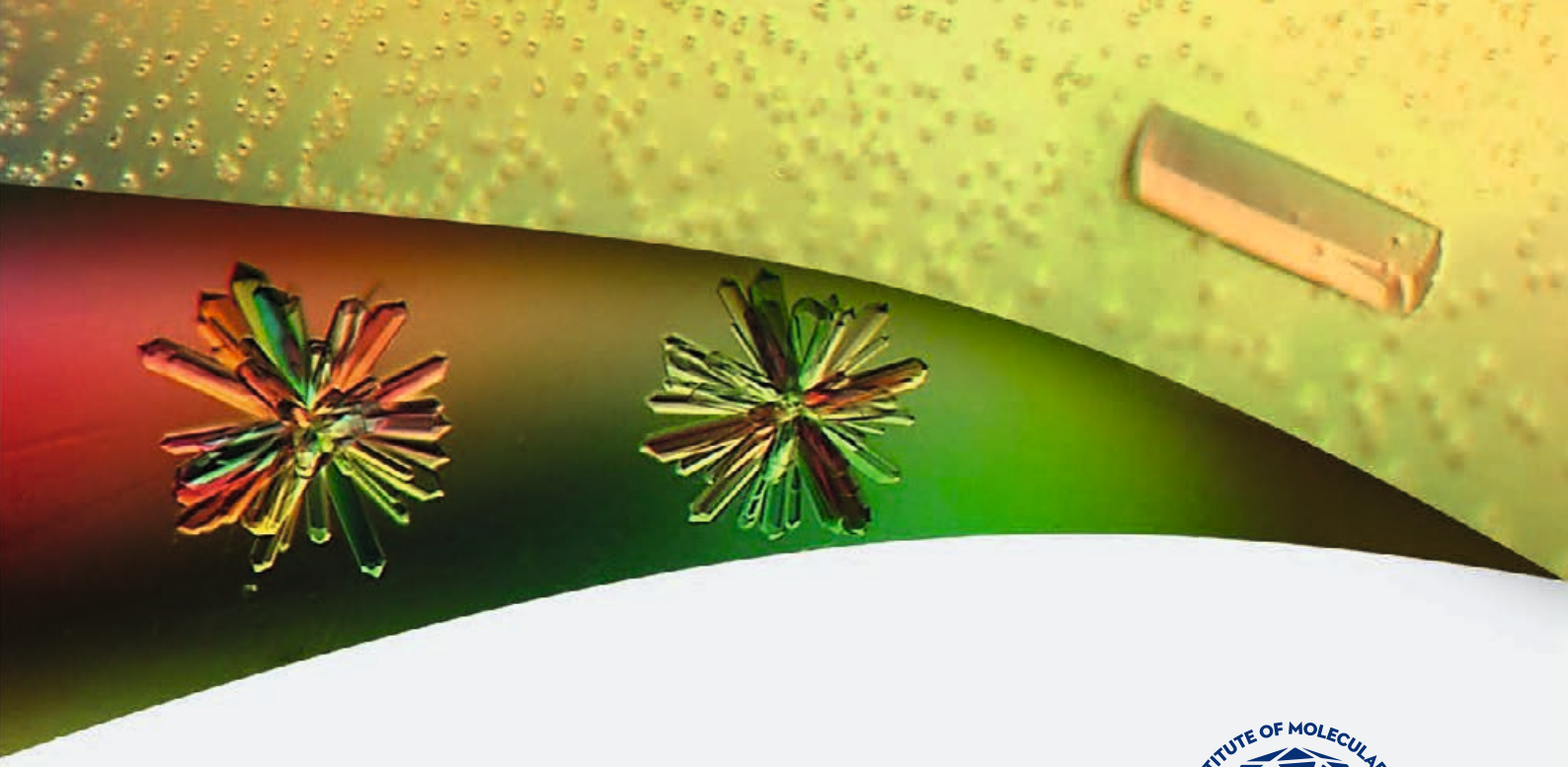

INTERNATIONAL INSTITUTE
OF MOLECULAR AND CELL BIOLOGY
IN WARSAW

# Annual Report

## January 2014 – April 2015

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Publication supported by the European Commission under FP7 GA No 316125 and by the Ministry of Science and Higher Education
fishmed.iimcb.gov.pl

**ISBN 978-83-917905-8-8**

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# Directors' Note

Another year has passed – already the sixteenth year in the history of our Institute's scientific endeavors. Like any year in the past, it brought the usual mix of many challenges and successful achievements as well as some obstacles and unfulfilled hopes. However, as you will learn from the following pages, it may generally be summarized as having been very successful. It is with great satisfaction that we present you with the current Annual Report in which we describe last year's most important events and the achievements of our staff.

The UN General Assembly declared 2014 the International Year of Crystallography, under the auspices of UNESCO and the International Union of Crystallography. The opening ceremony was held at the UNESCO headquarters in Paris and one of those invited was Dr. habil. Marcin Nowotny, Head of the Laboratory of Protein Structure at the Institute. Dr. Nowotny was among 8 young talents and delivered a speech during the Young Crystallographers Session. Not only did he represent our Institute and Poland, but he was also the representative of the CEE region. To celebrate this success, we decided on crystallography as the leading theme for the 2014/Q1 2015 report.

In 2014 the Institute was evaluated by a committee comprising members of two divisions of the Polish Academy of Sciences (Medical Sciences Division and Biological & Agricultural Sciences Division). The assessment result was very positive and confirmed the results of a ranking of universities and academic research institutions, done by the Ministry of Science and Higher Education, in which the IIMCB was assigned category 'A+', i.e. it was listed among 37 'flagship institutions' out of a total of 963 institutions from all fields of science. According to earlier declarations by the Ministry of Science, A+ institutions were to receive better funding for their chartered activities. The Ministry made good on that promise and at the start of 2015 the Institute received a formal decision on an increase in funding, valid through till the next edition of the ranking, i.e. most likely till 2017. This puts us in a comfortable position, allowing us to implement the majority of our plans aimed at expanding the Institute's scientific potential by increasing the number of research teams, among other things. Currently there is a competition in progress for the position of a research team leader, and it is spectacular with regard to the number of outstanding candidates. In mid-May this year a special session will be held at the Institute, during which four top candidates from abroad (two men and two women) will face an audience comprising the staff of the Institute and members of the International Advisory Board, and deliver presentations of their achievements and research plans. The person(s) who is recommended by the Board for the position of team leader will then negotiate with the Director the terms of his/her employment at the Institute. It is important that the quality of applicants, their research fields and the financial position of the Institute allows us to consider the formation of more than one new research team.

The only constraint to this are considerations concerning the available office and lab space. In order to develop and grow, the Institute must overcome these constraints and move to a new, larger facility. It has been estimated that a stable institution of an appropriate critical mass will be created only when there are about 15 research teams. Nine research teams do not provide enough momentum to keep the Institute at the top of rankings if, for example, two research groups decided to leave the Institute at the same time – and this sort of mobility and freedom is part of the Institute's philosophy. We may approach a moment when another team leader may decide to leave the Institute and migrate to an institution which will provide similar conditions for academic work, but will also provide stability of employment. Employment stability is not in the Institute's design, according to the founding Act. The Institute is a research facility where young, outstanding scientists, selected in open international competitions, can form their own, and usually first, independent research team. As shown by the history of the past 16 years we can correctly identify these 'young stars' and nurture their talents.

Being included in the Polish Roadmap for Research Infrastructures represented another successful milestone for the Institute. It was the result of a two-stage competition featuring several hundred institutions from across Poland. The final list included 46 institutions, of which 10 were associated with life sciences. This year a new competition for funding amounting to a total of approximately

PLN 800 million will be announced to support the development of these Research Infrastructures, and its results will be known in mid-2016. Success in this competition would provide funding for a new headquarters facility of the Institute.

At present, we employ over 200 people at our headquarters, of whom 137 are permanent or contract employees and 43 are PhD students. Because of these numbers, and in relation to our plans to further increase employment, we had to reorganize our administration, so as to allow for a more precise allocation of tasks and duties between the Directors and various administrative departments. The new administrative structure of the Institute was implemented as of September 2014 and, half a year later, we may say it was a change for the better. The creation of the LAP position (Laboratory-Administrative Partner) was of specifically high importance. Those employed or transferred to these positions were highly-qualified people and their job is to provide administrative support to team leaders. Because of regular meetings introduced between administrative staff and LAPs, the flow of information has improved and any problems may be discussed and solved as they emerge. Still, some elements of the new structure needed modification, which was done recently. Some changes were introduced regarding promotional materials including stationery, logo and business cards. Thanks to the organizational improvements we can now provide professional management for the Institute, and we are well posed to face even difficult and unexpected situations.

The success record of the Institute should also feature the results of our academic research projects. A significant majority of our publications are published in journals in the first quartile of the Institute for Scientific Information list (Thomson Reuters) and their percentage stays at a level of about 70%. However, papers published in the top 10% of journals on the list are still rare, and they most often result from cooperation between individual researchers and teams from international academic centers. The coming years will show whether these figures will change to our advantage. The problem of difficulties concerning the publication in the leading journals of excellent papers from lesser-known institutions has finally been recognized as serious and, following this, the group of Nature journals introduced the possibility of having papers reviewed without disclosing the authors' names and affiliations.

Even though basic research is our primary mission, some of our findings have turned out to have the potential for application. An important role in the identification of this potential is played by the BioTech-IP Division – a unit which deals, among other things, with the development of patent applications and with finding funding for these activities and their commercialization. In order to strengthen these procedures, the Institute established BioTech-IP, Ltd., a company in which it holds a 100% share. There are plans to establish another subsidiary in the coming months, in which a partial share will be held by the Institute and another part by an Institute employee – the author of the discovery that forms the basis of the new company.

The Institute is also active in the area of disseminating the awareness of modern biological sciences, especially molecular biology, by being a major financing partner of BioCEN – the Centre for Innovative Bioscience Education. The BioCEN labs have been visited by 19 000 children, but as of mid-2014 the space assigned to BioCEN activities had to be transferred to the newly established Zebrafish Developmental Genomics laboratory. In view of this, we have decided that we will finance the lease of new BioCEN facilities in a secondary school nearby. This means that we will be able to further pursue this string of very beneficial educational activities. Moreover, under the FishMed project, we run a series of educational activities addressed to children under the slogan "Be as Healthy as a Fish".

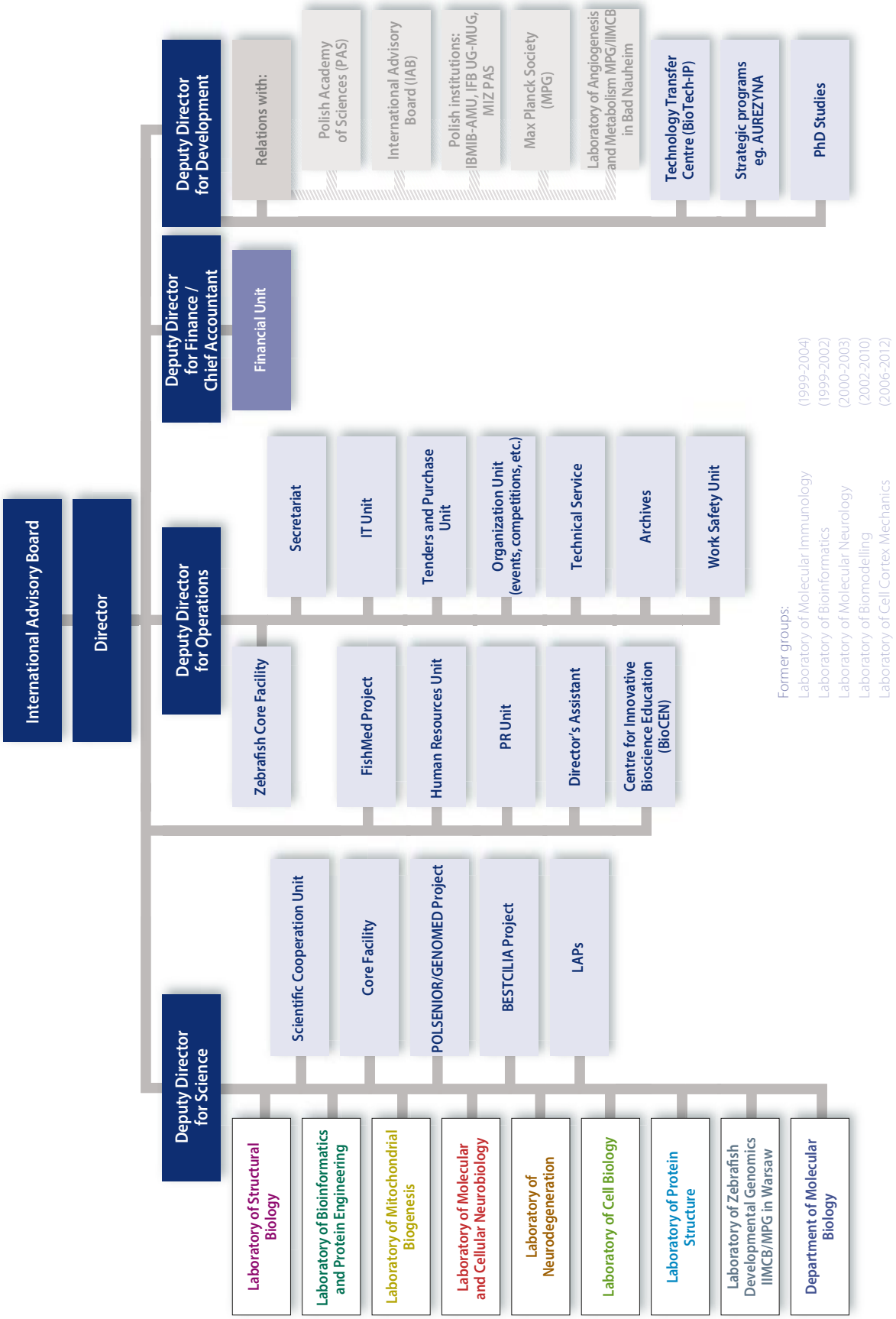
The FishMed project, developed under the RegPot 7PR programme, has been running well and the decision to extend the research to the zebrafish model proved to be beneficial for all. Thanks to funding from this project, we were able to employ 30 people for 42 months, primarily under full-time employment contracts: 17 academic researchers (post-docs and technicians), 5 employees of the Zebrafish Core Facility and 8 supporting staff (5 at Biotech-IP, 2 at the grants office and one PR specialist). We have also purchased exceptional equipment, including a LightSheet (SPIM) microscope. When it comes to equipment, and following our purchase of a full Next Generation Sequencing set, including Illumina NextSeq, financed by the Ministry of Science and Higher Education, our Institute will not be outranked by the best research facilities.

When we come to concluding our review of 2014 and Q1 2015, we have to stop and ask ourselves the question: what next? What will the Institute be like in the next 4 years and in the subsequent 4 years to come? Will it maintain its prominent position among Polish life sciences academic institutes? Will it pursue its growth path and will it be recognized as a unique institution on a European scale? I strongly believe that, despite the risks resulting from a limited number of teams and the inability to ensure their full stability at the Institute, there are reasons to consider our Institute a strong player with a full development potential. To bring this potential to full use, we need a new base with more available space and more research teams headed by young leaders. The first issue is within the competences of the authorities of the Capital City of Warsaw and the management at the Ministry of Science and Higher Education. The second issue is to be pursued in cooperation with the International Advisory Board who assist us in finding suitable talents and support their development through the evaluation of their research activities. We find the commitment of IAB members and their deep understanding of the local situation to be the best indicator of our future concerted efforts towards further development of the Institute and successes of its people.

Warsaw, April 2015

Jacek Kuźnicki

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



# 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 (PAS) 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. Involvement of the International Advisory Board, the rules of recruitment of all faculty members through international competition and lack of tenure employment make it unique among Polish research institutions. The principles of organization of the Institute differ from other research institutes in the country: an important body of the Institute is the IAB, 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 IAB. 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 European Research Council, Framework Programs of EU, Howard Hughes Medical Institute, European Molecular Biology Organisation, Wellcome Trust, Polish Swiss Research Programme, International Centre for Genetic Engineering and Biotechnology, Ministry of Science and Higher Education, National Science Centre, National Centre for Research and Development, Foundation for Polish Science, Structural Funds, etc.

IIMCB has adhered to the "Pact for Horizon 2020". Initiated by Prof. Lena Kolarska-Bobińska, Minister of Science and Higher Education, the "Pact" aims to mobilize Polish researchers to obtain EU funds from Horizon 2020, the EU framework program for research and innovation. Its seven-year budget for 2014-2020 amounts to almost 80 billion euros. In turn, academic and research institutes signing the "Pact" undertake to: build effective organizational and administrative infrastructure to support researchers applying to Horizon 2020; take the participation in Horizon 2020 into account in the process of evaluation and promotion of research staff; initiate and support partnerships between scientists and industry; adhere to and follow the rules of the European Charter and Code of Conduct.

In 2014 about 70% 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 63).

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. 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 ten research groups with full research equipment and core facilities including a zebrafish facility. The space occupied by IIMCB is not sufficient for the optimum functioning of the Institute and this is a major limiting factor as it prevents the establishment of new research groups. Consequently, it thwarts the innovative potential of activities undertaken.

## 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, who supervises the organization and activities of the Institute. The President of PAS nominates members of International Advisory Board and the Institute's Director. The IIMCB uses a building loaned to it by the PAS. It also played a crucial role as a party to the agreement with the Max Planck Society which made it possible to organize joint laboratories.

## The organization of research at IIMCB

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

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

1. Structural and biochemical studies of DNA methylation and hydroxymethylation (Bochtler group).
2. Experimental and theoretical studies on structures of RNAs and proteins and 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).
3. Biogenesis of mitochondrial proteins, cellular protein homeostasis, protein transport mechanisms, and redox processes in mitochondria (Chacińska group).
4. 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).
5. Studies of calcium and β-catenin signaling in the brain and molecular mechanisms of neurodegenerative and psychiatric diseases (Kuźnicki group).
6. Interdependence between endocytic transport, intracellular signal transduction, and transcriptional regulation (Miączyńska group).
7. Structural and biochemical studies of nucleic acid enzymes (Nowotny group).
8. Complex transcriptional regulatory mechanism of embryonic cardiac development *in vivo* (Winata group).
9. 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).

## Awards and Honors

IIMCB as a Consortium Leader with its project entitled ***Research Infrastructure of Molecules and Cells (IN-MOL-CELL)*** is one of the 10 institutions placed on the **Polish Roadmap for Research Infrastructures** in strategic research area: *Health strategy and an increase in effectiveness of health promotion activities*. According to Ministry of Science and Higher Education strategic research infrastructure are those research facilities, which are unique on the national, European or international level, and are crucial to the development of scientific research, R&D, and the scientific IT infrastructure.

President of the Republic of Poland Bronisław Komorowski awarded **Prof. Janusz Bujnicki Knight's Cross of the Order of Polonia Restituta**. Prof. Bujnicki was honored for outstanding achievements in scientific research work.

**Dr. Agnieszka Chacińska**, Head of the Laboratory of Mitochondrial Biogenesis and **Dr. Jacek Jaworski**, Head of the Laboratory of Molecular and Cellular Neurobiology received the professorial title from President of the Republic of Poland Bronisław Komorowski.

**Prof. Janusz Bujnicki** was one of three winners of the **National Science Centre (NCN) Awards 2014**. They are awarded for significant scientific achievements made in the context of basic research conducted in the Polish scientific institution, documented by publications.

**Prof. Janusz Bujnicki** was nominated by the Minister of Science and Higher Education one of the new **members of the Scientific Policy Committee** which is made up of the representatives of various fields of science and socioeconomic life, acting as a Minister's advisory body. He replaced Prof. Jacek Kuźnicki, whose 3-years term was completed. The committee consists of 12 members.

**Prof. Janusz Bujnicki** has been selected as one of **"25 leaders for the next 25 years" by the "Teraz Polska" magazine of the Polish Promotional Emblem Foundation.** The next stage of the project "25/25. Young leaders to start" will be a meeting of all awardees to develop a manifest of future leaders and a system that will popularize their ideas. The aim of the project is not to keep the list of 25 names fixed and closed, but to update the list of leaders depending on their activities and accomplishments.

**Prof. Janusz Bujnicki** became one of the eleven Ambassadors of Science within the **campaign "Occupation Scientist"** of the Ministry of Science and Higher Education. The campaign "Occupation Scientist" is to inform, what is the profession of researcher, how to be realized through scientific work and promote the selection of just such a career way. The campaign has been carried out as a series of promotional activities that will show to young people the profession of scientist, to encourage them to become interested in science. The symbol of the campaign is Apple Newton - a sign of ingenuity and innovation.

**Dr. Elżbieta Nowak** from the Laboratory of Protein Structure was awarded with the **L'Oreal Poland – UNESCO Award for Women in Science**, in the 14th edition of this program. Dr. Nowak received the scholarship for the project entitled *Structural and biochemical studies on proteins involved in nucleotide excision repair and reverse transcription*. L'Oréal Poland Awards for Women in Science with the support of the Polish Committee for UNESCO grants scholarships to women who are no more than 35 years (PhD student) and no more than 45 years with Ph.D. degree - and conduct research in the following areas: biology, biochemistry, biotechnology, agriculture, medicine, pharmacy and physiology.

**Foundation for Polish Science within the SKILLS project** awarded **Prof. Janusz Bujnicki** with the 1st prize, for the project *Commercialization of the eRNase technology - restriction enzymes for RNA*, and **Katarzyna Kamińska**, Prof. Bujnicki's PhD student, with the 3rd prize, for the project *Optimization of precursors for new anti-flu drugs: inhibitors of the influenza virus nuclease*. **Dr. Izabela Sabała** is one of the laureates of the second edition of the IMPULS competition organized within the SKILLS project by the Foundation for Polish Science. Her project is entitled *Enzymatic chimeras with bacteriolytic activity*. The aim of the IMPULS competition is to promote applied research and development of skills in commercialization of scientific results, and it is based on the evaluation of proposals of innovative ideas and research projects with a potential for commercialization.

**Katarzyna Kamińska**, PhD student in Laboratory of Bioinformatics and Protein Engineering received scholarship for outstanding young scientists conducting high-quality research and with impressive scientific achievements funded by Ministry of Science and Higher Education.

## Participation in Main Events

**Dr. Marcin Nowotny**, Head of Laboratory of Protein Structure represented Eastern Europe during the Opening Ceremony of the UNESCO **International Year of Crystallography**. Dr. Nowotny and his research were presented in a session *Young talented crystallographers of the World*. The Opening Ceremony was held in Paris on 20-21 January, 2014 at the UNESCO headquarters.

**Dr. Anna Bartosik,** a young IIMCB scientist **participated in the 64th Lindau Nobel Laureate Meeting, Germany**. The conference was dedicated to physiology and medicine. Nobel Laureates have been invited to lectures on a topics of the wide range of research fields. With 38 Nobel Laureates and approximately 600 young scientists from more than 80 countries, the 64th Lindau Nobel Laureate Meeting was a landmark on the agenda of the international scientific dialogue.

**Prof. Michał Witt** participated in an **Experts' Seminar on Legislative Solutions in the Field of Bioethics.** Prof. Witt held a lecture entitled *Designing legal regulations of genetic testing, biomedical research and biobanking in Poland*. The purpose of the seminar was to exchange good practices in legislation on bioethics between British and Polish experts in the context of the ratification of the Convention. The seminar was organized by the Government Plenipotentiary for Equal Treatment and the British Embassy.

**Prof. Jacek Kuźnicki** gave the presentation following the Prof. Robert Huber's lecture at the opening of the scientific conference *Challenges of Biotechnology in 21st Century*. This was an official opening of the Centre of Biotechnology at the Jagiellonian University. Prof. Kuźnicki's lecture was on *Brain diseases as a challenge for contemporary biomedicine*.

## Visits to IIMCB

IIMCB hosted **a meeting of the Life, Environmental and Geo Sciences (LEGS) panel of Science Europe**. Science Europe is an association of European Research Funding Organisations (RFO) and Research Performing Organisations (RPO), based in Brussels. Science Europe promotes the collective interests of the RFOs and RPOs. It supports its Member Organisations in their efforts to foster European research. The LEGS Scientific Committee informs and supports Science Europe in science policy activities. The Committee comprises 15 European academics representing a wide range of research disciplines, including Prof. Janusz Bujnicki from IIMCB (who was nominated by the Polish National Science Center / NCN, as a representative in the area of bioinformatics). LEGS is chaired by Prof. Dirk Inzé who is Scientific Director of the Department of Plant Systems Biology, Vlaams Instituut voor Biotechnologie in Belgium.

IIMCB hosted **Prof. Venki Ramakrishnan** from the Structural Studies Division, MRC Laboratory of Molecular Biology, Cambridge, UK. As a part of the event *'Meet the ribosome',* Prof. Ramakrishnan

*'Meet the ribosome'* organizers with Prof. Venki Ramakrishnan

delivered two lectures entitled *Structural biology of the elongation cycle of the ribosome* and *Use of recent advances in electron microscopy to study the ribosome at high resolution*. Prof. Venki Ramakrishnan shared the 2009 Nobel Prize in Chemistry for studies of the structure and function of the ribosome.

IIMCB was visited by **Adam Struzik,** Marshal of the Mazowieckie Voivodeship, Katarzyna Krężlewicz, Director of the Office of the Marshal and Konrad Wojnarowski, Assistant of the Marshal. The guests learnt about the history and achievements of the Institute from Prof. Jacek Kuźnicki and visited Zebrafish Core Facility, Laboratory of Mitochondrial Biogenesis and the newly created Laboratory of Zebrafish Developmental Genomics, IIMCB/Max Planck Research Group.

Visit from Adam Struzik, Marshal of the Mazowieckie Voivodeship, 13.06.2014

Visit from the members of the German Science Journalists' Association WPK, 2.06.2014

IIMCB was visited by the members of the **German Science Journalists' Association WPK (Wissenschafts-Pressekonferenz).** The guests were hosted by Prof. Jacek Kuźnicki; they also met with Dr. Agnieszka Chołuj, Head of the Centre for Innovative Bioscience Education and Hubert Ludwiczak, representative of the Technology Transfer Centre BioTech-IP.

# Cooperation with Other Institutions

## Domestic Cooperation

### Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University (IBMIB-AMU), Poznań

In January 2015, an agreement between the International Institute of Molecular and Cell Biology in Warsaw (IIMCB) and Adam Mickiewicz University in Poznań (AMU) was signed by **Prof. Jacek Kuźnicki**, Director of the Institute, **Prof. Michał Witt**, Deputy Director of the Institute, **Prof. Bronisław Marciniak**, Rector of the University, **Prof. Bogdan Jackowiak**, Dean of the Faculty of Biology at the University and **Prof. Zofia Szweykowska-Kulińska**, Head of the Institute of Molecular Biology and Biotechnology.

The aim of the agreement is to establish a new research group in the field of bioinformatics affiliated with both AMU and IIMCB. The laboratory in bioinformatics will be located at the Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznań. The group leader will be selected during an open international competition organised jointly by both institutions.

### Biocentrum Ochota (www.biocentrumochota.gov.pl), Warsaw

In January 2008, the scientific activities of the Biocentrum Ochota Consortium of the Polish Academy of Sciences were launched as 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 PAS
3. Nałęcz Institute of Biocybernetics and Biomedical Engineering PAS
4. Nencki Institute of Experimental Biology PAS
5. Mossakowski Medical Research Centre PAS
6. Institute of Fundamental Technological Research PAS

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 within the Horizon 2020 framework and expected in 2015 Operational Programmes of structural funds.

### Intercollegiate Faculty of Biotechnology (IFB UG-MUG), Gdańsk

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 mentor top and innovative research in the field of systems; the agreement to establish a new joint laboratory has been signed between the two institutions and a recruitment process for the relevant Lab Leader is under way. This cooperation is also very promising in the field of medical biology and molecular diagnostics.

### Museum and Institute of Zoology PAS (MIZ), Warsaw

The formal consortial agreement was signed to set up a joint sequencing platform (Seq4All) between IIMCB and Museum and Institute of Zoology PAS. The successful grant application to the Polish Ministry of Science and Higher Education resulted in funds of about 5 mln PLN for a purchase of two next generation sequencers. As a result of the tendering procedure, the Consortium has purchased two instruments: Illumina NextSeq 500 and MiSeq sequencers. Laboratories are equipped with advanced computer system for data analysis and complementary equipment. Areas of common interest of both institutions are de novo sequencing and assembly, RNAseq, CHIPseq, and the sequencing of modified or ancient DNA.

### Cooperation with companies and patients' organization

IIMCB actively collaborates with pharmaceutical and biotechnology companies such as Adamed and OncoArendi to develop new therapies in neurology and oncology. The Institute's Technology Transfer Unit Biotech-IP supports scientists in their work on applicable R&D projects and IP protection. At the end of 2014 the IIMCB established BioTech-IP Ltd - company dedicated to create and support spin-off companies devoted to commercialize scientific results coming from the Institute. Moreover, BioTech-IP Ltd is going to offer to external partners the portfolio of services in the field of business consulting and R&D. IIMCB was instrumental in establishing a spin-out company Proteon Pharmaceuticals Ltd. Creation of the second Spin-off 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, Germany

**First cooperation programme**

The cooperation started in 2001 as an initiative of the Max Planck Society (MPG) and Polish Academy of Sciences (PAS). According to the agreement, the Junior Research Group, with **Dr. Matthias Bochtler** as Lab Leader, selected in an open international competition run jointly by MPG and PAS, 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 staphopainstaphostatin system, has discovered a novel cysteine peptidase inhibitor mechanism. Furthermore the group has focused on eukaryotic protein degradation system. Dr. Bochtler's laboratory was provided 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 far more advanced X-ray generator (Proteum Bruker) equipped with a CCD detector (Platinum 135) and cryosystem (Cryostream series 700). 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 PAS in Warsaw as a full professor.

The Laboratory of Cell Cortex Mechanics MPG/PAS, headed by **Dr. Ewa Paluch** as a twin laboratory of Matthias Bochtler's MPG/PAS 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 Higher 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 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 finances 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. In 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.*

The mirror position in Warsaw has been filled by **Dr. Cecilia Winata**, who runs 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 focuses on transcriptional regulatory network of heart development and on epigenome profile of heart development. The group bases mainly on agenomics approach. This is the first research laboratory in Poland which, together with an extensive experience of the Zebrafish Core Facility, displays 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 has full access to MPI-HLR equipment, animal facilities, and genetic modification techniques for zebrafish and mice (for details see page 50).

### Institute of Molecular Biology and Genetics, Kiev, Ukraine

Since 2011, IIMCB actively cooperates with the Institute of Molecular Biology and Genetics in Kiev (IMBG), Ukraine by implementing the COMBIOM project entitled, "Strengthening Cooperation in Molecular Biomedicine between EU and Ukraine" (01.12.2011-31.05.2015), 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, 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.

Despite the political situation in Ukraine, IIMCB support to IMBG was quite intense in 2014. A number of IMBG young scientists visited IIMCB laboratories to develop research, use state-of-the-art equipment, learn new techniques and methods. They were hosted by Laboratories of Prof. Miączyńska, Prof. Jaworski, Prof. Bochtler, and Dr. Winata. Additionally, two IMBG scientists participated in IIMCB scientific meeting "Heart of Europe: Zebrafish Meeting", in September 2014, and eight took part in the Opening of the Academic Year of Ochota Biocentre in October, 2014. Moreover, in September 2014, a group of fourteen IMBG scientists benefited from the multidisciplinary, practical weekly training on IPR, Project Management and Equipment organized in Warsaw by BioTech-IP.

The project has been extended for a six-month period until 31 May, 2015. The final report session in April 2015 is being attended by Prof. Jacek Kuźnicki (IIMCB coordinator of COMBIOM), Prof. Matthias Bochtler and Dr. Cecilia Winata.

# ‘HR Excellence in Research’ logo and Lab Leader Competitions

The International Institute of Molecular and Cell Biology in Warsaw is acknowledged by the prestigious ‘HR Excellence in Research’ logo for the implementation of the principles of the European Charter for Researchers and the Code of Conduct for the Recruitment of Researchers. It’s a symbolic element of a demanding process of implementing the European Charter and Code policies and practices. The general idea of this process is to render the Institute an attractive place for researchers to work at.


Following analysis of Institute’s practices vis-a-vis rules of the European Charter and Code, in 2014 IIMCB focused on the implementation of actions aiming at improving IIMCB employment and working conditions such as: organization of soft-skills and career-development training for researchers, distributing information on job opportunities, involvement of researchers in decision-making process. Following the need for an Ombudsman for researchers the HR working group recommended **Dr. Urszula Białek-Wyrzykowska** for this position. Her official nomination is the next step on the agenda.

On 3 March 2015, **Prof. Jacek Kuźnicki** and **Dorota Libiszowska** participated in a celebration of the 10th anniversary of the ‘European Charter for Researchers and a Code of Conduct for the Recruitment of Researchers’ in Brussels, Belgium. At the event IIMCB received a symbolic ‘HR Excellence in Research’ statuette.

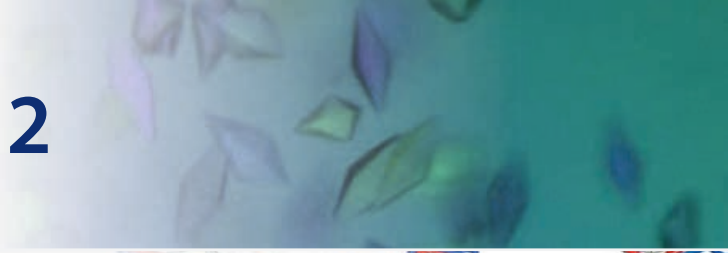
The European Charter for Researchers and the Code of Conduct, adopted by the European Commission in 2005, specify the role, rights and duties of researchers, their employees and funding agencies. Until now, nearly 190 research institutions from 28 European countries have been honored with the ‘HR Excellence in Research’ logo, which identifies particularly attractive work environments. The IIMCB has received this recognition as the third institution in Poland, following the Foundation for Polish Science and the Nencki Institute of Experimental Biology. Currently, there are five institutions in Poland having received the Commission acknowledgement.

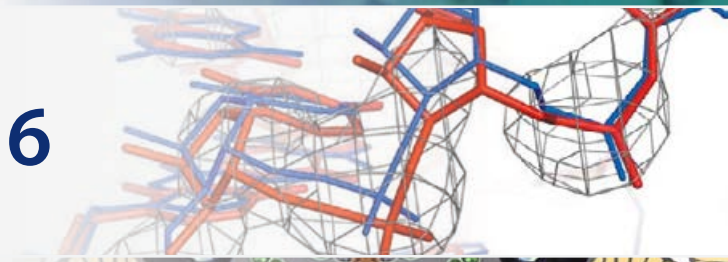
In line with the above mentioned rules, international competitions for lab leaders’ positions are considered at IIMCB as an essential mechanism for ensuring proper intake of talented young researchers to the Institute. This procedure is mandatory, unquestionably leading to a continuous improvement in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

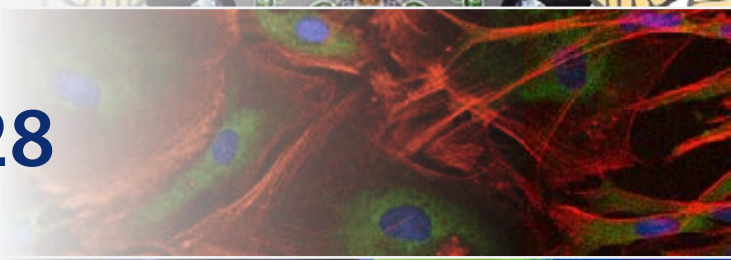
As a rule, every Lab Leader competition is advertised in internationally visible media (NatureJobs, Euraxess, IIMCB web page). The applicants are initially screened formally at the Institute. Later, they get evaluated by the Selection Committee made up of several members of the International Advisory Board. 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 technologies 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.

