp Gerontol***. 2013; 48(4):401-7.	3,705	Geriatrics & Gerontology	10/47
4	**Biro M**, Romeo Y, **Kroschwald S**, Bovellan M, **Boden A**, Tcherkezian J, Roux PP, Charras G, **Paluch EK**. Cell Cortex Composition and Homeostasis Resolved by Integrating Proteomics and Quantitative Imaging. ***Cytoskeleton (Hoboken)***. 2013; 70(11):741-54	2,865	Cell Biology	100/185
5	**Bragoszewski P, Gornicka A, Sztolsztener ME, Chacinska A**. The Ubiquitin-Proteasome System Regulates Mitochondrial Intermembrane Space Proteins. ***Mol Cell Biol***. 2013; 33(11):2136-48.	5,745	Biochemistry & Molecular Biology	50/290
6	**Bujnicki JM**, Tiuryn J. Bioinformatics and Computational Biology in Poland. ***PLoS Comput Biol***. 2013; 9(5):e1003048.	5,939	Mathematical & Computational Biology	4/47
7	Chon H, Sparks JL, **Rychlik M, Nowotny M**, Burgers PM, Crouch RJ, Cerritelli SM. RNase H2 roles in genome integrity revealed by unlinking its activities. ***Nucleic Acids Res***. 2013; 41(5):3130-43.	8,055	Biochemistry & Molecular Biology	27/290
8	Christou M, Crochemore M, Iliopoulos C.S, Kubica M, Solon PP, Radoszewski J, Rytter W, Szreder B, **Walen T**. Efficient seed computation revisited. ***Theor Comput Sci***. 2013; 483(SI):350-363.	0,697	Computer Science, Theory & Methods	78/100
9	**Clark AG**, Dierkes K, **Paluch EK**. Monitoring Actin Cortex Thickness in Live Cells. ***Biophys J***. 2013; 105(3):570-80.	3,975	Biophysics	17/72
10	Crochemore M, Iliopoulos CS, Kociumaka T,Kubica M, Pachocki J, Radoszewski J, Rytter W, Tyczynski W, **Walen T**. A note on efficient computation of all Abelian periods in a string. **Inform Process Lett**. 2013; 113(3):74-77.	0,606	Computer Science, Information Systems	102/132
11	**Czeredys M, Gruszczynska-Biegala J**, Schacht T, Methner A, **Kuznicki J.** Expression of genes encoding the calcium signalosome in cellular and transgenic models of Huntington's disease. ***Front Mol Neurosci***. 2013; 6:42.	0	N/A	0
12	Czeredys M, Samluk L, Michalec K, Tułodziecka K, **Skowronek K**, Nałęcz KA. Caveolin-1 - a novel interacting partner of organic cation/carnitine transporter (octn2): effect of protein kinase C on this interaction in rat astrocytes. ***PLoS One***. 2013; 8(12):e82105.	4,244	Multidisciplinary Sciences	7/56
13	Dudzik J, Chang WC, Kannan AM, **Filipek S**, Viswanathan S, Li P, Renugopalakrishnan V, Audette GF. Cross-linked glucose oxidase clusters for biofuel cell anode catalysts. ***Biofabrication***. 2013; 5(3):035009.	3,524	Engineering, Biomedical	8/79
14	Esteras N, Alquézar C, Bermejo-Pareja F, **Bialopiotrowicz E, Wojda U**, Martín-Requero A. Downregulation of extracellular signal-regulated kinase 1/2 activity by calmodulin KII modulates p21(Cip1) levels and survival of immortalized lymphocytes from Alzheimer's disease patients. ***Neurobiol Aging***. *2013*; 34(4):1090-100.	6,098	Geriatrics & Gerontology	1/47
15	Golanska E, Sieruta M, Corder E, Gresner SM, Pfeffer A, Chodakowska-Zebrowska M, Sobow TM, Klich I, **Mossakowska M, Szybinska A**, Barcikowska M, Liberski PP. The prion protein M129V polymorphism: Longevity and cognitive impairment among Polish centenarians. ***Prion***. 2013; 7(3):244-7.	2,71	Biochemistry & Molecular Biology	198/290

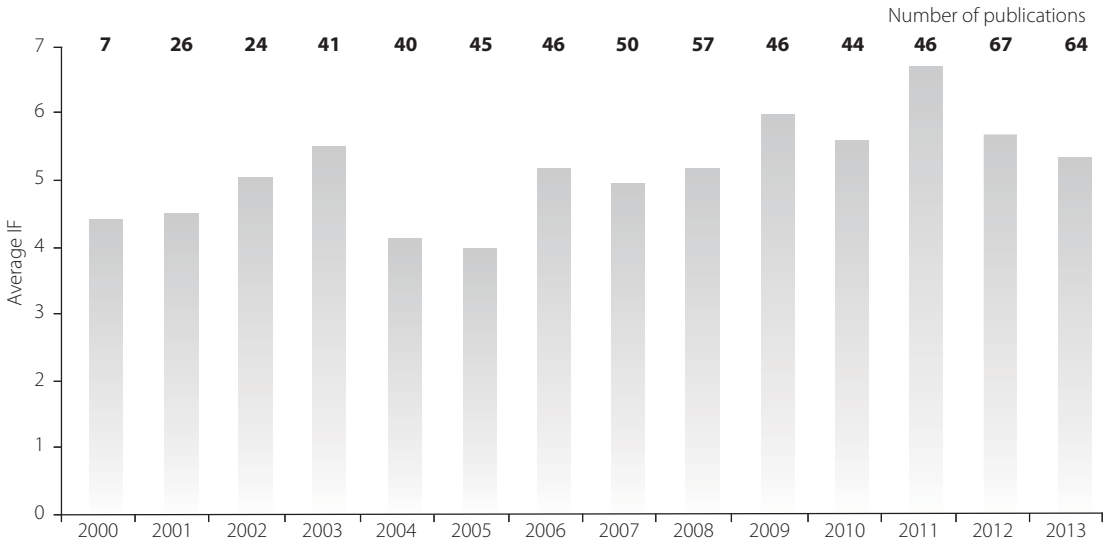
No	Publication	5-Year Impact Factor	Journal Category	Journal Rank in Category / Total Journals in Category
16	Golanska E, Sieruta M, Gresner SM, Pfeffer A, Chodakowska-Zebrowska M, Sobow TM, Klich I, **Mossakowska M, Szybinska A**, Barcikowska M, Liberski PP. APBB2 genetic polymorphisms are associated with severe cognitive impairment in centenarians. ***Exp Gerontol***. 2013; 48(4):391-4.	3,705	Geriatrics & gerontology	10/47
17	**Górecka KM, Komorowska W, Nowotny M**. Crystal structure of RuvC resolvase in complex with Holliday junction substrate. ***Nucleic Acids Res***. 2013; 41(21):9945-55.	8,055	Biochemistry & Molecular Biology	27/290
18	**Gruszczynska-Biegala J, Kuznicki J**. Native STIM2 and ORAI1 proteins form a calcium-sensitive and thapsigargin-insensitive complex in cortical neurons. ***J Neurochem***. 2013; 126(6):727-38.	4,25	Neurosciences	71/252
19	Gutmajster E, Witecka J, Wyskida M, Koscinska-Marczewska J, Szwed M, Owczarz M, **Mossakowska M**, Milewicz A, Puzianowska-Kuznicka M, Zejda J, Wiecek A, Chudek J, Sieron A.L. Telomere Length in Elderly Caucasians Weakly Correlates with Blood Cell Counts. ***ScientificWorldJournal***. 2013; 2013:153608.	1,603	Multidisciplinary Sciences	13/56
20	**Haniewicz P**, De Sanctis D, Büchel C, Schröder WP, Loi MC, Kieselbach T, **Bochtler M, Piano D**. Isolation of monomeric photosystem II that retains the subunit PsbS. ***Photosynth Res***. 2013; 118(3):199-207.	3,57	Plant Sciences	31/197
21	Honarnejad K, Daschner A, Giese A, Zall A, Schmidt B, **Szybinska A, Kuznicki J**, Herms J. Development and Implementation of a High-Throughput Compound Screening Assay for Targeting Disrupted ER Calcium Homeostasis in Alzheimer's Disease. ***PLoS One***. 2013; 8(11):e80645.	4,244	Multidisciplinary Sciences	7/56
22	Honarnejad K, Kirsch AK, Daschner A, **Szybinska A, Kuznicki J**, Herms J. FRET-Based Calcium Imaging: A Tool for High-Throughput/Content Phenotypic Drug Screening in Alzheimer Disease. ***J Biomol Screen***. 2013; 18(10):1309-20.	2,089	Biotechnology & Applied Microbiology	72/160
23	Iakoubov L, **Mossakowska M**, Szwed M, Duan Z, Sesti F, Puzianowska-Kuznicka M. A Common Copy Number Variation (CNV) Polymorphism in the CNTNAP4 Gene: Association with Aging in Females. ***PLoS One***. 2013; 8(11):e79790.	4,244	Multidisciplinary Sciences	7/56
24	Janusz A, Milek J, **Perycz M**, Pacini L, Bagni C, Kaczmarek L, Dziembowska M. The fragile x mental retardation protein regulates matrix metalloproteinase 9 mRNA at synapses; ***J Neurosci***. 2013; 33(46):18234-41.	7,869	Neurosciences	22/252
25	Jaremko M, Jaremko L, Nowakowski M, **Wojciechowski M, Szczepanowski RH**, **Panecka R**, Zhukov I, **Bochtler M**, Ejchart A. NMR structural studies of the first catalytic half-domain of ubiquitin activating enzyme. ***J Struct Biol***. 2014; 185(1):69-78.	3,549	Biochemistry & Molecular Biology	104/290
26	**Jaworska A**, Dzbek J, Styczynska M, **Kuznicki J.** Analysis of calcium homeostasis in fresh lymphocytes from patients with sporadic Alzheimer's disease or mild cognitive impairment. ***BBA-Mol Cell Res***. 2013; 1833(7):1692-9.	4,947	Biochemistry & Molecular Biology	60/290
27	**Jaworski J**. Uchwytny kształt pamięci. (Perceptible shape of memories). ***Biologia w szkole (Biology at school)***. 2013; 2:4-13.	0	N/A	0
28	Kapitein LC, van Bergeijk P, **Lipka J**, Keijzer N, Wulf PS, Katrukha EA, Akhmanova A, Hoogenraad CC. Myosin-V Opposes Microtubule-Based Cargo Transport and Drives Directional Motility on Cortical Actin. ***Curr Biol***. 2013; 23(9):828-34.	10,445	Biochemistry & Molecular Biology	19/290
29	Kapustian LL, Vigontina OA, Rozhko OT, Ryabenko DV, **Michowski W**, Lesniak W, Filipek A, Kroupskaya IV, Sidorik LL. Hsp90 and its co-chaperone, Sgt1, as autoantigens in dilated cardiomyopathy. ***Heart Vessels***. 2013; 28(1):114-9.	1,702	Cardiac & Cardiovascular Systems	63/124
30	Kraszewska MD, Dawidowska M, Kosmalska M, Sdek A, Grzeszczak W, Kowalczyk JR, SzczepaDski T, Witt M. BCL11B, FLT3, NOTCH1 and FBXW7 mutation status in T-cell Acute Lymphoblastic Leukemia patients. ***Blood Cell Mol Dis***. 2013; 50(1):33-8.	2,316	Hematology	39/67