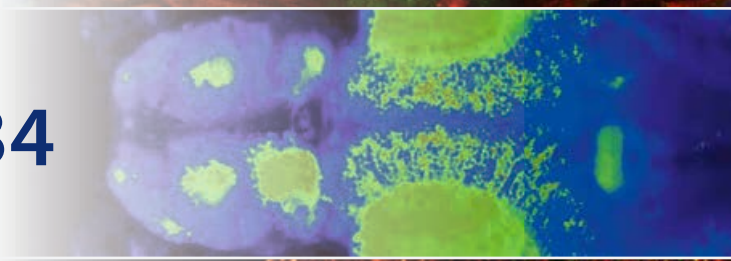
| Competition | Year | Number of candidates | **Winners employed at IIMCB** |
|---|---|---|---|
| I | 1998 | 6 | **Jarosław Dastych** |
| II | 1999 | 3 | **Maciej Żylicz** |
| III | 2000 | 6 | **Michał Hetman** |
| IV[1)] | 2000 | 7 | **Matthias Bochtler, Leszek Rychlewski** |
| V | 2002 | 9 | **Janusz 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[4)] | 2012[3)], 2012[3)] | 18 & 15 | – |
| XVI[4)] | 2013 | 14 | **Cecilia Lanny Winata** |
| XVII | 2015 | 17 | **?** |

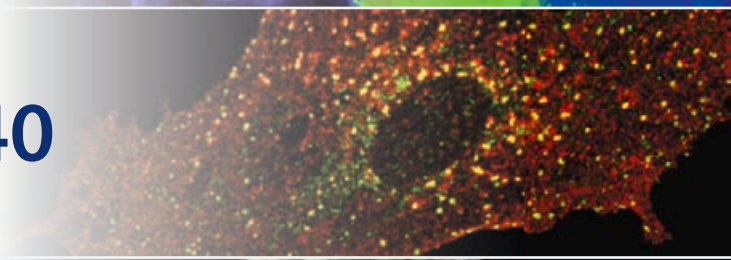
[1)] these competitions fulfilled the MPG/PAS agreement
[2)] no result
[3)] the winner did not accept the offer
[4)] this competition fulfilled the IIMCB/MPG agreement

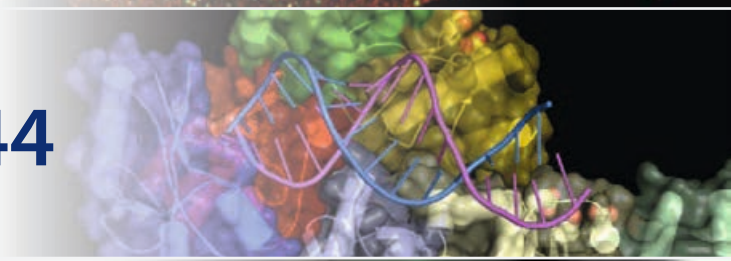


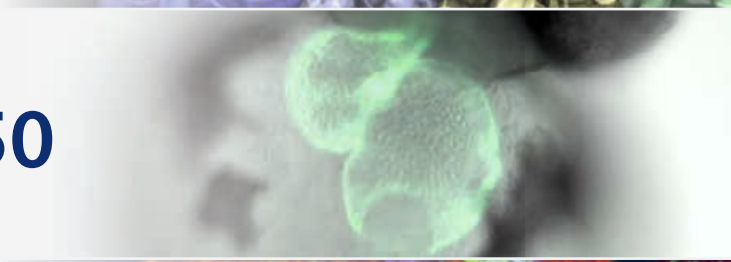


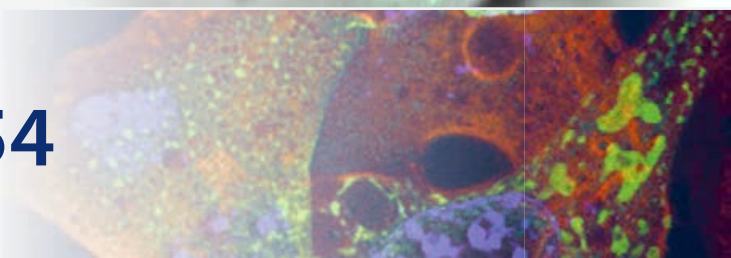












# Laboratory of Structural Biology

**Vice Head:**
Honorata Czapińska, PhD

**Postdoctoral Fellows:**
Humberto Fernandes, PhD (IBB PAS)
Anna Fricke (Piasecka), PhD (IBB PAS)
Agnieszka Kolano, PhD, (FishMed)
Monika Kowalska, PhD (on maternity leave)
Joanna Krwawicz, PhD (IBB PAS)
Katarzyna Misztal, PhD
Małgorzata Perycz, PhD (IBB PAS)
Dario Piano, PhD
Marek Wojciechowski, PhD

**FishMed Research Assistants:**
Marta Wawrzyniak, PhD (until October 2014, part-time)
Thomas Fricke, PhD (since January 2015, part-time)

**PhD Students:**
Patrycja Haniewicz, MSc
Asgar Abbas Kazrani, MSc
Marlena Kisiała, MSc (IBB PAS)
Karolina Mierzejewska, MSc
Michał Pastor, MSc (IBB PAS)
Dominik Rafalski, MSc
Karthik Shanmuganandam, MSc (until Nov. 2014)
Wojciech Siwek, MSc (until Dec. 2014)

**MSc Students:**
Marta Szychowska, Eng (until Oct. 2014)
Patrycja Wawrzyniecka, BSc (until March 2014)

**Technician:**
Agnieszka Olszewska (part-time)

**Laboratory-Administrative Partners (LAPs):**
Izabela Zacharek, MSc (part-time, until Feb. 2014)
Paulina Okafor (part-time, from Nov. 2014)

# 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-PAS 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 PAS, 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 |

Cover photo: Crystals of PvuRts1I. Author: Asgar Abbas Kazrani.

# Selected Recent Publications

## Protein-nucleic acid interactions

- **Mierzejewska K, Siwek W, Czapinska H**, Kaus-Drobek M, Radlinska M, **Skowronek K, Bujnicki JM**, Dadlez M, **Bochtler M**. Structural basis of the methylation specificity of R.DpnI. ***Nucleic Acids Res***, 2014; 42(13): 8745-54
- **Wojciechowski M, Rafalski D**, Kucharski R, **Misztal K**, Maleszka J, **Bochtler M**, Maleszka R. Insights into DNA hydroxymethylation in the honeybee from in-depth analyses of TET dioxygenase. ***Open Biol***, 2014; 4(8):140110
- **Kazrani AA, Kowalska M, Czapinska H, Bochtler M**. Crystal structure of the 5hmC specific endonuclease PvuRts1I. ***Nucleic Acids Res***, 2014; 42(9):5929-36
- Gallagher JM, Yamak A, Kirilenko P, Black S, **Bochtler M**, Lefebvre C, Nemer M, Latinkic B. Carboxy terminus of GATA4 transcription factor is required for its cardiogenic activity and interaction with CDK4. ***Mech Dev***, 2014; 134:31-41
- **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 Ecl18kI acquire specificities for different DNA targets. ***Nucleic Acids Res***, 2008; 36:6101-8
- Sukackaite R, Grazulis S, **Bochtler M**, Siksnys V. The recognition domain of the BpuJI restriction endonuclease in complex with cognate DNA at 1.3-A resolution. ***J Mol Biol***, 2008; 378:1084-93
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- **Sokolowska M, Kaus-Drobek M, Czapinska H**, Tamulaitis G, **Szczepanowski RH**, Urbanke C, Siksnys V, **Bochtler M**. Monomeric restriction endonuclease BcnI in the apo form and in an asymmetric complex with target DNA. ***J Mol Biol***, 2007; 369:722-34
- **Kaus-Drobek M, Czapinska H, Sokolowska M**, Tamulaitis G, **Szczepanowski RH**, Urbanke C, Siksnys V, **Bochtler M**. Restriction endonuclease MvaI is a monomer that recognizes its target sequence asymmetrically. ***Nucleic Acids Res***, 2007; 35:2035-46
- **Bochtler M, Szczepanowski RH**, Tamulaitis G, Grazulis S, **Czapinska H**, Manakova E, Siksnys V. Nucleotide flips determine the specificity of the Ecl18kI restriction endonuclease. ***EMBO J***, 2006; 25:2219-29
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## Other

- **Sabala I, Jagielska E**, Bardelang PT, **Czapinska H**, Dahms SO, Sharpe JA, James R, Than ME, Thomas NR, **Bochtler M**. Crystal structure of the antimicrobial peptidase lysostaphin from Staphylococcus simulans. ***FEBS J***, 2014; 281(18):4112-22
- 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
- **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
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- **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 seeks to understand the mechanistic aspects of DNA methylation and hydroxymethylation and role of these modifications in (relatively) simple model organisms.

To better understand how proteins bind specifically to DNA with a 6-methyladenine (6mA) base, we studied the 6mA-dependent restriction endonuclease R.DpnI. The enzyme cleaves DNA at GATC sites with high efficiency when the adenines of both strands are methylated and much lower efficiency when only one is methylated. Unmethylated DNA is not a substrate of the protein. Using crystallographic methods, we previously showed that R.DpnI consists of a catalytic and non-catalytic (winged helix) domain, which are separately specific for DNA sequence and modification status. However, remaining to be clarified is how the individual domains achieve 6mA specificity. With an additional crystal structure of R.DpnI with DNA bound to both domains in hand, we noticed that the adenine methyl groups in an R.DpnI DNA substrate are so close together in space that the DNA has to be deformed relative to ideal B-DNA or non-methylated DNA to avoid a clash. R.DpnI binds to the deformed conformation and hence also reads DNA indirectly. This mechanism is specific to adenine methylation, and one nucleotide stagger can operate together with more conventional mechanisms of methyl-specific binding, such as desolvation effects and favorable van der Waals interactions (Mierzejewska et al., 2014).

As a model of the specific binding of proteins to DNA that contain the 5-hydroxymethylcytosine (5hmC) base, we chose the PvuRts1I endonuclease, which has become a tool for 5hmC mapping. Although we could only grow PvuRts1I crystals without DNA, these crystals were very informative. They showed that PvuRts1I consists of an N-terminal, atypical PD-(D/E)XK catalytic domain, and C-terminal SRA domain that might accommodate a flipped 5hmC or 5ghmC base. Changes to predicted catalytic residues of the PD-(D/E)XK domain or putative pocket for a flipped base abolish catalytic activity. However, fluorescence changes that are indicative of base flipping are not observed when PvuRts1I is added to DNA substrates that contain pyrrolocytosine in place of 5hmC. Despite this caveat, the structure suggests a model of PvuRts1I activity, thus presenting opportunities for protein engineering to alter the enzyme properties for biotechnological applications (Kazrani et al., 2014; Fig. 1).

In addition to structural work on 5hmC, we also made some efforts to develop animal models of 5hmC that are simpler than the available mouse models and might not be as confounded by the many different and overlapping roles of 5hmC in mammals. In vertebrates, 5hmC is generated by the oxidation of 5-methylcytosine in DNA (not in free nucleosides or nucleotides) by oxoglutarate-dependent TET dioxygenases.

The honeybee contains a single gene that is clearly orthologous to mammalian TET genes. In collaboration with Prof. Ryszard Maleszka at Australian National University, we demonstrated that the honeybee TET 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. *In vivo*, the levels of 5hmC are condition-dependent and relatively low, but in testes and ovaries, 5hmC is present at approximately 7-10% of the total level of 5mC, which is comparable to the levels reported for certain mammalian cell types. Honeybee TET is alternatively spliced and highly expressed throughout development and in adult tissues, with the highest expression found in adult brains. Altogether, our data show that the honeybee might be an attractive model organism with unique biology to study TET-driven DNA hydroxymethylation (Wojciechowski et al., 2014).

Zebrafish DNA also contains the 5hmC base, but its role is unclear. Some findings suggest a possible role in DNA demethylation. However, other studies (some published before the discovery of the activity of TET enzymes in vertebrates) point to the deaminative rather than oxidative remodeling of DNA methylation. This claim is controversial because this pathway should be highly mutagenic. Our own data indicate that TET genes in zebrafish are bona fide orthologs of their mammalian counterparts. Moreover, we have tentative evidence of a much earlier appearance of the 5hmC base in the zebrafish embryo than previously reported in the literature. Gene inactivation studies that used TALEN- and CRISPR-based genome editing are currently underway (unpublished, in collaboration with Olov Andersson, Karolinska Institute, Stockholm).

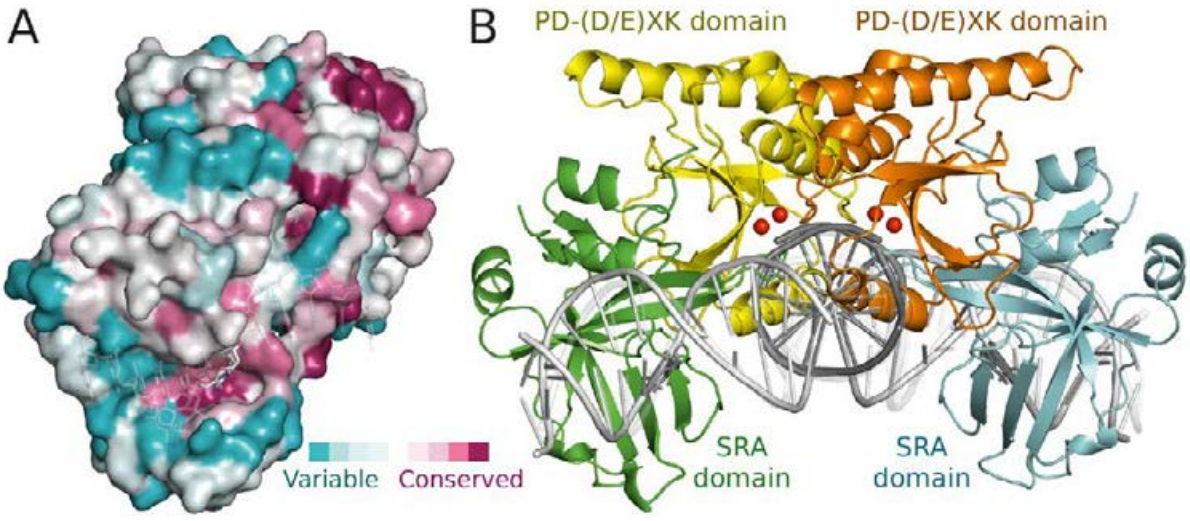



**Fig. 1. Crystal structure of PvuRts1I with DNA modeled in the orientation that is found in other complexes of SRA domains with DNA.** (A) Monomer, illustrating the conservation of amino acids in PvuRts1I. (B) Modeled dimer, with DNA bound also to the catalytic domain. The dimerization mode, which is not observed in the crystal, was deduced from the cleavage stagger.

# Laboratory of Bioinformatics and Protein Engineering

**Postdoctoral Fellows and Research Associates:**
Michał Boniecki, PhD
Grzegorz Chojnowski, PhD
Justyna Czarnecka, PhD
Stanisław Dunin-Horkawicz, PhD
Wayne Dawson, PhD (FishMed)
Jarosław Kijek, PhD
Maciej Maciejczyk, PhD
Filip Stefaniak, PhD
Martyna Nowacka, PhD
Radosław Pluta, PhD
Elżbieta Purta, PhD
Agata Sulej, PhD

**PhD Students:**
Catarina Alneida, MSc
Astha, MSc
Magdalena Byszewska, MSc
Dawid Głow, MSc
Elżbieta Jankowska, MSc
Magdalena Machnicka, MSc
Marcin Magnus, MSc
Paweł Piątkowski, MSc
Krzysztof Szczepaniak, MSc
Diana Toczydłowska, MSc

**Associate researcher, working extramurally:**
Joanna Kasprzak, PhD

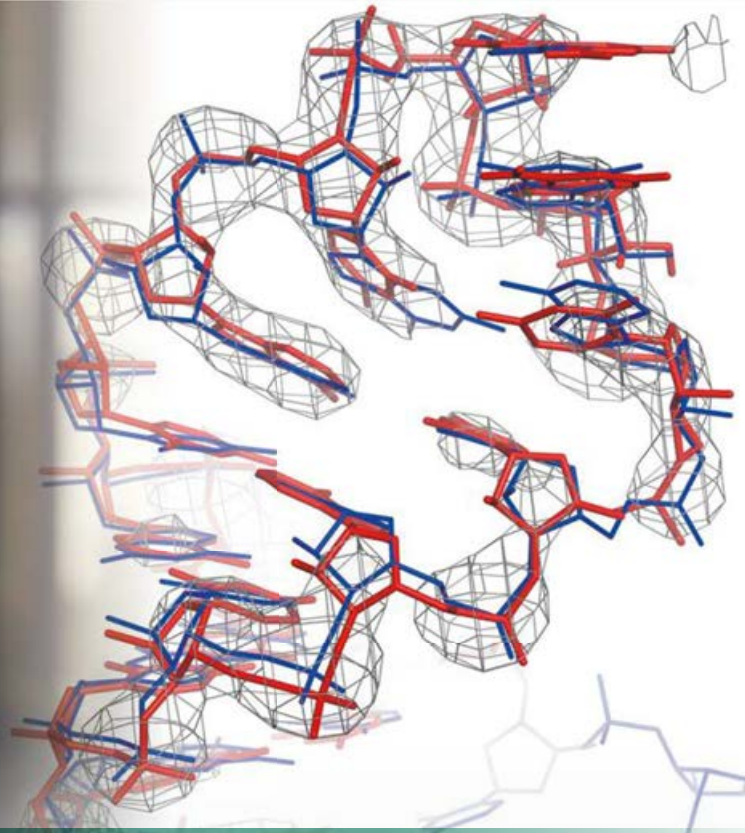
**Research Technicians:**
Małgorzata Kurkowska, MSc
Sylwia Panek, MSc

**Technician:**
Iwona Ptasiewicz (part-time)

**Laboratory-Administrative Partner (LAP):**
Agnieszka Faliszewska, MSc

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

**Volunteer:**
Gaja Dreszler, BSc



# 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 | Professor, Head of Laboratory of Bioinformatics and Protein Engineering, IIMCB, Warsaw, Poland (100% appointment) |
| 2006 | Associate Professor (extraordinarius) Bioinformatics Laboratory, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland (currently 25% appointment) |
| 2010-2011 | Deputy Director, IIMCB, Warsaw, Poland (1 year rolling position) |
| 2008 | Visiting Professor, University of Tokyo, Japan (sabbatical) |
| 2004-2006 | Assistant Professor, Bioinformatics Laboratory, Faculty of Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland |
| 2001-2002 | Group Leader, Laboratory of Bioinformatics2001 Visiting Scientist, Computational Biology Branch, National Center for Biotechnology Information, National Institutes of Health, Bethesda, Maryland, USA (with Dr. E.V. Koonin) |
| 1999-2001 | 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

- Scientific Policy Committee – advisory body of the Polish Minister of Science and Higher Education (selected in 2014)
- 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)
- Science Promotion Council, Polish Academy of Sciences, RUN-PAS (2013-2014)
- Academy of Young Scientists, Polish Academy of Sciences, AMU-PAS (elected in 2011)
- Committee for Evolutionary and Theoretical Biology, Polish Academy of Sciences (elected member, 2008-2011, 2011-2014)
- Committee for Biochemistry and Biophysics, Polish Academy of Sciences (ex officio AMU-PAS member, 2011-2014)
- 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)
- Editorial board member of *Nucleic Acids Research* (2005-2012), *Journal of Applied Genetics* (2004-Present), *Database Journal* (2008-Present), *Biology Direct* (2013-Present)

Cover photo: Comparison of published coordinates of the GCGA tetraloop from the group II intron IC subdomain (blue) and crystal structure model built using Brickworx (red). The model was fitted into the experimentally phased map (3.1 Å resolution) shown contoured at 3.0σ. (*Figure taken from Chojnowski et al., 2015*).

### Selected awards of the lab leader

| | |
|---|---|
| 2014 | Award of the Polish National Research Center (NCN) |
| 2014 | MISTRZ Award from the Foundation for Polish Science |
| 2014 | Award of the Prime Minister for Outstanding Research Achievements |
| 2014 | Selected as one of "25 leaders for the next 25 years" by "Teraz Polska" magazine of the Polish Promotional Emblem Foundation |
| 2014 | Award of the Knight's Cross of the Order of Polonia Restituta |
| 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) |
| 2012 | Award for Outstanding Research Achievements, Ministry of Science and Higher Education |
| 2011, 2013-14 | Adam Mickiewicz University Rector Special Award for Top Performance in Publishing High Impact Research Articles |
| 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) |
| 2007 | Adam Mickiewicz University Rector Award for Research Achievements (Team 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 |
| 2002-2005 | 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 and Sigma-Aldrich |

### 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, Matelska D.

### 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 Ph.D. Students (Marshall of the Masovia Province): Machnicka M, Magnus M
- 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)

## Recent Publications since January 2014


- **Chojnowski G, Walen T, Piatkowski P, Potrzebowski W, Bujnicki JM**. Brickworx builds recurrent RNA and DNA structural motifs into medium and low-resolution electron density maps. ***Acta Crystallogr D Biol Crystallogr***. 2015 Mar 1;71(Pt 3):697-705
- Philips A, **Lach G, Bujnicki JM**. Computational methods for prediction of RNA interactions with metal ions and small organic ligands. ***Methods Enzymol***. 2015;553:261-85
- **Glow D, Pianka D, Sulej A, Kozlowski L, Czarnecka J, Chojnowski G, Skowronek KJ, Bujnicki JM.** Sequence-specific cleavage of dsRNA by Mini-III RNase ***Nucleic Acids Res***. 2015 Mar 11;43(5):2864-73
- **Byszewska M, Smietanski M, Purta E, Bujnicki JM**. RNA methyltranserases involved in 5′ cap biosynthesis. ***RNA Biol***. 2014;11(12):1597-607
- **Machnicka M, Olchowik A**, Grosjean H, **Bujnicki JM**. Distribution and frequencies of post-transcriptional modifications in transfer RNAs. ***RNA Biol***. 2014;11(12):1619-29
- Nowis D, Malenda A, Furs K, Oleszczak B, Sadowski R, Chlebowska J, Firczuk M, **Bujnicki JM**, Staruch AD, Zagozdzon R, Glodkowska-Mrowka E, Szablewski L, Golab J. Statins impair glucose uptake in tumor cells. ***BMJ Open Diabetes Res Care***. 2014 Apr 26;2(1):e000017.
- **Szczepaniak K, Lach G, Bujnicki JM, Dunin-Horkawicz S**. Designability landscape reveals sequence features that define axial helix rotation in four-helical homo-oligomeric antiparallel coiled-coil structures. ***J Struct Biol***, 2014; 188(2):123-133
- RNAcentral Consortium (Petrov AI, Kay SJE, Gibson R, Kulesha E, Staines D, Bruford EA, Wright MW, Burge S, Finn R, Kersey PJ, Cochrane G, Bateman A, Griffiths-Jones S, Harrow J, Chan PP, Lowe TM, Zwieb CW, Wower J, Williams KP, Hudson CM, Gutell R, Clark MB, Dinger M, Cheng X, **Bujnicki JM**, Chua N, Liu J, Wang H, Skogerbø G, Zhao Y, Chen R, Zhu W, Cole JR, Chai B, Huang H, Huang H, Cherry JM, Pruitt KD). RNAcentral: an international database of ncRNA sequences. ***Nucleic Acids Res***. 2015 Jan;43(Database issue):D123-9
- Grabowska AD, **Wywiał E**, **Dunin-Horkawicz S**, Lasica AM, Wösten MM, Nagy-Staroń A, Godlewska R, Bocian-Ostrzycka K, Pieńkowska K, Laniewski P, **Bujnicki JM**, van Putten JP, Jagusztyn-Krynicka EK. Functional and bioinformatics analysis of two Campylobacter jejuni homologs of the thiol-disulfide oxidoreductase, DsbA. ***PLoS One***, 2014; 9(9):e106247
- **Walen T**, **Chojnowski G, Gierski P**, **Bujnicki JM**. ClaRNA: a classifier of contacts in RNA 3D structures based on a comparative analysis of various classification schemes. ***Nucleic Acids Res***, 2014; 42(19):e151
- Ramos-Molina B, Lambertos A, Lopez-Contreras AJ, **Kasprzak JM**, Czerwoniec A, **Bujnicki JM**, Cremades A, Penafiel R. Structural and degradative aspects of ornithine decarboxylase antizyme inhibitor 2. ***FEBS Open Bio***, 2014; 4:510-521
- Sierocka I, **Kozlowski LP**, **Bujnicki JM**, Jarmolowski A, Szweykowska-Kulinska Z. Female-specific gene expression in dioecious liverwort Pellia endiviifolia is developmentally regulated and connected to archegonia production. ***BMC Plant Biol***, 2014; 14(1):168
- **Magnus M**, **Matelska D**, **Lach G, Chojnowski G**, **Boniecki MJ**, **Purta E**, **Dawson W**, **Dunin-Horkawicz S**, **Bujnicki JM**. Computational modeling of RNA 3D structures, with the aid of experimental restraints. ***RNA Biol***, 2014; 11(5):522-536

- **Rother K**, Rother M, Skiba P, **Bujnicki JM**. Automated modeling of RNA 3D structure. ***Methods Mol Biol***, 2014; 1097:395-415
- **Majorek KA**, **Dunin-Horkawicz S**, Steczkiewicz K, Muszewska A, Nowotny M, Ginalski K, **Bujnicki JM**. The RNase H-like superfamily: new members, comparative structural analysis and evolutionary classification. ***Nucleic Acids Res***, 2014; 42(7):4160-79
- Toczyłowska-Mamińska R, Olszewska A, Laskowski M, Bednarczyk P, **Skowronek K**, Szewczyk A. Potassium channel in the mitochondria of human keratinocytes. ***J Invest Dermatol***, 2014; 134(3):764-72
- Crochemore M, Iliopoulos CS, Kubica M, Radoszewski J, Rytter W, Stencel K, **Walen T**. New simple efficient algorithms computing powers and runs in strings. ***Discrete Applied Mathematics***, 2014; 163(3):258–267
- **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
- 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
- Plotka M, Kaczorowska AK, Stefanska A, Morzywolek A, Fridjonsson OH, **Dunin-Horkawicz S, Kozlowski L**, Hreggvidsson GO, Kristjansson JK, Dabrowski S, **Bujnicki JM**, Kaczorowski T. Novel Highly Thermostable Endolysin from Thermus scotoductus MAT2119 Bacteriophage Ph2119 with Amino Acid Sequence Similarity to Eukaryotic Peptidoglycan Recognition Proteins. ***Appl Environ Microbiol***, 2014; 80(3):886-895
- **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***, 2014; 65(3):310-319
- **Mierzejewska K, Siwek W**, Czapinska H, Kaus-Drobek M, Radlinska M, **Skowronek K**, **Bujnicki JM**, Dadlez M, Bochtler M. Structural basis of the methylation specificity of R.DpnI**.** 2014. ***Nucleic Acids Res.***, 42(13), 8745-54
- **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, doi:10.1038/ncomms4004


# 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/), statistical potentials for predicting the structure of RNAprotein 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 the 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 the 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 elucidating 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, the 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 that are used for model building.

# Recent highlights

## Identification and characterization of a nuclease that sequence-specifically cleaves dsRNA

Molecular biology has made tremendous progress in the 20th century, owing to the discovery of restriction endonucleases that cleave double-stranded DNA molecules in specific sequences. However, no "restriction enzymes for double-stranded RNA" have yet been found in nature or engineered in the laboratory, thus limiting the progress of RNA research. Numerous ribonucleases (RNases) exist that cut RNA internally and exhibit substrate specificity, but their target sites are usually limited to one or a few specific nucleotides in single-stranded RNA and often within a context of a particular three-dimensional structure of the substrate. Members of the RNase III superfamily that have been characterized to date cleave double-stranded RNA but mainly recognize the structure of their substrates, whereas the substrate sequence plays a minor role. Until now, there are no reports of any member of the RNase III superfamily that exhibits substantial sequence preference that would make it suitable for the precise fragmentation of long regular dsRNA molecules. The availability of enzymes that are able to cleave double-stranded RNA in a sequence-dependent manner could potentially facilitate the development of new nucleic acid manipulation techniques.

In a recent study, we discovered and characterized the ability of a member of the RNase III superfamily from *Bacillus subtilis*, called BsMinIII, to sequence-specifically cleave double-stranded RNA. The experimental analysis was prompted by a bioinformatics analysis that identified a certain position in the structure of RNase III as a potential site for insertions that could cause the enzyme to make sequence-specific contacts with the RNA. Although our initial intention was to create such an enzyme by protein engineering, BsMinIII was found to possess a natural variant of such an insertion compared with sequence-nonspecific RNase III. Our group analyzed the sites that were cleaved by the BsMinIII enzyme in a limited digest of bacteriophage Φ6 dsRNA and identified a nucleotide sequence that was cleaved with high preference. We defined nucleotide residues within that sequence that affected cleavage efficiency. We also determined a crystal structure of the BsMinIII enzyme and constructed a model of the protein-RNA complex. We studied the insertion that distinguishes MinIII enzymes from other members of the RNase III family. It is structurally disordered in the absence of RNA and essential for specific cleavage but not dsRNA binding.

Our results suggest that BsMinIII may serve as a prototype of sequence-specific dsRNase that could possibly be developed toward a "restriction enzyme for RNA."

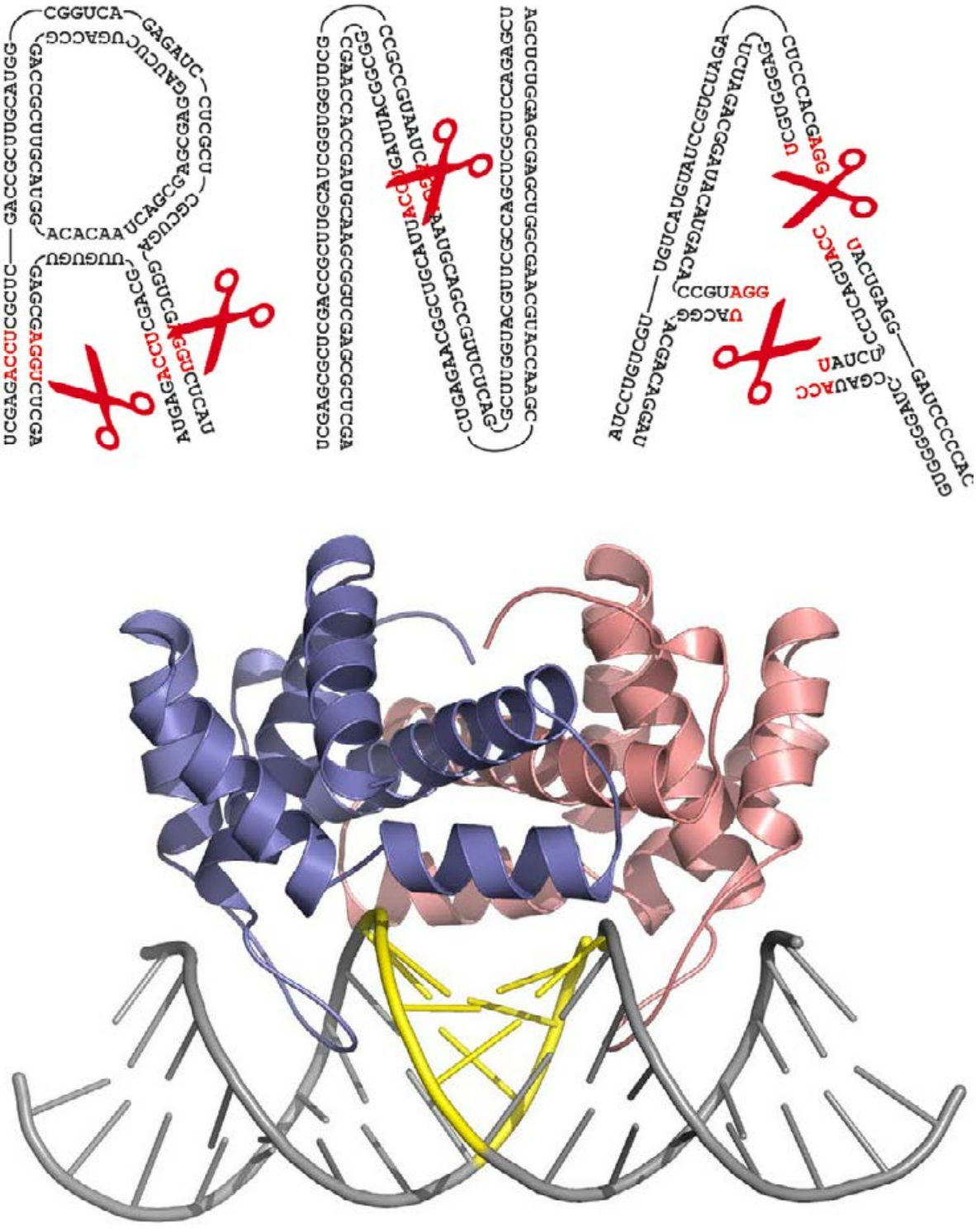
## Development of computational methods for structural analyses of RNA molecules and RNA-protein complexes

The number of experimentally determined structures of nucleic acid molecules, including nucleic acid-protein complexes, is increasing rapidly, in line with recent discoveries and growing interest in biological functions that are exerted by nucleic acids, beyond their protein-coding capacity. In general, the method-of-choice for studies of macromolecular structures is X-ray crystallography. However, nucleic acid crystallography, unlike protein crystallography, still lacks sufficient methodology to facilitate straightforward crystal structure determination. In particular, computational tools that automatically build a crystal structure model into an experimental electron-density map are markedly less developed for nucleic acids than for proteins.

We developed BrickworX, a computer program that builds crystal structure models of nucleic acid molecules using recurrent motifs, including double-stranded helices. It builds on the RNA Bricks database (http://iimcb.genesilico.pl/rnabricks) that was previously developed by our group, which stores information about recurrent RNA 3D motifs and their interactions that are found in experimentally determined RNA structures and RNA-protein complexes. The advantage of Brickworx compared with other available methods is that it can process poor-quality electron density maps, in which only a fraction of ribonucleotide residues can be modeled with confidence. For instance, if only a fraction of the phosphate group positions can be detected, then a correctly placed complete motif that matches these phosphates can be built into the electron-density map. In the first step, Brickworx searches for electron-density peaks that may correspond to the phosphate groups. It can also take into account phosphate group positions that are provided by the user. Subsequently, based on comparisons of the 3D patterns of the phosphorus atom with the database of nucleic acid fragments, it finds matching positions of double-stranded helical motifs (A-RNA or B-DNA) in the unit cell. If the target structure is RNA, then the helical fragments are further extended with recurrent RNA motifs from a fragment library that contain single-stranded segments. Finally, the matched motifs are merged and refined in real-space to find the most likely conformations, including the fit of sequence to the electron density map.

The Brickworx program is available for download and as a web server at http://iimcb.genesilico.pl/brickworx

## Molecular scissors for dsRNA



**The model of the BsMiniIII-dsRNA complex structure.** The dimer of the experimentally determined BsMiniIII structure is shown in cartoon representation. The pale pink and slate blue colors indicate the monomers. The homology-modeled dsRNA 20-mer is shown as grey sticks, with the BsMiniIII target site ACCU/AGGU colored in yellow. The coordinates of the model are available from ftp://genesilico.pl/iamb/models/RNases/eRNase_BsMiniIII/.


# Laboratory of Mitochondrial Biogenesis

**Postdoctoral Fellows:**
Piotr Brągoszewski, PhD
Beata Drabarek, PhD (since February 2015)
Elżbieta Januszewicz, PhD (since September 2014)
Łukasz Samluk, PhD (since November 2014)
Anna Sokół, PhD, (FishMed)
Małgorzata Sztolsztener, PhD (until December 2014)
Ulrike Topf, PhD
Michał Wasilewski, PhD

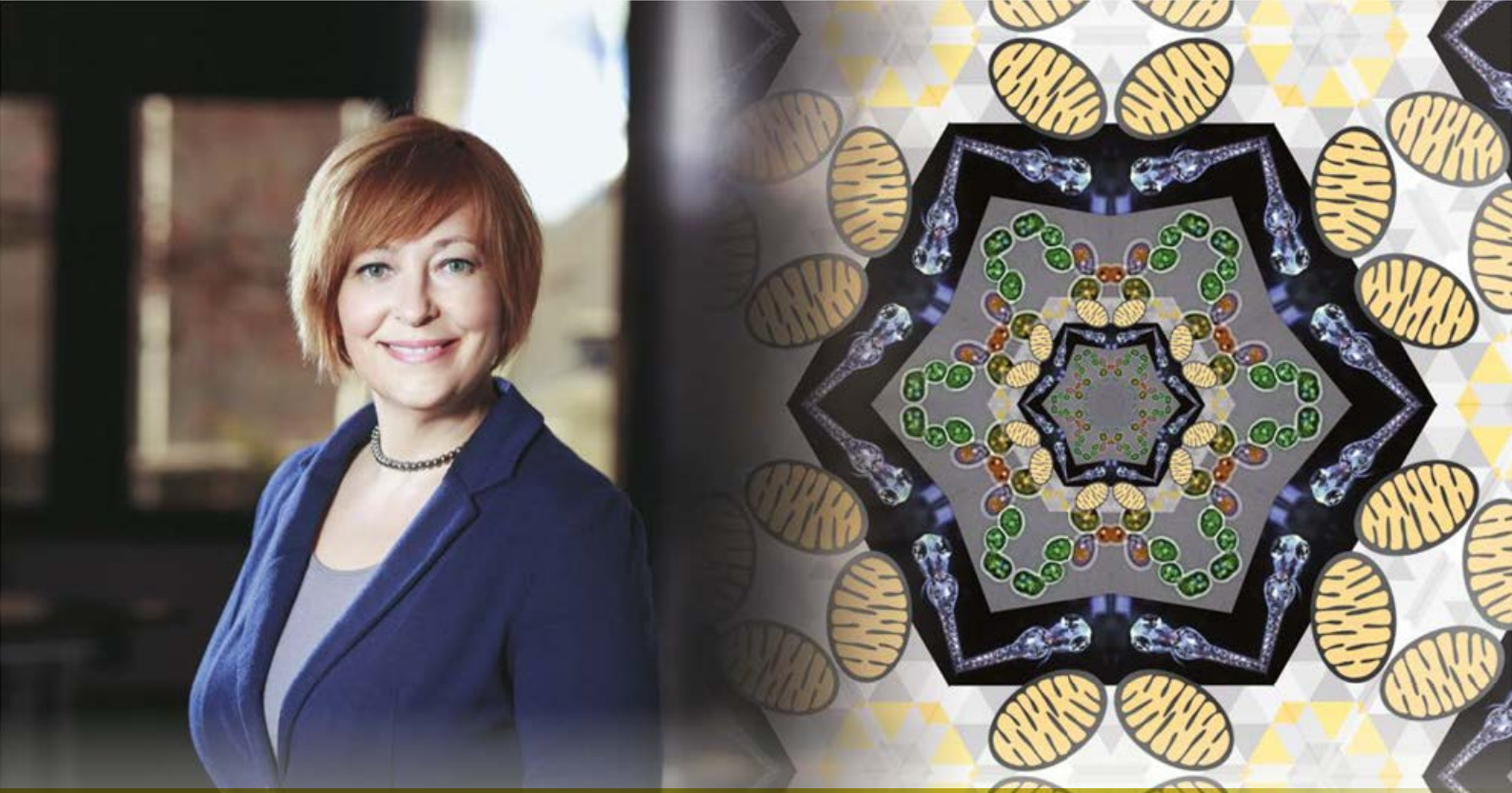
**PhD Students:**
Magdalena Chojnacka, MSc Eng
Piotr Chrościcki, MSc
Agnieszka Górnicka, MSc (until September 2014)
Karthik Mohanraj, MSc
Paulina Sakowska, MSc Eng
Krzysztof Tarasiuk, MSc Eng (since October 2014)
Aksana Varabyova, MSc (until October 2014)
Lidia Wróbel, MSc Eng

**Laboratory-Administrative Partner (LAP):**
Aleksandra Matusiak, MSc Eng

**FishMed Research Assistant:**
Michał Bazała, MSc (joint with Neurodegeneration Laboratory)

**Technician:**
Elżbieta Grzelak

**Undergraduate Students:**
Jakub Dominowski
Aleksandra Fergin



# Lab Leader: **Agnieszka Chacińska**, PhD, Professor

## Education and Degrees

| | |
|---|---|
| 2014 | Professor of Biological Sciences, nomination by the President of the Republic of Poland |
| 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 |
| 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 Laboratory of Mitochondrial Biogenesis, International Institute of Molecular and Cell Biology, Warsaw, Poland |
| 2008-2009 | Associate Member of Excellence Cluster BIOSS, Centre for Biological Signalling Studies, University of Freiburg, Germany |
| 2007-2009 | Member of the Board, Collaborative Research Centre (SFB 746) |
| 2007-2010 | Project Leader in Collaborative Research Centre (SFB 746) |
| 2004-2009 | Group Leader (German equivalent of Assistant Professor), Institute for Biochemistry and Molecular Biology, University of Freiburg, Germany |
| 2001-2004 | Postdoctoral Fellow, Laboratory of Prof. Nikolaus Pfanner, University of Freiburg, Germany |
| 1999 | Visiting Scientist, Laboratory of Prof. Sabine Rospert, Max Planck Research Unit, Halle, Germany |
| 1997 | Visiting Scientist, Laboratory of Prof. Gottfried Schatz, Biozentrum, University of Basel, Switzerland |
| 1994-2000 | Doctoral research with Prof. Magdalena Boguta, Institute of Biochemistry and Biophysics, Warsaw, Poland |

Cover photo: Kaleidoscope presenting objects of our interest: mitochondria, yeast and Danio rerio fish. Author: Agata Trojanowska

# Publications 2011-2014

- Ieva R, Schrempp SG, Opaliński L, Wollweber F, Höß P, Heißwolf AK, Gebert M, Zhang Y, Guiard B, Rospert S, Becker T, **Chacinska A**, Pfanner N, van der Laan M. Mgr2 functions as lateral gatekeeper for preprotein sorting in the mitochondrial inner membrane. ***Mol Cell***, 2014; 56:641-652
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Co-workers affiliated with IIMCB are given in bold

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- 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
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- 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

# 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.

Our long-standing interests concern (but are not limited to) the mitochondrial intermembrane space assembly (MIA) pathway that utilizes the transfer of disulfide bonds and is dedicated to the import and biogenesis of proteins, residents of the intermembrane space of mitochondria. We are interested in the following aspects of mitochondrial biology:

- Redox-related protein biogenesis events driven by MIA in yeast and higher eukaryotes.
- Cross-talk between mitochondrial architecture and dynamic events that are involved in mitochondrial protein biogenesis.
- Impact of protein transport pathways on mitochondrial and cellular protein homeostasis.

Our research seeks to understand the biogenesis of proteins localized in the intermembrane space of mitochondria (Fig. 1). To be entrapped in the intermembrane space of mitochondria, proteins utilize catalyzed thiol-disulfide exchange that is driven by mitochondrial MIA machinery. Our research aims to understand the biogenesis of proteins that are localized in the intermembrane space of mitochondria. We completed our investigation of the early stages of mitochondrial intermembrane space biogenesis. We identified an alternative version of the TOM complex that consists of Tom40 but not other core subunits of the classic TOM complex, Tom22 and Tom5. Consistent with this finding, the import of proteins to the intermembrane space of mitochondria can be inhibited by blocking a lumen of the putative protein channel within Tom40 (Gornicka et al., *Mol Biol Cell*, 2014).

One interesting mechanistic aspect under debate is the mode of cooperation between Mia40 and Erv1, two major components of the MIA pathway (Fig. 1). 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 that is governed by MIA involves a spatially and temporally coordinated chain of events (for review, see Stojanovski et al., *Biochim Biophys Acta*, 2012). Interestingly, our preliminary data from human cells show that the interaction between Mia40 and Erv1 is less transient and more stable, suggesting that the ternary complex mode is more efficiently used in the mitochondria of higher eukaryotes.

In the search for the non-canonical functions of MIA, we investigated inner mitochondrial membrane proteins. Surprisingly, a multispanning membrane protein that is 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 not only serves as an oxidoreductase but also 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 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.

Within the mitochondrial inner membrane, the inner boundary membrane and cristae membranes that are separated by the crista junctions can be distinguished. MICOS, a recently discovered large protein complex (von der Malsburg et al., *Dev Cell*, 2011), is crucial for establishing and maintaining the proper inner membrane architecture. Furthermore, MICOS components were reported to interact with the TOM, SAM, and Mia40 translocases to facilitate the biogenesis of mitochondrial proteins. The mitochondrial protein and copper chaperone Cox17 has been known to be involved in the assembly of cytochrome *c* oxidase. We found an unexpected link between Cox17 and the MICOS complex. Cox17 interacts with Mic60 and modulates the integrity of the MICOS complex. Moreover, the cooperation of Cox17 with Mic60 is supported by copper ions. We propose that Cox17 is a newly identified player in maintaining the architecture and function of the MICOS complex (Chojnacka et al., submitted).

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 degrading the proteins that are 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 (the proteasome) in the cytosol (Fig. 1). Interestingly, the proteasome competes with mitochondrial protein import machinery. Our study 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).

Intermembrane space proteins utilize thiol-disulfide exchange that is driven by the MIA pathway as a mechanism for trapping proteins in mitochondria. This implies that unfolded proteins that are no longer oxidized can leak out from mitochondria. Our results demonstrate the existence of retro-translocation. This mode serves as an important surveillance mechanism that involves the proteasome and regulates the abundance of intermembrane space proteins in response to changes in cellular physiology (Bragoszewski et al., submitted).

We also performed a global proteome analysis to identify changes that are caused by the defective import of proteins into mitochondria caused by MIA dysfunction (in collaboration with Dr. Bettina Warscheid, 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.

We further exploited the advantages of yeast cells, including the ease of genetic manipulation, advanced biochemistry, and suitability for genetics screens and other high throughput non-biased approaches. However, we are extremely interested and active in expanding our studies to higher eukaryotes. The components of the MIA pathway, similar to other mitochondrial protein translocases, are conserved, but they have remained only poorly understood (for review, see Sokol et al., *FEBS Lett*, 2014). We reconstituted the human MIA pathway in yeast. We exchanged the essential yeast counterparts for the human proteins MIA40 and ALR (the sulfhydryloxidase, yeast Erv1 homolog) and established the mechanistic principles of the human MIA pathway. Importantly, using our "humanized" yeast, we addressed the exact molecular defect that is caused by the pathogenic mutant variant of ALR (Sztolsztener et al., *Traffic*, 2013). Currently in our lab, cultured human cells and the zebrafish *Danio rerio* have been established. The studies, based on cultured mammalian cells, broaden our perspective because we are learning about specific and universal aspects of mechanisms that safeguard the passage of mitochondrial precursor proteins and the molecular basis of pathology. We are also actively pursuing research in the zebrafish *Danio rerio* within the FishMed project to uncover the deleterious consequences of disrupting mitochondrial protein biogenesis at the level of the vertebrate organism.

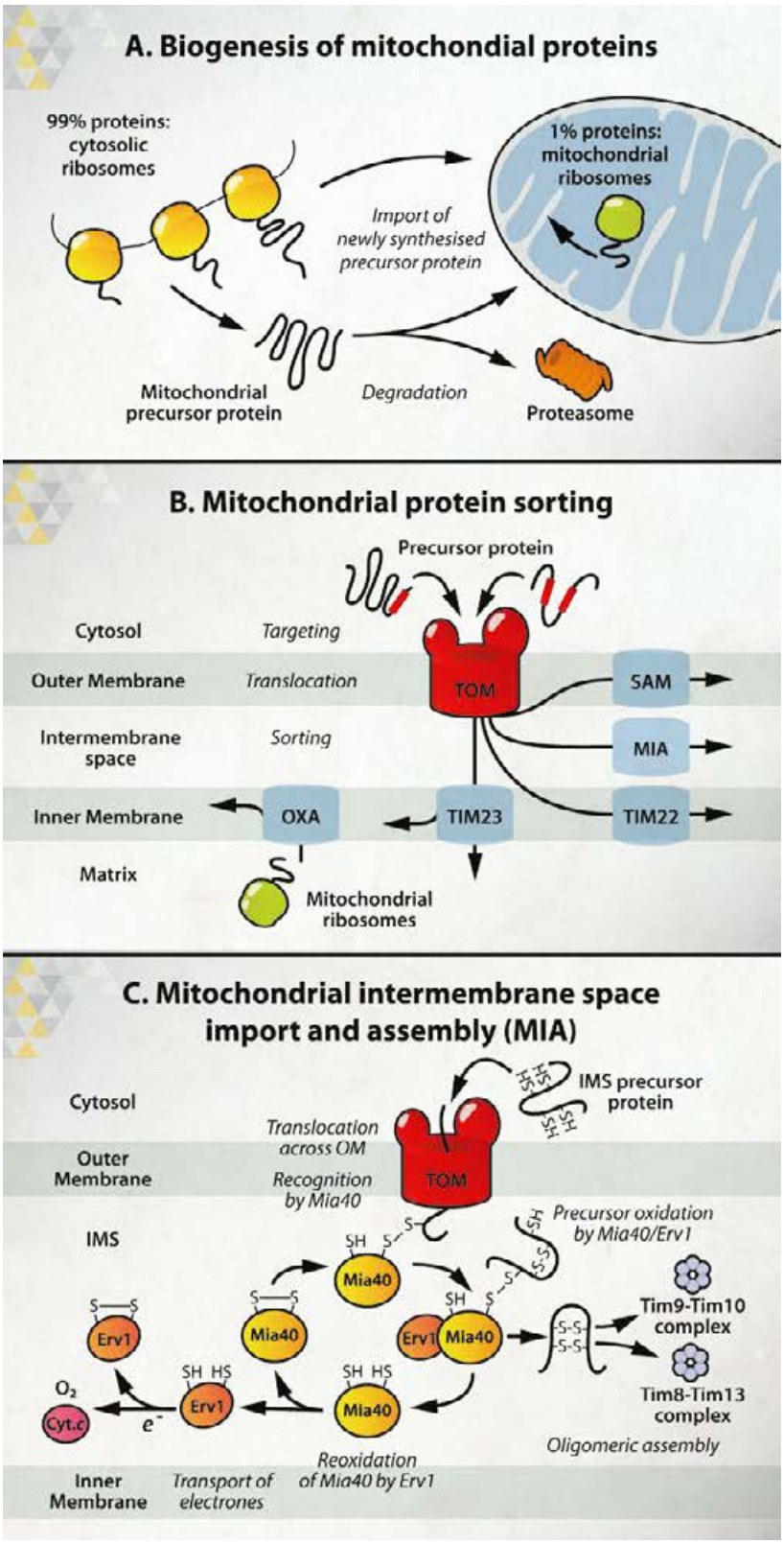



**Fig. 1.** (A) **A large majority of mitochondrial proteins are synthesized on cytosolic ribosomes and enter mitochondria via a main gate that is formed by the TOM complex.** The possibility exists that protein synthesis is coupled to protein transport. Non-imported mitochondrial proteins are cleared 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, 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).

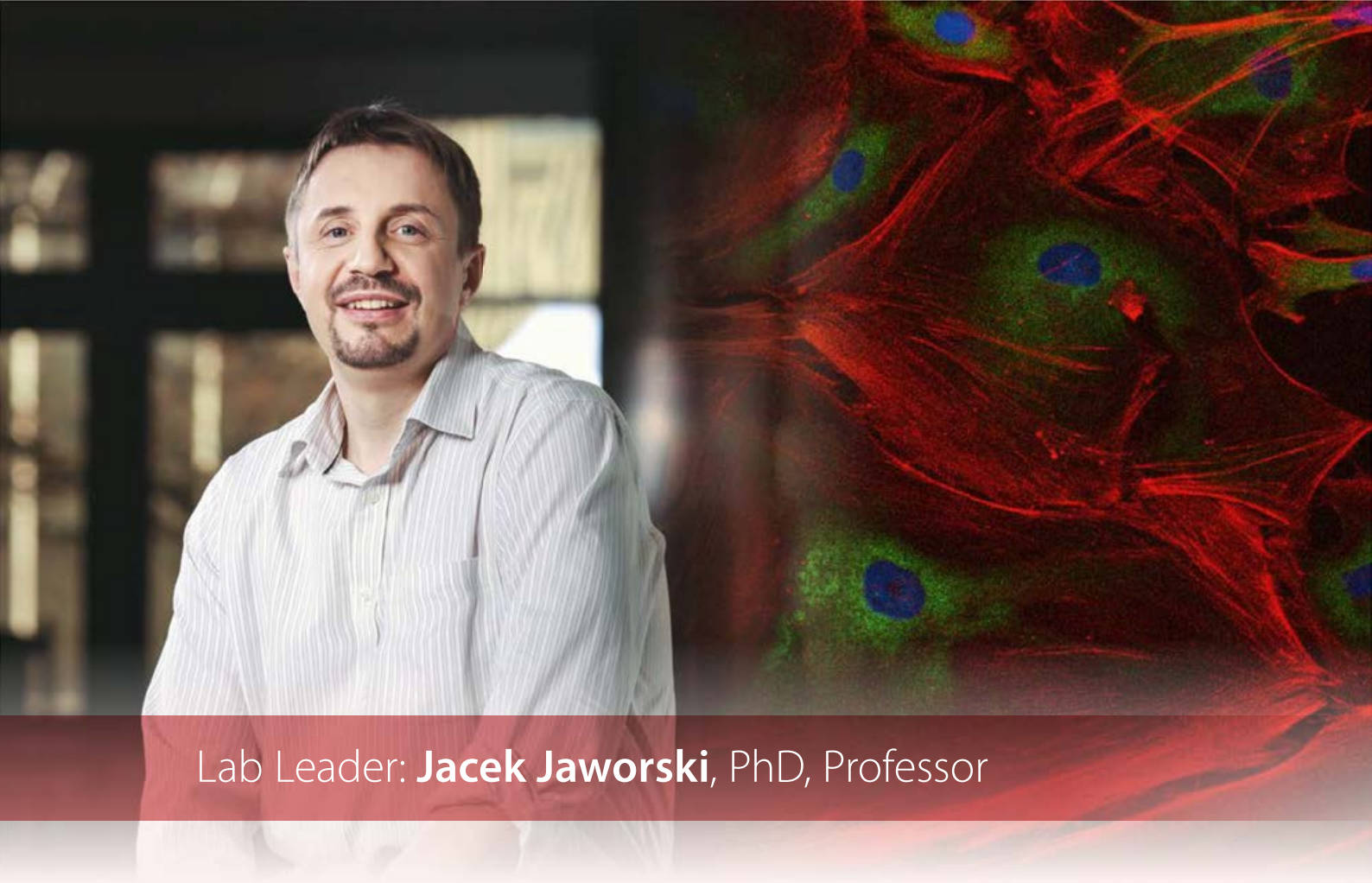
# Laboratory of Molecular and Cellular Neurobiology

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

**Junior Researchers:**
Joanna Lipka, MSc (MPD student)
Agnieszka Kolka, MSc
Alicja Kościelny, MSc (since Oct. 2014)
Marcelina Pieprzyk, MSc
Katarzyna Rydz, (until June, 2014)
Aleksandra Tempes, MSc
Agnieszka Skałecka, MSc
Katarzyna Świtoń, MSc
Anna Urbańska, MSc
Małgorzata Urbańska, MSc
Kamila Jączyńska, MSc

**FishMed Research Assistant:**
Lidia Wolińska-Nizioł, PhD 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. C.C. Hoogenraad, Erasmus Medical Center, Rotterdam, Holland |
| 2002-2005 | Postdoctoral Associate with Prof. Morgan Sheng, Picower Center for Learning and Memory, Massachusetts Institute of Technology and Howard Hughes Medical Institute, Cambridge, MA, USA |
| 2000 | Research training (1 month) with Dr. J. Guzowski, ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, USA |
| 1997-2001 | Research training (7 months) with Prof. J. Mallet, Laboratoire de Genetique Moleculaire de la Neurotransmission et des Processus Neurodegeneratifs (LGN), UMR 9923, Centre National de la Recherche Scientifi que, 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

| | |
|---|---|
| 2014 | Foundation for Polish Science Professorial Subsidy "Mistrz" |
| 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 for Young Investigators (1 year scholarship) |
| 2000 | EMBO Short-Term Fellowship |
| 1999 | Polish Network for Cell and Molecular Biology UNESCO/ PAS Scholarship |
| 1997 | French Government Scholarship |

## Membership in Scientific Societies, Organizations, and Panels

| | |
|---|---|
| 2015 | Scientific Advisory Board to the Nencki Institute of Experimental Biology, PAS, Member |
| 2011 | Neurobiology Committee of the Polish Academy of Sciences, Member |

## Awards, Honors and Titles (Lab members – 2013-2014)

| | |
|---|---|
| 2014 | M.Sc. degree to Katarzyna Rydz |
| 2013 | L'Oreal 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 |

Cover photo: SEGA-derived cells cultured *in vitro*. Nuclei are stained blue, phosphorylated S6 - green, F-actin - red. Author: Anna Malik

# Selected publications

## Publications in 2014

- Kulcenty K, Wróblewska J, Mazurek S, **Liszewska E, Jaworski J.** Molecular mechanisms of induced pluripotency. ***Contemp Oncol***, 2015; 19(1A):A22-9
- Skupien A, Konopka A, Trzaskoma P, Labus J, Gorlewicz A, **Swiech L**, Babraj M, Dolezyczek H, Figiel I, Ponimaskin E, Wlodarczyk J, **Jaworski J**, Wilczynski GM, Dzwonek J. CD44 regulates dendrite morphogenesis through Src tyrosine kinase-dependent positioning of the Golgi. ***J Cell Sci***, 2014; 127(23):5038-5051
- Geiger JC, **Lipka J**, Segura I, Hoyer S, Schlager MA, Wulf PS, Weinges S, Demmers J, Hoogenraad CC, Acker-Palmer A. The GRIP1/14-3-3 Pathway Coordinates Cargo Trafficking and Dendrite Development. ***Dev Cell***, 2014; 28(4):381-393

## Other selected publications

- **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
- 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 BR, **Malik AR**, Wulf PS, Hoogenraad CC, **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. ***BBA – Proteins & 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 fl uorescence 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

Mammalian/mechanistic target of rapamycin (mTOR) is a serine-threonine kinase that is 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 the regulation of mTOR-dependent translation 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 that are involved in the processes of dendritic branching and synapse formation and the 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 the 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 noncanonical 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., 2013a). Collectively, these findings

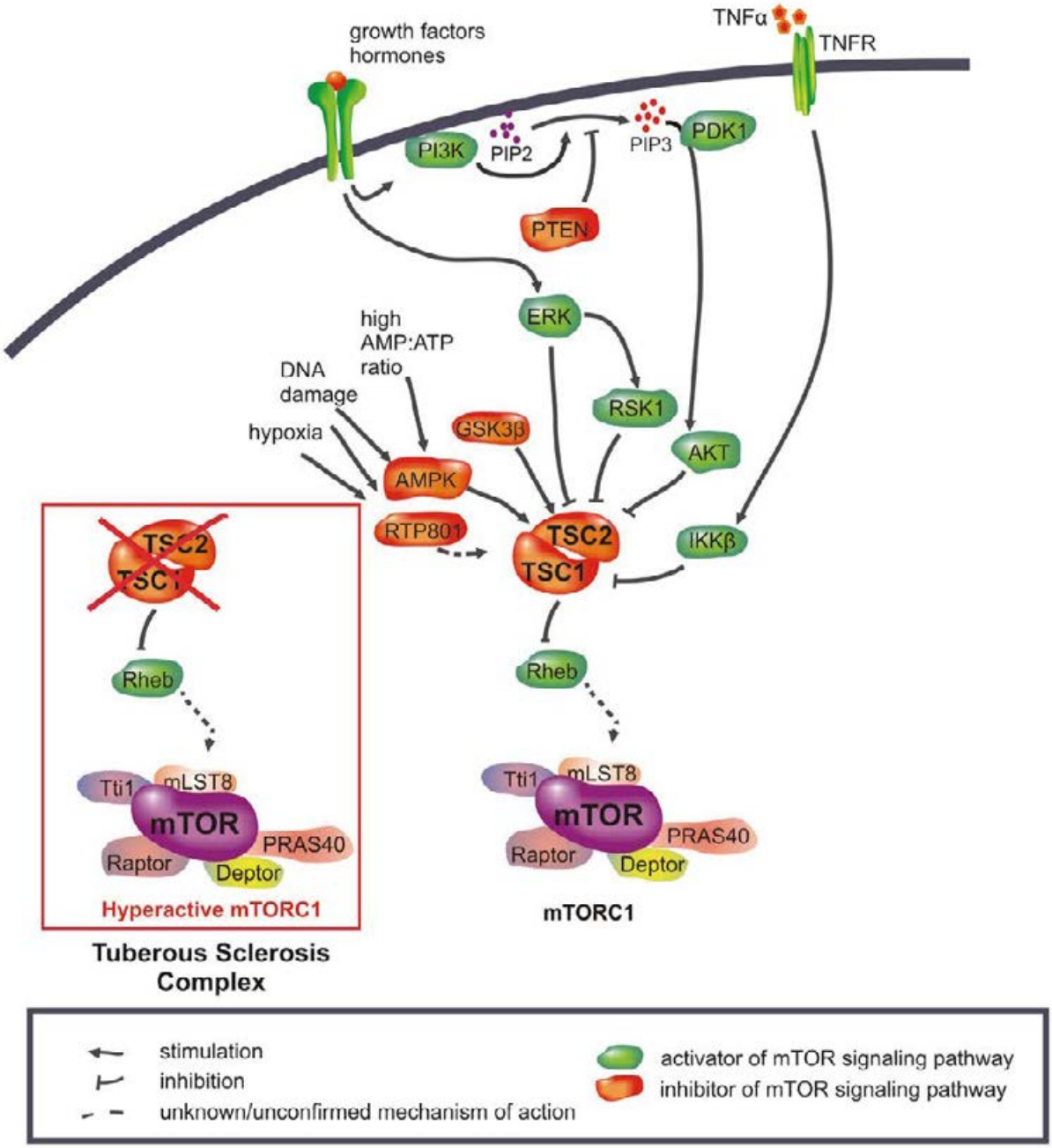



**Fig. 1. Schematic diagram of current understanding of mTOR complexes 1 (mTORC1) control.** Trophic factors, hormones and other molecules acting on cell surface receptors signal to mTORC1 via tuberous sclerosis complex (TSC) and Rheb. Inset: mechanism of mTORC1 hypeactivation in Tuberous Sclerosis Complex. For more details see Malik et al., 2013, BBA.

suggest that we are still far from revealing the full complexity of the mTOR signaling network, both in neurons and in nonneuronal cells. However, the results of our shRNA screens, combined with the results of proteomic analyses of mTOR interactions at the subcellular level, allowed us to narrow our research toward identifying the cellular compartment-specific regulation and functions of mTOR in neurons, with a special focus on membrane trafficking events. 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 (iPSCs) from patients with tuberous sclerosis (TSC), a disease that is most likely caused by mTOR hyperactivation. In 2014, significant efforts were made in investigating these clinically relevant topics.

Tuberous sclerosis is a multiorgan disease that is caused by mutations in the mTORC1 inhibitors hamartin and tuberin (TSC1 and TSC2, respectively) (Fig. 1) and characterized by epilepsy, autism, and the formation of benign tumors in the brain (e.g., cortical tubers, SEGA), 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. To discover such new mechanisms, Dr. Anna Malik performed shRNA screens in hypertrophic neuronal cells that were experimentally devoid of TSC2 expression. We identified several proteins that possibly contribute to TSC-related hypertrophy. Among them, we focused on the role of glutamate-cysteine ligase catalytic subunit (GCLC), a protein that is important for the control of oxidative stress levels (Fig. 2). We characterized this protein as a potential target in the pharmacotherapy of TSC. GCLC inhibition increased cellular stress in TSC2-depleted neurons and SEGA-derived cells. Moreover, cortical tubers and SEGAs that were resected from patients' brains showed elevated GCLC and stress marker expression. Finally, GCLC inhibition led to growth arrest and the loss of SEGA-derived cells

(Malik et al., submitted). Based on this evidence, we concluded that GCLC is a part of redox adaptation in TSC that is needed for the overgrowth and survival of mutant cells and thus could be a novel target for SEGA treatment (Fig. 2). Currently, our research focuses on additional cellular processes that are linked to positive hits of our screen. Toward this end, in addition to TSC2-lacking neurons and SEGA cells, we utilize TSC patients iPSC-derived neuroprecursors of TSC patients, developed in our laboratory by Dr. Ewa Liszewska.

Our another approach to investigate TSC is to study mutant zebrafish retina as an easily accessible *in vivo* model with regard to TSC pathology-related problems with neuroprecursor proliferation, migration, and differentiation. In 2014, during the course of the FishMed project, we began to characterize the TSC2vu242 zebrafish mutant line, which lacks functional TSC2. Thus far, we have introduced the line to our main system and confirmed hyperactivation of the mTORC1 signaling pathway in both the retina and brain of our mutants (Fig. 3).

animal heterogeneity-related variability. Our system recapitulates *in vivo* responses to KA, namely the induction of (*i*) c-Fos expression and mTOR, (*ii*) neuronal cell death and astrogliosis, and (*iii*) aberrant axon rearrangements. Using this system, we showed that rapamycin does not globally change the transcription profile induced by KA. Yet, a more detailed inspection of the microarray results revealed that levels of some genes (e.g., Elmo1, Abra, Gprc5a, Nr4a3, Npas4, Vgf, and Tubb6) differed between the KA and KA+rapamycin groups. Intriguingly, three of these gene are known to be involved in cytoskeleton regulation and could be involved in long-term morphological rearrangements of neurons. Indeed, our current results support the role of Elmo-1, a positive regulator of Rac1 and cdc42, in axonal growth.

Lastly, to better understand the link between TSC and epileptogenesis, our laboratory joined the EPISTOP project consortium in 2013 (http://www.epistop.eu). The main aim of EPISTOP is to identify the molecular predictors of the progression of epileptogenesis in TSC patients. Our team, together with others,

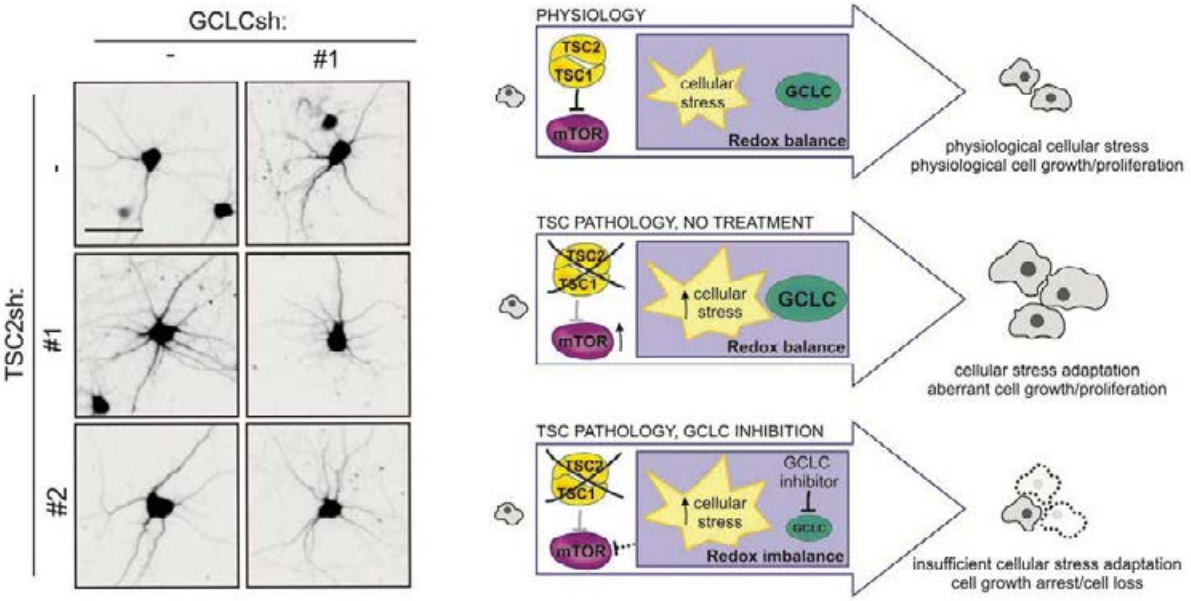



**Fig. 2. Tuberous sclerosis complex-deficient cells require glutamate-cysteine ligase for aberrant growth and survival.** Left panel: GCLC was found in our shRNA screen for molecules needed for aberrant growth of TSC-lacking cortical neurons. photo by Dr. Anna Malik. Right panel: Proposed model of GCLC contribution to TSC-related tumors development. Based on Malik et al., submitted.

One symptom of TSC is epilepsy. Several lines of evidence point to mTOR hyperactivity as a molecular trigger of epileptogenesis. However, mTOR effectors in this process are still undefined. Epileptogenesis is well known to require substantial transcriptome changes that further lead to gross rearrangements of neuronal circuits. mTOR is known to regulate transcription by all three RNA polymerases (Malik et al., 2013b). To date, however, no research has investigated whether its transcriptional activity contributes to epileptogenesis. Intriguingly, our previous research (Macias et al., 2013) revealed that kainic acid (KA)-induced status epilepticus leads to an increase in the presence of phosphorylated, presumably active, mTOR in the nucleus. Thus, we decided to search for a link between mTOR transcription and the actions of KA. We utilized microarray technology to investigate how rapamycin, an inhibitor of mTOR, affects the KA-induced transcriptome. We developed a simplified system for this analysis, namely an organotypic hippocampal slice culture, to reduce

is searching for potential markers of epileptogenesis that were 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 2014, to support progress within the EPISTOP project, we introduced to our lab novel technology of gel-free, blot-free, medium-scale Western blot analysis (Sally Sue from ProteinSimple). This capillary-based system is fully automated and allows the reliable detection and comparison of protein expression levels from small amounts of material (e.g., unique patients' tissue). In 2015, in collaboration with EPISTOP clinical centers, we began an analysis of blood and cortical tubers of TSC patients to find molecular signatures of TSC pathology.

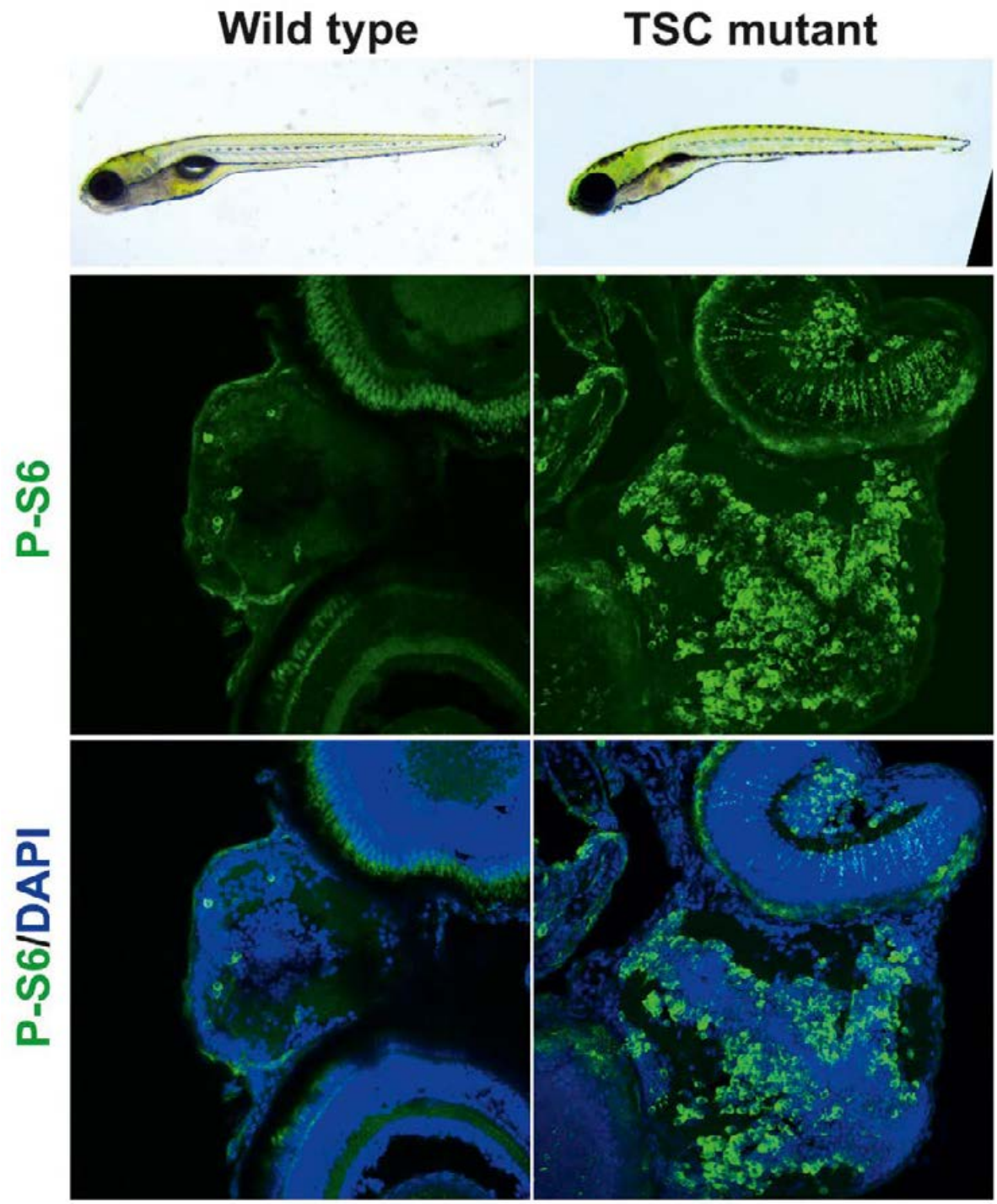



**Fig. 3. Increased mTORC1 activity is characteristic for TSC2 mutant fish.** Retinas and brains of wild type and TSC-model fish were immunofluorescently stained for phosphorylated form of ribosomal protein S6 (green), an indirect indicator of mTORC1 activity. DAPI staining highlights nuclei of imaged cells. Photo by: Dr. Justyna Jezierska and Dr. Lidia Wolińska-Nizioł.