No	Publication	5-Year Impact Factor	Journal Category	Journal Rank in Category / Total Journals in Category
31	Kubica M, Kulczyński T, Radoszewski J, Rytter W, **Waleń T**. A linear time algorithm for consecutive permutation pattern matching. ***Inform Process Lett***. 2013; 113(12):430–33.	0,606	Computer Science, Information	102/132
32	Kuzniewska B, Rejmak E, **Malik AR, Jaworski J,** Kaczmarek L, Kalita K. Brain-derived neurotrophic factor induces matrix metalloproteinase 9 expression in neurons via the serum response factor/c-Fos pathway. ***Mol Cell Biol***. 2013; 33(11):2149-62.	5,745	Biochemistry & Molecular Biology	50/290
33	**Latek D**, **Pasznik P**, Carlomagno T, Filipek S. Towards Improved Quality of GPCR Models by Usage of Multiple Templates and Profile-Profile Compariso. ***PLoS One***. 2013; 8(2):e56742.	4,244	Multidisciplinary Sciences	7/56
34	**Lipka J**, Kuijpers M, **Jaworski J**, Hoogenraad CC. Mutations in cytoplasmic dynein and its regulators cause malformations of cortical development and neurodegenerative diseases. ***Biochem Soc T***. 2013; 41(6):1605-12.	3,267	Biochemistry & Molecular Biology	165/290
35	**Liszewska E, Jaworski J**. Why do we need induced pluripotent stem cells in neurobiology? ***Postepy Biochem. (Progress in Biochemistry)***. 2013; 59(2):164-174.	0	N/A	0
36	Lukasiak P, Antczak M, Ratajczak T, **Bujnicki JM**, Szachniuk M, Adamiak RW, Popenda M, Blazewicz J. RNAlyzer--novel approach for quality analysis of RNA structural models. ***Nucleic Acids Res***. 2013; 41(12):5978-90.	8,055	Biochemistry & Molecular Biology	27/290
37	**Machnicka MA, Milanowska K**, Osman Oglu O, **Purta E, Kurkowska M, Olchowik A, Januszewski W**, Kalinowski S, **Dunin-Horkawicz S, Rother KM**, Helm M, **Bujnicki JM**, Grosjean H. MODOMICS: a database of RNA modification pathways: 2013 update. ***Nucleic Acids Res***. 2013; 41(D1):D262-7.	8,055	Biochemistry & Molecular Biology	27/290
38	**Macias M, Blazejczyk M**, Kazmierska P, Caban B, **Skalecka A, Tarkowski B**, Rodo A, Konopacki J, **Jaworski J**. Spatiotemporal Characterization of mTOR Kinase Activity Following Kainic Acid Induced Status Epilepticus and Analysis of Rat Brain Response to Chronic Rapamycin Treatment. ***PLoS One***. 2013; 8(5):e64455.	4,244	Multidisciplinary Sciences	7/56
39	Maître JL, Berthoumieux H, Gabriel Krens SF, Salbreux G, Jülicher F, **Paluch E**, Heisenberg CP. Cell adhesion mechanics of zebrafish gastrulation. ***M S-MED SCI***. 2013; 29(2):147-50.	0,467	Medicine, Research & Experimental	102/121
40	**Malik AR, Urbanska M, Gozdz A, Swiech LJ, Nagalski A, Perycz M, Blazejczyk M, Jaworski J.** Cyr61, a Matricellular Protein, Is Needed for Dendritic Arborization of Hippocampal Neurons. ***J Biol Chem***. 2013; 288(12):8544-59.	5,023	Biochemistry & Molecular Biology	62/290
41	**Malik AR, Urbanska M, Macias M, Skalecka A, Jaworski J**. Beyond control of protein translation: What we have learned about the non-canonical regulation and function of mammalian target of rapamycin (mTOR). ***Biochim Biophys Acta-Proteins and Proteomics***. 2013; 1834(7):1434-48.	3,012	Biophysics	16/72
42	**Matelska D, Purta E, Panek S, Boniecki MJ, Bujnicki JM, Dunin-Horkawicz S**. S6:S18 ribosomal protein complex interacts with a structural motif present in its own mRNA. ***RNA***. 2013; 19(10):1341-8.	4,43	Biochemistry & Molecular Biology	54/290
43	**Miaczynska M.** Effects of membrane trafficking on signaling by receptor tyrosine kinases. ***CSH Perspect Biol***. 2013; 5(11):a009035.	10,367	Cell Biology	20/185
44	Milanowska K, Mikolajczak K, Lukasik A, Skorupski M, Balcer Z, **Machnicka MA, Nowacka M**, Rother KM, **Bujnicki JM**. RNApathwaysDB—a database of RNA maturation and decay pathways. ***Nucleic Acids Res***. 2013; 41(D1):D268-72.	8,055	Biochemistry & Molecular Biology	27/290
45	Nadrowski P, Chudek J, Grodzicki T, **Mossakowska M**, Skrzypek M, Wiecek A, Zdrojewski T, Kozakiewicz K. Plasma level of N-terminal pro brain natriuretic peptide (NT-proBNP) in elderly population in Poland - The PolSenior Study. ***Exp Gerontol***. 2013 Sep;48(9):852-7.	3,705	Geriatrics & Gerontology	10/47
46	**Nagalski A**, Irimia M, Szewczyk L, Ferran JL, **Misztal K, Kuznicki J, Wisniewska MB**. Postnatal isoform switch and protein localization of LEF1 and TCF7L2 transcription factors in cortical, thalamic, and mesencephalic regions of the adult mouse brain. ***Brain Struct Funct***. 2013; 218(6):1531-49.	6,821	Anatomy & Morphology	1/21

No	Publication	5-Year Impact Factor	Journal Category	Journal Rank in Category / Total Journals in Category
47	**Nowak E**, Potrzebowski W, Konarev PV, Rausch JW, Bona MK, Svergun DI, **Bujnicki JM**, Le Grice SF, **Nowotny M**. Structural analysis of monomeric retroviral reverse transcriptase in complex with an RNA/DNA hybrid. ***Nucleic Acids Res***. 2013; 41(6):3874-87.	8,055	Biochemistry & Molecular Biology	27/290
48	**Paluch EK**, Raz E. The role and regulation of blebs in cell migration. ***Curr Opin Cell Biol***. 2013; 25(5):582-90.	12,034	Cell Biology	15/185
49	**Pawlowski M, Bogdanowicz A, Bujnicki JM**. QA-RecombineIt: a server for quality assessment and recombination of protein models. ***Nucleic Acids Res***. 2013; 41(Web Server issue):W389-97.	8,055	Biochemistry & Molecular Biology	27/290
50	**Philips A, Milanowska K, Lach G, Bujnicki JM**. LigandRNA: computational predictor of RNA-ligand interactions. ***RNA***. 2013; 19(12):1605-16.	5,43	Biochemistry & Molecular Biology	54/290
51	Pulawski W, Filipek S, **Zwolinska A**, Debinski A, **Krzysko K**, Garduño-Juárez R, Viswanathan S, Renugopalakrishnan V. Low-temperature molecular dynamics simulations of horse heart cytochrome c and comparison with inelastic neutron scattering data. ***Eur Biophys J***. 2013; 42(4):291-300.	2,361	Biophysics	42/72
52	**Puton T, Kozlowski LP, Rother KM, Bujnicki JM**. CompaRNA: a server for continuous benchmarking of automated methods for RNA secondary structure prediction. ***Nucleic Acids Res***. 2013; 41(7):4307-23.	8,055	Biochemistry & Molecular Biology	27/290
53	**Pyrzynska B, Banach-Orlowska M, Teperek-Tkacz M**, Miekus K, Drabik G, Majka M, **Miaczynska M**. Multifunctional protein APPL2 contributes to survival of human glioma cells. ***Mol Oncol***. 2013; 7(1):67-84.	6,379	Oncology	20/197
54	Qiu J, Wenz LS, Zerbes RM, Oeljeklaus S, Bohnert M, Stroud DA, Wirth C, Ellenrieder L, Thornton N, Kutik S, Wiese S, Schulze-Specking A, Zufall N, **Chacinska A**, Guiard B, Hunte C, Warscheid B, van der Laan M, Pfanner N, Wiedemann N, Becke T. Coupling of Mitochondrial Import and Export Translocases by Receptor-Mediated Supercomplex Formation. ***Cell***. 2013; 154(3):596-608.	34,366	Biochemistry & Molecular Biology	1/290
55	Roszkowska-Gancarz M, Bartoszewicz Z, Polosak J, Kurylowicz A, Jonas M, **Mossakowska M**, Franek E, Puzianowska-Kuźnicka M. Total and high molecular weight adiponectin and level-modifying polymorphisms of ADIPOQ in centenarians. ***Endokrynologia Polska***. 2013; 63(6):439-446.	0	Endocrinology & Metabolism	105/122
56	**Sadowski Ł, Jastrzębski K**, Kalaidzidis Y, Heldin CH, Hellberg C, **Miaczynska M**. Dynamin Inhibitors Impair Endocytosis and Mitogenic Signaling of PDGF. ***Traffic***. 2013; 14(6):725-36.	5,076	Cell Biology	54/185
57	**Sztolsztener ME, Brewinska A**, Guiard B, **Chacinska A**. Disulfide bond formation: sulfhydryl oxidase ALR controls mitochondrial biogenesis of human MIA40. ***Traffic***. 2013; 14(3):309-20.	5,076	Cell Biology	54/185
58	van Spronsen M, Mikhaylova M, **Lipka J**, Schlager MA, van den Heuvel DJ, Kuijpers M, Wulf PS, Keijzer N, Demmers J, Kapitein LC, Jaarsma D, Gerritsen HC, Akhmanova A, Hoogenraad CC. TRAK/Milton Motor-Adaptor Proteins Steer Mitochondrial Trafficking to Axons and Dendrites. ***Neuron***. 2013; 77(3):485-502.	16,403	Neurosciences	5/252
59	**Varabyova A**, Stojanovski D, **Chacinska A**. Mitochondrial protein homeostasis. ***IUBMB Life***. 2013; 65(3):191-201.	3,508	Biochemistry & Molecular Biology	148/290
60	**Varabyova A, Topf U, Kwiatkowska P, Wrobel L, Kaus-Drobek M, Chacinska A**. Mia40 and MINOS act in parallel with Ccs1 in the biogenesis of mitochondrial Sod1. ***FEBS J***, 2013; 280(20):4943-59.	3,6	Biochemistry & Molecular Biology	74/290
61	**Wegierski T.** Mikroskopowe metody rejestracji wewnątrzkomórkowych odpowiedzi wapniowych. ***Kosmos***. 2013; Tom 62; Nr 2(299):193-203.	0	N/A	0
62	**Wisniewska MB**. Physiological Role of beta-Catenin/TCF Signaling in Neurons of the Adult Brain. ***Neurochem Res***. 2013; 38(6):1144-55.	2,427	Neurosciences	169/252
63	**Wojciechowski M, Czapinska H, Bochtler M**. CpG Underrepresentation and the Bacterial CpG Specific DNA Methyltransferase M.Mpel. ***Proc Natl Acad Sci USA***. 2013; 110(1):105-10.	10,583	Multidisciplinary Sciences	4/56
64	**Wojda U, Kuznicki J**. Alzheimer's Disease Modeling: Ups, Downs, and Perspectives for Human Induced Pluripotent Stem Cells. ***J Alzheimers Dis***. 2013; 34(3):563-88.	4,394	Neurosciences	64/252