# Laboratory of Neurodegeneration

**Vice Head:**
Łukasz Majewski, PhD

**Senior Scientist:**
Tomasz Węgierski, PhD

**Senior Postdoctoral Fellow:**
Joanna Gruszczyńska-Biegała, PhD

**Postdoctoral Fellows:**
Magdalena Czeredys, PhD
Smijin Karthully Soman, PhD (FishMed)

**PhD Students:**
Kinga Gazda, MSc in Engineering
Anna Jaworska, MSc (continuing the international PhD studies program in Munich)
Dagmara Kaczyńska, MSc (July 2014 – March 2015)
Łukasz Szewczyk,MSc (until February 2014)
Aleksandra Szybińska, MSc (until December 2014)

**FishMed Research Assistant:**
Michał Bazała, MSc (joint with Laboratory of Mitochondrial Biogenesis)

**MSc Students:**
Aleksandra Kurek (not in the picture)
Filip Maciąg (not in the picture)
Maria Śladowska

**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, NanoVelos sp. z o.o.
- Adam Sobczak, postdoctoral research fellow, Institute of Biochemistry and Biophysics PAS, and Bio&Technology Innovations Platform (BioTech-IP) of Ochota Biocentre Consortium
- Łukasz Szewczyk, PhD Student at the Laboratory of Molecular Neurobiology headed by Dr. Marta B. Wiśniewska, CeNT, University of Warsaw
- Marta B. Wiśniewska, PhD, DSc Habil, Professor at the University of Warsaw, research group leader, Laboratory of Molecular Neurobiology, CeNT, University of Warsaw
- Urszula Wojda, PhD, Professor, research group leader, Laboratory of Advanced Preclinical Studies, Neurobiology Centre at the Nencki Institute of Experimental Biology PAS

# 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 (PAS), Poland |
| 1980 | PhD in Biochemistry, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland |
| 1976 | MSc in Biochemistry, Warsaw University, Poland |

## Postdoctoral Training

| | |
|---|---|
| July 2014 | Visiting Professor, partnership Laboratory (Prof. B. E. Snaar-Jagalska) within the *FishMed* project, Leiden University, Leiden, The Netherlands |
| 1992-1995 | Visiting Professor, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland, USA |
| 1981-1984 | Visiting Fellow (postdoc), Laboratory of Cell Biology (Head: E.D. Korn), National Institutes Health, Bethesda, Maryland, USA |

## Professional Employment

| | |
|---|---|
| 2002-Present | Director of the Institute and Head of the Laboratory of Neurodegeneration, IIMCB |
| 2000-2001 | Director, Centre of Excellence for Studies on Mechanisms of Neurodegeneration Phare Sci-Tech II, Nencki Institute of Experimental Biology PAS, Warsaw, Poland |
| 1999-2001 | Acting Director, IIMCB; Organizer and Director, Centenarian Program |
| 1996-2002 | Head, Laboratory of Calcium Binding Proteins, professor 2002-2014, Nencki Institute of Experimental Biology PAS, Warsaw, Poland |
| 1991-1992 | Deputy Scientific Director, Nencki Institute of Experimental Biology PAS, Warsaw, Poland |
| 1986-1992 | Associate Professor and Head, Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology PAS, Warsaw, Poland |
| 1984-1985 | Research Associate, Nencki Institute of Experimental Biology PAS, Warsaw, Poland |
| 1980-1981 | Postdoctoral Fellow, Nencki Institute of Experimental Biology PAS, Warsaw, Poland |
| 1976-1980 | PhD Student, Nencki Institute of Experimental Biology PAS, Warsaw, Poland |

## Membership in Scientific Societies, Organizations, and Panels

| | |
|---|---|
| 2014-Present | Member of Working Group for National Smart Specializations, Ministry of Economy in Poland |
| 2014-Present | Member of Management Committee in Action BM1406, acronym: IONCHAN-IMMUNRESPON, European Cooperation in Science and Technology (COST) |
| Jul 1, 2013 - | Dec. 31, 2013 President, Ochota Biocentre Consortium (rotating presidency) |
| 2012-2015 | Expert, National Science Centre |
| Jul 1, 2012 - | Dec. 31, 2012 President of the Science Policy Committee, Ministry of Science and Higher Education (rotating presidency); member 2011-2014 |
| 2012-Present | Board Member of Marceli Nencki's Foundation for Support of Biological Sciences |
| 2011-2014 | Member, Scientific Council of the Nencki Institute of Experimental Biology PAS and the Mossakowski Medical Research Centre PAS |
| 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 PAS, Scientific Units (units operating under the name of |

Cover photo: Neurons in zebrafish head tagged with GCaMP3 calcium probe. This picture, taken in vivo in Zeiss Z.1 lightsheet microscope is a maximum intensity projection of couple of hundreds of stacks, showing the most active areas like optic tectum, telencephalon and olfactory regions. Signal comes from GFP in neurons. Image taken by Michał Bazała.

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|---|---|
| | the Department of Antarctic Biology PAS and Institute of Biochemistry and Biophysics PAS) |
| 2011-2014 | Member, BIO-IMAGINE Steering Committee, 7th Framework Program at the Nencki Institute of Experimental Biology PAS |
| Jul 1, 2010- -Dec. 31, 2010 | President, Ochota Biocentre Consortium (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 |
| 2006-2011 | Member, Advisory Group of the 7th Framework Program for Health, European Commission |
| 2004-Present | Member, Polish Academy of Sciences |
| 2004-Present | Honorary chairman, one of the founders, BioEducation Foundation |
| 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 |
| 1996-1998, 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 PAS) |
| 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 biochemist) |
| 1976 | MSc, Magna cum laude, University of Warsaw, Poland |

# Selected publications


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- **Majewski L**, **Kuznicki J**. SOCE in neurons: signaling or just refilling? ***BBA Mol Cell Res***, 2015. Jan 31. pii: S0167-4889(15)00034-8
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- **Gruszczynska-Biegala J**, **Kuznicki J**. Native STIM2 and ORAI1 proteins form a calcium-sensitive and thapsigargin-insensitive complex in cortical neurons. ***J Neurochem***, 2013 Sep;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 Jul;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
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***** no IIMCB affiliation


# Current Projects

We are interested in the molecular mechanisms that are involved in neurodegeneration and psychiatric diseases, with a special emphasis on the role of calcium homeostasis and signaling, and β-catenin pathways. These processes are being studied at the genomic, proteomic, and cellular levels using zebrafish and mice as model organisms. Our major present 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. Regulation of β-catenin in mature neurons.

## 1. Dysregulation of calcium homeostasis in neurodegenerative diseases

Calcium dyshomeostasis is an early event in the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). In AD, calcium dyshomeostasis is an early event that precedes other disease symptoms and can affect many cellular processes. Drugs with the ability to restore calcium homeostasis to values that are observed in healthy control cells could be applied as therapeutics in AD. In collaboration with Prof. Jochen Herms and Dr. Kamran Honernajad from 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 investigate their putative mechanism of action, almost 160 of the best compounds were chosen for an enzyme-linked immunosorbent assay (ELISA) to determine γ-secretase activity, whose gain of function is believed to be a major factor in the pathology of familial AD. 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; *J Biomol Screen*, 2013). We also identified tetrahydrocarbazoles as novel multifactorial drug candidates for the treatment of AD (*Transl Psychiatry,* 2014).

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. Disturbances in calcium handling were found by many research groups in immortalized human B-lymphocytes that were derived from patients with an inherited form of AD (i.e., familial AD), but observations of similar changes in cells that were 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 store-operated calcium entry (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 Mol Cell Res*, 2013).

The study of changes in calcium homeostasis in AD lymphocytes was broadened by an analysis of the regulation of apoptosis in lymphocytes from SAD and MCI patients, immortalized with the Epstein-Baar virus or unmodified, freshly isolated B-cells. The research was performed in collaboration with the Laboratory of Preclinical Studies of Higher Standards, headed by Prof. Urszula Wojda (now at the Neurobiology Center of the Nencki Institute of Experimental Biology PAS in Warsaw). The results demonstrated that (*i*) compared with aging donors, immortalized B-lymphocytes and unmodified B-cells from SAD patients displayed alterations in apoptosis that was evoked by 2-deoxyribose (2dRib)-induced redox stress, and the alterations were associated with increased levels of p21, which is known to be the effector of the calcium-calmodulin pathway (*Neurobiol Aging*, 2013), (*ii*) the response to redox stress differentiated lymphocytes from SAD and FAD patients, and (*iii*) alterations in the apoptotic response and an increase in p21 protein levels occurred in early AD and MCI lymphocytes, indicating that p21 may represent a potential early marker of AD.

The genetic manipulation of proteins that are 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 (reviewed by Majewski and Kuźnicki, *BBA Mol Cell Res,* 2015).

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 that are 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 both overexpression systems and the ablation of gene expression by RNA interference in various cell lines. We are analyzing the regulation of SOCE complexes using quantitative co-localization (Fig. 1) and calcium measurements with the aid of the calcium indicator Fura-2 (work in progress).

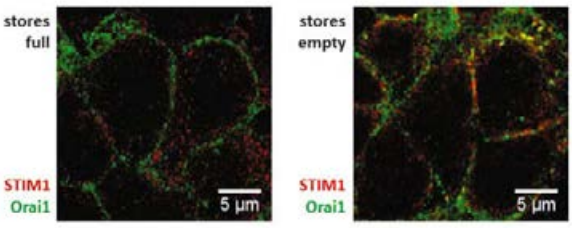


**Fig. 1. An experimental model to study the quantitative co-localization of SOCE machinery.** The fraction of calcium sensor STIM1 (in red) that co-localizes with SOCE channel Orai1 (in green) about 6-fold following the depletion of calcium stores.

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 that are 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 (reviewed by Wojda and Kuźnicki, *J Alzheimers Dis*, 2013). 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).

We analyzed the expression of calcium-related genes in transgenic mouse models of HD. We hypothesized that mutated huntigtin might affect the expression of components of calcium homeostasis and signaling pathways, thereby initiating or propagating the neurodegenerative processes of HD. To test this hypothesis, we analyzed mRNA levels in the brains of transgenic 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 (*Front Mol Neurosci*, 2013). We are now analyzing the effect of selected proteins in the regulation of SOCE and their role in other signaling pathways in HD pathology using medium spiny neuron (MSN) cultures from the striatum of HD transgenic mice (Fig. 2).

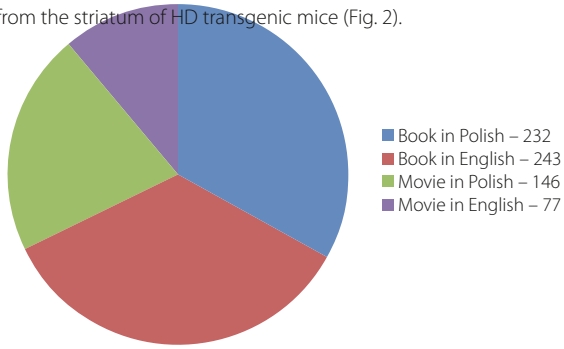


**Fig. 2. Analysis of SOCE in medium spiny neurons (MSN) from mouse Huntington's disease model (YAC128).**

Parkinson's disease is a neurodegenerative disease that is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), leading to motor and cognitive deficits. The cause of PD is believed to be multifactorial, with genetic predisposition that possibly interacts with environmental factors. In this project, in collaboration with Prof. Oliver Bandmann from the University of Sheffield, we used a *pink1* mutant (pink$^{-/-}$) zebrafish line with a premature stop mutation (Y431*) in the Pink1 kinase domain (*Ann Neurol* 2013). There was a loss of dopamine neurons in *pink1*$^{-/-}$ mutant larvae at 5 days post fertilization (dpf), which then further progressed through adulthood, and impairment of mitochondrial

function and morphology at 5 dpf. The knockdown of *mcu* rescued dopaminergic neurons in *pink1* mutant zebrafish. To confirm the results from morpholino-based knockdown, we treated the experimental groups of zebrafish with ruthenium red (RR), a pharmacological inhibitor of Mcu and performed WISH using a TH riboprobe. We observed the rescue of dopamine neurons in RR-treated *pink1*$^{-/-}$ zebrafish, thereby confirming the results from the morpholino study. This restoration of the number of dopaminergic neurons in *pink1*$^{-/-}$ zebrafish implies that the inhibition of *mcu* decreases mitochondrial calcium overload-based toxicity, leading to viable dopamine neurons. We also studied the possible role of Vdac1 in the manifestation of mitochondrial calcium overload during pink1 deficiency. The knockdown of vdac1 did not rescue dopamine neurons in pink1 mutant zebrafish. This indicates that Mcu is a better target for altering mitochondrial calcium influx (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 puncta-like colocalization of YFP-STIM1 and ORAI1 but not YFP-STIM2 or 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 (*J Neurochem*, 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 that were 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 trying to establish whether this interaction occurs in specific parts of neuronal cells.

## 3. Regulation of β-catenin in mature neurons

β-catenin is an activator of LEF/TCF transcription factors in the canonical Wnt pathway that is involved in early brain patterning and neurogenesis. Although Wnt/β-catenin signaling has been associated with psychiatric diseases (e.g., major depression disorder, bipolar disorder, and schizophrenia) and neurodegenerative diseases (e.g., AD, HD, and PD), little is known about the physiological role of Wnt/β-catenin in the adult brain. Several years ago, 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 regulates the transcription of the *Cacna1g* gene that encodes the Cav3.1 subunit of voltage-gated calcium channels. Recently, by combining bioinformatics and experimental approaches, we identified other genes that are involved in neuronal excitability as a β-catenin target (*BMC Genomics*, 2012), suggesting that β-catenin might contribute to electrical signal propagation in thalamic neurons. Additionally, we comprehensively analyzed LEF1/TCF protein localization in the adult mouse brain and the expression profile of their isoforms in cortical, thalamic, and midbrain regions. This analysis revealed a developmentally coordinated transition in the isoform 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 (*Brain Struct Funct*, 2013). As a continuation of these projects, we focused on the role of lithium in β-catenin stabilization in neurons of the adult brain. Lithium is an effective mood stabilizer in bipolar disorder, but it exerts severe toxic effects even with slight overdose. The mechanism of its therapeutic action remains unclear, limiting the discovery of better treatments. Lithium inhibits GSK3α/β, which negatively regulates β-catenin, but unknown is whether its therapeutic levels stabilize β-catenin in the brain. Our results demonstrated that therapeutically relevant doses of lithium selectively activate Wnt/β-catenin signaling in thalamic neurons. The mechanism of this selectivity depends on the TCF7L2 transcription factor, which in the brain is specifically expressed in thalamic neurons and facilitates the nuclear shift of β-catenin. Our study points to the possible role of the thalamus and TCF7L2 in the underlying pathophysiology of bipolar disorder (paper submitted, March 2015). This project was initiated in our laboratory and currently is a collaborative effort together with the Laboratory of Molecular Neurobiology at CeNT, University of Warsaw, headed by a former lab member, Dr. Marta B. Wiśniewska. Moreover, in collaboration with Prof. Shernaz X. Bamji from the Brain Research Center, University of British Columbia, Vancouver, Canada, we participated in a paper on the effects of β-catenin stabilization *in vivo* on cognitive flexibility and long-term synaptic depression (*Proc Natl Acad Sci U S A*, 2014).

We are also interested in the consequences of the impaired polysialylation of neuronal cell adhesion molecule (NCAM), the cytoplasmic domain of which is bound under certain conditions to the protein complex that consists of GSK3 and β-catenin. Using Western blot and immunohistochemical, histological, and ultrastructure analyses, we found that myelin content was decreased and axons showed some features of degeneration in the brains of mice that are deficient in ST8SIA2 but not ST8SIA4 (two polysialyltransferases). In particular, axonal pathologies were reflected by low levels of neurofilaments, a substantial increase in TAU phosphorylation, and changes in axonal shape and myelin sheath thinning. The number of oligodendrocyte lineage cells in the cortex, estimated by immunostaining for OLIG2 and NG2, was approximately two-fold lower in *St8sia2* knockout mice compared with controls. Because axonal pathology and oligodendrocyte dysfunction have been suggested to be features of schizophrenia, we can conclude that *St8sia2*$^{-/-}$ mice exhibit a novel, schizophrenia-related phenotype (paper to be submitted, Spring 2015).

# Laboratory of Cell Biology

**Postdoctoral Fellows:**
Anna Bartosik, PhD (FishMed, until March 2015)
Noga Budick-Harmelin, PhD (maternity leave since February 2015)
Jarosław Cendrowski, PhD
Agnieszka Mamińska, PhD (maternity leave since January 2015)
Beata Pyrzyńska, PhD (part-time until June 2014)
Ewelina Szymańska, PhD
Daria Zdżalik, PhD (since June 2014)

**PhD Students:**
Kamil Jastrzębski, MSc
Sam D. Stephen, MSc (until July 2014)

**FishMed Research Assistant:**
Lidia Wolińska-Nizioł, PhD (joint with Laboratory of Molecular and Cellular Neurobiology)

**Undergraduate Students:**
Katarzyna Kuźmicz, BSc (since November 2014)
Rafał Sejdak, Eng (until September 2014)
Richard Welten, BSc (since November 2014)

**Trainees:**
Krzysztof Gajos, MSc (April-October 2014)
Agata Mieżaniec, Eng (since November 2014)
Rafał Sejdak, MSc (since October 2014)

**Laboratory-Administrative Partner (LAP):**
Paulina Okafor, MSc (since November 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 (2006-2012) |
| 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 |

Cover photo: Platelet-derived growth factor receptor (green) that colocalizes with cholera toxin subunit B (red) on endosomes in a human fibroblast cell (Author: Kamil Jastrzębski).

# Selected publications


- **Banach-Orłowska M, Szymańska E, Miaczynska M**. APPL1 endocytic adaptor as a fine tuner of Dvl2-induced transcription. ***FEBS Lett***, 2015; 589:532-9
- **Sadowski Ł, Jastrzębski K, Purta E**, Hellberg C, **Miaczynska M**. Labeling of platelet-derived growth factor by reversible biotinylation to visualize its endocytosis by microscopy. ***Methods Enzymol***, 2014; 535:167-77
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- **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 corepressor 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
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- **Miaczynska M**, Stenmark H. Mechanisms and functions of endocytosis. ***J Cell Biol***, 2008; 80:7-11
- **Pilecka I, Banach-Orlowska M, Miaczynska M.** Nuclear functions of endocytic proteins. ***Eur J Cell Biol***, 2007; 86:533-547
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- ***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

We study how intracellular signal transduction and membrane trafficking in endocytosis are integrated at the molecular level. In particular, we focus on proteins with established roles in endocytosis to investigate their involvement in various signaling pathways and their impact on patterns of gene expression. Initially, our efforts were focused on studying the endosomal and nuclear roles of APPL proteins, while in recent years we broadened our interests to other multifunctional proteins that act in endocytosis and signaling. The specific projects that are developed by our group follow two general lines of investigation (Fig. 1), 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.

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; Sadowski et al., *Experimental Cell Research*, 2009). Moreover, several endocytic proteins can undergo nucleocytoplasmic shuttling and interact with nuclear molecules that are involved in transcription or chromatin remodeling, changing their localization or activity, and thus may directly modulate the levels or specificity of gene transcription (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).

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

We have been studying the endocytic trafficking of platelet-derived growth factor (PDGF) with the aim of evaluating the impact of endocytosis on PDGF-dependent signaling events. By employing reversible biotinylation, we developed a novel tool to track internalized PDGF-BB using confocal microscopy (Sadowski et al., *Methods in Enzymology*, 2014). This tool allowed us to uncover two modes of PDGF endocytosis: dynamin-dependent and -independent internalization (Sadowski et al., *Traffic*, 2013). Intriguingly, although these routes appeared to be functionally equivalent for the endocytic sorting of PDGF, they differed with respect to the mitogenic signaling of PDGF. The dynamin-mediated endocytosis of PDGF was required for signaling via the STAT3 transcription factor, expression of *MYC*, and mitogenic response of cells. More recently, we characterized the dynamin-independent internalization of PDGF, which involves small GTPases of the Rho family and contributes to PDGF-induced gene expression (Jastrzebski et al., in preparation).

## 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 as those that activate TCF/LEF, AP-1, NF-κB, and STAT transcription factors. All of these pathways can be induced by extracellular ligands that bind appropriate plasma membrane receptors that undergo internalization, but the way in which endocytosis affects the ultimate signaling responses remains poorly investigated and controversial. 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 candidate regulators that function as activators or inhibitors of a given pathway. After initial validation, we delineated the molecular mechanisms of action of a newly identified negative regulator of Wnt signaling (Torun et al., in revision), an endocytic complex involved in NF-κB signaling (Maminska et al., in revision), and an endocytic regulator that affects the expression of AP-1 target genes (Szymanska et al., in preparation). Although cultured mammalian cells remain our main model, we validated some of our findings using zebrafish embryos. Further ongoing work to characterize the impact of endocytic proteins on patterns of gene expression is financed by a MAESTRO grant from the National Science Center and a project funded under the Polish-Swiss Research Programme.

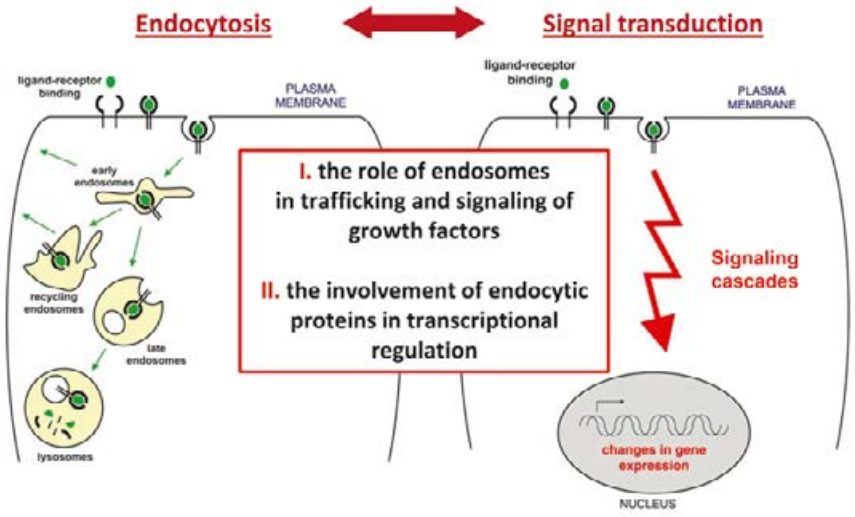


**Fig. 1. Endocytosis and signal transduction as the two processes of interest studied in the Laboratory of Cell Biology, with two main research lines (boxed).** Author: Marta Miączyńska

# Laboratory of Protein Structure

**Postdoctoral Fellows:**
Małgorzata Figiel, PhD
Vineet Gaur, PhD
Karolina Górecka, PhD
Marcin Jaciuk, PhD
Elżbieta Nowak, PhD
Agnieszka Topolska-Woś, PhD

**PhD Students:**
Deepshikha Malik
Michał Rażew
Mirosław Śmietański

**Laboratory-Administrative Partner (LAP):**
Paweł Kustosz

**Research Technicians:**
Agnieszka Gołąb
Jakub Gruchota
Weronika Komorowska
Marzena Nowacka
Justyna Studnicka

**Technician:**
Iwona Ptasiewicz

# 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 |

Cover photo: Crystal structure of Ty3 reverse transcriptase. Author: Elżbieta Nowak.

# Selected publications


- **Gaur V**, Wyatt HDM, **Komorowska W**, **Szczepanowski RH**, de Sanctis D, **Gorecka KM**, West SC, **Nowotny M**, Structural and Mechanistic Analysis of the Slx1-Slx4 Endonuclease, ***Cell Reports*** 2015, pii: S2211-1247(15)00165-5
- **Miętus M**, **Nowak E**, **Jaciuk M**, **Kustosz P**, **Studnicka J**, **Nowotny M**, Crystal structure of the catalytic core of Rad2: insights into the mechanism of substrate binding. ***Nucleic Acids Res***. 2014; 42(16):10762-75
- **Figiel M**, **Nowotny M**, Crystal structure of RNase H3-substrate complex reveals parallel evolution of RNA/DNA hybrid recognition. ***Nucleic Acids Res***. 2014; 42(14):9285-94
- **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; 21(4):389-96; [§]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; 5:3004; [§]corresponding authors, [*]equally contributing
- **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
- **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
- **Figiel M**, Chon H, Cerritelli SM, **Cybulska M**, Crouch RJ, **Nowotny M**, The structural and biochemical characterization of human RNase H2 complex reveals the molecular basis for substrate recognition and Aicardi-Goutieres syndrome defects. ***J. Biol. Chem.*** 2011; 286:10540-50
- **Jaciuk M**, **Nowak E**, Skowronek K, **Tanska A**, **Nowotny M**, Structure of UvrA nucleotide excision repair protein in complex with modified DNA. ***Nat. Struct. Mol. Biol.*** 2011; 18:191-197
- **Rychlik MP**, Chon H, Cerritelli SM, **Klimek P**, Crouch RJ, **Nowotny M**, Crystal structures of RNase H2 in complex with nucleic acid reveal the mechanism of RNA-DNA junction recognition and cleavage. ***Mol. Cell*** 2010; 40:658-670
- **Nowotny M**, Yang W, Structural and functional modules in RNA interference (review). ***Curr Opin Struct Biol.*** 2009; 19(3):286-93
- **Nowotny M**, Retroviral integrase superfamily: the structural perspective (review). ***EMBO Rep.*** 2009; 10(2):144-51

**Without IIMCB affiliation:**

- **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**, Gaidamakov SA, Crouch RJ, Yang W, Crystal structures of RNase H bound to an RNA/DNA hybrid: substrate specificity and metal-dependent catalysis. ***Cell*** 2005; 121:1005-16


# 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 concern nucleases and and reverse transcriptases.

## RNases H

We have had a long standing interest in the mechanism of RNases H. These are nucleases that cleave ribonucleotides in the context of DNA. For example, RNase H1 cleaves the RNA strand of RNA/DNA hybrids and requires a stretch of at least four ribonucleotides. RNase H2 preferentially cleaves RNA-DNA junctions in double-stranded nucleic acids, even when only a single ribonucleotide is present. It is the only known enzyme that can initiate the clean removal of single ribonucleotides from genomic DNA. This is an important function because replicative polymerases misincorporate millions of ribonucleotides in each round of DNA replication in mammalian cells. We reported the first crystal structures of bacterial RNase H2 in complex with a DNA fragment that harbors a single ribonucleotide (Rychlik M, et al., *Mol Cell*, 2010). They revealed that RNA-DNA junction recognition occurs at and around the active site. The 2'-OH group of RNA forms a network of interactions with the protein, and the ribose of the DNA residue of the junction forms a stacking interaction with a conserved tyrosine residue. This stacking excludes the presence of a 2'-OH, which selects for DNA in the second position of the

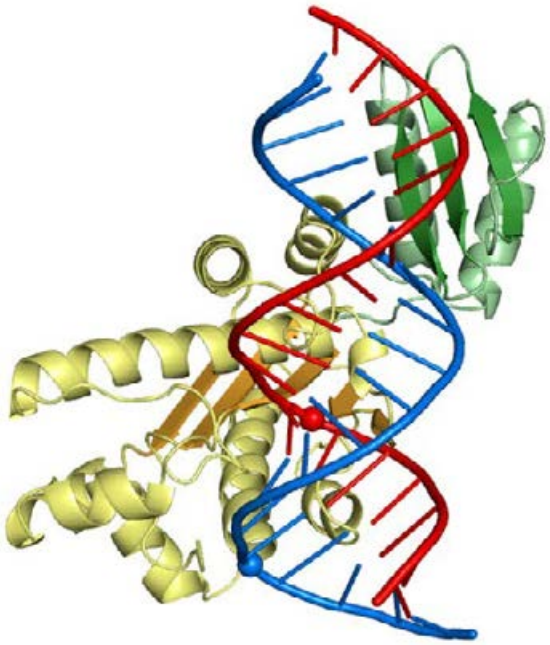

**Fig. 1. Crystal structure of RNase H3 (catalytic domain in yellow and N-domain in green) interacting with an RNA/DNA hybrid (RNA in red and DNA in blue).** The cleaved phosphate is shown as a red sphere, and the phosphate group of the deformed DNA residue is shown as a blue sphere.

junction. The substrate is deformed, so the phosphate of the RNA-DNA junction can participate in coordinating a critical metal ion at the active site. Therefore, we discovered that the substrate is used to assemble the active site for its own cleavage. This is a unique and novel mechanism that we termed "substrate-assisted catalysis," which endows RNase H2 with exquisite specificity for RNA-DNA junctions.

RNases H3 are present in some species of bacteria. They are closely related to RNases H2, but their biochemical properties more closely resemble RNases H1—they prefer to cleave RNA/DNA hybrids. Their unique feature is the N-terminal substrate-binding domain related to TATA-binding proteins. To explain the mechanism of action of RNases H3, we solved its crystal structure in complex with an RNA/DNA hybrid (Fig. 1; Figiel M, et al., *Nucleic Acid Res*, 2014). At the active site, the tyrosine that is conserved in RNases H2 and  which selects against 2'-OH groups, is replaced by glutamate, which binds the 2'-OH. This explains why RNase H3 prefers to cleave stretches of RNA. Moreover, one of the phosphate groups of the DNA strand is tightly bound in a pocket on the protein surface, leading to deformation of the DNA, which requires B-form sugar pucker. This conformation is only available for DNA, which serves to specifically recognize the non-cleaved strand as DNA. Interestingly, a very similar feature is observed in RNases H1, but there the pocket comprises different residues, suggesting that this structural element evolved independently in RNases H1 and H3. The N-terminal domain of RNase H3 specifically recognizes the hybrid by forming interactions with two 2'-OH groups and stacking interactions between aromatic rings of a tyrosine and a histidine residue and ribose rings of two deoxyribonucleotides. This mode of substrate binding is highly reminiscent of the one observed for the hybrid-binding domain of RNases H1, although the two hybrid-binding modules are structurally unrelated. This constitutes another example of the parallel evolution of hybrid recognition.

Our studies of RNases H suggest a universal set of elements that are used by proteins to discriminate RNA from DNA. Ribonucleotides are recognized by contacts with the 2'-OH group and DNA residues either by deformation to a B-form conformation or stacking interactions between aromatic amino acid side chains and ribose rings that exclude the presence of 2'-OH groups.

## Reverse transcriptases

In 2014 we reported a crystal structure of an RT from Ty3 retrotransposon (a yeast retroelement from the Gypsy class that is thought to comprise the direct ancestors of retroviruses). This is the first reported structure of a retrotransposon RT, and it revealed unexpected homodimerization of Ty3 RT induced by substrate binding (Fig. 2; Nowak E, et al., *Nat Struct Mol Biol*, 2014). The Ty3 RT homodimer is asymmetric. One subunit (subunit A) has a canonical DNA polymerase conformation and interacts with the RNA/DNA substrate in a way that is conducive to DNA synthesis. The other subunit (subunit B) has an altered conformation, with the active site of the polymerase blocked. The RNase H domains from neither subunit A nor B interact with the substrate, so we postulated that one of them undergoes a substantial conformational change to be able to bind and cleave the RNA. Based on the structure and biochemical experiments, we demonstrated that subunit B contributes the RNase H activity. This, in turn, demonstrates that dimerization evolved to correctly position the RNase H domain for RNA hydrolysis. The overall architecture of Ty3 and HIV RTs is quite similar. This includes

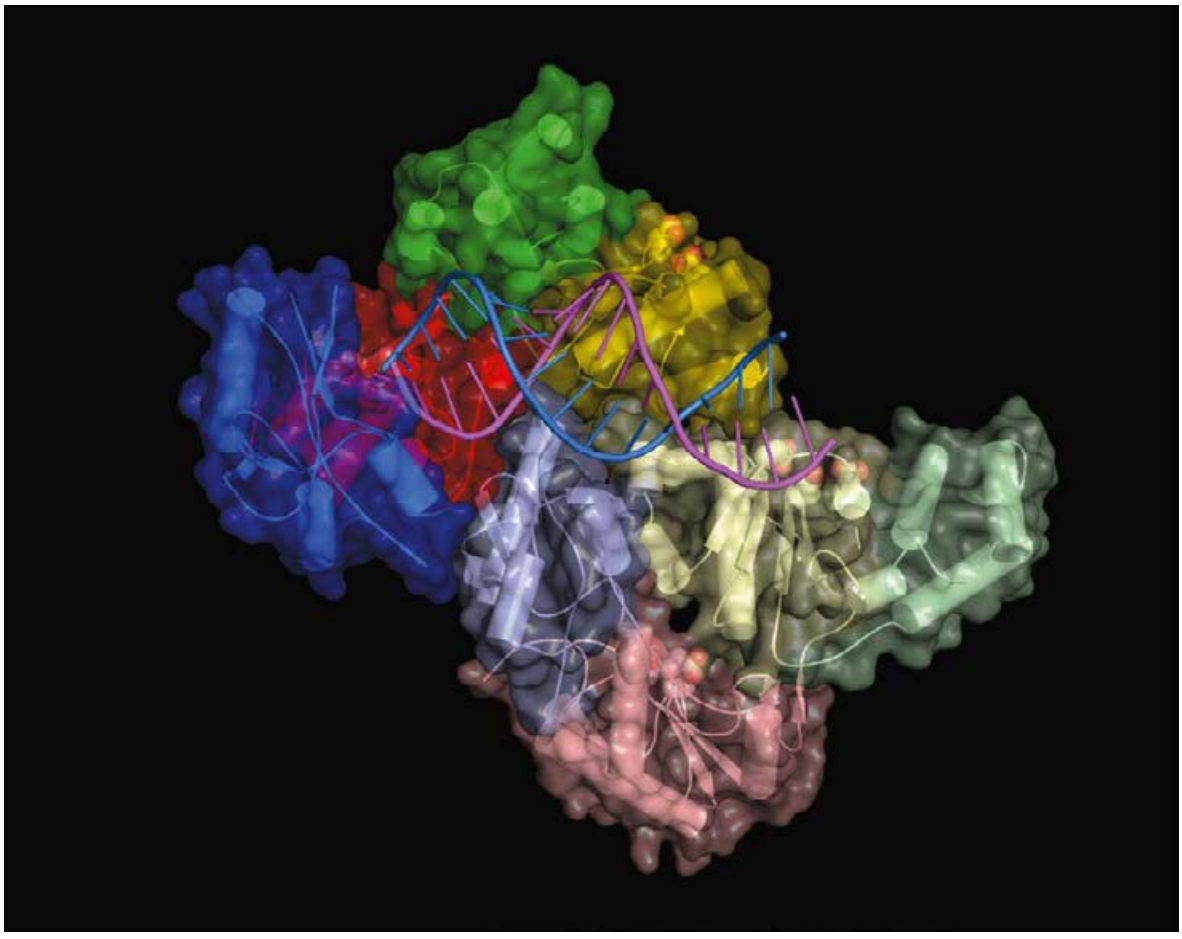

**Fig. 2. Crystal structure of Ty3 reverse transcriptase.** The subunit with a polymerase-competent configuration is shown in darker color (blue, fingers subdomain; red, palm; green, thumb; yellow, RNase H domain), and the subunit with an altered conformation is shown in lighter shades of the same colors. The RNA template strand is in purple, and the DNA primer strand is in blue. Active-site residues for polymerase and RNase H domain are shown as spheres.

the altered conformation of the structural subunit of HIV RT (p51) and subunit B of Ty3 RT. There are, however, important differences. HIV is a constitutive heterodimer. Its larger subunit has acquired a new RNase H domain while the ancestral domain was converted to a structural "connection" domain without catalytic activity. Therefore, in HIV RT, both polymerase and RNase H activities reside in one subunit. In contrast, Ty3 RT is a substrate-induced homodimer, with the two activities residing in two separate subunits. These results provide interesting insights into the evolution of retroviral RTs from their retrotransposon ancestors.

The overall picture that emerges from our studies is that although different classes of RTs catalyze very similar reactions, they are quite diverse in their architecture and mechanism. They can form homo- or heterodimers or function as monomers. A very important element of the RT mechanism is the fine-tuning of RNase H activity that is essential, for example, for the proper generation and removal of the polypurine tract (PPT) primers required for the synthesis of the second DNA strand. This is achieved in three different ways: (i) for retroviral dimeric HIV-1 RT, RNase H is regulated by conformational changes of the substrate, (ii) for retroviral monomeric XMRV RT by the mobility of the RNase H domain, and (iii) for Ty3 RT by conformational changes of this domain.

Our studies of reverse transcriptases have been performed in collaboration with Dr. Stuart Le Grice (National Cancer Institute, NIH, USA).