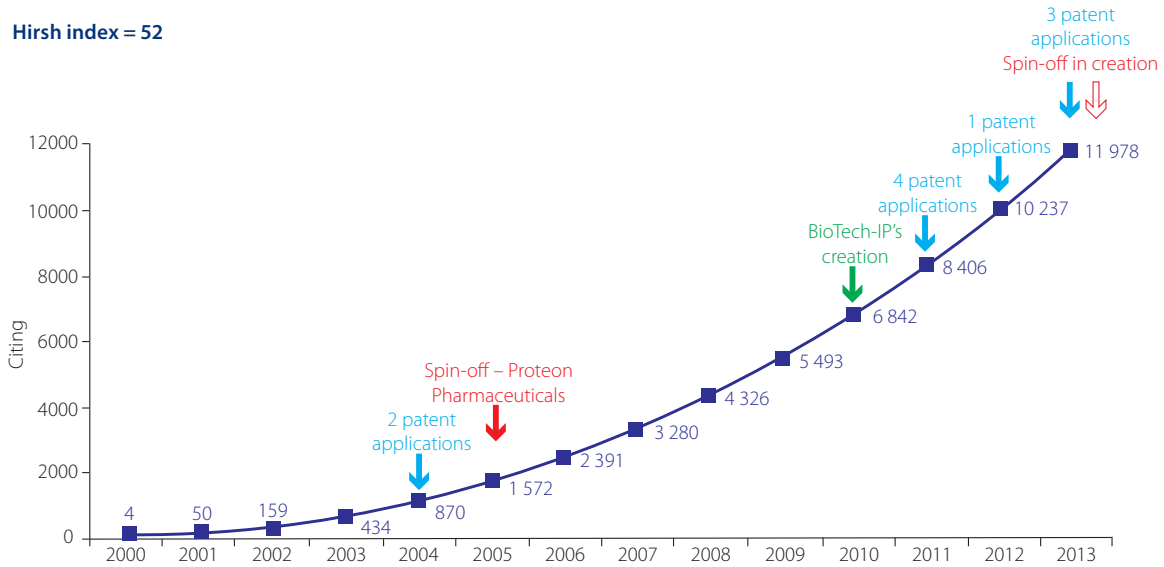
No	Publication	5-Year Impact Factor	Journal Category	Journal Rank in Category / Total Journals in Category
65	**Wrobel L, Trojanowska A, Sztolsztener ME, Chacinska A**. Mitochondrial protein import: Mia40 facilitates Tim22 translocation into the inner membrane of mitochondria. ***Mol Biol Cell***. 2013; 24(5):543-54.	5,429	Cell Biology	51/185
66	Wu Y, **Yuan S**, Chen S, Wu D, Chen J, Wu J. Enhancing the production of galacto-oligosaccharides by mutagenesis of Sulfolobus solfataricus beta-galactosidase. ***Food Chem***. 2013; 138(2-3):1588-95.	4,072	Food Science & Technology	10/124
67	Xu C, Liu R, Mehta AK, Guerrero-Ferreira RC, Wright ER, **Dunin-Horkawicz S**, Morris K, Serpell LC, Zuo X, Wall JS, Conticello VP. Rational Design of Helical Nanotubes from Self-Assembly of Coiled-Coil Lock Washers. J Am Chem Soc. 2013 Oct 16;135(41):15565-78.	10,237	Chemistry, Multidisciplinary	11/152
68	Zariwala MA, Gee HY, **Kurkowiak M**, Al-Mutairi DA, Leigh MW, Hurd TW, Hjeij R, Dell SD, Chaki M, Dougherty GW, Adan M, Spear PC, Esteve-Rudd J, Loges NT, Rosenfeld M, Diaz KA, Olbrich H, Wolf WE, Sheridan E, Batten TF, Halbritter J, Porath JD, Kohl S, Lovric S, Hwang DY, Pittman JE, Burns KA, Ferkol TW, Sagel SD, Olivier KN, Morgan LC, Werner C, Raidt J, Pennekamp P, Sun Z, Zhou W, Airik R, Natarajan S, Allen SJ, Amirav I, Wieczorek D, Landwehr K, Nielsen K, Schwerk N, Sertic J, Köhler G, Washburn J, Levy S, Fan S, Koerner-Rettberg C, Amselem S, Williams DS, Mitchell BJ, Drummond IA, Otto EA, Omran H, Knowles MR, Hildebrandt F. ZMYND10 Is Mutated in Primary Ciliary Dyskinesia and Interacts with LRRC6. ***Am J Hum Genet***. 2013; 93(2):336-45	12,512	Genetics & Heredity	7/161

Number of IIMCB publications and average impact factor of journals in which they were published



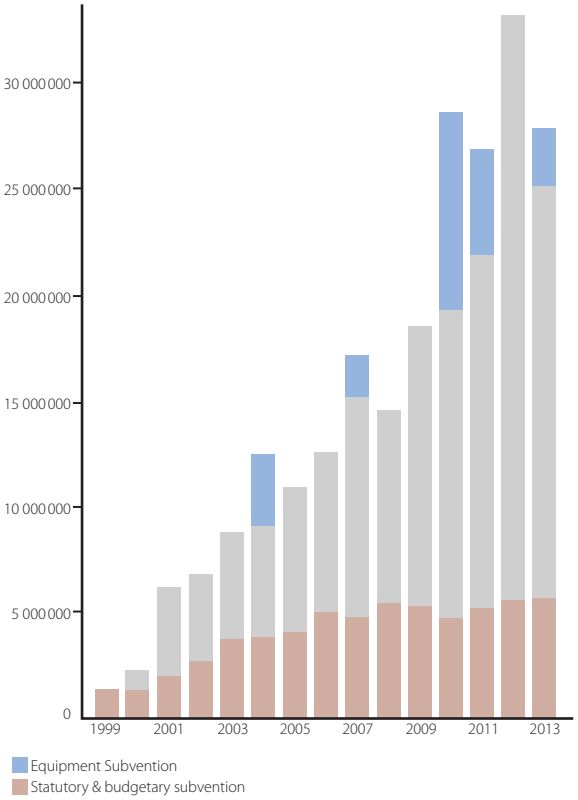
Cumulative citations, patent applications and spin-offs (2000–2013)

Hirsh index = 52



Diversity of Funding IIMCB'2013

Annual income (in PLN)



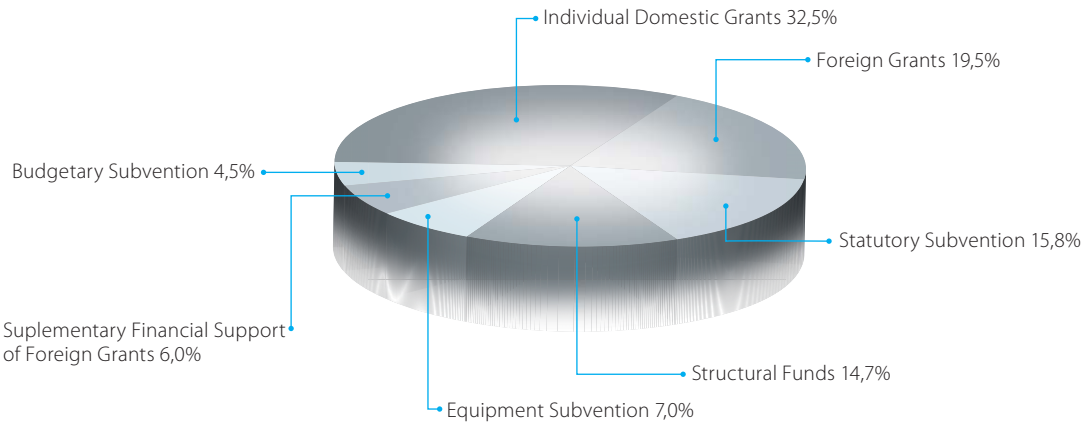
Profit & loss statement (amounts in PLN)

	amounts in PLN
A. Net revenue on sales and equivalents*	31 284 351
B. Operational activity costs:	31 896 406
Depreciation (equipment)	903 289
Research materials	11 415 064
Utilities	402 316
Services	3 237 182
Fees and taxes	1 160 486
Salaries and wages	10 370 000
Social and health insurance	2 729 346
Other operational expenses, in this:	1 678 723
business trips	949 021
property insurance	18 092
fellowships	711 360
others	250
C. Other operational income (subventions)	617 335
D. Other operational expenses	212
E. Financial income (interests)	257 085
F. Financial expenses (others)	722
Profit on business activity (A-B+C-D+E-F)	**261 431**