## XPG/Rad2 DNA repair nuclease

Nucleotide excision repair is a general DNA repair pathway able to detect and correct a wide variety of unrelated lesions. The general steps of the pathway include the damage detection and verification and incision of the DNA on both sides of the lesion, so that the modified DNA fragment can be removed and replaced. In eukaryotes the nuclease that is responsible for the cut on the 3′ side of the lesion is XPG (Rad2 in yeast). It belongs to the flap endonuclease family, along with FEN1 (which is involved in DNA replication), EXO1 (which participates in DNA repair), and GEN1 (which resolves Holliday junctions [HJs]). Among these enzymes, only XPG/Rad2 is able to cleave DNA bubbles (i.e., structures with an unpaired DNA region flanked by double-stranded DNA). Cleavage occurs at the junction between single-stranded and double-stranded DNA (ss/dsDNA junction). The DNA bubble structure corresponds to the nucleic acid in the NER complex. To elucidate the molecular basis of the unique substrate specificity of XPG/Rad2, we solved four crystal structures of the catalytic core of Rad2 in complex with DNA (Fig. 3; Mietus M, et al., 2014). The structures showed that the protein does not bind the single-stranded portion of DNA. Instead, the main specificity determinant is an element called a "helical wedge", which forms a flat surface that interacts with the last exposed base pair of the double-stranded DNA, which allows Rad2 to specifically recognize the ss/dsDNA junction. An important element of flap endonucleases is a so-called "helical arch." In FEN1 and EXO1, it blocks the exit from the active site. The arch has been proposed to fold to clamp the substrate at the active site, once its 5′-end is threaded through the protein. No such 5′-end is present in DNA bubbles, but the helical arch in Rad2 adopts a different structure compared with FEN1 and EXO1. It comprises only one helix that is positioned differently and does not block access to the active site. Therefore, the active site is more accessible, thus allowing the accommodation of DNA bubbles, which would explain the unique substrate specificity of XPG/Rad2. We are currently performing additional biochemical experiments to further verify this model.

Mutations of XPG protein in humans lead to severe genetic diseases (e.g., xeroderma pigmentosum and Cockayne syndrome) with predisposition to skin cancer and developmental abnormalities. We were able to map the positions of the patient mutations on the structure of Rad2 (most of the mutated residues are conserved between Rad2 and XPG). This analysis showed that nearly all of the mutations disrupt the overall structure of XPG/Rad2 rather than affect the functional residues. We also tested the effect of alanine substitutions of DNA-binding residues identified in our structure *in vitro* and *in vivo* using nuclease assays and yeast complementation experiments, respectively. Several variants showed defects *in vitro*, confirming for example the importance of helical wedge interactions. *In vivo*, all the alanine substitution variants were proficient in NER, showing that residual Rad2 activity is sufficient for DNA repair.

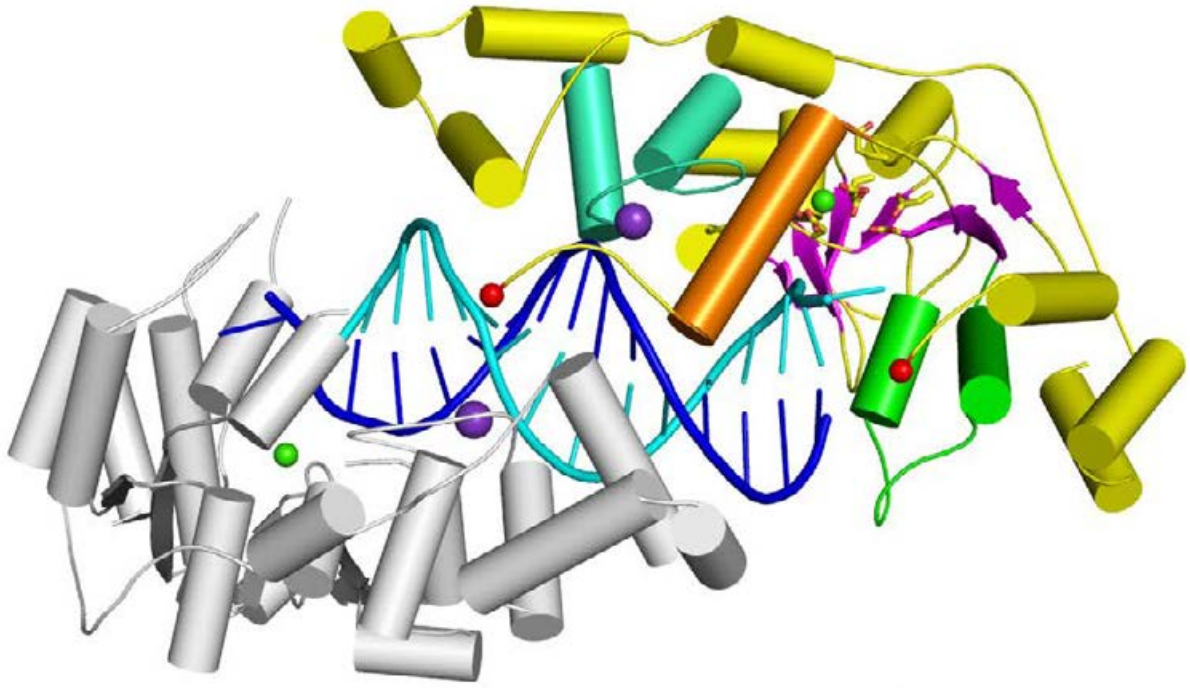

**Fig. 3. Crystal structure of Rad2-DNA complex.** The complex contains two independent protein molecules. One is shown in color: cyan for H2TH motif, green for hydrophobic wedge, and orange for helical arch. The DNA is shown in cyan and blue. The potassium ion is shown as a purple sphere, and the calcium ion at the active site is shown as a green sphere.

# Laboratory of Zebrafish Developmental Genomics

**Postdoctoral Fellows:**
Katarzyna Nieścierowicz, PhD (FishMed, since April 2014)
Michał Pawlak, PhD (FishMed, since October 2014)
Leszek Pryszcz, PhD (since January 2015)

**FishMed Research Assistants:**
Monika Rychlik (until February 2015)
Sreedevi Sugunan (since March 2015)

**Research Assistants:**
Maciej Łapiński

**Technican:**
Agnieszka Olszewska

# 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 Alestrom, 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 |

Cover photo: The two-chambered zebrafish heart viewed in transgenic line Tg(myl7:EGFP) with myocardial-specific egfp expression. Author: Maciej Łapinski.

# Selected Recent Publications


- ***Winata CL**, Kondrychyn I, Korzh V. Changing Faces of Transcriptional Regulation Reflected by Zic3. 2015. ***Current Genomics***, 16(2), 117-127
- Kraus P, **Winata CL**, Lufkin T. BAC transgenic zebrafish for transcriptional promoter and enhancer studies. ***Meth Mol Biol***, 2015; 1227:245-258
- Utami KH, **Winata CL**, Hillmer AM, Aksoy I, Long HT, Liany H, Chew EG, Mathavan S, Tay SK, Korzh V, Sarda F, Davila S, Cacheux V. Impaired development of neural-crest cell derived organs and intellectual disability caused by MED13L haploinsufficiency. ***Hum Mutat***, 2014; 35(11):1311-1320
- Aanes H, **Winata CL**, Moen LF, Ostrup O, Mathavan S, Collas P, Rognes, T, Alestrom P. Normalization of RNAsequencing data from samples with varying mRNA levels. ***PLoS One***, 2014; 9(2):e89158
- **Winata CL**, Kondrychyn I, Kumar V, Srinivasan KG, Orlov Y, Ravishankar A, Prabhakar S, Stanton LW, Korzh V, Mathavan S. (2013) Genome-wide analysis reveals Zic3 interaction with distal regulatory elements to regulate zebrafi sh developmental genes. ***PLoS Genet***, 9(10): e1003852
- Aanes H*, **Winata CL***, Lin CH, Chen JP, Srinivasan KG, Lee SG, Lim AY, Hajan HS, Collas P, Bourque G, Gong Z, Korzh V, Alestrom P, Mathavan S. (2011) Zebrafish mRNA sequencing deciphers novelties in transcriptome dynamics during maternal to zygotic transition. ***Genome Res***, 21(8): 1328-1338. (*equal contribution)
- Lindeman LC, Andersen IS, Reiner AH, Li N, Aanes H, Ostrup O, **Winata C**, Mathavan S, Muller F, Alestrom P, Collas P. (2011) Prepatterning of developmental gene expression by modified histones before zygotic genome activation. ***Dev Cell***, 21(6):993-1004
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- Korzh S, **Winata CL**, 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. ***Dev Biol***, 359(2): 262-276
- Yin A, Korzh S, **Winata CL**, 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)
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- Yin A, **Winata CL**, 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 Expr Patterns***, 10(7-8):338-44
- Ung CY, Lam SH, Hlaing MM, **Winata CL**, 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
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- Lam SH, Mathavan S, Tong Y, Hu J, **Winata CL**, Lee S, Miller LD, Liu ET, and Gong Z. (2004) Preliminary microarray analyses of gene expression in zebrafish treated with xenobiotic and bioactive compounds. ***Mar Biotechnol***, 6: S468-S474


*This publication is with 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 of our research is to understand the dynamics of gene regulation during embryonic development *in vivo*.

The research in our lab focuses on two levels of gene regulation: transcriptional and translational. At the level of transcriptional regulation, we seek to understand the mechanism by which transcription factors (TFs) and epigenetic landscape interact to regulate heart development. At the level of translation, we investigate the mechanism of translational control of maternal mRNAs though cytoplasmic polyadenylation during early embryonic development.

## 1. Elucidating the genome-wide regulatory landscape of heart development

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 on molecular mechanism and downstream targets of cardiac TFs. 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

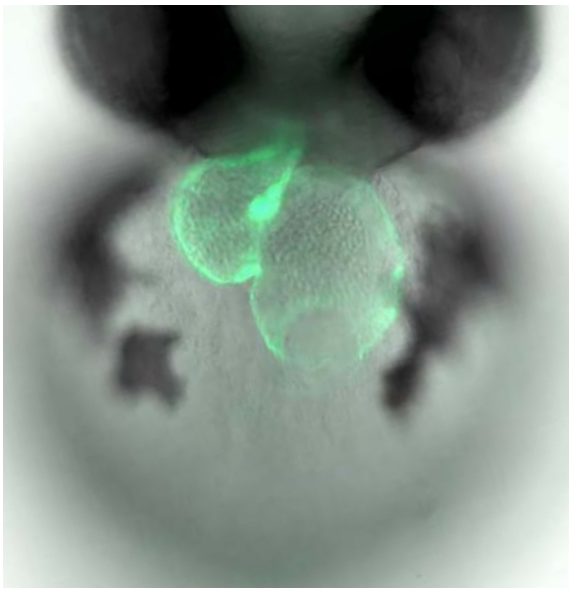


**Fig. 1. The two-chambered zebrafish heart viewed in transgenic line Tg(*myl7*:EGFP) with myocardial-specific egfp expression.** Picture taken with Zeiss Axio Imager.M2 by Maciej Łapinski.

aspects of cardiac development. Such 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.

*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.

Building upon our experience in using ChIP-seq on zebrafish whole embryos and FACS-sorted cells to study transcriptional regulation during zebrafish development (Winata et al., 2013), we are focusing our current effort to characterize the downstream regulatory network of cardiac TFs during key phases of heart development. In parallel to this, we are developing tools for tissue-specific analysis of transcriptional regulation in the form of transgenic lines expressing fusion tagged TFs.

*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.

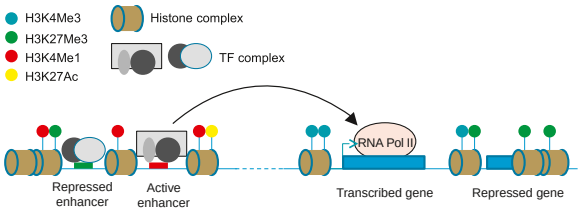
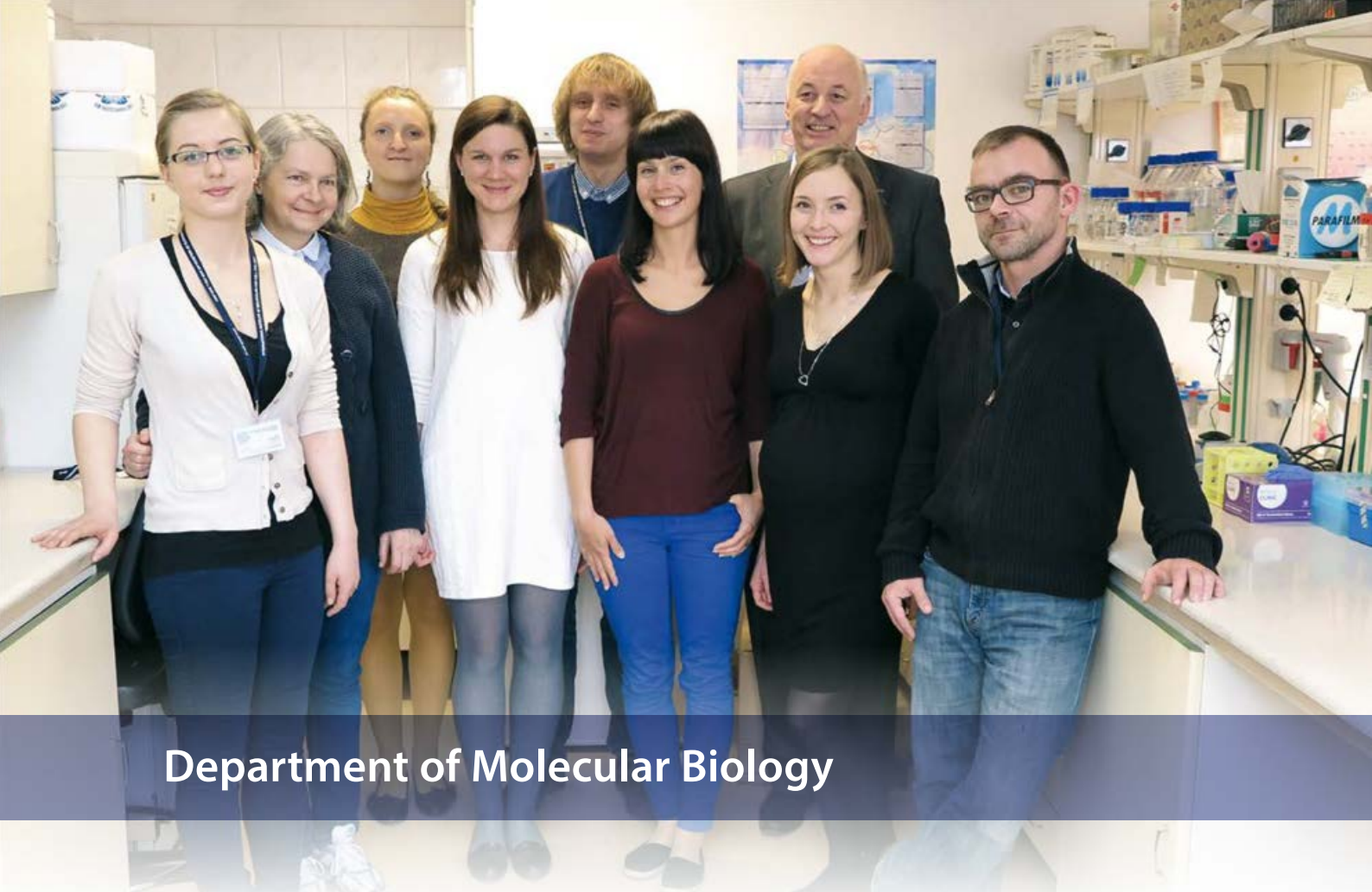



**Fig. 2. A simplified model of transcriptional regulation of gene expression.** The binding of transcription factors (TFs) to regulatory elements such as enhancers result in the transcription of a target gene. Epigenetic factors in the form of histone modifications in turn modulate the accessibility of regulatory elements to TFs, adding another layer of gene expression regulation.

## 2. Molecular mechanism of maternal developmental control through cytoplasmic polyadenylation of mRNAs

During embryogenesis, a silent transcriptional period exists from the moment of fertilization up to the time of zygotic genome activation known as the maternal to zygotic transition (MZT). During this period of transcriptional silence, development is regulated by maternally deposited mRNAs which consisted of two different subpopulations: those which exist in a polyadenylated form and those with very short or no poly(A) tail at fertilization and are gradually polyadenylated with developmental progression (Aanes & Winata et al., 2011). The latter cohort of maternal mRNAs is thought to undergo a form of translational control known as cytoplasmic polyadenylation, which involves its initial de-adenylation at the point of fertilization and subsequent re-adenylation to activate its translation. In support of this, the 3'UTR of this cohort of maternal mRNAs contain signals known to indicate delayed cytoplasmic polyadenylation. We observed that pan-embryonic inhibition of cytoplasmic polyadenylation resulted in the inability of the embryo to undergo MZT, suggesting that this process is a crucial mechanism underlying the maternal control of pre-MZT development.

Our research in this topic focuses on two lines of investigation: (1) to characterize the molecular mechanism of cytoplasmic polyadenylation during pre-MZT development, and (2) to understand how cytoplasmic polyadenylation contributes to the regulation of MZT. In our previous RNA-seq data (Aanes & Winata et al., 2011), the transcripts of at least three different cytoplasmic element binding proteins (CPEBs) were present during pre-MZT period. These factors are known to regulate both cytoplasmic polyadenylation and translation initiation. Currently, functional study of the CPEBs in zebrafish embryogenesis is in progress, as well as the development of methods and tools for the analysis of RNA binding by these factors.



# Department of Molecular Biology

**Postdoctoral Fellows:**
Maciej Olszewski, PhD (FishMed)
Milena Wiech, PhD

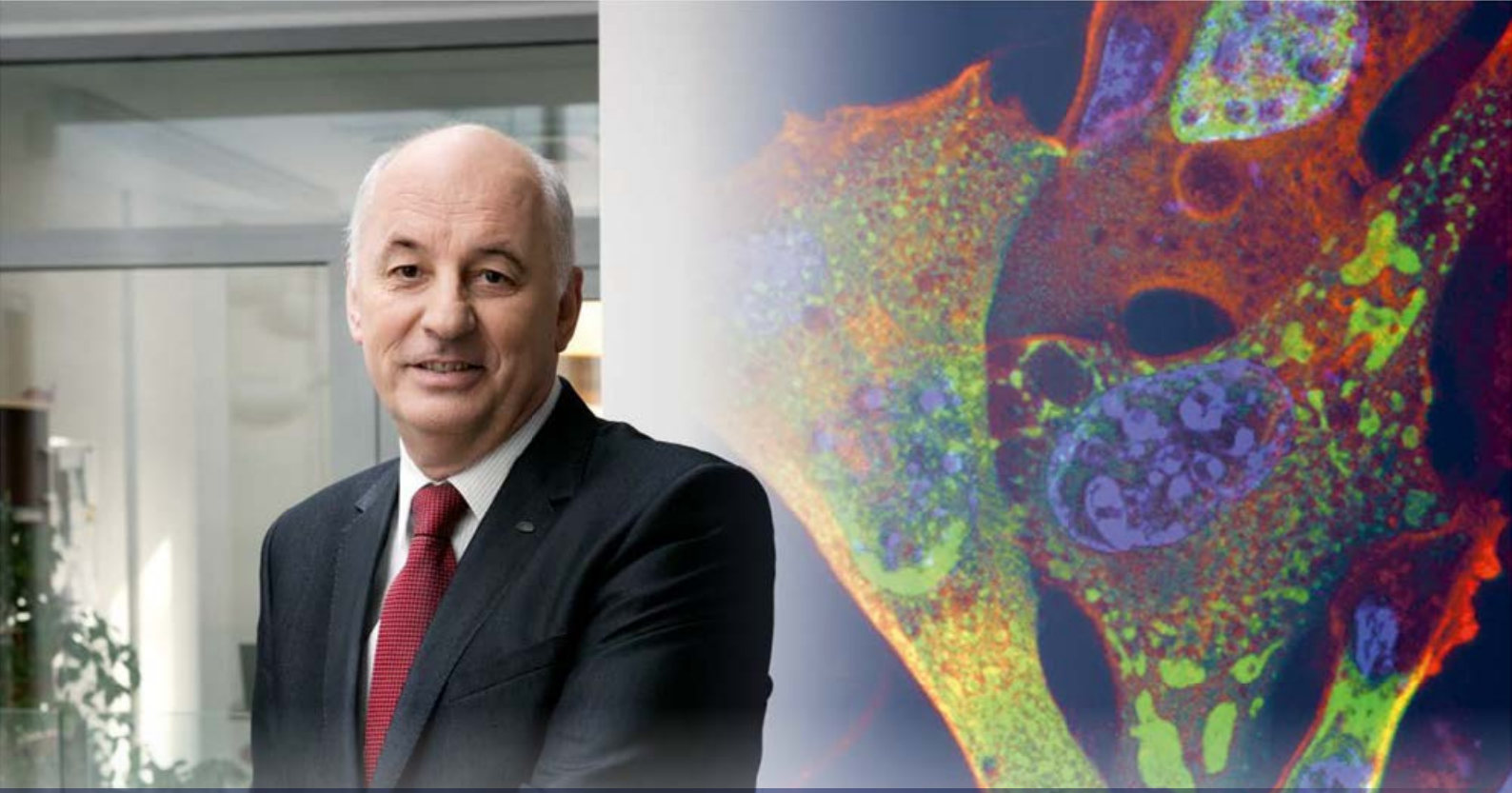
**FishMed Research Assistants:**
Marta Wawrzyniak, PhD (till October 2014, joint with Laboratory of Structural Biology)
Magdalena Pruszko, MSc (since November 2014)

**PhD Students:**
Marcin Herok, MSc
Marta Małuszek, MSc
Zuzanna Tracz-Gaszewska, MSc

**Laboratory-Administrative Partner (LAP):**
Grażyna Orleańska, MSc

**Undergraduate Student:**
Julia Zdzieszyńska

**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 Gdansk, Poland |
| 1977 | MSc in Physics, University of Gdansk, 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 Gdansk, 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 Gdansk, Poland |
| 1991-1994 | Head, Department of Molecular Biology, University of Gdansk, Poland |
| 1993-1994 | Visiting Professor, University of Utah, Medical Center, Institute of Oncology, Salt Lake City, Utah, USA |
| 1990-1993 | Vice President, University of Gdansk, Poland |
| 1988-1991 | Associate Professor, Department of Molecular Biology, University of Gdansk, Poland |
| 1981-1988 | Assistant Professor, Department of Biochemistry, University of Gdansk, 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

Cover photo: MEF cells were transfected with plasmid encoding p73 alone or together with p53 R175H and HSP70 WT. 48 hours post-transfection cells were fixed and labeled with specific antibodies. Immunostaining revealed that p73 is present in the HSP70-dependent cytoplasmic aggregates of p53 R175H. Author: Milena Wiech

- 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 Gdansk |
| 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 Gdansk) |
| 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, Wawrzynow A, Banecki B, Bieganowski P

### Professor Titles Received

Liberek K, Marszalek J, Konieczny I, Wawrzynow A

### Publications

Over 80 publications in primary scientific journals, including two papers published in *Cell, six in EMBO J*, six in *Proc Natl Acad Sci USA*, and more than 30 in J Biol Chem. These papers were cited more than 6 000 times (including 22 papers cited more than 100 times).

## 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
- Kirkegaard T, Roth AG, Petersen NH, Mahalka AK, Olsen OD, Moilanen I, **Zylicz A**, Knudsen J, Sandhoff K, Arenz C, Kinnunen PK, Nylandsted J, Jaattela 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
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- Wawrzynow B, Pettersson S, **Zylicz A**, Bramham J, Worrall E, Hupp TR, Ball KL. A function for the RING fi nger 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
- 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
- **Kudla G**, **Lipinski L**, **Caffin F**, **Helwak A**, **Zylicz M**. High guanine and cytosine content increases mRNA levels in mammalian cells. ***PLoS Biology***, 2006; 4:0933-42
- **Walerych D**, **Kudla G**, **Gutkowska M**, **Wawrzynow B**, Muller L, **King FW**, **Helwak A**, **Boros J**, **Zylicz A**, **Zylicz M**. Hsp90 chaperones wild-type p53 tumor suppressor protein. ***J Biol Chem***, 2004; 279: 48836-45
- Dworakowska D, Jassem E, Jassem J, Peters B, Dziadziuszko R, **Zylicz M**, Jakobkiewicz-Banecka J, Kobierska-Gulida G, Szymanowska A, Skokowski J, Roessner A, Schneider-Stock R. MDM2 gene amplification: a new independent factor of adverse prognosis in non-small cell lung cancer (NSCLC) ***Lung Cancer***, 2004; 43:285-295
- **Kudla G**, **Helwak A**, **Lipinski L**. Gene conversion and GC content evolution in mammalian Hsp70. ***Mol Biol Evol***, 2004; 21:1438-44
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- **King FW**, **Wawrzynow A**, Hohfeld J, **Zylicz M**. Cochaperones Bag-1, Hop and Hsp40 regulate Hsc70 and Hsp90 interactions with wild-type or mutant p53. ***EMBO J***, 2001; 20:6297-305

# Summary of work

The research conducted in the Department of Molecular Biology mainly focuses on the activity of molecular chaperones in mammalian cells, including cell transformation. Using highly purified recombinant human proteins, we previously showed that wildtype and mutant p53 tumor suppressor form different types of complexes with molecular chaperones. 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 37°C. We also elucidated the role of ATP in that reaction (Walerych et al., *J Biol Chem*, 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 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 (Walerych et al., *J Biol Chem*, 2010). We also provided 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 37°C, whereas the requirement for the HSP70-HSP40 system and its cooperation with HSP90 increased under heat shock conditions (Walerych et al., *Oncogene*, 2009).

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). In acidic environments, HSP70 binds with high affinity and specificity to BMP, thereby facilitating the BMP binding and activity of acid sphingomyelinase (ASM). Inhibition of the HSP70-BMP interaction by BMP antibodies or a point mutation in HSP70 (Trp90Phe) and the pharmacological and genetic inhibition of ASM effectively reversed the HSP70-mediated stabilization of lysosomes. Notably, the reduced ASM activity in cells from patients with Niemann-Pick disease (NPD) A and B (i.e., severe lysosomal storage disorders that are caused by mutations in the sphingomyelin phosphodiesterase 1 [*SMPD1*] gene that encodes ASM) was also associated with a marked decrease in lysosomal stability, and this phenotype could be effectively corrected by treatment with recombinant HSP70. Altogether, these data open exciting possibilities for the development of new treatments for lysosomal storage disorders and cancer with compounds that enter the lysosomal lumen through the endocytic delivery pathway (Kirkegaard et al., *Nature*, 2010).

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. We have shown 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 (Wiech et al., *PLoS One*, 2012). In cancer cells, where the level of endogenous HSP70 is elevated, nuclear aggregates that contain mutant p53 and TAp73α are formed. In the presence of MDM2, these aggregates are additionally stabilized and upon proteasome inhibition form nuclear amyloid-like structures. The refolding kinetics of p53 indicated that HSP70 caused transient exposure of the p53 aggregate-prone domains, which can interact with MDM2 and form stable aggregates (Wiech et al., *PLoS One*, 2012). MDM2 protein was previously shown to interact with more than 100 client proteins. We discovered that MDM2, in addition to its E3-ubiquitin ligase activity, exhibited molecular chaperone-like 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* that was coexpressed with wildtype *TP53* stimulated efficient p53 protein folding *in vivo*, and such an effect was abrogated with an ATP binding-defective form of MDM2 (Wawrzynow et al., *J Biol Chem*, 2007).

Elevated levels of MDM2 oncoprotein are often detected in cancer cells. As shown previously, amplification of the *MDM2* gene correlated with a decreased survival rate in lung cancer patients (Dworakowska et al., 2004). Recently, we demonstrated that the inhibition of endogenous HSP70 impeded the formation of nuclear amyloid-like structures that contain TAp73α, p53R175H, and MDM2. We elucidated the role of HSP70/HSP40 chaperone machinery in the formation of this complex and showed that the multichaperone complex that contains HSP40, HSP70, and HSP90 stabilized the TAp73α-p53R175H complex. These events keep TAp73α-dependent apoptosis inhibited, resulting in cancer cell chemoresistance to cisplatin or etoposide treatment. At increased levels, MDM2 oncoprotein can replace the chaperones in that complex and trigger the formation of the three-body TAp73α-p53R175H-MDM2 complex (Fig. 1), which significantly amplifies cancer cell chemoresistance. These findings, underlying a new gain-of-function mechanism of mutant p53, have therapeutic implications and support the idea that the evolutionarily ancient role of heat shock proteins in helping cells to adapt, survive, and proliferate is co-opted by cancer cells (Tracz-Gaszewska et al., submitted).

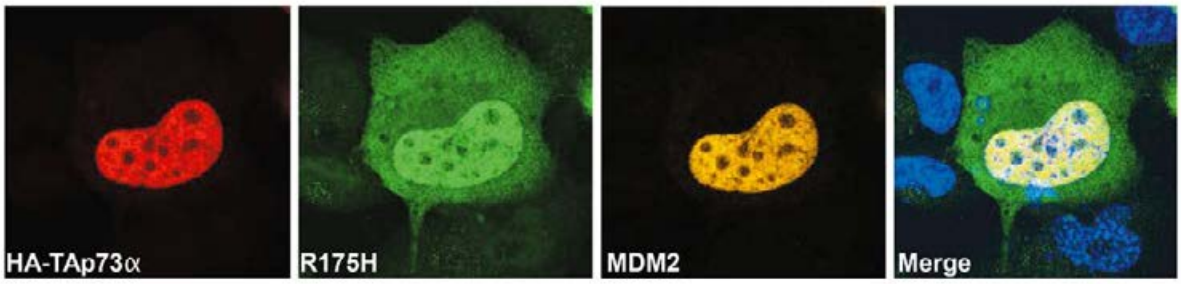



**Fig. 1. MDM2 forms a three body complex with TAp73α and p53 R175H**. Immunostaining of H1299 cells transfected with plasmids encoding p53 R175H, HA-TAp73α and MDM2 revealed co-localization of TAp73α and p53 R175H with MDM2 in the nucleus.

# Core Facilities

# Core Facility

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

Vice Head:
Roman Szczepanowski, PhD

Senior Staff Scientists:
Katarzyna Misztal, PhD
Krzysztof Skowronek, PhD, DSc Habil
Tomasz Węgierski, PhD

Radiation Safety Officer:
Piotr Brągoszewski, PhD

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 50 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 two crystallization robots and ~1,000 crystallization conditions (buffers). Crystallization process is carried out in a crystallization hotel at 4°C or 18°C, and the progress is tracked by a CCD camera. Finally the crystals are analyzed using an X-ray generator (Proteum Bruker) equipped with a CCD detector (Platinum 135) and cryosystem (Oxford 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 (VP-DSC and VP-ITC), analytical ultracentrifugation (Beckman Coulter ProteomeLab XI-I), and surface plasmon resonance (Biacore 3000). 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). In addition to fast proteomics applications (protein identification and protein integrity assays) for internal users which are vital for crystallography projects we provide non-standard analyses of macromolecules other than proteins, 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. Newest acquisitions include Andor Revolutions XD spinning-disk for real-time confocal microscopy and Zeiss Lightsheet Z.1 single-plane illumination microscope (SPIM) for imaging of large objects, such as fluorescently labeled zebrafish larvae. The latter system is unique in Poland.

5. Recently, IIMCB has acquired a new NexSeq 500, New Generation Sequencing (NGS) system from Illumina. The purchase was supported by the Polish Ministry of Science and Higher Education equipment grant, for the scientific consortium of IIMCB and Museum and Institute of Zoology PAS. The NextSeq 500 Sequencing System delivers the power of high-throughput sequencing with the speed, simplicity, and affordability of a desktop NGS system. The fast, integrated, sample-to-results workflow enables many sequencing applications—including exomes, whole genomes, and transcriptomes—in a single run. The NGS service is provided by Dr. Katarzyna Misztal.

The Core Facility provides sufficient assistance with methodological principles, experimental design, initial training, the procedures needed for an experiment, data analysis, and final interpretation and acts as a link between scientists and state of the art technology. 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. Biophysical part of the Core Laboratory is one of the founding member of the Association of Resources for Biophysical Research in Europe (ARBRE).


X-ray generator Bruker Proteum

# Zebrafish Core Facility

Head: **Małgorzata Wiweger**, PhD

Veterinarian:
Piotr Korzeniowski, DVM

Technicians:
Olga Chojnacka, MSc (since September 2014)
Maciej Mańk, MSc
Maciaj Ochnio, MSc (since October 2014)
Krzysztof Surga, MSc
Monika Turniak, MSc (until October 2014)

The Zebrafish Core Facility (ZCF) is a breeding and research facility (license number PL14656251) that is entitled to keep wild type and genetically modified zebrafish (*Danio rerio*; licences: GMO: 01-101/2012 and GMO: 01-105/2013) and use them for FishMed and other projects. ZCF has been established in 2012. All the research activities at ZCF are carried out in compliance with fundamental ethical principles (authorizations 339/2012, 354/2012, 356/2012, 457/2013, 458/2013, 611/2014, 661/2015, and 663/2015) and in compliance with the relevant European and international 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, namely Article 22 of Animal Experiments Act (January 21st, 2005)). Since 2013, ZCF is 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.

This state-of-the-art facility includes a water plant, as well as a stand-alone quarantine unit and the main system manufactured by Tecniplast. In 2013, appx 6000 fish / 30 lines were kept in 300 tanks. By the end of 2014, this number increased to 7,000 fish / 38 different lines (5 wild type, 19 mutants and 14 transgenics). Also the aquatic system has been expanded and now fish are housed in 470 tanks. Further extension of this system has been planned for 2015.

Beside the aquaria room, which is a restricted area, ZCF has a laboratory space available to all users. Alongside incubators, microscopes, and injectors, PCR machine, the laboratory also is equipped with a needle puller and beveller, suited for production of micro needles suitable for injection of cells, zebrafish, Drosophila and other organisms. The personnel is available if users would like to discuss zebrafish biology, husbandry and research or seek a help in solving technical issues.

ZCF is open to the internal and external users with priority given to the FishMed researchers and other IIMCB employees. In 2014, 9 research groups from IIMCB and 7 external users from: the Nencki Institute, CENT, University of Warsaw, and Warsaw Medical University used the facility. For past 2 years, the cost of the fish and access to ZCF equipment was free of charge for the academic users. Similar rule should apply in 2015.

ZCF is actively involved in publicizing the zebrafish model. Among many actions in 2014, ZCF was involved in the organization of an academic event "Heart of Europe: Zebrafish Meeting (HEZ)", which was held in Warsaw on September 17-19. During this event, experts in this field and young researchers presented extensive use of zebrafish model in many fields of science, such as: modelling of human diseases, developmental biology, neurology, bioinformatics, toxicology, etc (http://zebrafish.pl/index.php/programme). Nearly 170 people from 25 countries: Austria (4), Belgium (5), Croatia (5), Czech Republic (6), Denmark (1), Finland (4), France (4), Germany (15), Greece (4), Hungary (9), Ireland (1), Italy (7), The Netherlands (7), Norway (2), Poland (68), Portugal (2), Russian Federation (1), Singapore (1), Slovenia (2), Spain (2), Sweden (1), Switzerland (1), Ukraine (2), United Kingdom (13) and USA (4) attended this meeting. The idea of having the HEZ meeting as a returning event met positive response and the next meeting was scheduled for March 2016. ZCF also was involved in the organization of national and international network group(s) that gather zebrafish researcher in Eastern and Central Europe and e.g. the aquatic section of the Polish

Laboratory Animals Science Association has been constituted and now accepts new members.

In 2014, ZCF offered several in-house trainings that covered fish handling, bases of zebrafish husbandry, basic and more advanced methods and techniques used in zebrafish research. We also taught zebrafish during the XX Biotechnology Summer School in Stegna, on the X course for animal care-takers in Warsaw and on a course for veterinary students. As a result of our activity an article - „Zebrafish as a laboratory animal – principles of nursing and veterinary care" has been published in Życie Weterynaryjne (2014), 89(9): 750-756 (in Polish). ZCF also actively supports the "Be Healthy as a Fish" program (http://www.iimcb.gov.pl/archive-30/items/akcja-edukacyjna-badz-zdrow-jak-ryba.html).

Zebrafish are freshwater, small (3-5cm) tropical fish with a life-cycle of approximately 3-4 months. External fertilization, translucent body, small body size, large mutant/transgenic collection and availability of various genetic tools make zebrafish an excellent organism to study multiple aspects of human diseases. Furthermore, zebrafish as a lower vertebrate is an attractive alternative to mice and rats and can be used for implementation of "3R" (reduction, replacement and refinement) ethical guidance at the Ochota Campus.