Sources of Funding

	amounts in PLN	amounts in EUR(1)
Statutory Subvention	4 412 720	1 064 024
Budgetary Subvention	1 274 000	307 195
Individual Domestic Grants	9 118 063	2 198 607
Structural Funds	4 104 850	989 788
Supplementary Financial Support of Foreign Grants	1 694 888	408 682
Foreign Grants	5 452 568	1 314 759
Equipment Subvention	1 950 000	470 197
Total	**28 007 089**	**6 753 253**

(1) 1 EUR - 4,1472 @ 31st Dec'2013



Education

Educational Activities

IIMCB continues its doctoral program in partnership with other research and educational institutes of the Ochota Campus. Currently 48 PhD students are on board within the doctoral programs of the Institute of Biochemistry and Biophysics, of the Nencki Institute, of the University of Poznań and of the Foundation for Polish Science. The PhD students of IIMCB are self-organized as a group with the representative Marcin Magnus. They have regular working seminars every two months. The postdoctoral fellows are similarly self-organized with group representatives Elżbieta Purta and Karolina Górecka. The "Postdoc's seminars" are devoted to the presentation of personal experience of the postdoc, being complementary to regular IIMCB seminars. Both groups representatives participate in meetings with Directors, Lab Leader's meetings, etc.

International PhD Programme

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

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

Support for bio-tech-med scientists in technology transfer

In 2013 IIMCB was running several projects to support technology transfer in Biocentrum Ochota consortium.

Projects:

- "Effective Technology Transfer in Biotechnology, ETTBio", supported with the funds from the INTERREG IVC programme.
- "Support for bio-tech-med scientists in technology transfer through scholarships, training courses and internships", sponsored by Operational Programme - Human Capital; 8.2.1.
- "Support for the protection of industrial property generated in scientifi c entities as a result of R&D work", supported by Operational Programme – Innovative Economy 1.3.2.
- Innovativenes Creator, the programme of the National Centre for Research and Development. Several activities were made possible within these projects:
  - research stipends for innovative projects for PhD students working in Biocentrum Ochota institutes,
  - two-month practices for Biocentrum Ochota scientists at industrial sites,
  - training courses on issues such as: R&D project management, raising a company, commercialization of R&D results, Intelectual Property Rights (IPR), negotiations in business,
  - joint science to business projects
  - funds secured for patent protection
  - management of IP and commercialization of R&D results.

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

IIMCB has joined LiSMIDoS program with a major goal to participate in activities of independent Doctoral School, with real influence on its educational curriculum: in this framework IIMCB faculty will run courses, summer schools, etc. Some of them will be performed in a teleconference format. The major objective is to provide a programme of interdisciplinary training to PhD students that will allow them to work in today's competitive scientific environment that very often requires crossdisciplinary expertise.

Theses defended in 2013

Michał Piętal, PhD thesis: „New tools, methods, and models for analyzing intra- and intermolecular contacts in proteins, nucleic acids and their complexes", Thesis advisor: J.M. Bujnicki, 19.12.2013, Institute of Biochemistry and Biophysics PAN, Warsaw, Poland

Milena Wiech, PhD thesis: „HSP70 in stabilization of mutants p53 in cancer cells", Thesis advisor: A. Żylicz, 19.11.2013, Nencki Institute of Experimental Biology PAN, Warsaw, Poland

Kaja Milanowska, PhD thesis: „New bioinformatics methods for prediction of metal- and ligand-binding sites in RNA structures" *Magna cum laude*, Thesis advisor: J.M. Bujnicki, 15.11.2013, Adam Mickiewicz University, Poznań, Poland

Anna Philips, PhD thesis: „Development of new biological databases of nucleic acids metabolism", Thesis advisor: J. M. Bujnicki, 15.11.2013, Adam Mickiewicz University, Poznań, Poland

Shuguang Yuan, PhD thesis: „The Activation Mechanisms of m-Opioid, Lipid and Formyl Peptide G-Protein-Coupled Receptors", Thesis advisor: S. Filipek, 14.06.2013, Nencki Institute of Experimental Biology PAN, Warsaw, Poland

Agata Sulej (Kamaszewska), PhD thesis: „Engineering of a sequence-specific ribonuclease H" *Magna cum laude*, Thesis advisor: J.M. Bujnicki, 28.05.2013, Institute of Biochemistry and Biophysics PAN, Warsaw, Poland

Umesh Ghoshdastider, PhD thesis: „Cell-free expression and molecular modeling of the γ-secretase complex and G-protein-coupled receptors", Thesis advisor: S. Filipek, 27.05.2013, Goethe University, Frankfurt, Germany

Maria Werner, PhD thesis: „Characteristics of human methyltransferases involved in RNA cap formation" *Magna cum laude*, Thesis advisor: J. M. Bujnicki, 14.05.2013, Institute of Biochemistry and Biophysics PAN, Warsaw, Poland

Łukasz Kozłowski, PhD thesis: „An integrated bioinfrmatics service for protein analysis. Prediction of domains and intrinsically disordered regions", Thesis advisor: J.M. Bujnicki, 07.05.2013, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

Irina Tuszyńska, PhD thesis: „Prediction of protein-RNA complexes structure and interactions" *Magna cum laude*, Thesis advisor: J.M. Bujnicki, 09.04.2013, Institute of Biochemistry and Biophysics PAN, Warsaw, Poland

Małgorzata Figiel, PhD thesis: „Structural and biochemical studies of Rnase H function in larger protein assemblies" *Magna cum laude*, Thesis advisors: M. Nowotny and J. Kuźnicki, 27.02.2013, Nencki Institute of Experimental Biology PAN, Warsaw, Poland

Iga Korneta, PhD thesis: „Bioinformatics analysis of the human spliceosomal proteome", Thesis advisor: J.M. Bujnicki, 03.01.2013, Nencki Institute of Experimental Biology PAN, Warsaw, Poland

Stipends received in 2013

Irina Tuszyńska and **Marcin Jaciuk** were awarded stipend grants for young researchers given by Foundation for Polish Science (FNP) **under the START Programme**.

Anna Sara Urbańska is a laureate of 2013 edition of the project implemented by the Mazovia Province authorities, entitled "Scientific potential for the economy of Mazovia – scholarships for PhD students".

'More Good Science'

Prof. Janusz M. Bujnicki, Head of the Laboratory of Bioinformatics and Protein Engineering at the International Institute of Molecular and Cell Biology in Warsaw has initiated an action dubbed 'More Good Science' ("Więcej Dobrej Nauki" in Polish). Its purpose is to offer free help to researchers struggling with scientific grant applications. The action specifically targets inexperienced scientists, whose scientific projects applications have been refused funding and who may experience problems when addressing adverse critical reviews. The main idea is to identify projects based on good concepts, which were underappreciated, and to bring in experts who can provide help to support the authors in their efforts towards the development of better grant applications.

'More Good Science' consists of three stages:

1. February/March/April 2014: A series of talks given by Prof. Bujnicki in small and medium-sized academic centers in Poland: Częstochowa, Siedlce, Szczecin, Olsztyn, and Białystok. Prof. Bujnicki will introduce basic rules and "DO's and DONT's" pertinent to grant applications, in particular with respect to basic research and grant programs offered by the Polish National Science Centre to junior and mid-career scientists.
2. May 2014: Workshops/consultations in Warsaw (at the International Institute of Molecular and Cell Biology), devoted to discussions between the participants and experts, and with the focus on improving the unsuccessful grant applications.
3. Early in 2015: Second round of consultations, following the evaluation of proposals submitted in stage 2 (as many of these are expected not to be funded successfully, despite the authors' work towards improvement).

More information (in Polish): http://iimcb.genesilico.pl/werwa.html

Centre for Innovative Bioscience Education (BioCEN)

The aim of the Centre for Innovative Bioscience Education (BioCEN), is to reduce the gap between science and society in Poland by conducting educational activities popularizing biology: open lectures, workshops for students and courses for biology teachers. All activities are focused on improving biology education and the awareness of biology in society. The co-founders of the Centre for Innovative Bioscience Education are: the International Institute of Molecular and Cell Biology (IIMCB), Nencki Institute of Experimental Biology PAN (IBD), the Institute of Biochemistry and Biophysics PAN (IBB), University of Warsaw's Faculty of Biology, and the BioEducation Foundation. IIMCB houses the BioCEN laboratory, office and administration. BioCEN also coordinates a second laboratory at the Warsaw University of Life Sciences. In 2013 alone, over 4,000 young participants attended laboratory workshops and over 50 biology teachers participated in Symposium. In the span of 12 years of its activity BioCEN was visited by 17,000 students who attended workshops on various topics.

Laboratory workshops

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

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

Courses for biology teachers

Since we strongly encourage teachers to implement practical protocols at schools, we equip them with classroom scenarios and affordable experimental kits that can be used in school laboratories. The proposed teaching materials exemplify a state of-the-art approach towards innovative biology education. They allow for the development of practical skills and introduce a teaching approach based on project development by a team of students. Last but not least, our educational procedures improve the ability of analytical thinking. During our workshops we popularize a method known as Inquiry Based Science Education. "Inquiry" is defined as "a search for truth, information, or knowledge" – seeking information by questioning.

In 2013, as part of teacher training, the 12th BioCEN and Nencki Institute Symposium for teachers was organized.

The 50th anniversary of the discovery of the DNA

As a part of celebration of the 50th anniversary of the discovery of the DNA structure BioCEN carried out the following events:

- a happening organized together with the Association of Rare Diseases in April 2013 at the entrance of the subway station "CENTRUM" in Warsaw. During the event, at the BioCEN stand it was possible to isolate participant's own DNA as well as to obtain information on the structure and role of DNA in the human body
- two biological quizzes were presented at screens of Warsaw metro trains, for a total of about three weeks. The first one contained questions based on the a survey of the Europeans' knowledge of life sciences. The second quiz aimed to popularize knowledge about DNA. Questions and answers concerned basic facts about the structure and function of DNA and research that lead to better understanding of this molecule.

BioCEN in the media

In 2013, our employees, using BioCEN laboratory contributed to an educational offer also in the media, by completion of the following projects:

- Episode of "Mr. Albert Offer" of educational portal of Polish National Television entitled „Dirt, compass and spiders". During, the episode Jacek Patryn talked about the presence of bacteria in the environment and the way to culture bacteria in a laboratory.
- Episode of „How it works?" of the TVP1 (first channel of Polish National TV) concerning microbiology. During, the episode Maciej Lirski presented the techniques used in microbiological laboratories
- The movie „Transformation of bacteria in a few steps"; supplemental material to Piotr Kossobudzki article in Gazeta Wyborcza, a main national newspaper, available on the GW website (http://wyborcza.pl/1,75400,14198699,Jak_zrobic_swiecace_bakterie__Pokaz_krok_po_kroku.html). Anna Wasążnik presented the procedure of bacteria transformation.

17th Science Picnic (June 15, 2013)

As in previous years, the BioEducation Foundation and BioCEN organized an exhibition and science show during the 17th Science Picnic in Warsaw. The motto for 2013 was "Life". One of our demonstrations was related to DNA and the variety of methods used in molecular biology research:

- Necklaces with your own DNA – isolation of DNA from a cheek swab
- In connection with this year's theme of the Science Picnic, we also presented various species of protozoa, which were made available to us by the Nencki Institute of Experimental Biology PAN.

2nd Congress of Polish Education (June 15-16, 2013)

This two-day meeting of teachers and educators from across the Polish, which was held in Warsaw on 15 and 16 June 2013, was attended by over 1,300 people. In addition to the plenary sessions of the congress, 12 thematic sessions, devoted to various aspects of education, from pre-school education to the professional and higher education, were organized. During the Congress BioCEN presented its activities targeted at students and teachers.

17th Science Festival (September 20-29, 2013)

The objective of the Warsaw Science Festival is to enhance public awareness of science and technology. Over 500 activities take place in different formats (seminars, debates, guided tours, workshops, performances, contests, films), representing various fields of science.

They are aimed at different target groups (young children, primary school, high school, general public) and are run for one week at various venues: universities, scientific research institutions and museums. In 2013 BioCEN organized open laboratory workshops for the public:

- *See DNA?* a workshop for students
- *On the trail of DNA* a workshop for students.

8th Children's Science Festival (September 28, 2013)

It was the third time when BioCEN participated in the Children's Science Festival. During several hours of workshops children learned about DNA. Several hundred children took part in the workshop.

6th Industrial Picnic in Stalowa Wola (October 10, 2013)

BioCEN organized presentation and practices how to design scientific experiment. In frame of this initiative all interested participants could design their dream experiment according to scientific standards.

Family laboratory workshops

In 2013 we continued laboratory workshops for younger children, accompanied by their guardians. Many years of working with children has enabled us to develop a unique program of educational workshops tailored to their age. During our workshop the little scientists perform each experiment themselves, under the supervision of an experienced tutor. Guardians accompany the children and take part in carrying out experiments. Currently, we offer four different workshop topics to families who visit our laboratory, including:

- *How many vitamins are there in a candy?*
- *Why don't plants need to eat?*

The interactive map of informal education in Poland presented by the Institute for Educational Research (IBE)

In 2012, an evaluation of our educational offer was carried out by the Institute for Educational Research. We were chosen for the analysis out of 348 educational centres across Poland.

The evaluation indicated that our activities met the majority of Best Practices in Education requirements, defined by the IBE. These include:

- providing significant support for schools in implementing the requirements of the new core curriculum, e.g. explaining the rules of "scientific thinking",
- implementing experiments and observations,
- learning to draw conclusions,
- determining cause-and-effect relationships,
- working in small groups,
- offering classes conducted by young and enthusiastic animators.

In 2013, on the webpage of the Institute for Educational Research an interactive map of informal education centers has been published (http://eduentuzjasci.pl/pl/badania/110-badanie/556-dobre-praktyki-w-przyrodniczej-edukacji-pozaformalnej-badania-oferty-zajec-przyrodniczych.html?showall=&start=6). This map allows finding an institution that offers extra activities for the pupils, e.g. in a form of workshops. Few centers that have undergone a thorough evaluation, including BioCEN, are indicated by separate color.

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

"The Students' Academy" is an initiative which brings together 300 junior high schools and 35,000 students from five regions of Poland. During workshops students design and carry out experiments and team projects and make observations in accordance with scientific procedures. Teachers from participating schools have access to Internet-based coaching and participate in professional training, focusing on the preparation of scientific observations and experiments for students, guidance for student projects, and approaches to motivate learning. The project started in 2010 and BioCEN tasks ended in 2013. Currently, in preparation for the publication are protocols of experiments in biology, physics, and mathematics for pupils of junior high schools. Agnieszka Chołuj who served as a biology expert in the project "Akademia Uczniowska" (Pupils' Academy) has developed a section on biological protocols.

Staff and co-workers

Persons who coordinate and administrate BioCEN are: **dr Agnieszka Chołuj**, Aleksandra Kot-Horodyńska, Karolina Kurzela, Anna Wasążnik, Marcin Wiśniewski (as a coordinator at Warsaw University of Life Sciences). Animators: Piechnik Aleksandra, Strumiłło Marta, Patryn Jacek, Spanier Michał, Malanowska Kaja, Kulka Marek, Krzyczmonik Katarzyna, Matuszko Gabriela, Andrzejewski Kryspin, Lirski Maciej, Mrozek Paulina, Strzelecka Dominika, Łepeta Katarzyna, Klecza Anna, Rejch Ada, Stachowicz Nina, Sobańska Zuzanna, Franczuk Artur, Kępska Marta, Skowron Waldemar, Dudek Agata, Kulecka Maria, Pogorzelska Róża, Szczepańska Izabela, Sytek Piotr, Więcek Karolina, Szymańska Katarzyna, Komorowski Łukasz, Agnieszka Góral. Co-workers: Piotr Horodyński, Kamil Koper, Emilia Rejmak-Kozicka.