## Zebrafish lines that are kept in stock at ZCF:

| | name | afected gene | mutation type |
|---|---|---|---|
| **wild type** | AB | | wild type |
| | ABTL | | wild type |
| | TL | | wild type |
| | TU | | wild type |
| | WIK | | wild type |
| **mutants** | *albino* | *slc45a2* | *unknown* |
| | *casper* | *(roy x nacre)* | *unknown* |
| | *dackel* | *ext2* | *to273b* |
| | *gata5* | *gata5* | *tm236a* |
| | *hand2* | *hand2* | *56cx* |
| | *hi307* | *b3gat3* | *hi307Tg* |
| | *hi954* | *uxs1* | *hi954Tg* |
| | *hi1002* | *csnk1a1* | *hi1002Tg* |
| | *knypek* | *glypican 4* | *u34.8* |
| | *nacre* | *mitfa* | *unknown* |
| | *pink-1* | *pink-1* | *sh397* |
| | *pinscher* | *slc35b2* | *to216z* |
| | *roy* | *unknown* | *unknown* |
| | *siberblick* | *wnt11* | *tx226* |
| | *tbx5* | *tbx5* | *21A* |
| | *trilobite* | *vangl2* | *m209* |
| | *tsc2* | *tsc2* | *vu242* |
| | *ztor* | *ztor* | *xu015* |
| **transgenics** | *Tg(Ath5:gapRFP/Ptf1a:cytGFP/Crx:gapCFP) - SoFa* | | |
| | *Tg(Brn3c:mGFP)* | | |
| | *Tg(Cmlc2:mRFP)* | | |
| | *Tg(Fli:eGFP)* | | |
| | *Tg(Flt1BAC:YFP)* | | |
| | *Tg(HuC:GCaMP3)* | | |
| | *Tg(HuC:GCaMP5G)* | | |
| | *Tg(Kdr-l:mCherry-CAAX)* | | |
| | *Tg(Kop:EGFP-UTRnanos3)er1* | | |
| | *Tg(Mnx1:TagRFP-T)* | | |
| | *Tg(Myl7:eGFP)* | | |
| | *Tg(Nkx2.5:eGFP)* | | |
| | *Tg(Vas:eGFP)* | | |
| | *Tg(Xla.Eef1a1:mlsEGFP)* | | |
| | Wet-Aqua pink | | |

# Bio&Technology Innovations Platform (BioTech-IP)

BIO&TECHNOLOGY innovations platform


Head: **Magdalena Powierża**, Msc (FishMed)

Senior Expert:
Leszek Lipiński, PhD (FishMed)

Project Manager:
Adam Sobczak, PhD (FishMed)

Specialists:
Hubert Ludwiczak, MSc (FishMed)
Piotr Potepa, MSc (FishMed)


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 Biocentrum Consortium in Warsaw in such areas as biotechnology, biomedicine, bioinformatics, bioengineering, material technologies, and bionanotechnology (www.biotech-ip.pl).

## Main tasks of 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 Biocentrum institutes

## 2014 events and achievements

**Completion of the ETTBio project**

The "Effective Technology Transfer in Biotechnology" (ETTBio) project, with IIMCB/BioTech-IP as a project partner, was completed by the end of 2014. The aim of the project was to identify, exchange, and share good practices to enable successful and effective technology transfer in biotechnology among 10 partners from seven European regions. The final results and outputs of the project for IIMCB included the preparation of implementation plans of selected good practices: "Establishment of a bioincubator in Warsaw" and "Improvement of technology transfer mechanisms (SPV) for Ochota Biocentrum." The project resulted in publication of a book by Imperial College Press comprising of the best practices from each region. BioTech-IP described three of them in the following publications:


- Powierża M, Potepa P. The creation of a new technology transfer office
- Powierża M, Potepa P. Public funds for patenting, valorization and science-industry collaboration
- Powierża M, Potepa P, Ludwiczak H. Education for scientists

The project was co-financed by the European Union (European Regional Development Fund) and made possible by the INTERREG IVC Programme. The ETTBio budget was more than €2 million for the 2012-2014 period.

**PhD scholarships**

Seven PhD students from six Ochota Biocentrum institutes were granted scholarships for their research projects. The 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. In total 30 scholarships were granted for the students of Ochota Biocentre consortium.

**Internship program**

Three scientific researchers were supported by internships that took place in MTZ Clinical Research, Adamed Group, and Genomed and 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." In total 9 scholarships were granted for PhD students and scientists of Ochota Biocentre consortium.

**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, which were attended by a total of 41 participants.

**Science-to-business brunches**

BioTech-IP organized four science-to-business brunches, during which Ochota Biocentre scientists presented their research findings to invited entrepreneurs and investors. The brunches were attended by a total of 72 people.

**BioTech-IP Ltd – SpinTech project by NCRD**

After completion of a project funded by NCRD called SpinTech IIMCB finalized the formal preparation of setting up a Purpose Vehicle Company. At the end of 2014 the IIMCB established BioTech-IP Ltd - company owned by IIMCB dedicated to create and support Spin-off companies devoted to commercialize scientific results coming from the Institute. Moreover, BioTech-IP Ltd is also going to offer a range of services in the field of business consulting and R&D to external partners. Creation of a Spin-off company based on the ERC Proof of Concept grant by **Prof. Janusz Bujnicki** is currently being negotiated.

**Initiation of collaboration with GlaxoSmithKline**

The BioTech-IP team initialized a collaboration with GlaxoSmithKline (GSK) within the GSK programme Discovery Partnerships with Academia (DPAc). During the science-to-business meeting, organized by BioTech-IP, 13 scientists had the opportunity to present their projects to experts from GSK and receive constructive feedback. The quests form GSK expressed their interest in organizing another science-to-business meeting in 2015.

**International collaboration in Technology Transfer field**

BioTech-IP broadened its network of international collaborations with technology transfer offices and companies that are dedicated to technology commercialization.

Within the ENTENTE Professional Exchange Programme, BioTech-IP established professional links with Direction Administration et de la Valorisation de la Recherche (AVRE), the technology transfer office at the University of Mons (Belgium). BioTech-IP also established collaborations with GWT GmbH and HZDR Innovations GmbH, companies dedicated to the commercialization of technologies from Technical University in Dresden and institutes of the Leibniz Society.

**Promotion and dissemination**

Team members of BioTech-IP attended several international meetings concerning the commercial exploitation of IP, where inventions from institutes of the Biocentrum Ochota campus were promoted. Moreover, members of BioTech-IP co-authored "Effective Technology Transfer in Biotechnology: Best Practice Case Studies in Europe," published by Imperial College Press.

**FishMed project**

BioTech-IP began 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.

BioTech-IP participated in the Bionection partnering conference on technology transfer and commercialization of research results, which took place on October 9-10, 2014, in Dresden, Germany. The two-day event was organized by Biosaxony e.V., the association of the biotechnology and life sciences industries in the Free State of Saxony. Its aim was to present excellent scientific projects that are ready for transfer to potential economic partners. BioTech-IP provided assistance to five researchers from Ochota Biocentrum who presented their technologies and inventions, including **Justyna Czarnecka**, PhD, and **Elżbieta Jagielska**, PhD, from IIMCB. Dr. Czarnecka from the Laboratory of Bioinformatics and Protein Engineering presented technology for the sequence-specific cleavage of RNA, and Dr. Jagielska introduced the LytM enzyme as a new weapon against *Staphylococcus*.

**Management of Intelectual Properties**

The results of AriaDNA project lead to development of innovative methods of identification of ethnic origin of biological material based on novel genetic markers. These discoveries became the foundation of three patent applications co-authored by **Dr. Izabela Sabała** in cooperation with scientific institutes in Poland. WIPO has published the international patent application "Methods of identification of ethnic origin based on differentiated transcription profiles and genetic markers used in those methods" under the number WO 2015/008245.

Moreover, a European patent was granted (EP 2 699 254) on the basis of an application by **Prof. Matthias Bochtler** and **Dr. Izabela Sabała** – "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".

Polish patent P.395923 was granted to the invention by "Warianty endonukleazy restrykcyjnej MwoI o zmienionej specyficzności substratowej" by **Prof. Janusz Bujnicki** and **Dr. Krzysztof Skowronek**.

# IT Unit

Head: **Roman Szczepanowski**, PhD

IT Specialist:
Jakub Skaruz

System Administrator:
Michał Romiszewski

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.

To improve the quality of network services, in 2014 IT Unit has launched the following services:

1. Virtualization of servers providing key network services (DNS, antispam, file services)
2. New file server:
   - Personal network drive with 10 Gigabytes of storage for each user
   - Shared network drive available for departments and project groups
   - Previous Versions - snapshots of network files are created of documents saved on the new file server
3. New version of the backup and archive software, which will provide better support for offsite backup, archive and replication.

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 Laboratory of Bioinformatics and Protein Engineering: 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 Laboratory of Protein Structure and the Laboratory of Structural Biology and storage servers for the data from ZEISS Lightsheet SPIM microscope are also located in the server room. This is where the databases of the PolSenior centenarians' project can be accessed.

# 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 attain 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 have introduced a new research model: zebrafish. The FishMed Center, 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 diseases. European Union and national funding is used to finance the employment of over 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.

## 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 who is 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 Center and research with zebrafish models among scientific and non-scientific communities, including promotion of the project's innovative results.

## Management

The FishMed project is coordinated by **Prof. Jacek Kuźnicki**, who created the idea of setting up the FishMed Center at IIMCB. He is advised by the International Advisory Board and FishMed Committee. The project management team consists of a project manager, **Dr. Urszula Bialek-Wyrzykowska**, and Workpackage Leaders: **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).

The FishMed Committee is an executive body that assesses work progress, makes strategic decisions, and reacts to major delays in the project. The Committee consists of a Project Coordinator (Jacek Kuźnicki), European Partners (Oliwer Bandmann, Thomas Braun, Marcos Gonzalez-Gaitan, William Harris, Carl-Philipp Heisenberg, Ewa Snaar-Jagalska, Herman Spaink, and Didier Stainier), IIMCB Laboratory Leaders (Matthias Bochtler, Janusz Bujnicki, Agnieszka Chacińska, Jacek Jaworski, Marta Miączyńska, Cecilia Lanny Winata, and Maciej Żylicz), and Workpackage Leaders.

In 2014, IIMCB organized meetings of the International Advisory Board (May 23-24) and FishMed Committee (September 18) as a satellite event to The Heart of Europe: Zebrafish Meeting.

## Twinning and research

The FishMed Center 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 Center 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**, the 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 (until October 2014)
**Research Assistant**: Thomas Fricke, PhD (since January 2015)

5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) result from the modification of cytosine in DNA. Both DNA modifications are essential for normal development. 5mC is considered a repressive chromatin marker. The role of 5hmC is not fully understood. In higher animals, 5hmC has been associated with tissue differentiation. The 5hmC base has also been described as an intermediate of an oxidative DNA demethylation pathway. At least in this role, 5hmC should be associated with gene reactivation.

Mammalian Ten-Eleven-Translocation (TET) proteins are a group of dioxygenases that catalyze the conversion of 5mC to 5hmC and further oxidation to formylcytosine and carboxylcytosine. In mammals, 5hmC plays a role in zygotic genome activation, primordial germ cell formation, hematopoiesis, and learning and memory.

Fish have three TET dioxygenase genes (zTET1-zTET3) that are orthologous to their mammalian TET1-3 counterparts. However, the biological role of TET proteins in fish has not been clearly established. Our qRT-PCR experiments showed that the transcript levels of all TET genes are low in zygotes but increase over time until 5 days post-fertilization (5dpf). Transcript levels were higher for TET enzymes than for enzymes of the deamination pathway, suggesting that DNA demethylation in zebrafish might be mediated by TET-dependent oxidation.

Zebrafish TET dioxygenases are active and convert 5mC to 5hmC. The 5hmC base is present in zebrafish gDNA at different stages of embryogenesis (ZGA, 1-5 dpf). This observation was confirmed by dot-blot analysis, immunofluorescence staining, and a 5hmC-glucosylation assay. To estimate the 5hmC content of genomic DNA independently of the 5hmC-specific antibody at different time points of zebrafish development, we will use phage β-glucosyltransferase to convert genomic 5hmC to azidoglucose-5hmC using UDP-azidoglucose as the carbohydrate donor. The azide group will then serve as the site of attachment for DBCO-Cy3, which can react with azido-glucose 5hmC in a copper-free Click reaction. Our preliminary experiments showed that this method works with genomic DNA. Currently, we are setting up the methods for fixed embryos.

To evaluate the role of TET proteins and 5hmC in the zebrafish genome, we are in the process of performing TALEN - and Cas9/CRISPR-mediated knockout experiments. Among five different TALEN nucleases that we have tested, we have chosen the one that leads to a mutation in the zTET3 gene. We also tested different single-guide RNA that are designed to target zTET1 and zTET2 genes by a Cas9/CRISPR system and selected the ones that work. From the mosaic F0 generation, we chose fish that transmitted mutations through the germ line. To date, we have raised the F1 generation with a TALEN-mediated mutation in the zTET3 gene (leading to a reading-frame shift) and F1 generation with a Cas9/CRISPR-mediated mutation in the zTET1 and zTET2 genes. We are currently focused on genotyping these fish and raising heterozygous and homozygous F2 generations.

Part of the work described above was presented as posters at the 11th International Conference on Zebrafish Development and Genetics in Madison, Wisconsin, United States, and at The Heart of Europe: Zebrafish Meeting in Warsaw in September 2014.

**Janusz 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
**Research Assistant**: Michał Dyzma, MSc (until September 2014)

The goal of the work in the Bujnicki group within the FishMed project is to develop computational methods for analyzing the effects of mutations on protein and RNA structures. Our current studies are oriented toward developing viable research tools to study RNA secondary structures and three-dimensional structures, particularly in the case of the structural stability of mutations and effects of RNA interference (RNAi), including micro-RNA (miRNA) and silencing RNA (siRNA). We are also interested in applications to evolution and the stability of RNA structures. These are likely to be important for understanding why zebrafish embryos show far more "off-target" responses to RNAi techniques than *Arabidopsis thaliana*, *C. elegans*, *Drosophila melanogaster*, and murine and human cell lines where RNAi techniques have been applied.

We have been developing and refining a three-dimensional structural modeling and prediction tool (SimRNA) and employing and modifying other support tools that help predict restraints in the secondary structure and pseudoknot topologies of RNA properly. These studies include single-stranded RNA (ssRNA) in mRNA sequences and the corresponding secondary structure that is developed in double-stranded RNA (dsRNA) when it involves partially complementary sequences, such as when "off-target" effects of RNAi (particularly with high doses of siRNA) and open reading frame (ORF) interactions occur. We are also interested in 3'-untranslated region interactions, which appear to be more favorable to yielding the gene-specific control of expression in zebrafish embryos.

Currently, we are further developing and refining the SimRNA program to show more representative behavior in the effect of mutations on the structure of RNA and RNA-RNA interactions. Simply predicting the correct structure is insufficient; one must also understand the stability and dynamics of RNA, disordered regions of RNA, RNA flexibility, and the various suboptimal structures that are present in the structural ensemble of RNA molecules.

Publication: "Computational modeling of RNA 3D structures, with the aid of experimental restraints," Marcin Magnus, Dorota Matelska, Grzegorz Łach, Grzegorz Chojnowski, Michal J Boniecki, Elzbieta Purta, Wayne Dawson, Stanislaw Dunin-Horkawicz, Janusz M Bujnicki, *RNA Biology*, 2014.

**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 undeniably essential for life. This fact is supported by numerous human pathologies that are a consequence of mitochondrial dysfunction, which are often fatal or incurable. Our research focuses on specific pathways that precisely target nuclearly encoded proteins into specific mitochondrial compartments. We use *Danio rerio* as a model system to study how defects in these pathways and faulty mitochondrial protein homeostasis influence the development of a vertebrate organism. Our research seeks to contribute new findings to the field of mitochondrial protein biogenesis that can be used to understand the molecular basis of human diseases in the future.

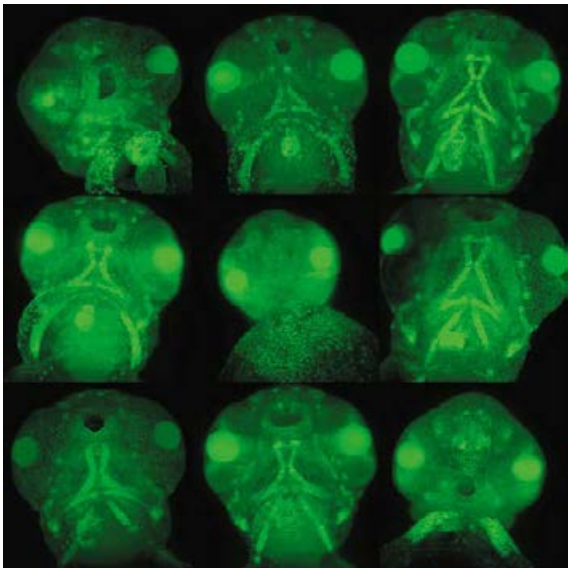


**Fig. 1. Maximum intensity projections of mitochondria in zebrafish heads.** Image presents different developmental stages and anatomy changes after depletion of proteins involved in the mitochondria biogenesis. The eye lenses, jaw muscles and heart structures can be easily distinguished. For these experiments, the Tg(Xla.Eef1a1:mlsEGFP) line was used, in which mitochondria are labeled with the green fluorescent protein (GFP). Picture was taken by Michał Bazała on Zeiss Lightsheet Z.1 microscope.

The mitochondrial intermembrane space assembly (MIA) pathway is involved in oxidation-dependent protein targeting to the intermembrane space of mitochondria. Its effectiveness strongly relies on the enzymatic activity of the MIA40 oxidoreductase. MIA40 protein is evolutionarily conserved from yeast to humans. We utilized a commonly used approach that is based on antisense Morpholino particles to decrease the levels of MIA40 protein and one of its substrates, TIMM9. We used our previously achieved expertise in biochemical and imaging techniques to accomplish a detailed

analysis of how knocking down these proteins influences vertebrate development. This research was performed using a wildtype zebrafish line and a transgenic line with mitochondria that were labeled with a green fluorescent marker. To provide a more detailed analysis and confirm the specificity of our findings, we are currently applying novel techniques that involve CRISPRi to transiently decrease the levels of our proteins of interest.

According to our bioinformatics analysis, zebrafish have two paralogues of MIA40. In collaboration with our twinning partner, Prof. Didier Stainier (Max Planck Institute, Bad Nauheim, Germany), we have used a reverse-genetics approach in conjunction with the TALEN technique to generate a genetic mutant of the MIA40 gene. We successfully obtained zebrafish lines with various alleles for both MIA40 paralogues separately. We are currently in the process of analyzing the phenotype of the F1 generation incrosses, which yielded a pool of 25% bi-allelic mutants. Additionally, we are establishing the F2 generations on a transgenic background. To complete our research, we plan to obtain and analyze the fish lines with mutations in both paralogues of the MIA40 gene.

Publication: "Mitochondrial protein translocases for survival and wellbeing," Anna Magdalena Sokol, Malgorzata Eliza Sztolsztener, Michal Wasilewski, Eva Heinz, Agnieszka Chacinska, *FEBS Letters*, 2014.

**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, PhD

mTOR is a very important serine-threonine kinase that participates in many cellular processes, including growth and development. Our aim is to study mTOR function in neurons *in vivo* and unravel the mechanisms by which mTOR regulates dendritic morphology. To achieve this aim, two Zebrafish mutant strains are used. One is depleted of mTOR protein (carrying a *xu015* mutation in the *mTOR* gene [1]), and the other exhibits an excess of mTOR activity (due to disruption of the upstream inhibitor of mTOR, *TSC2* [2]). These strains will provide information on the mTOR kinase and its molecular pathways in neurons. Preliminary phenotyping of the mutant strains shows that both heterozygotes and homozygotes are viable until the larval stage (7 dpf), but mTOR$^{-/-}$ mutants have smaller eyes and a smaller head, and TSC2$^{-/-}$ mutants have a shorter body. Moreover, we confirmed that the mutant homozygotes (both mTOR$^{-/-}$ and TSC2$^{-/-}$) lack a swimming bladder and do not swim as well as controls. The eyes of both mutant strains constitute all retinal layers, suggesting normal gross morphology. Moreover, the S6 pathway is highly activated in the TSC2$^{-/-}$ and TSC$^{+/-}$ fish, confirming the overactivation of mTOR.

We seek to unravel mTOR-dependent neuronal function *in vivo*, and the methodology to visualize single neuronal cells in the retina of zebrafish has been developed in collaboration with the Prof. William Harris laboratory (Department of Physiology, Development, and Neuroscience, Cambridge University, United Kingdom). This method employs retinal lineage tracers that are coupled with fluorescent protein expression and microinjections or blastomere transplantations, enabling us to record single isolated neuronal cells within the native tissue (whole-mount imaging) in detail and subsequently allowing us to three-dimensionally compare neuronal dendritic morphologies between wildtype and mutant fish. Moreover, using single-plane illumination microscopy, we are able to record dendritic arbors of single neuronal cells over time.

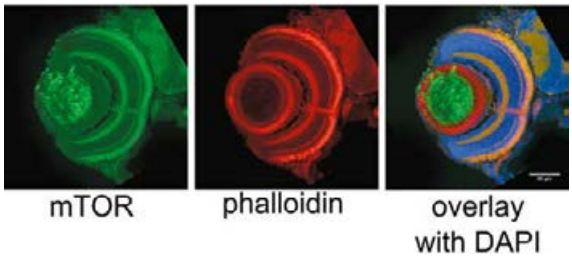



**Fig. 2. mTOR kinase localization in Zebrafish retina.**
Overall gross localization of mTOR (in green) in the whole eye of wild type Zebrafish. All retinal layers could be distinguished by phalloidin (the plexiform layers and optic nerve; in red) and DAPI (the retinal ganglion cell layer and nuclear layers; in blue) stainings. Maximum projections of confocal images from LSM5. Obj. 20x scale bar 50 um. Photo by Justyna Jezierska.

References
1. Ding Y, Sun X, Huang W et al. Haploinsufficiency of target of rapamycin attenuates cardiomyopathies in adult zebrafish. *Circ Res* 2011; 109: 658-669.
2. Kim SH, Speirs CK, Solnica-Krezel L, Ess KC. Zebrafish model of tuberous sclerosis complex reveals cell-autonomous and noncell-autonomous functions of mutant tuberin. *Dis Model Mech* 2011; 4: 255-267.

**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**: Michał Bazała, MSc

Parkinson's disease is a neurodegenerative disease that is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), leading to motor and cognitive deficits. The cause of Parkinson's disease is believed to be multifactorial, with genetic predisposition that possibly interacts with environmental factors. In this project, in collaboration with Prof. Oliver Bandmann (University of Sheffield), we used a pink1 mutant (pink$^{-/-}$) zebrafish line with a premature stop mutation (Y431*) in the Pink1 kinase domain (Bandmann et al., 2013). There is loss of dopaminergic neurons in pink1$^{-/-}$ mutant larvae at 5 dpf, which further progresses through adulthood. These mutants also exhibit impairment of mitochondrial function and morphology at 5 dpf. The knockdown of mcu rescued dopaminergic neurons in pink1 mutant zebrafish. To confirm the results of the morpholino-based knockdown, we treated the experimental groups of zebrafish with a pharmacological inhibitor of Mcu (ruthenium red) and performed WISH using a tyrosine hydroxylase riboprobe. Ruthenium red treatment rescued dopaminergic neurons in pink1$^{-/-}$ zebrafish, thus confirming the results of the morpholino study. This restoration of the number of dopaminergic neurons in pink1$^{-/-}$ zebrafish implies that mcu inhibition decreases mitochondrial calcium overload-induced toxicity, leading to viable dopaminergic neurons. We also studied the possible role of Vdac1 in the manifestation of mitochondrial calcium overload during pink1 deficiency. The knockdown of vdac1 did not rescue dopaminergic neurons in pink1 mutant zebrafish. This

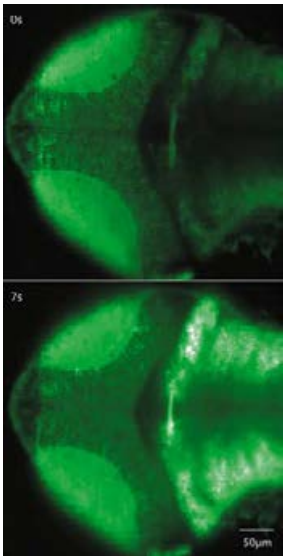



**Fig. 3. Calcium efflux observed in neurons of zebrafish brain during live exposure to glutamic acid.** GCaMP3 probe in Tg(HuC:GCaMP3) fish line emits bright fluorescence in presence of calcium ions. Top view. Green signal comes from GCaMP3 probe in neurons. Picture was taken by Michał Bazała on Zeiss Lightsheet Z.1 microscope.

indicates that Mcu is a better target for altering mitochondrial calcium influx (work in progress).

Manuscript: "MCU silencing leads to restored dopaminergic neurons in pink1 mutant zebrafish model of Parkinson's disease," Smijin Soman, Marcs Da Costa, Jacek Kuznicki, Oliver Bandmann (in preparation).

**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 signalling and transcriptional regulation in zebrafish.
**Postdoctoral Fellow**: Anna Bartosik, PhD
**Research Assistant**: Lidia Wolińska-Nizioł, PhD

The Laboratory of Cell Biology collaborates with Prof. Marcos Gonzalez-Gaitan (University of Geneva) 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. Using unbiased RNAi screens that were 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. Among the screening hits, in 2014 we described a novel negative regulator of canonical Wnt signaling and delineated its molecular mechanism of action (Torun et al., manuscript in revision). Our studies were performed in parallel in cultured mammalian cells and during early embryonic zebrafish development. The latter was achieved thanks to a collaboration with Prof. Gonzalez-Gaitan's group. The results allowed us to conclude that the function of a newly identified regulator in the canonical Wnt pathway is evolutionarily conserved between fish and humans.

In a second project, we have been characterizing another novel player that was identified in RNAi screens, an endocytic protein complex that negatively regulates NF-κB signaling (Maminska et al., manuscript in revision). In this case, we performed parallel investigations in cultured mammalian cells and zebrafish embryos. In both systems, we described the expression of NF-κB target genes and their alterations upon depletion of the novel regulator, providing *in vivo* validation of our initial data that were obtained in cell cultures.

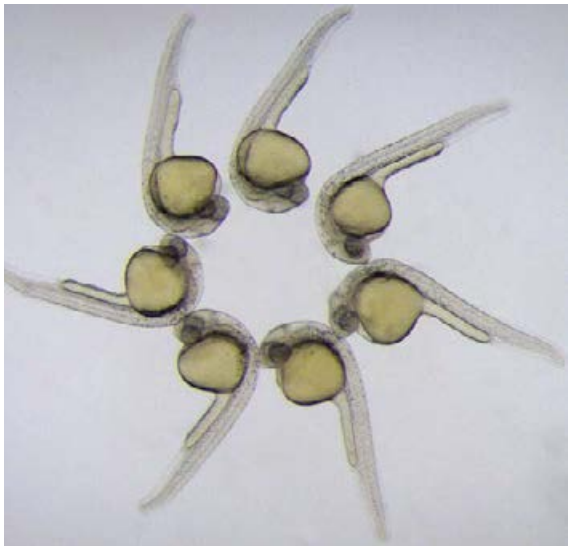


**Fig. 4. Lateral views of wild-type (WT) zebrafish embryos at 24 h post fertilization.** Photo by Lidia Wolińska-Nizioł.

Publication: "Effects of membrane trafficking on signaling by receptor tyrosine kinases," Marta Miaczynska, *Cold Spring Harbor Perspectives in Biology*, 2013.

**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 (until October 2014)
**Research Assistant**: Magdalena Pruszko, MSc (since November 2014)

More than 50% of human cancers carry a mutation in the TP53 gene. Analyses of the frequency of the mutation in human cancers clearly demonstrate that some amino acid residues are mutated disproportionately more frequently than others. Many of these so-called "hot-spot" mutations are the gain-of-function type (i.e., they not only lose their natural function but also gain new functions that they did not possess previously). For a number of years, various aspects of mutant p53 biology have been investigated in the Department of Molecular Biology, and the FishMed project enabled us to extend this research to an animal model.

Mutations in the TP53 gene have been suggested to result in the altered splicing of some mRNAs, including vascular endothelial growth factor (VEGF) mRNA. Zebrafish have been successfully used as a model for assaying the angiogenic potential of xenotransplanted cancer cells. We created cell lines that stably express VEGF splice reporter constructs and various versions of p53 and injected these cells into the perivitelline space of developing zebrafish embryos. The number of abnormally growing blood vessels allowed us to quantify the angiogenic potential of the transplanted cells. This, in turn, reflects the alternative splicing of the VEGF reporter that results from the co-expression of various p53 mutant proteins.

Another topic of our research that utilizes zebrafish as a model is tumor invasiveness. *In vitro* settings have demonstrated that certain mutations in p53 increase cellular migration. We are seeking to determine whether these mutations increase the invasiveness of cancer cells in an animal host. In this assay, tumor cells are intravenously injected into the zebrafish embryo, and the distribution of these cells within the fish is analyzed at regular time intervals. This allows us to observe the stages of tumor invasion, including extravasation and tissue infiltration. Additional genetic modifications that alter the expression of chemokines or their receptors in the injected cells or fish embryos shed light on the role of particular tumor-host interactions in tumor invasiveness. This model has been successfully employed by our twinning partner in Leiden and is now implemented at IIMCB with the important advance of including selective plane illumination microscopy, which allows the imaging of developing tumors in fish embryos at high resolution with low photo damage.

## Zebrafish research group

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

**Project**: Transcriptional regulatory landscape of heart development
**Postdoctoral Fellow**: Katarzyna Nieścierowicz, PhD
**Postdoctoral Fellow**: Michal Pawlak, PhD

**Research Assistant**: Monika Rychlik (until February 2015)
**Research Assistant**: Sreedevi Sugunan (since March 2015)

The advent of next-generation sequencing (NGS) technology has brought a revolution in many fields of biology. Developmental biology has seen revelations of the complexity of regulatory mechanisms that govern developmental processes, which renders almost meaningless the study of a single factor in isolation. At the very heart of developmental regulation is the selective activation and inactivation of gene transcription through complex interactions between different transcription factors (TFs) and regulatory elements. The Laboratory of Zebrafish Developmental Genomics (LZDG) was founded with the mission to dissect this biological complexity and answer a central question regarding how transcription is regulated to achieve specific patterns at the level of organismal morphology. To answer this question, our current project focuses on the early development of the heart, which in the embryonic stage consists of a relatively uniform cellular composition and performs mostly mechanistic functions during the early stages of development. Nevertheless, the study of heart development is often hindered by the fact that this organ is essential for an organism's survival, such that the loss of function of molecular regulators of its development often causes early lethality. The zebrafish alleviates this problem by its ability to survive without a functioning heart up to a relatively late stage of development, which greatly facilitates studies of heart morphogenesis. Capitalizing on this beneficial feature, we seek to determine the molecular mechanisms that regulate heart development at the transcriptional level by applying NGS to profile the binding sites of key cardiac TFs, including Nkx2.5, Tbx5, Gata5, and Hand2, as well as epigenetic markers of active/inactive promoters (H3K4Me3/H3K27Me3), active/inactive enhancers (H3K27Ac/H3K4Me1), and RNA polymerase II. The application of genomics has the advantage of providing a global view of regulation that considers multiple interacting factors that are involved in heart morphogenesis.

The first employees of the LZDG funded by FishMed, Dr. Katarzyna Niescierowicz and Ms. Monika Rychlik, officially began their work in April 2014 to focus on preparatory work to set up the laboratory. With the arrival of the group leader in June 2014, the laboratory was officially operational and well equipped with basic laboratory equipment and fluorescent stereomicroscopes for live observations of transgenic zebrafish embryos. Through FishMed funding, the laboratory also acquired an upright Zeiss Axio Imager.M2 equipped with fluorescence and the Apotome system, which is ideal for imaging complex biological samples, including live embryos. Current laboratory activities include experiments to validate antibodies for ChIP-seq, the optimization of a cell-sorting protocol to isolate cardiomyocytes from the transgenic lines Tg(*nkx2.5*:EGFP) and Tg(*myl7*:EGFP), and the establishment of important heart transgenic and mutant lines in the IIMCB zebrafish core facility for use in the study. Our efforts are also directed toward developing new methods and resources to improve the tissue-specific analysis of transcriptional regulation in the form of transgenic lines that express fusion-tagged TFs.

Publication: "Changing Faces of Transcriptional Regulation Reflected by Zic3", Cecilia Lanny Winata, Igor Kondrychyn, and Vladimir Korzh, Current Genomics, 2015.

## Research visits

**Smijin Soman** visited **Oliver Bandmann's** laboratory in the Department of Neuroscience, Sheffield Institute of Translational Neuroscience, University of Sheffield, United Kingdom.

**Justyna Jezierska** visited **William Harris's** laboratory in the Department of Physiology, Development, and Neuroscience, Cambridge University, United Kingdom.

**Maciej Olszewski** and **Jacek Kuźnicki** visited **Ewa Snaar-Jagalska's** laboratory in the Department of Molecular Cell Biology, Institute of Biology, Leiden University, The Netherlands.

**Anna Sokół** visited **Didier Steinier's** laboratory in the Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany.

**Cecilia Lanny Winata**, **Katarzyna Misztal**, and **Karolina Mierzejewska** visited **Thomas Braun's** laboratory in the Department of Cardiac Development and Remodeling, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany.

## Zebrafish Core Facility

**Leader**: Małgorzata Wiweger, PhD
**Technician**: Maciej Mańk, MSc in biology
**Technician**: Krzysztof Surga, MSc in biology
**Technician**: Monika Turniak, Ing. in zootechnics (until October 2014)
**Veterinarian**: Piotr Korzeniowski, DVM

In 2014, our state-of-the-art facility was fitted with three new standalone tanks (manufactured by Tecniplast, purchased from FishMed funds). This increased the number of tanks that are being used for fish housing from 300 to 470 and allowed consequent expansion of the fish stock. In 2014, 12 fish lines were imported, including eight transgenic lines and three mutant lines that were requested


by FishMed groups. By the end of 2014, a total of 7,000 fish from 38 different lines (five wildtypes, 19 mutants, and 14 transgenics) were kept in-house. Thanks to support from FishMed, other investments have also been made, including a new water purification system that secured the functionality of the old system and provided a basis for further expansion of the Zebrafish Core Facility and a behavioral analysis system (ZebraCube and ZebraBox manufactured by ViewPoint) that supports projects that are run by FishMed groups and external users.

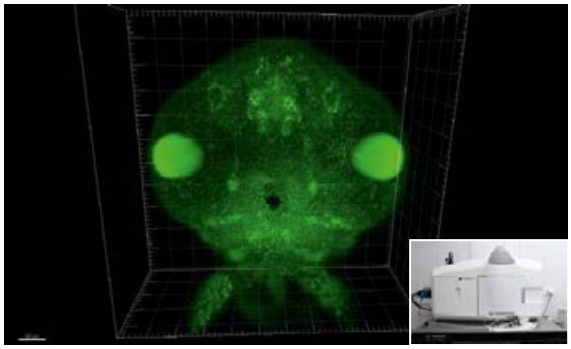
ZCF was actively involved in publicizing FishMed and the zebrafish model. Among many actions in 2014, ZCF was involved in organizing an academic event, Heart of Europe: Zebrafish Meeting (HEZ), which was held in Warsaw on September 17-19. We offered several in-house training sessions for FishMed and other users, including topics on fish handling, the basis of zebrafish husbandry, and basic and more advanced methods and techniques that are used in zebrafish research. ZCF also actively supports the "Be Healthy as a Fish" program (http://www.iimcb.gov.pl/archive-30/items/

akcja-edukacyjna-badz-zdrowjak-ryba.html; accessed April 16, 2015), in which elementary school students learn about research that is done with the zebrafish model.

## Research equipment

IIMCB laboratories are equipped with state-of-the-art scientific equipment. Several large pieces of equipment have been purchased specifically for zebrafish-related research. The best example is Lightsheet.Z1, a state-of-the-art fluorescence microscope that is manufactured by Zeiss. It works on the principle of single-plane illumination microscopy, in which the illuminating planar sheet of light is perpendicular to the detection axis. This allows for the much more efficient usage of illumination light, thereby reducing unwanted effects associated with 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 long-term live imaging of medium-sized objects, such as zebrafish larvae.

The total amount of project resources that has been invested in equipment exceeds 3.7 million PLN.



**Zebrafish head, 2 days after fertilization.** Visualization in 3D of GFP localized in mitochondria. Picture was taken by Michał Bazała on Zeiss Lightsheet Z.1 microscope.

## Bio&Technology Innovations Platform

In response to the growing potential of IIMCB, a separate unit, referred to as the Bio&Technology Innovations Platform (BioTech-IP), was established in March 2010 to manage intellectual property (IP) that is generated by scientists who work at IIMCB. BioTech-IP's main task is to find, protect, and commercialize IP with strong market potential.

In 2014, BioTech-IP began cooperating with technology transfer experts from the United States, United Kingdom, Germany, and Spain. One of them assisted with the commercialization of technology that was developed at IIMCB and later deployed by a spin-off company, Proteon Pharmaceuticals. Another consultant was involved in negotiating with investors to establish a spin-off on the basis of new technology that was developed in Prof. Bujnicki's laboratory. BioTech-IP's team also received consultation from experts from Germany and Spain on best practices in the implementation of technology transfer-supporting tools, such as a special purpose vehicle and bioincubator.

**Networking events**

In 2014, BioTech-IP organized five networking meetings, two of which were financially supported by the FishMed project. The FishMed brunch, which took place on September 18, 2014, hosted two guest lecturers from abroad, Ron Dirks, PhD (ZF-screens), and Jan de Sonneville, PhD (Life Science Methods), both from The Netherlands. Both scientists-turned-bioentrepreneurs gave a short overview of the services that their companies provide, which specialize in zebrafish toxicology screens. Additionally, Małgorzata Wiweger, PhD (IIMCB), talked about opportunities for collaborative research and business projects with the institute's Zebrafish Core Facility. On November 3, 2014, BioTech-IP hosted a networking event that featured GlaxoSmithKline's Discovery Partnerships with Academia (GSK-DPAc) Programme. It was an opportunity to learn about GSK's approach and case studies in searching for collaborative research projects at very early stages of drug discovery.

**Bionection conference, Dresden**

On October 9-10, 2014, a representative from BioTech-IP participated in Bionection, the first international technology transfer conference held in Dresden, Germany. The two-day event was organized by Biosaxony, the association of biotechnology and life sciences industries in the Free State of Saxony. Its aim was to present scientific projects that are ready to be transferred to potential economic partners. The conference was an excellent opportunity to get in touch with industry representatives and present BioTech-IP's technology offerings at the biopartnering session.

**BioForum trade fair, Łodź**

On May 28-29, 2014, two representatives from BioTech-IP participated in the 13th BioForum trade fair, the Central European Forum of biotechnology and innovative bioeconomy in Łodź, Poland.

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 public relation activities at the Institute, focusing on both the research community (by promoting the zebrafish model and disseminating scientific results) and wider society (by inspiring it to take an interest in researching and developing a dialogue with the scientific community).

## Discussion platform on zebrafish usage

**IIMCB OPEN SEMINARS**

- *Regeneration in Planaria: Why some can while others can't*, **Jochen Rink** (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany)
- *Wnt non-canonical signaling patterns cardiac form and function*, **Daniela Panakova** (Max-Delbruck-Center for Molecular Medicine, Berlin, Germany)
- *A dual role of ESCRT proteins in the formation and function of ciliated organs*, **Maximilian Fuerthauer** (Valrose Institute of Biology, Nice, France)
- *Zebrafish as a model for cancer*, **Marina Mione** (Karlsruhe Institute of Technology, Karlsruhe, Germany)
- *Novel Signaling Mechanisms Controlling Collective Cell Migration*, **Darren Gilmour** (European Molecular Biology Laboratory, Heidelberg, Germany)

**BRAINSTORMING SESSION on TALEN and CRISPR/Cas9 based methods for genome modification**; July 18, 2014, IIMCB, Warsaw, Poland

Event initiated by: Anna Sokoł, Laboratory of Mitochondrial Biogenesis, IIMCB

- *TALEN based methods for gene disruption and editing in Danio rerio,* **Zacharias Kontarakis** (Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany)
- *CRISPR/Cas9 and CRISPRi methods for gene modifications and control of gene expression in Danio radio*, **Andrea Rossi** (Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany)

– *Genotyping tools: CEL1 and T7EN1 in action*, **Joanna Krwawicz** (Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland)
– *Genome modifications in Nasonia vitripennis model organism*, **Małgorzata Perycz** (Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland)

**HEART OF EUROPE: ZEBRAFISH MEETING**, September 17-19, 2014, IIMCB, Warsaw, Poland

FishMed lectures:

– *Zebrafish models of neurodegenerative diseases - past, present and future*, **Oliver Bandmann** (University of Sheffield, United Kingdom)
– *Cell and tissue mechanics in zebrafish gastrulation*, **Carl-Philipp Heisenberg** (Institute of Science and Technology Austria, Austria)
– *Modeling and inhibition of human breast cancer in zebrafish xenograft embryos*, **Ewa Snaar-Jagalska** (Leiden University, The Netherlands)
– *Targeting MCU leads to enhanced dopaminergic neuronal functional status in pink -/- mutant zebrafish*, **Smijin Soman** (International Institute of Molecular and Cell Biology, Poland)
– *At the crossroads of immunity and metabolism: novel insights from zebrafish screening technologies*, **Herman Spaink** (Leiden University, The Netherlands)
– *Cardiovascular development in zebrafish*, **Didier Stainier** (Max Planck Institute for Heart and Lung Research, Germany)
– *A genomics approach to study zebrafish development*, **Cecilia Winata** (International Institute of Molecular and Cell Biology, Poland)

## Dissemination of scientific results

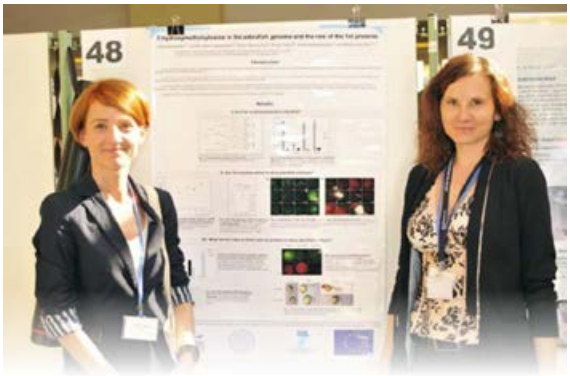
The active presence of FishMed researchers in the international research scene facilitated the dissemination of knowledge on the project and its scientific results:

– **Anna Sokół** and **Michał Wasilewski**, Euromit 2014 & Young Mitoscientists' Forum, June 2014, Tampere, Finland
– **Agnieszka Kolano**, 11th International Conference on Zebrafish Development, June 2014, Madison, Wisconsin, United States
– **Wayne Dawson**, Bioinformatics in Toruń 2014, June 2014, Toruń, Poland
– **Agnieszka Kolano** and **Anna Sokół**, Zebrafish Development & Genetics course, Marine Biological Laboratory, August 2014, Woods Hole, Massachusetts, United States
– **Katarzyna Nieścierowicz**, The Genome Access Course, September 2014, New York, United States
– **Wayne Dawson**, 1st Congress of the Polish, Biochemistry, Cell Biology, Biophysics and Bioinformatics Societies, BIO 2014, September 2014, Warsaw, Poland
– **Michał Bazała**, 1st LightSheet Fluorescence Microscopy International Conference, September 2014, Barcelona, Spain
– **Małgorzata Wiweger**, Improving Zebrafish Husbandry Towards Better Research and Animal Welfare, November 2014, Lisbon, Portugal
– **Katarzyna Nieścierowicz**, **Michał Pawlak** and **Cecilia Lanny Winata**, Keystone Symposium Heart Disease and Regeneration: Insights from Development, March 2015, Copper Mountain, Colorado, United States

**HEART OF EUROPE: ZEBRAFISH MEETING**, September 17-19, 2014, IIMCB, Warsaw, Poland

FishMed posters:

– "The Lightsheet microscope in the analysis of dynamic *in vivo* processes using fluorescent zebrafish transgenic lines" **Michal Bazała**, **Anna Sokół**, Tomasz Węgierski, **Jacek Kuźnicki**, **Agnieszka Chacińska**
– "SimRNA: Using contact-related entropy reranking and calibration in 3D RNA structure prediction" **Wayne Dawson**, Michał Boniecki, **Janusz Bujnicki**
– "Meta-predictor for assessing the effects of RNA mutations" Łukasz Rączkowski, Marcin Magnus, **Janusz Bujnicki**
– "The development of the zebrafish visual system as an *in vivo* model to study zTOR function and dysfunction in neurons" **Justyna Jezierska**, **Lidia Wolińska-Nizioł**, **Agata Góźdź**, **Jacek Jaworski**
– "Studying the gene regulatory network of the heart development in *Danio rerio* using genomics approach" **Katarzyna Nieścierowicz**, **Cecilia Winata**
– "Fishing in mitochondrial biogenesis: the MIA pathway during vertebrate development" **Anna Sokół, Michał Bazała**, **Didier Stainier**, **Agnieszka Chacińska**
– "The ESCRT activity limits basal NF-κB signaling" **Agnieszka Mamińska**, **Anna Bartosik**, Magdalena Banach-Orłowska, **Irinka Castanon**, Morgane Poulain, **Maximilian Fürthauer**, **Marcos González-Gaitán**, **Marta Miączyńska**
– "Gain-of-function p53 mutations in cancer invasiveness" **Maciej Olszewski**, **Marta Wawrzyniak**, **Alicja Żylicz**, **Maciej Żylicz**
– "5-hydroxymethylcytosine in the zebrafish genome and the role of the Tet proteins" **Agnieszka Kolano**, **Marta Wawrzyniak**, Marek Wojciechowski, Karthik Shanmuganandam, Michał Pastor, **Matthias Bochtler**



Dr. Agnieszka Kolano and Dr. Marta Wawrzyniak presenting the poster.

## Activating and initiating a dialogue with wider society

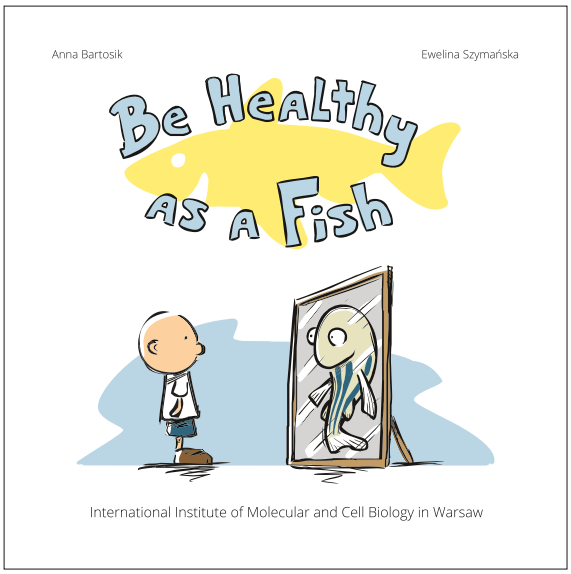
As a new facet of dissemination activities, FishMed introduced a visibility strategy that targets circles outside academia.

The **"Be Healthy as a Fish"** campaign was initiated by IIMCB during the Festival of Science. Elementary school children were the first group to participate in the workshops, which is being


Dr. Urszula Białek-Wyrzykowska presenting zebrafish at the "Be Healthy as a Fish" workshop

held throughout the 2014/2015 school year. The objective of the program is to teach children basic knowledge about the life of fish and the possibilities of their use in studies of certain human diseases. Modern science and FishMed project are introduced to children in a

friendly and accessible manner. The workshops are directed toward children from the 5th and 6th grades of elementary school. During the workshops, children watch the *Be Healthy as a Fish* educational movie, perform simple biological experiments, observe zebrafish under a microscope, and take part in discussions about the genetic similarities of humans and fish. They also receive notebooks, *Be Healthy as a Fish* books and bookmarks. The workshops are free of charge. Since September 2014, IIMCB has conducted 16 workshops, with 327 participants from 16 schools (as of March 31, 2015).



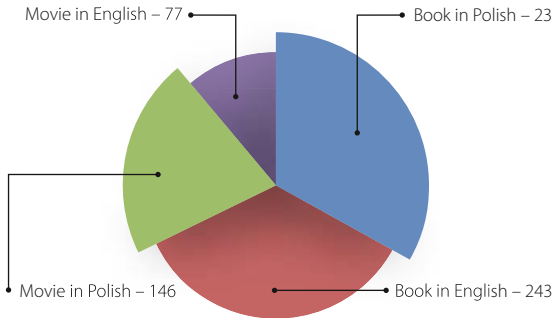

*Be Healthy as a Fish* book


*Be Healthy as a Fish* movie

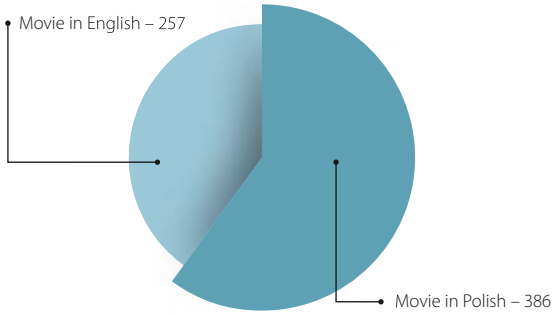
*Be Healthy as a Fish* educational materials (books and movies) are available in English and Polish versions online on the IIMCB web page, FishMed web page, and IIMCB YouTube channel.

To date, more than 500 people have received the *Be Healthy as a Fish* book in English, and nearly 750 people have received it in Polish.

## The downloads statistics




"Be Healthy as a Fish" books and movies downloaded from the web page (as of March 31, 2015)




Views of the "Be Healthy as a Fish" movie on the IIMCB YouTube channel (as of March 31, 2015)

## Cooperation with media

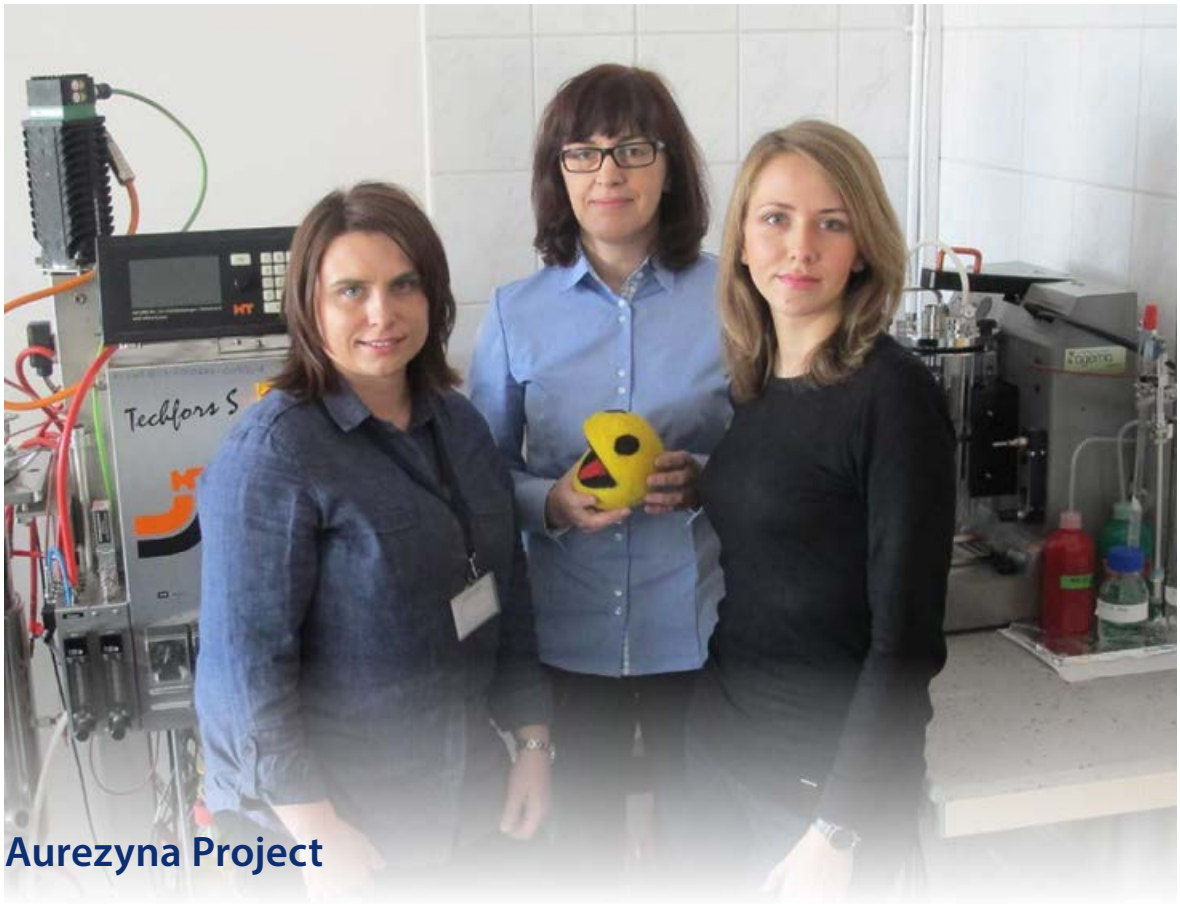
An IIMCB database concerning Polish media has been created and is updated after every press release (to date, the database contains 220 positions in three categories: national media, science media, and local media). Since the beginning of the project, 39 media releases have been published.

## Other FishMed popularization activities involved:

- Organization of workshop for talented youth in cooperation with the Polish Children's Fund (IIMCB, March 2014)
- Presentation at the "Experience of the REGPOT-PL projects in improving competence in the future EC projects' management" mini-symposium (Warsaw University of Life Sciences, March 2014)
- Organization of workshop for students from the Warsaw University of Life Sciences (IIMCB, May 2014)
- Visit from Adam Struzik, Marshal of the Mazowieckie Voivodeship (IIMCB, June 2014)
- Organization of a festival lesson within the XVIII Festival of Science (IIMCB, September 2014)
- Presentation at the "Health in the main role" conference (Warsaw Centre for Socio-Educational Innovation and Training, October 2014)
- Organization of workshop for children during the Days of Education organized by Polish Association Supporting People with Inflammatory Bowel Disease "J-elita" (IIMCB, December 2014)

# Projects outside research lab teams

As a complement to the basic activities of regular IIMCB laboratories, two smaller independent research teams operate at the Institute, running their own research projects. The creation of these groups, in parallel to the normal recruitment policy of IIMCB, arose from the need to use the existing expertise and highly qualified researchers and to enable the development of topics relevant to the Institute and of particular importance for domestic science. Each of these groups carries out highly-funded research projects, which are an important part of IIMCB's research policy.




## Aurezyna Project

Head: **Izabela Sabała**, PhD

Postdoctoral Fellow:
Elżbieta Jagielska, PhD

PhD Student:
Maja Grabowska, MSc

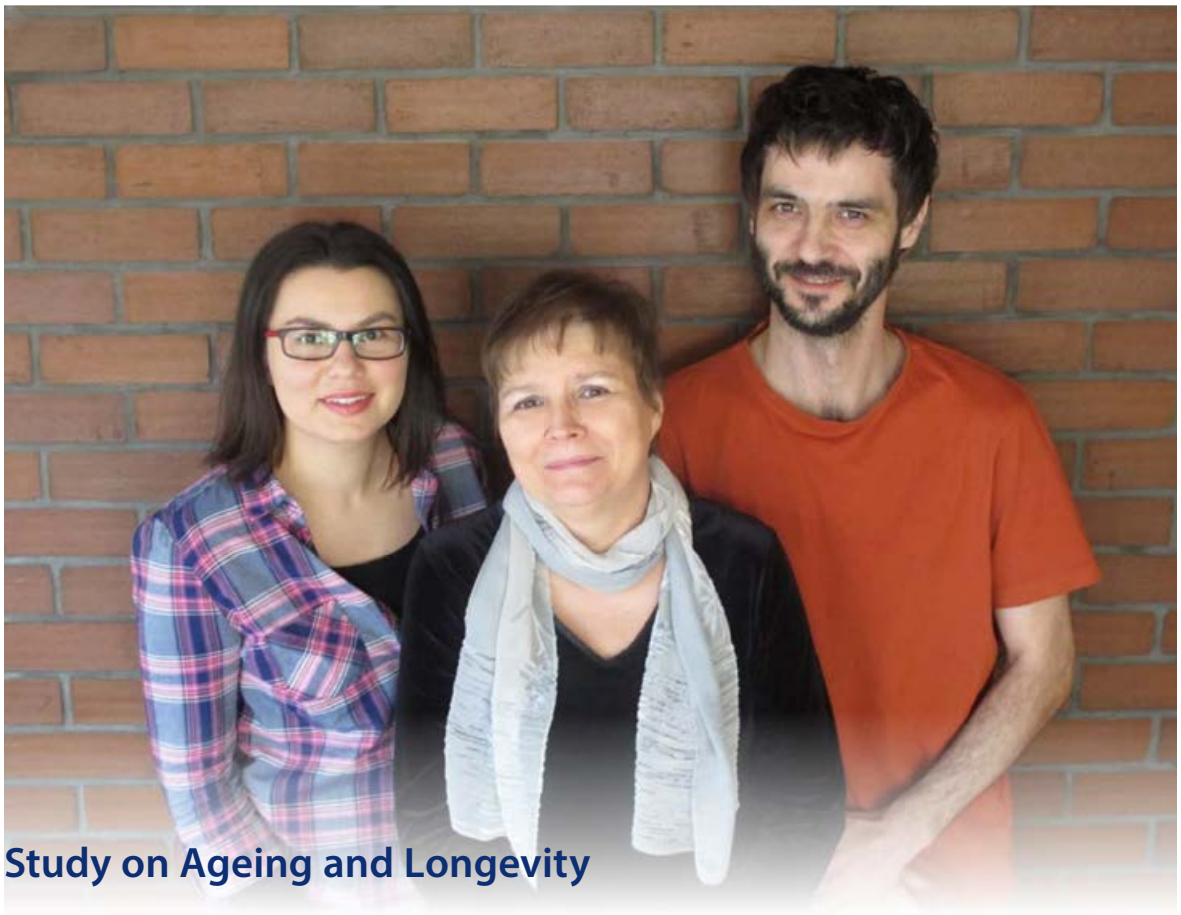
Research Assistant:
Paweł Mitkowski, MSc

A group headed by Dr. Izabela Sabała carries out a 3-year project *Biotechnological applications of bacteriolytic protein (Aurezyna)* which is financed by the National Center for Research and Development under the Applied Research Program. The funding was awarded to a consortium established by IIMCB (project leader) and A&A Biotechnology (commercial partner). While working on structural and biochemical characterization of an autolysin from *Staphylococcus aureus*, unusual and commercially valuable features of the enzyme were discovered: very efficient lysis of staphylococcal cells in unique environmental conditions of low temperature and exceptionally low ionic strength. The aim of the project is to explore commercial applications of the enzyme ranging from staphylococcal cell lysis allowing isolation of cellular components, through diagnostic tests, to a wide range of bacteriostatic and bacteriolytic applications, e.g. to eliminate staphylococci from food and hospital environment. In parallel, basic research focused on further structural and biochemical characterization of the protein is carried out to broaden our knowledge on regulation of activity and determination of enzyme specificity. Moreover, it will also guide enzyme engineering to expand its tolerance to environmental conditions and modulate the specificity.

As a result of the orchestrated efforts of our team together with internal (Bochtler Lab, Bujnicki Lab) and external collaborating groups (Nottingham University UK, Fritz Lipmann Institute, Jena, Germany) we have published a paper in the special issue of FEBS Journal presenting new information on structural organization of the studied enzyme (1) and presented these results on international meetings. Structural work is in progress bringing more information on the molecular mechanisms of substrate binding and recognition which is of particular importance for ongoing enzyme specificity engineering.

Moreover, a new line of research has emerged from the project. It is focused on generating new characteristics of bacteriolytic enzymes by combining various domains from different proteins. Such chimeric enzymes might be superior to natural enzymes in their specificity, efficiency and other characteristics. In 2014 this project was awarded by the Foundation for Polish Science Impuls programme supporting the commercial exploitation of research and promoting applied research.

**(1) Sabala I**, **Jagielska E**, Bardelang PT, Czapinska H, Dahms SO, Sharpe JA, James R, Than ME, Thomas NR, Bochtler M. Crystal structure of the antimicrobial peptidase lysostaphin from *Staphylococcus simulans*. ***FEBS J***. 2014; 281(18):4112-22.



# Study on Ageing and Longevity

Head: **Dr. Małgorzata Mossakowska**, DSc Habil

Project Assistant:
Aleksandra Szybalska

IT Specialist:
Przemysław Ślusarczyk

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 people aged 100+. Research on Polish centenarians was proposed by **Prof. Ewa Sikora** from the Nencki Institute of Experimental Biology of the Polish Academy of Sciences (PAS) 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 the 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 the 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 at 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 impacting 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 the Polish National Health Fund (NFZ), the project also contributed 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 the 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 foundation for pursuing these policies. The results of *PolSenior* were published in more than 30 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)*, lead by Genomed and financed by the National Centre for Research and Development (NCBR). The project is carried out in cooperation with the Mossakowski Medical Research Centre PAS (Department of Human Epigenetics) and 24 Godziny LLC. The project aims to determine 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 is carried out with the use of the biological material and clinical data yielded by the *PolStu* and the *PolSenior* projects. In 2013, the databases of the aforementioned projects were searched for a selection of healthy long-living individuals (aged 95 years or more). To enhance the previous study, a group of 300 centenarians and nonagenarians from Warsaw took part in the *PLGen* project, which continued till the end of September 2014.

Whole genome sequences from 130 individuals, of high quality and coverage (30x), were obtained from centenarians and nonagenarians, who were included in the *PLGen* project. These sequences were analyzed using a bioinformatic pipeline, allowing a parallel analysis of multiple genome sequences, designed in the project frame. The resulting information on single nucleotide and deletion/insertion variants is currently used to create records of the *Polish Reference Genome Database*.

**Selected publications:**


PolStu project

- **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; 69(10):1269-75

PolSenior project

- Kocelak P, Olszanecka-Glinianowicz M, Owczarek A, Bozentowicz-Wikarek M, Brzozowska A, **Mossakowska M**, Skalska A, Wiecek A, Chudek J. *Plasma visfatin/nicotinamide phosphoribosyltransferase (visfatin/NAMPT) concentration is not related to kidney function in elderly subjects.* ***Clin Chem Lab Med***. 2014. doi: 10.1515/cclm-2014-0574
- Olszanecka-Glinianowicz M, Owczarek A, Bożentowicz-Wikarek M, Brzozowska A, **Mossakowska M**, Zdrojewski T, Grodzicki T, Więcek A, Chudek J. *Relationship between circulating visfatin/NAMPT, nutritional status and insulin resistance in an elderly population – results from the PolSenior substudy.* ***Metabolism***. 2014; 63(11):1409-18
- Skalska A, Wizner B, Więcek A, Zdrojewski T, Chudek J, Klich-Rączka A, Piotrowicz K, Błędowski P, **Mossakowska M**, Michel JP, Grodzicki T. *Reduced functionality in everyday activities of patients with self-reported heart failure hospitalization – population-based study results.* ***Int J Cardiol***. 2014; 176(2):423-9
- Klich-Rączka A, Piotrowicz K, **Mossakowska M**, Skalska A, Wizner B, Broczek K, Wieczorowska-Tobis K, Grodzicki T. *The assessment of cognitive impairment suspected of dementia in Polish elderly people: results of the population-based PolSenior Study.* ***Exp Gerontol***. 2014; 57:233-42
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PLGen project

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# 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 **Prof. 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.

A consortium led by **Dr. Marcin Nowotny** was distinguished by the National Science Centre with a prestigious grant within the **SYMFONIA 2 Programme**. Dr. Nowotny received a funding for a project entitled *Mitochondrial RNA decay and surveillance - comprehensive interdisciplinary studies*. The project will be carried out in a consortium with the Institute of Biochemistry and Biophysics (Group Leader: Dr. Roman Szczęsny), Faculty of Biology, University of Warsaw (Group Leader: Prof. Paweł Golik) and Faculty of Mathematics, Informatics and Mechanics, University of Warsaw (Group Leader: Dr. Bartosz Wilczyński). SYMFONIA is a funding opportunity intended for exceptional established researchers wanting to carry out interdisciplinary or cross-domain research in collaboration with teams representing different areas of research.

**Prof. Janusz Bujnicki and Prof. Jacek Jaworski** are beneficiaries of the **MASTER/MISTRZ programme of the Foundation for Polish Science**. Prof. Bujnicki received funding for the project entitled *Functional studies on human and viral methyltransferases involved in RNA cap biosynthesis: from in silico docking and in vitro validation, to RNA methylation inhibition in human cells.* Prof. Jacek Jaworski have been awarded a grant for the project entitled *mTOR kinase and protein sorting by retromer and trans-Golgi network*. This year the academic grants for professors were awarded to eight leading researchers from the life sciences. The objective of the MISTRZ/MASTER programme is to support distinguished scholars by awarding them grants designed either to intensify the research they are already conducting or to explore new fields of research.

## 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.

### 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 Prof. Andrzej Dziembowski (IBB PAS) 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.

### eRNAza project within Applied Research Program, NCBR

A consortium led by **Prof. Janusz Bujnicki** won the competition of the National Centre for Research and Development (NCBR) for applied research projects (**Program Badań Stosowanych, PBS**). Prof. Bujnicki's project entitled *Development of new biotechnology products based on innovative technique of ribonucleic acid cleavage* received the top score among 120 competing proposals in track A competition in biological, agricultural, forest, and veterinary sciences. Planned research will be carried out in a consortium with A&A Biotechnology S.C., a Polish company in Gdynia (Group leader: Dr. Sławomir Dąbrowski). "Program Badań Stosowanych" is a funding opportunity intended for researchers interested in turning the results of their research to practical applications and supports collaboration between the academia and industry.

# Facts & Figures

# Grants

## 7th Framework Programme

### ERC Grants

- **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**
- **MorphoCorDiv,** "The inherent morphological potential of the actin cortex and the mechanics of shape control during cell division" ERC Starting Grant; (311637); 1,500,000 EUR; 2013-2018; **E. Paluch** (grant implemented at University College London, UK)

### 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; 2011-2015; **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**
- **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 regulationin 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**

## 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; 2009-2015; **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 zebrafi sh" (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 **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**

### 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; 2008–2015; **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" (UDAPOIG.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; 2009-2015; **J.M. Bujnicki** and **S. Filipek**

## National Centre for Research and Development (NCBR) Research Grants

- **Applied Research Programme (PBS)** "Development of new biotechnology products based on innovative technique of ribonucleic acid cleavage" (245550); 2,829,000 PLN (total grant budget: 3,316,441 PLN); 2015-2018, **J.M. Bujnicki**
- **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 PAS
- **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.

## National Science Centre (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**
- **SYMFONIA** "Mitochondrial RNA decay and surveillance - comprehensive interdisciplinary studies" (2014/12/W/NZ1/00463); 2,953,248 PLN (total grant budget: 6,879,968 PLN); 2014-2019; Coordinator **M. Nowotny**
- **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** "Coupling of synthesis and transport for proteins targeted to the mitochondria" (2013/11/B/NZ3/00974); 1,165,520 PLN; 2014-2017; **A. Chacińska**
- **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** (transfered to CENT, UW)
- **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; 2012-2016; **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; 2011-2015; **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** "Extramitochondrial factors regulating turnover of mitochondrial intermembrane space proteins" (2013/11/D/NZ3/02294); 796,100 PLN; 2014-2017; **P. Brągoszewski**
- **SONATA** "Patient-specific iPS cells as a novel approach to study patophysiology of mTOR related neurodvelopmental disorders" (2013/11/D/NZ3/01079); 700,000 PLN; 2014-2017; **E. Liszewska**
- **SONATA** "Identifi cation of post-transcriptional modifications in RNA sequences through mass spectrometry" (2012/05/D/ST/6/0382); 493,125 PLN; 2013-2014; **B. Kluge**
- **SONATA** "Determination of composition structure and substrate specifi city 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. Mierzejewska**
- **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; 2011-2015; **A. Urbańska**
- **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 diff erentiation into neurons?" (2012/04/S/NZ3/00264); 608,100 PLN; 2012-2015; **B. Tarkowski**

## Ministerial Research Grants

### Ideas Plus

- Coupling of synthesis and transport for proteins targeted to the mitochondria (000263); 3,156,000 PLN; 2014-2017; **A. Chacińska**

### 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**

### 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**
- "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**

# Scientific Meetings and Lectures

## Conferences organized

HEART of EUROPE ZEBRAFISH MEETING

17–19 September 2014, Warsaw, Poland

IIMCB organized a conference entitled ***Heart of Europe: Zebrafish Meeting*, chaired by Dr. Małgorzata Wiweger**. The meeting was attended by nearly 170 people from 25 countries **including some FishMed Partners**. The objective of this conference was to present academic research carried out with an extensive use of zebrafish (*Danio rerio*) in many areas of science. The main topics covered: behaviour, cancer and disease models, cell biology and cell migration, development and organogenesis, emerging technologies, husbandry and health, neurology, 'omics and bioinformatics, toxicology and chemical screens.

IIMCB, together with the Warsaw Medical University were co-organizers of the **V. CePT Conference *CePT project as a model of scientific synergy***. The conference summarized the whole CePT project; Profs. **Jacek Kuźnicki** and **Michał Witt** were the chairmen. The project *Centre for Preclinical Research and Technology (CePT)* is the biggest biomedical and biotechnological undertaking in Central and Eastern Europe. The aim of this project is to create a dynamic scientific centre in Warsaw consisting of closely cooperating environmental research centres, conducting research on the most common civilizational diseases, especially: neoplastic, neurological and vascular, as well as ageing and age – related diseases.

An international conference and workshop ***Primary ciliary dyskinesia (PCD) – modern diagnostic approaches*** was held in Cracow. The conference was organized within the European multi-centered project **BESTCILIA**, whose partner in Poland is **Prof. Michał Witt**. During the conference, lectures of internationally-recognized PCD experts were presented and a unique hands-on workshop on the most important methods used in PCD diagnostics was organized. The conference was attended by 65 participants from Europe and USA, including 35 clinicians from Poland.

IIMCB hosted ***BRAINSTORMING SESSION on TALEN and CRISPR/Cas9 based methods for genome modification*** organized by Anna Sokół. Speakers were: Zacharias Kontarakis, Andrea Rossi, Joanna Krwawicz and Małgorzata Perycz.

IIMCB hosted workshop within Polish-French-Ukrainian EU **project COMBIOM *Practical Training on IPR, Project Management and Equipment***. Young researchers from Ukraine practiced IPR, patenting and commercialization of scientific results; funding opportunities under the current EU Framework Programme - Horizon 2020, and projects management. Principles and on-site training of Mass Spectrometry and Fluorescence microscopy and BioCEN good practices in popularization of science were presented.


COMBIOM *Practical Training on IPR, Project Management and Equipment*, 22-26.09.2014.

**IIMCB hosted a meeting of the Life, Environmental and Geo Sciences (LEGS) panel of Science Europe**. Science Europe is an association of European Research Funding Organisations (RFO) and Research Performing Organisations (RPO), based in Brussels. Science Europe promotes the collective interests of the RFOs and RPOs.