Administration & Staff

Administration

Scientific Office

Dominika Dubicka-Boroch Director's Assistant
Agnieszka Wagner-Ziemka Domestic Cooperation Manager
Katarzyna Nakielska Domestic Grants Administrator

Administration Unit

Agnieszka Karbowska Director's Representative for Administrative Matters (on maternity leave)
Daria Filipek PR Specialist
Dorota Makarewicz Director's Representative for Administrative Matters (since Oct. 2013)
Agnieszka Gwara Secretary
Tomasz Miętek Tenders Specialist
Roman Szczepanowski Director's Representative for Information Technology and Research Equipment
Robert Banasiak Maintenance Specialist
Anna Brzezińska Purchase &Tenders Specialist (not in the picture)

International Cooperation Unit

Dorota Libiszowska Foreign Grants Specialist
Aleksandra Nałęcz-Tolak International Cooperation Specialist
Marcin Ogonowski International Cooperation Specialist
Urszula Białek-Wyrzykowska International Cooperation Manager

Human Resources Unit

Beata Tkacz Human Resources Manager
Monika Domańska-Paśko Human Resources Specialist

Financial Unit

Renata Knyziak Accounting Specialist
Monika Nowicka Payroll Specialist
Hanna Iwaniukowicz Financial Manager
Agnieszka Kuna Accounting Specialist
Mariola Arkuszewska Accounting Specialist

Staff at IIMCB (as of 31 March 2014)

Administration		Funding
Jacek Kuźnicki	Director	IIMCB
Michał Witt	Deputy Scientific Director	IIMCB(1/2)
Hanna Iwaniukowicz	Financial Manager	IIMCB
Monika Nowicka	Payroll Specialist	IIMCB
Renata Knyziak	Accounting Specialist	IIMCB
Agnieszka Kuna	Accounting Specialist	IIMCB/Structural Funds
Mariola Arkuszewska	Accounting Specialist	IIMCB/Structural Funds
Monika Domańska-Paśko	Human Resources Specialist	IIMCB
Beata Tkacz	Human Resources Specialist	IIMCB
Urszula Białek-Wyrzykowska	International Cooperation Manager	IIMCB(1/2)
Dorota Wasiak-Libiszowska	Foreign Grants Manager	FNP/EU
Marcin Ogonowski	International Cooperation Specialist	IIMCB/Structural Funds/EU
Agnieszka Wagner-Ziemka	Planning and Reporting Manager	IIMCB
Agnieszka Karbowska	Director's Representative for Administrative Matters (maternity leave)	IIMCB
Dorota Makarewicz	Director's Representative for Administrative Matters	IIMCB
Dominika Dubicka- Boroch	Director's Assistant	IIMCB
Anna Brzezińska	Tender Specialist	IIMCB
Robert Banasiak	Maintenance Specialist	IIMCB
Aleksandra Nałęcz-Tolak	International Cooperation Specialist	IIMCB/EU (3/4)
Katarzyna Nakielska	Domestic Grants Administrator	IIMCB
Agnieszka Gwara	Secretary	IIMCB
Roman Szczepanowski	Director's Representative for Information Technology and Research Equipment	IIMCB
Daria Filipek	PR Specialist, FishMed	EU
Dudzin Magdalena	HS Specialist	IIMCB (1/4)

Cell Biology Laboratory		
Marta Miączyńska	Head	IIMCB
Anna Bartosik	Post-doctoral Fellow, FishMed	EU
Beata Pyrzyńska	Post-doctoral Fellow	Polish-Swiss Res. Programme (1/4)
Ewelina Szymańska	Post-doctoral Fellow	FNP POMOST
Lidia Wolińska- Nizioł	FishMed Technical Assistant	EU (1/2)
Jarosław Cendrowski	Post-doctoral Fellow	NCN
Kamil Jastrzębski	Junior Researcher	FNP
Agnieszka Mamińska	Junior Researcher	IIMCB
Sam D. Stephen	Junior Researcher	EU
Łukasz Sadowski	Junior Researcher	Volunteer
Noga Budick-Harmerlin	PhD	Volunteer
Anna Toruń	Junior Researcher	Volunteer
Rafał Sejdak	Undergraduate student	FNP

Neurodegeneration Laboratory		
Jacek Kuźnicki	Head	IIMCB
Tomasz Węgierski	Senior researcher	IIMCB
Magdalena Czeredys	Post-doctoral Fellow	NCBR
Joanna Gruszczyńska-Biegała	Post-doctoral Fellow	IIMCB/NCN
Łukasz Majewski	Post-doctoral Fellow	NCN
Smijin Karthully, Soman	Post-doctoral Fellow	EU
Kinga Gazda	Junior researcher	NCN
Anna Jaworska	Junior researcher	FNP (MPD Project)
Andrzej Nagalski	Junior researcher	NCN (till Dec. 2013)
Aleksandra Szybińska	Junior researcher	IIMCB
Michal Bazała	FishMed Technical Assistant	EU (1/2)
Łukasz Szewczyk	Junior researcher	Volunteer
Aleksandra Kurek	Undergraduate student	IIMCB
Robert Drozd	Junior Researcher (Intern)	IIMCB

Bioinformatics and Protein Engineering Laboratory		
Janusz M. Bujnicki	Head	IIMCB/ERC
Michał Boniecki	Post-doctoral Fellow	ERC
Grzegorz Chojnowski	Post-doctoral Fellow	IIMCB/NCN
Dawson Wayne	Senior Researcher, FishMed	EU
Stanisław Dunin-Horkawicz	Post-doctoral Fellow	NCN/EU
Dyzma Michał	Technician, FishMed	EU
Kasprzak Joanna	Post-doctoral Fellow	ERC
Bogusław Kluge	Post-doctoral Fellow	NCN (1/2)
Grzegorz Łach	Programmer	IIMCB
Martyna Nowacka	Post-doctoral Fellow	NCN
Radosław Pluta	Junior Researcher- Assistant	NCN
Elżbieta Purta	Technician	IIMCB
Krzysztof Skowronek	Senior Researcher	IIMCB/ERC
Justyna Czarnecka	Technician	ERC-PoC
Piotr Bentkowski	Junior Researcher- Assistant	NCN
Krzysztof Szczepaniak	Junior Researcher	NCN/MNiSW
Sylwia Panek	Technician	ERC
Astha	Junior Researcher	NCN
Magdalena Byszewska	Junior Researcher	NCN
Ilona Domagała	Junior Researcher	Bio&Techn.Innov.
Dawid Głów	Junior Researcher	NCN
Katarzyna H. Kamińska	Junior Researcher	IIMCB
Łukasz Kozłowski	Post-doctoral Fellow	ERC
Małgorzata Kurkowska (Durawa)	Junior Researcher/ Technician	NCN
Magdalena Machnicka (Mika)	Junior Researcher	TEAM FNP/NCN
Marcin Magnus	Junior Researcher	TEAM FNP/Mazovia fellowship
Dorota Matelska	Junior Researcher	TEAM FNP
Anna Olchowik	Junior Researcher	MPD FNP
Jakub Jopek	Junior Researcher (co-supervision)	PhD school of Warsaw University
Paweł Piątkowski	Junior Researcher	TEAM FNP
Jankowska Elżbieta	Junior Researcher	NCN
Agata Sulej (Kamaszewska)	PhD	ERC-PoC
Juliusz Stasiewicz	Junior Researcher	TEAM FNP/ NCN
Irina Truszyńska	Post-doctoral Fellow	ERC
Albert Bogdanowicz	MSc Student	TEAM FNP
Katarzyna Grudziąż	MSc Student	NCN
Rafał Zaborowski	MSc Student	TEAM FNP
Andrzej Lichaczewski	MSc Student	TEAM FNP / NCN
Mateusz Dobrychłop	MSc Student	NCN
Przemysław Gierski	MSc Student	IIMCB
Dawid Podgórski	MSc Student	Volunteer
Agnieszka Faliszewska	Office Manager	IIMCB/ TEAM FNP
Jan Kogut	Computer Administrator/Programmer (part time)	BIOCENTRUM
Tomasz Jarzynka	Computer Administrator/Programmer (part time)	BIOCENTRUM
Łukasz Munio	Computer Administrator	BIOCENTRUM

Structural Biology MPG/PAN Laboratory		
Matthias Bochtler	Head	IIMCB
Honorata Czapińska	Post-doctoral Fellow	TEAM FNP (3/4)
Monika Kowalska (Sokołowska)	Post-doctoral Fellow	MNiSW
Marek Wojciechowski	Post-doctoral Fellow	IIMCB
Katrzyna Misztal	Post-doctoral Fellow	IIMCB
Agnieszka Kolano	Post-doctoral Fellow	EU
Dario Piano	Post-doctoral Fellow	IIMCB
Marta Wawrzyniak	FishMed Technical Assistant	EU (1/2)
Patrycja Haniewicz	Junior Researcher	NCN
Asgar Abbas Kazrani	Junior Researcher	FNP
Karolina Mierzejewska	Junior Researcher	FNP
Dominik Rafalski	Junior Researcher	FNP
Karthik Shanmuganandam	Junior Researcher	FNP
Wojciech Siwek	Junior Researcher	NCN
Małgorzata Perycz	PhD	IBB Volunteer
Humberto Fernandes	PhD	IBB Volunteer
Anna Fricke	PhD	IBB olunteer
Michał Pastor	Junior Researcher	IBB Volunteer
Marlena Kisiała	Junior Researcher	IBB Volunteer

Mitochondrial Biogenesis Laboratory		
Agnieszka Chacińska	Head	IIMCB
Piotr Brągoszewski	Post-doctoral Fellow	Welcome FNP/IIMCB
Anna Sokół	Post-doctoral Fellow, FishMed	EU
Małgorzata Sztolsztener	Post-doctoral Fellow	Welcome FNP/EMBO IG
Ulrike Topf	Post-doctoral Fellow	Fellowship from Swiss National Science Foundation
Michał Wasilewski	Post-doctoral Fellow	NCN/IIMCB
Magdalena Chojnacka	PhD Student	Welcome FNP
Piotr Chrościcki	PhD Student	Welcome FNP
Agnieszka Górnicka	PhD Student	Welcome FNP
Karthik Mohanraj	PhD Student	Welcome FNP
Paulina Sakowska	PhD Student	NCN/EMBO IG
Aksana Varabyova	PhD Student	NCN
Lidia Wróbel	PhD Student	NCN
Aleksandra Matusiak	Lab Manger	Wellcome FNP
Michał Bazała	FishMed Technical Assistant	EU (1/2)
Aleksandra Fergin	Student	Volunteer
Arianna Barchesi	Student	Volunteer/Erasmus

Molecular and Cellular Neurobiology Laboratory		
Jacek Jaworski	Head	IIMCB
Magdalena Błażejczyk	Post-doctoral Fellow	EU/IIMCB
Iwona Cymerman	Post-doctoral Fellow	NCN
Agata Góźdź	Post-doctoral Fellow	NCN/ EU
Justyna Jezierska	Post-doctoral Fellow, FishMed	EU
Aleksandra Janusz	Post-doctoral Fellow	NCN
Matylda Macias	Post-doctoral Fellow	NCN
Ewa Liszewska	Post-doctoral Fellow	Era-Net Neuron
Bartosz Tarkowski	Post-doctoral Fellow	NCN
Anna Malik	Post-doctoral Fellow	NCN/ FNP Pomost
Lidia Wolińska-Nizioł	FishMed Technical Assistant	EU (1/2)
Joanna Lipka	Junior researcher	FNP
Agnieszka Skałecka	Junior researcher	NCN/ IIMCB
Katarzyna Świtoń	Junior researcher	NCN/ IIMCB
Anna Urbańska	Junior researcher	Mazovia Fellowship
Malgorzata Urbańska	Junior researcher	L'Oreal Award / MNiSW
Aleksandra Piechnik	Technician	EU
Marcelina Pieprzyk	Technician	NCN/ IIMCB
Agnieszka Kolka	MSc	IIMCB
Katarzyna Rydz	BSc	FNP
Janiszewska Alicja	MSc	NCN

Protein Structure Laboratory		
Marcin Nowotny	Head	Wellcome Trust/UE
Elżbieta Nowak	Post-doctoral Fellow	EU
Małgorzta Figiel	Post-doctoral Fellow	EU
Vinnet Gaur	Post-doctoral Fellow	Wellcome Trust
Karolina Górecka	Post-doctoral Fellow	Wellcome Trust
Jakub Gruchota	Technician	HHMI
Aleksandra Knapik	Post-doctoral Fellow	HHMI
Marcin Jaciuk	Junior Researcher	ERC
Michał Rażew	Junior Researcher	IIMCB
Mirosław Śmietański	Junior Researcher	HHMI
Michał Miętus	Junior Researcher	FNP
Marzena Nowacka	Technician	EU
Weronika Komorowska	Technician	NCBR
Paweł Kustosz	Technician	EU
Justyna Studnicka	Technician	Wellcome Trust

Molecular Biology Department		
Maciej Żylicz	Head	IIMCB
Maciej Olszewski	Post-doctoral Fellow, FishMed	EU
Milena Wiech	Post-doctoral Fellow	IIMCB
Marta Wawrzyniak	FishMed Technical Assistant	EU (1/2)
Marcin Herok	Junior Researcher	IBD/NCN
Marta Małuszek	Junior Researcher	IBB PhD School
Magdalena Pruszko	Junior Researcher	FNP International PhD programme
Zuzanna Tracz-Gaszewska	Junior Researcher	IBB PhD School/FNP Ventures
Grażyna Orleańska	Secretary	IIMCB (1/2)
Cell Cortex Mechanics MPG/ PAN Laboratory		
Ewa Paluch	Head	Free Leave
PolSenior Project		
Małgorzata Mossakowska	Coordinator	IIMCB
Aleksandra Szybalska	Project Assistant	NCBR
Przemysław Ślusarczyk	IT Specjalist	NCBR (1/2)
Katrzyna Wodzyńska	Assistant	NCBR (1/2)
Research Support Service		
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HEART of EUROPE ZEBRAFISH MEETING

September 17–19, 2014, Warsaw, Poland

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- Cell Biology
- Cell Migration
- Development and Organogenesis
- Emerging Technologies
- Husbandry and Health
- Neurology
- 'Omics and Bioinformatics
- Toxicology and Chemical Screens

Speakers:

Peter Aleström, Norway; **Claire Allen**, United Kingdom; **Oliver Bandmann**, United Kingdom; **Petr Bartunek**, Czech Republic; **Michael Brand**, Germany; **Marta Gajewska**, Poland; **Carl-Philipp Heisenberg**, Austria; **Corinne Houart**, United Kingdom; **Jean-Philippe Mocho**, United Kingdom; **Stephan Neuhauss**, Switzerland; **Stefan Schulte-Merker**, The Netherlands; **Didier Stainier**, Germany; **Ewa Snaar-Jagalska**, The Netherlands; **Herman Spaink**, The Netherlands; **Uwe Strähle**, Germany; **Béla Urbányi**, Hungary; **Cecilia Lanny Winata**, Poland.

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