## Regular IIMCB seminars

**Mariusz Jaskólski** (Institute of Bioorganic Chemistry, Polish Academy of Sciences, and Faculty of Chemistry, A. Mickiewicz University (UAM), Poznań, Poland) *New frontiers of macromolecular crystallography: ultrahigh resolution and superstructure modulation*, 09.01.2014

**Justyna Jezierska** (International Institute of Molecular and Cell Biology in Warsaw, Laboratory of Molecular and Cellular Neurobiology, Warsaw, Poland) *Elevated Dyn A levels cause Purkinje cells degeneration. Mechanisms underlying SCA23*, 16.01.2014

**Jochen C. Rink** (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany) *Regeneration in Planaria: Why some can while others can't*, 23.01.2014

**Bernd Bukau** (Zentrum fuer Molekulare Biologie der Universität Heidelberg (ZMBH), Heidelberg, Germany) *Cellular strategies for coping with protein aggregation*, 31.01.2014

**Daniela Panakova** (Max-Delbrück-Center for Molecular Medicine, Berlin, Germany) *Wnt non-canonical signaling patterns cardiac form and function*, 06.02.2014

**Piotr Wardęga** (NanoTemper Technologies GmbH, Munich, Germany) *Some like it hot – Biomolecule Analytics using Microscale Thermophoresis (MST)*, 07.02.2014

**Witold Konopka** (Laboratory of Animal Models Neurobiology Center, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland) *miR-103 protects against obesity*, 13.02.2014

**Michał Korostyński** (Department of Molecular Neuropharmacology Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland) *Regulation of inducible transcriptional networks in the brain*, 20.02.2014

**Maciej Olszewski** (International Institute of Molecular and Cell Biology in Warsaw, Department of Molecular Biology, Warsaw, Poland) *Beyond vesicles: clathrin, centrosome overduplication and senescence*, 27.02.2014

**Joanna Hoffmann** (University of Arts in Poznań; Leader of the Studio for Transdisciplinary Projects and Research, FAE/UAP; co-founder of CodersDojo Poznań, Leader of the Art & Science Node in Berlin) *Art in the Society of Knowledge*, 04.03.2014

**Magdalena Dziembowska** (Laboratory of Neurobiology, Nencki Institute of Experimental Biology & Centre of New Technologies, University of Warsaw, Poland) *Local translation at the synapse*, 06.03.2014

**Rafał Płoski** (Department of Medical Genetics, Centre for Biostructure, Medical University of Warsaw, Poland) *Next Generation Sequencing on Illumina HiSeq: Application in Clinical Genetics and in Analysis of DNA Methylation in Humans*, 13.03.2014

**Robert Hołyst** (Institute of Physical Chemistry Polish Academy of Sciences, Warsaw, Poland) *Protein mobility and cytoplasmatic viscosity in HeLa, Swiss 3T3 and E. coli cells and its influence on protein association and gene expression*, 20.03.2014

**Maria Siemionow** (Plastic Surgery Research and Microsurgical Training for Cleveland Clinic's Department of Plastic Surgery, Cleveland, USA) *Cell therapies in regenerative medicine and transplantation*, 26.03.2014

**Yegor Vassetzky** (CNRS research director, Head of the Laboratory of Chromatin, Development and Cancer, Institut Gustave Roussy, Paris, France) *Nuclear organization in lymphoid cells: implications for translocations and gene regulation*, 01.04.2014

**Rafal Ciosk** (Friedrich Miescher Institute for Biomedical Research Basel, Switzerland) *Regulation of pluripotency in animal development*, 03.04.2014

**Maximilian Fürthauer** (Valrose Institute of Biology, University of Nice Sophia-Antipolis, Nice, France) *A dual role of ESCRT proteins in the formation and function of ciliated organs*, 10.04.2014

**Andrzej M. Kierzek** (Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK) *Computer simulation of molecular cell biology*, 17.04.2014

**Christophe Lamaze** (Institute Curie, Paris, France) *Membrane dynamics and endosomal sorting in JAK/STAT signaling*, 24.04.2014

**Joanna Trylska** (Biomolecular Machines Laboratory, Centre of New Technologies, University of Warsaw, Poland) *Bacterial ribosome inhibitors*, 08.05.2014

**Stefano De Renzis** (European Molecular Biology Laboratory, Heidelberg, Germany) *Building cells and tissues during development*, 15.05.2014

**Ji-Joon Song** (Korea Advanced Institute of Science and Technology (KAIST), Department of Biological Sciences, Daejeon, Korea) *Mechanistic understanding on neurodegenerative disease proteins*, 29.05.2014

**Carlo Vascotto** (University of Udine, Department of Medical and Biological Sciences, Udine, Italy) *Molecular journey to unveil the secrets of a DNA repair protein*, 05.06.2014

**Heidi McBride** (Canada Research Chair and Killam Scholar Associate Professor, McGill University Department of Neurology and Neurosurgery Montreal, Canada) *Mitochondrial derived vesicles provide new insights into quality control and peroxisomal biology*, 13.06.2014

**Yiliang Ding** (Department of Cell and Developmental Biology, John Innes Centre, Norwich, UK) *Decipher the RNA structural code: A Transformative Platform Reveals Novel Regulatory Features*, 26.06.2014

**Charles Cantor** (Scripps Research Institute, La Jolla, and Sequenome Inc., USA) *Noninvasive personalized genomics part II*, 27.06.2014

**Stuart F.J. Le Grice** (RT Biochemistry Section, HIV Drug Resistance Program, National Cancer Institute – Frederick, Bethesda, USA) *HIV regulatory RNAs and their therapeutic targeting*, 23.07.2014

**Marina Mione** (Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany) *Zebrafish as a model for cancer*, 08.09.2014

**Dominika Borek** (University of Texas Southwestern Medical Center, Department of Biophysics, USA) *Challenges in molecular structure determination across different levels of cellular organization*, 25.09.2014

**Darren Gilmour** (European Molecular Biology Laboratory (EMBL) Heidelberg, Germany) *Novel Signaling Mechanisms Controlling Collective Cell Migration*, 09.10.2014

**Peter Rehling** (University Medical Centre Goettingen, Germany) *Biogenesis of mitochondrial membrane protein complexes*, 16.10.2014

**Paul Wyatt** (Drug Discovery Unit, College of Life Sciences, University of Dundee, UK) *The discovery of therapeutics for neglected diseases and the translation of novel biology through small molecule drug discovery*, 21.10.2014

**Franck Perez** (Department of Cell Biology, Institut Curie / CNRS Paris, France) *Systematic analysis of Golgi-dependent secretion in mammalian cells*, 23.10.2014

**Izabela Sumara** (Institute of Genetics and Molecular and Cellular Biology (IGBMC) Illkirch, France) *Mitotic ubiquitination pathways*, 20.11.2014

**Jean Gruenberg** (Biochemistry Department, University of Geneva, Switzerland) *Endosomal lipids in trafficking and signalling*, 11.12.2014

**Paweł Zawadzki** (David Sherratt's Lab, Department of Biochemistry, University of Oxford, UK) *Super-resolution microscopy and single-molecule FRET perspective on chromosome segregation in Escherichia coli*, 18.12.2014

## IIMCB Annual Report Session, 06.06.2014, Mierki, Poland

**Tomasz Węgierski** (Laboratory of Neurodegeneration) *Amyloid Precursor Protein in calcium homeostasis - How does it work?*

**Agnieszka Górnicka** (Laboratory of Mitochondrial Biogenesis) *TOM: Cruise of MIA-dependent proteins across the outer mitochondrial membrane*

**Asgar Abbas Kazrani** (Laboratory of Structural Biology) *Crystal structure of the 5hmC specific endonuclease PvuRts1I*

**Zuzanna Tracz- Gaszewska** (Department of Molecular Biology) *Molecular chaperones and chemoresistance of cancer cells with mutated TP53*

**Ewelina Szymańska** (Laboratory of Cell Biology) *Dynamin: a pinchase which guards the AP-1 activity*

**Ilona Domagala** (Laboratory of Bioinformatics and Protein Engineering) *Against bacterial resistance to antibiotics: Identification of novel inhibitors of ErmC' methyltransferase*

**Anna Malik** (Laboratory of Molecular and Cellular Neurobiology) *mTOR, S6-kinase and endocytosis – is there a link?*

**Karolina Górecka** (Laboratory of Protein Structure) *Structure and mechanism of RuvC Holliday junction resolvase*

**Jacek Kuźnicki** (Director), *Conclusions, Institute's matters*

# Publications in 2014/Q1 2015

## List of papers with IIMCB-affiliated main authors (first and/or corresponding)

| Lp. | Publication | 5 Year IF | Journal Category | Journal Rank in Category / Total Journals in Category | Quartile in Category |
|---|---|---|---|---|---|
| 1 | **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 Apr;21(4):389-96 | **12,338** | Biophysics | 2/74 | Q1 |
| 2 | **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; 5:3004 | **11,023** | Multidisciplinary Sciences | 3/55 | Q1 |
| 3 | **Chojnowski G, Walen T, Piatkowski P, Potrzebowski W, Bujnicki JM.** Brickworx builds recurrent RNA and DNA structural motifs into medium and low-resolution electron density maps. ***Acta Crystallogr D Biol Crystallogr*** 2015;71(Pt 3):697-705 | **9,416** | Crystallography | 1/23 | Q1 |
| 4 | **Głów D, Pianka D, Sulej AA, Kozłowski ŁP, Czarnecka J, Chojnowski G, Skowronek KJ, Bujnicki JM**. Sequence-specific cleavage of dsRNA by Mini-III RNase. ***Nucleic Acids Res.*** 2015;43(5):2864-73 | **8,378** | Biochemistry & Molecular Biology | 22/291 | Q1 |
| 5 | **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 | **8,378** | Biochemistry & Molecular Biology | 22/291 | Q1 |
| 6 | **Figiel M, Nowotny M**. Crystal structure of RNase H3-substrate complex reveals parallel evolution of RNA/DNA hybrid recognition. ***Nucleic Acids Res***. 2014; 42(14):9285-94 | **8,378** | Biochemistry & Molecular Biology | 22/291 | Q1 |
| 7 | **Kazrani AA, Kowalska M, Czapinska H, Bochtler M**. Crystal structure of the 5hmC specific endonuclease PvuRts1I. ***Nucleic Acids Res.*** 2014;42(9):5929-36 | **8,378** | Biochemistry & Molecular Biology | 22/291 | Q1 |
| 8 | **Majorek KA, Dunin-Horkawicz S**, Steczkiewicz K, Muszewska A, **Nowotny M**, Ginalski K, **Bujnicki JM.** The RNase H-like superfamily: new members, comparative structural analysis and evolutionary classification. ***Nucleic Acids Res.*** 2014;42(7):4160-79 | **8,378** | Biochemistry & Molecular Biology | 22/291 | Q1 |
| 9 | **Mierzejewska K, Siwek W, Czapinska H,** Kaus-Drobek M, Radlinska M, **Skowronek K, Bujnicki JM**, Dadlez M, **Bochtler M**. Structural basis of the methylation specificity of R.DpnI. ***Nucleic Acids Res.*** 2014;42(13):8745-54 | **8,378** | Biochemistry & Molecular Biology | 22/291 | Q1 |
| 10 | **Miętus M, Nowak E, Jaciuk M, Kustosz P, Studnicka J, Nowotny M.** Crystal structure of the catalytic core of Rad2: insights into the mechanism of substrate binding ***Nucleic Acids Res***. 2014;42(16):10762-75 | **8,378** | Biochemistry & Molecular Biology | 22/291 | Q1 |
| 11 | **Walen T, Chojnowski G**, Gierski P, **Bujnicki JM**. ClaRNA: a classifier of contacts in RNA 3D structures based on a comparative analysis of various classification schemes. ***Nucleic Acids Res***. 2014; 42(19):e151 | **8,378** | Biochemistry & Molecular Biology | 22/291 | Q1 |
| 12 | **Magnus M, Matelska D, Lach G, Chojnowski G, Boniecki MJ, Purta E, Dawson W, Dunin-Horkawicz S, Bujnicki JM**. Computational modeling of RNA 3D structures, with the aid of experimental restraints. ***RNA Biol***. 2014;11(5):522-36. | **5,488** | Biochemistry & Molecular Biology | 50/291 | Q1 |
| 13 | **Byszewska M, Smietanski M, Purta E, Bujnicki JM**. RNA methyl-transferases involved in 5'cap biosynthesis. ***RNA Biol.*** 2014;11(12):1597-607 | **5,448** | Biochemistry & Molecular Biology | 50/291 | Q1 |
| 14 | **Machnicka M, Olchowik A**, Grosjean H, **Bujnicki JM**. Distribution and frequencies of post-transcriptional modifications in tRNAs. ***RNA Biol.*** 2014;11(12):1619-29 | **5,448** | Biochemistry & Molecular Biology | 50/291 | Q1 |
| 15 | **Gornicka A, Bragoszewski P**, Chroscicki P, Wenz LS, Schulz C, Rehling P, **Chacinska A**. A discrete pathway for the transfer of intermembrane space proteins across the outer membrane of mitochondria. ***Mol Biol Cell***. 2014;25(25):3999-4009 | **5,154** | Cell Biology | 59/185 | Q2 |
| 16 | **Majewski L, Kuznicki J**. SOCE in neurons: Signaling or just refilling? ***BBA-Mol Cell Res***. 2015 Jan 31. pii: S0167-4889(15)00034-8. doi: 10.1016/j.bbamcr.2015.01.019 | **4,814** | Biochemistry & Molecular Biology | 52/291 | Q1 |
| 17 | **Wojciechowski M, Rafalski D**, Kucharski R, **Misztal K**, Maleszka J, **Bochtler M**, Maleszka R. Insights into DNA hydroxymethylation in the honeybee from in-depth analyses of TET dioxygenase. ***Open Biol.*** 2014;4(8). pii: 140110. | **4,556** | Biochemistry & Molecular Biology | 67/291 | Q1 |

| Lp. | Publication | 5 Year IF | Journal Category | Journal Rank in Category / Total Journals in Category | Quartile in Category |
|---|---|---|---|---|---|
| 18 | **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.*** 2014;65(3):310-9 | **4,197** | Biochemical Research Methods | 24/78 | Q2 |
| 19 | **Sabala I, Jagielska E**, Bardelang PT, **Czapinska H**, Dahms SO, Sharpe JA, James R, Than ME, Thomas NR, **Bochtler M**. Crystal structure of the antimicrobial peptidase lysostaphin from Staphylococcus simulans. ***FEBS J***. 2014;281(18):4112-22 | **3,673** | Biochemistry & Molecular Biology | 85/291 | Q2 |
| 20 | **Banach-Orlowska M, Szymanska E, Miaczynska M**. APPL1 endocytic adaptor as a fine tuner of Dvl2-induced transcription. ***FEBS Lett.*** 2015;589(4):532-9 | **3,47** | Biophysics | 25/74 | Q2 |
| 21 | **Sokol AM, Sztolsztener ME, Wasilewski M**, Heinz E, **Chacinska A**. Mitochondrial protein translocases for survival and wellbeing. ***FEBS Lett***. 2014;588(15):2484-95 | **3,47** | Biophysics | 25/74 | Q2 |
| 22 | **Szczepaniak K, Lach G, Bujnicki JM, Dunin-Horkawicz S**.Designability landscape reveals sequence features that define axial helix rotation in four-helical homo-oligomeric antiparallel coiled-coil structures. ***J Struct Biol.*** 2014; pii: S1047-8477(14)00193-2 | **3,407** | Biophysics | 24/74 | Q2 |
| 23 | **Winata CL,** Kondrychyn I, Korzh V.Changing Faces of Transcriptional Regulation Reflected by Zic3. ***Current Genomics***. 2015;16(2): 117-127 | **2,987** | Genetics & Heredity | 69/165 | Q2 |
| 24 | Philips A, **Lach G, Bujnicki JM**. Computational methods for prediction of RNA interactions with metal ions and small organic ligands**.** ***Methods Enzymol***. 2015;553:261-85 | **2,152** | Biochemical Research Methods | 42/78 | Q3 |
| 25 | **Sadowski Ł, Jastrzębski K, Purta E,** Hellberg C**, Miaczynska M**. Labeling of platelet-derived growth factor by reversible biotinylation to visualize its endocytosis by microscopy. ***Methods Enzymol.*** 2014;535:167-77 | **2,152** | Biochemical Research Methods | 42/78 | Q3 |

## List of papers without IIMCB-affiliated main authors (first and/or corresponding)

| Lp. | Publication | 5 Year IF | Journal Category | Journal Rank in Category / Total Journals in Category | Quartile in Category |
|---|---|---|---|---|---|
| 1 | Ieva R, Schrempp SG, Opaliński L Wollweber F, Höß P, Heißwolf AK, Gebert M, Zhang Y, Guiard B, Rospert S, Becker T, **Chacinska A**, Pfanner N, van der Laan MMgr2 functions as lateral gatekeeper for preprotein sorting in the mitochondrial inner membrane. ***Mol Cell.*** 2014 ;56(5):641-52 | **15,324** | Biochemistry & Molecular Biology | 5/291 | Q1 |
| 2 | Mathys H, Basquin J, Ozgur S, Czarnocki-Cieciura M, Bonneau F, Aartse A, Dziembowski A, **Nowotny M**, Conti E, Filipowicz W. Structural and biochemical insights to the role of the CCR4-NOT complex and DDX6 ATPase in microRNA repression. ***Mol Cell.*** 2014; 54(5):751-65 | **15,324** | Biochemistry & Molecular Biology | 5/291 | Q1 |
| 3 | Geiger JC, **Lipka J**, Segura I, Hoyer S, Schlager MA, Wulf PS, Weinges S, Demmers J, Hoogenraad CC, Acker-Palmer A.The GRIP1/14-3-3 Pathway Coordinates Cargo Trafficking and Dendrite Development. ***Dev Cell***. 2014;28(4):381-93 | **13,012** | Developmental Biology | 3/41 | Q1 |
| 4 | Mills F, Bartlett TE, Dissing-Olesen L, **Wisniewska MB**, **Kuznicki J**, Macvicar BA, Wang YT, Bamji SXCognitive flexibility and long-term depression (LTD) are impaired following β-catenin stabilization in vivo. ***P Natl Acad Sci USA.*** 2014;111(23):8631-6 | **10,727** | Multidisciplinary Sciences | 4/55 | Q1 |
| 5 | Pfanner N, van der Laan M, Amati P, Capaldi RA, Caudy AA, **Chacinska A**, Darshi M, Deckers M, Hoppins S, Icho T, Jakobs S, Ji J, Kozjak-Pavlovic V, Meisinger C, Odgren PR, Park SK, Rehling P, Reichert AS, Sheikh MS, Taylor SS, Tsuchida N, van der Bliek AM, van der Klei IJ, Weissman JS, Westermann B, Zha J, Neupert W, Nunnari J. Uniform nomenclature for the mitochondrial contact site and cristae organizing system. ***J Cell Biol***. 2014;204(7):1083-6 | **10,437** | Cell Biology | 21/185 | Q1 |
| 6 | Bovellan M, Romeo Y, Biro M, Boden A, Chugh P, Yonis A, Vaghela M, Fritzsche M, Moulding D, Thorogate R, Jégou A, Thrasher AJ, Romet-Lemonne G, Roux PP, **Paluch EK**, Charras G. Cellular control of cortical actin nucleation. ***Curr Biol.*** 2014 Jul 21;24(14):1628-35 | **10,227** | Biochemistry & Molecular Biology | 19/291 | Q1 |

| Lp. | Publication | 5 Year IF | Journal Category | Journal Rank in Category / Total Journals in Category | Quartile in Category |
|---|---|---|---|---|---|
| 7 | RNAcentral Consortium (Petrov AI, Kay SJE, Gibson R, Kulesha E, Staines D, Bruford EA, Wright MW, Burge S, Finn R, Kersey PJ, Cochrane G, Bateman A, Griffiths-Jones S, Harrow J, Chan PP, Lowe TM, Zwieb CW, Wower J, Williams KP, Hudson CM, Gutell R, Clark MB, Dinger M, Cheng X, **Bujnicki JM**, Chua N, Liu J, Wang H, Skogerbø G, Zhao Y, Chen R, Zhu W, Cole JR, Chai B, Huang H, Huang H, Cherry JM, Pruitt KD). RNAcentral: an international database of ncRNA sequences. ***Nucleic Acids Res***. 2015 Jan;43(Database issue):D123-9 | **8,378** | Biochemistry & Molecular Biology | 22/291 | Q1 |
| 8 | Zheng H, Shabalin I, **Handing K, Bujnicki JM,** Minor M. Magnesium binding architectures in RNA crystal structures: validation binding preferences, classification, and motif detection. ***Nucleic Acids Res***. 2015. pii: gkv225. [Epub ahead of print] | **8,378** | Biochemistry & Molecular Biology | 22/291 | Q1 |
| 9 | Tomecki R, Drazkowska K, Kucinski I, Stodus K, Szczesny RJ, **Gruchota J**, Owczarek EP, Kalisiak K, Dziembowski A. Multiple myeloma-associated hDIS3 mutations cause perturbations in cellular RNA metabolism and suggest hDIS3 PIN domain as a potential drug target ***Nucleic Acids Res***. 2014;42(2):1270-90 | **8,378** | Biochemistry & Molecular Biology | 22/291 | Q1 |
| 10 | Jentschura UD, **Łach G,** De Kieviet M, Pachucki K. One-Loop Dominance in the Imaginary Part of the Polarizability: Application to Blackbody and Noncontact van der Waals Friction. ***Phys Rev Lett.*** 2015;114(4):043001 | **7,411** | Physics, Multidisciplinary | 6/78 | Q1 |
| 11 | Toczyłowska-Mamińska R, Olszewska A, Laskowski M, Bednarczyk P, **Skowronek K**, Szewczyk APotassium channel in the mitochondria of human keratinocytes. ***J Invest Dermatol.*** 2014;134(3):764-72 | **6,113** | Dermatology | 1/61 | Q1 |
| 12 | Skupien A, Konopka A, Trzaskoma P, Labus J, Gorlewicz A, **Swiech L**, Babraj M, Dolezyczek H, Figiel I, Ponimaskin E, Wlodarczyk J, **Jaworski J**, Wilczynski GM, Dzwonek J. CD44 regulates dendrite morphogenesis through Src tyrosine kinase-dependent positioning of the Golgi. ***J Cell Sci.,*** 2014; 127(23):5038-5051 | **6,007** | Cell Biology | 46/185 | Q1 |
| 13 | **Kurkowiak M**, Ziętkiewicz E, **Witt M**. Recent advances in primary ciliary dyskinesia genetics. ***J Med Genet***. 2015;52(1):1-9 | **5,64** | Genetics & Heredity | 19/165 | Q1 |
| 14 | Melin J, Schulz C, Wrobel L, Bernhard O, **Chacinska A**, Jahn O, Schmidt B, Rehling P. Presequence recognition by the tom40 channel contributes to precursor translocation into the mitochondrial matrix. ***Mol Cell Biol.*** 2014;34(18):3473-85 | **5,614** | Biochemistry & Molecular Biology | 55/291 | Q1 |
| 15 | Skalska A, Wizner B, Więcek A, Zdrojewski T, Chudek J, Klich-Rączka A, Piotrowicz K, Błędowski P, **Mossakowska M**, Michel JP, Grodzicki T. Reduced functionality in everyday activities of patients with self-reported heart failure hospitalization - Population-based study results. ***Int J Cardiol.*** 2014;176(2):423-9 | **5,101** | Cardiac & Cardiovascular Systems | 11/125 | Q1 |
| 16 | **Mossakowska M** , Broczek K, Wieczorowska-Tobis K, Klich-Raczka A, Jonas M, Pawlik-Pachucka E, Safranow K, **Kuznicki J** , Puzianowska-Kuznicka MCognitive Performance and Functional Status Are the Major Factors Predicting Survival of Centenarians in Poland. **J Gerontol A-Biol**. 2014;69(10):1269-75 | **5,023** | Geriatrics & Gerontology | 3/49 | Q1 |
| 17 | Geremek M, Ziętkiewicz E, Bruinenberg M, Franke L, Pogorzelski A, Wijmenga C, **Witt M**. Ciliary genes are down-regulated in bronchial tissue of primary ciliary dyskinesia patients. ***PLoS One***. 2014; 9(2):e88216 | **5,015** | Multidisciplinary Sciences | 8/55 | Q1 |
| 18 | Grabowska AD, **Wywiał E, Dunin-Horkawicz S**, Lasica AM, Wösten MM, Nagy-Staroń A, Godlewska R, Bocian-Ostrzycka K, Pieńkowska K, Laniewski P, **Bujnicki JM**, van Putten JP, Jagusztyn-Krynicka EK. Functional and Bioinformatics Analysis of Two Campylobacter jejuni Homologs of the Thiol-Disulfide Oxidoreductase, DsbA. ***PLoS One.*** 2014;9(9):e106247 | **5,015** | Multidisciplinary Sciences | 8/55 | Q1 |
| 19 | Ziętkiewicz E, Rutkiewicz E, Pogorzelski A, Klimek B, Voelkel K, **Witt M**. CFTR Mutations Spectrum and the Efficiency of Molecular Diagnostics in Polish Cystic Fibrosis Patients. ***PLoS One.*** 2014;9(2):e89094 | **5,015** | Multidisciplinary Sciences | 8/55 | Q1 |
| 20 | Sierocka I, **Kozlowski LP, Bujnicki JM**, Jarmolowski A, Szweykowska-Kulinska Z Female-specific gene expression in dioecious liverwort Pellia endiviifolia is developmentally regulated and connected to archegonia production. ***BMC Plant Biol***. 2014;14(1):168 | **4,758** | Plant Sciences | 22/199 | Q1 |

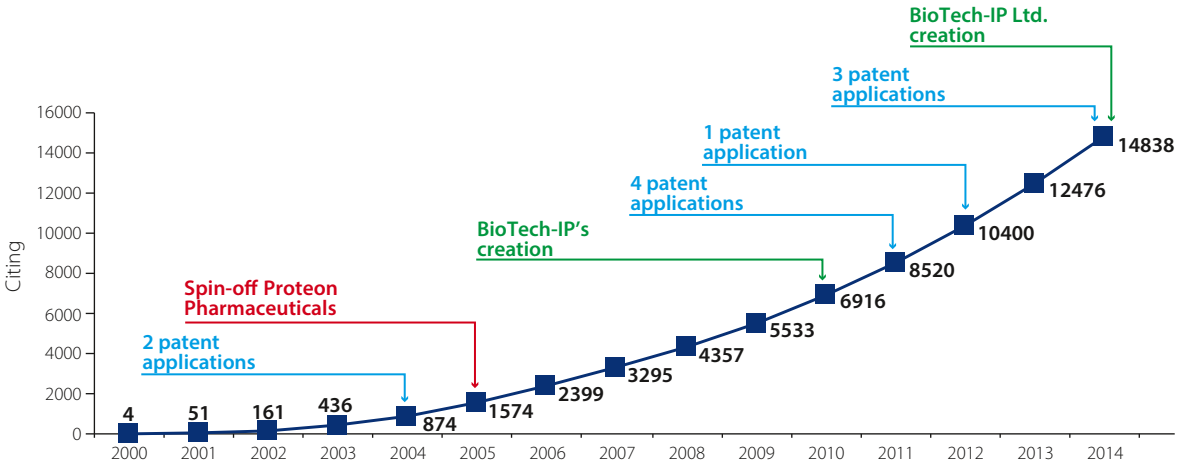
| Lp. | Publication | 5 Year IF | Journal Category | Journal Rank in Category / Total Journals in Category | Quartile in Category |
|---|---|---|---|---|---|
| 21 | Plotka M, Kaczorowska AK, Stefanska A, Morzywolek A, Fridjonsson OH, ***Dunin-Horkawicz S, Kozlowski L***, Hreggvidsson GO, Kristjansson JK, Dabrowski S, **Bujnicki JM**, Kaczorowski T. Novel highly thermostable endolysin from Thermus scotoductus MAT2119 bacteriophage Ph2119 with amino acid sequence similarity to eukaryotic peptidoglycan recognition proteins. ***Appl Environ Microbiol.*** 2014;80(3):886-95 | **4,486** | Biotechnology & Applied Microbiology | 30/165 | Q1 |
| 22 | Chudek J, Kocełak P, Owczarek A, Bozentowicz-Wikarek M, **Mossakowska M**. Fibroblast growth factor 23 (FGF23) and early chronic kidney disease in the elderly. ***Nephrol Dial Transplant***. 2014;29(9):1757-63 | **3,486** | Urology & Nephrology | 11/77 | Q1 |
| 23 | Honarnejad K, Daschner A, Gehring AP, Szybinska A, Giese A, **Kuznicki J**, Bracher F, Herms J. Identification of tetrahydrocarbazoles as novel multifactorial drug candidates for treatment of Alzheimer's disease. ***Transl Psychiatry***. 2014;4:e489 | **4,36** | Psychiatry | 24/136 | Q1 |
| 24 | Brendel M, **Jaworska A**, Grießinger E, Rötzer C, Burgold S, Gildehaus FJ, Carlsen J, Cumming P, Baumann K, Haass C, Steiner H, Bartenstein P, Herms J, Rominger A. Cross-Sectional Comparison of Small Animal [18F]-Florbetaben Amyloid-PET between Transgenic AD Mouse Models. ***PLoS One***. 2015 Feb 23;10(2):e0116678 | **4,015** | Multidisciplinary Sciences | 8/55 | Q1 |
| 25 | Kolanczyk M, Krawitz P, Hecht J, **Hupalowska A**, **Miaczynska M,** Marschner K, Schlack C, Emerich D, Kobus K, Kornak U, Robinson PN, Plecko B, Grangl G, Uhrig S, Mundlos S, Horn D. Missense variant in CCDC22 causes X-linked recessive intellectual disability with features of Ritscher-Schinzel/3C syndrome. ***Eur J Hum Genet.*** 2014; doi: 10.1038/ejhg.2014.109 | **3,943** | Genetics & Heredity | 38/165 | Q1 |
| 26 | Klich-Rączka A, Piotrowicz K, **Mossakowska M**, Skalska A, Wizner B, Broczek K, Wieczorowska-Tobis K, Grodzicki T. The assessment of cognitive impairment suspected of dementia in Polish elderly people: results of the population-based PolSenior Study. ***Exp Gerontol.*** 2014;57:233-42 | **3,797** | Geriatrics & Gerontology | 8/49 | Q1 |
| 27 | Laczmanski L, Milewicz A, Puzianowska-Kuznicka M, Lwow F, Kolackov K, Mieszczanowicz U, Pawlak M, Krzyzanowska-Swiniarska B, Bar-Andziak E, Chudek J, **Mossakowska M**. Interrelation between genotypes of the vitamin D receptor gene and serum sex hormone concentrations in the Polish elderly population: the PolSenior study. ***Exp Gerontol.*** 2014;57:188-90 | **3,797** | Geriatrics & Gerontology | 8/49 | Q1 |
| 28 | **Ghoshdastider U, Wu RL**, Trzaskowski B, Mlynarczyk K, Miszta P, Gurusaran M, Viswanathan S, Renugopalakrishnan V, Filipek S. Molecular effects of encapsulation of glucose oxidase dimer by graphene. ***RSC Adv***. 2015;5:13570-13578 | **3,708** | Chemistry, Multidisciplinary | 35/148 | Q1 |
| 29 | Jorstad A, Nigro B, Cali C, **Wawrzyniak M**, Fua P, Knott G. NeuroMorph: A Toolset for the Morphometric Analysis and Visualization of 3D Models Derived from Electron Microscopy Image Stacks. ***Neuroinformatics.*** 2015;13(1):83-92 | **2,972** | Computer Science, Interdisciplinary Applications | 12/102 | Q1 |
| 30 | 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 | **3,407** | Biophysics | 24/74 | Q2 |
| 31 | Iakoubov L, **Mossakowska M**, Szwed M, Puzianowska-Kuznicka M. A Common Copy Number Variation Polymorphism in the CNTNAP2 Gene: Sexual Dimorphism in Association with Healthy Aging and Disease. ***Gerontology***. 2015;61(1):24-31 | **3,114** | Geriatrics & Gerontology | 20/49 | Q2 |
| 32 | Olszanecka-Glinianowicz M, Owczarek A, Bożentowicz-Wikarek M, Brzozowska A, **Mossakowska M**, Zdrojewski T, Grodzicki T, Więcek A, Chudek J. Relationship between circulating visfatin/NAMPT, nutritional status and insulin resistance in an elderly population - results from the PolSenior substudy. ***Metabolism.*** 2014;63(11):1409-18 | **3,008** | Endocrinology & Metabolism | 44/124 | Q2 |
| 33 | Kocelak P, Olszanecka-Glinianowicz M, Owczarek A, Bożentowicz-Wikarek M, Brzozowska A, **Mossakowska M,** Zdrojewski T, Grodzicki T, Więcek A, Chudek J. Plasma visfatin/nicotinamide phosphoribosyltransferase levels in hypertensive elderly - results from the PolSenior substudy. ***J Am Soc Hypertens.*** 2015;9(1):1-8 | **2,53** | Peripheral Vascular Disease | 31/65 | Q2 |

| Lp. | Publication | 5 Year IF | Journal Category | Journal Rank in Category / Total Journals in Category | Quartile in Category |
|---|---|---|---|---|---|
| 34 | Tarnowski K, Fituch K, **Szczepanowski RH**, Dadlez M, Kaus-Drobek M. Patterns of structural dynamics in RACK1 protein retained throughout evolution: A hydrogen-deuterium exchange study of three orthologs. ***Protein Sci.*** 2014;23(5):639-51 | **3,029** | Biochemistry & Molecular Biology | 146/291 | Q3 |
| 35 | Gallagher JM, Yamak A, Kirilenko P, Black S, **Bochtler M**, Lefebvre C, Nemer M, Latinkić BV. Carboxy terminus of GATA4 transcription factor is required for its cardiogenic activity and interaction with CDK4. ***Mech Develop.*** 2014;134:31-41 | **2,426** | Developmental Biology | 26/41 | Q3 |
| 36 | Laczmanski L, Lwow F, **Mossakowska M,** Puzianowska-Kuznicka M, Szwed M, Kolackov K, Krzyzanowska-Swiniarska B, Bar-Andziak E, Chudek J, Sloka N, Milewicz A. Association between vitamin D concentration and levels of sex hormones in an elderly Polish population with different genotypes of VDR polymorphisms (rs10735810, rs1544410, rs7975232, rs731236). ***Gene***. 2015;559(1):73-6 | **2,246** | Genetics & Heredity | 106/165 | Q3 |
| 37 | Roszkowska-Gancarz M, Jonas M, Owczarz M, Kurylowicz A, Polosak J, Franek E, **Slusarczyk P, Mossakowska M,** Puzianowska-Kuznicka M. Age-related changes of leptin and leptin receptor variants in healthy elderly and long-lived adults. ***Geriatr Gerontol Int.*** 2015 Mar;15(3):365-71. doi: 10.1111/ggi.12267. Epub 2014 Feb 26 | **1,724** | Geriatrics & Gerontology | 30/49 | Q3 |
| 38 | Bednarska-Makaruk M, Rodo M, Szirkowiec W, **Mossakowska M**, Puzianowska-Kuźnicka M, Skalska A, Zdrojewski T, Ryglewicz D, Wehr H. Paraoxonase 1 activity and level of antibodies directed against oxidized low density lipoproteins in a group of an elderly population in Poland - PolSenior study. ***Arch Gerontol Geriatr***. 2015;60(1):153-61 | **1,694** | Geriatrics & Gerontology | 31/49 | Q3 |
| 39 | Crochemore M, Iliopoulos CS, Kubica M, Radoszewski J, Rytter W, Stencel K, **Walen T**. New simple efficient algorithms computing powers and runs in strings. ***Discrete Applied Mathematics***. 2014; 163(3):258–267 | **0,838** | Mathematics, Applied | 146/251 | Q3 |
| 40 | Prajsner A, Chudek J, **Szybalska A,** Piotrowicz K, Zejda J, Więcek A, PolSenior Study Group. Socioeconomic profile of elderly Polish men treated for benign prostate hyperplasia: Results of the population-based PolSenior study. ***European Geriatric Medicine.*** 2015, 6:53-57 | **0,613** | Geriatrics & Gerontology | 47/49 | Q4 |
| 41 | Labuz-Roszak B, Skrzypek M, Pierzchała K, Machowska-Majchrzak A, **Mossakowska M,** Chudek J, Mańka-Gaca I, Bartman W, Więcek ASecondary prevention of stroke in elderly people in Poland-Results of PolSenior study. ***Neurol Neurochir Pol***. 2014;48(2):85-90 | **0,587** | Clinical Neurology | 178/194 | Q4 |

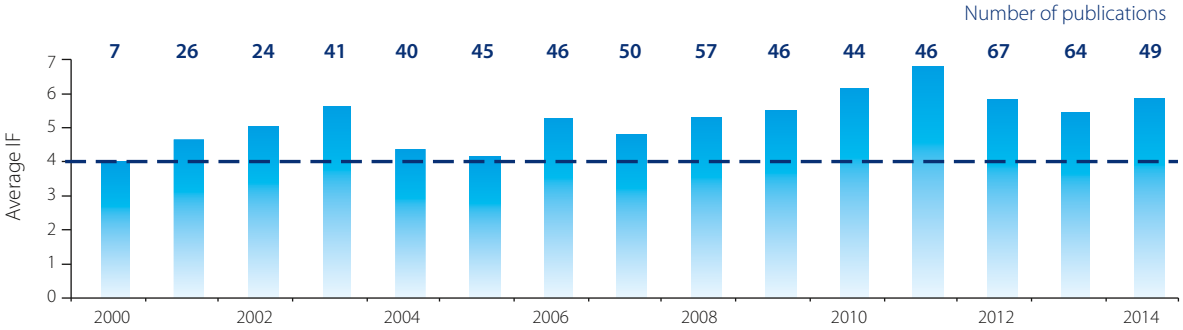
## List of other papers

| Lp. | Publication |
|---|---|
| 1 | Nowis D, Malenda A, Furs K, Oleszczak B, Sadowski R, Chlebowska J, Firczuk M, **Bujnicki JM**, Staruch AD, Zagozdzon R, Glodkowska-Mrowka E, Szablewski L, Golab J. Statins impair glucose uptake in human cells. ***BMJ Open Diabetes Res Care***. 2014;2(1):e000017 |
| 2 | Ramos-Molina B, Lambertos A, Lopez-Contreras AJ, Kasprzak JM, Czerwoniec A, **Bujnicki JM**, Cremades A, Penafiel R. Structural and degradative aspects of ornithine decarboxylase antizyme inhibitor 2. ***FEBS OpenBio*** 2014;4:510-21 |
| 3 | **Rother K,** Rother M, Skiba P, Bujnicki JM. Automated modeling of RNA 3D structure. ***Methods Mol Biol***. 2014;1097:395-415 |
| 4 | Skubiszewska A, Wodzyńska K, **Szybalska A**, Ślusarczyk P, Broczek K, Puzianowska-Kuźnicka M, Olędzka G, **Mossakowska M**. Ocena sprawności funkcjonalnej warszawskich stulatków w zakresie podstawowych czynności życia codziennego – wyniki wstępne. ***Gerontologia Polska*** 2014, 3:151-5 |
| 5 | **Liszewska E, Jaworski J**. Czy można zrobić mózg ze skóry? Wszechświat 2014, 115 (1-3): 24-29 |
| 6 | Roszkowska-Gancarz M, Kurylowicz A, Polosak J, **Mossakowska M**, Franek E, Puzianowska-Kuźnicka M. Functional polymorphisms of the leptin and leptin receptor genes are associated with longevity and with the risk of myocardial infarction and of type 2 diabetes mellitus. ***Endokrynol Pol.*** 2014;65(1):11-6 |

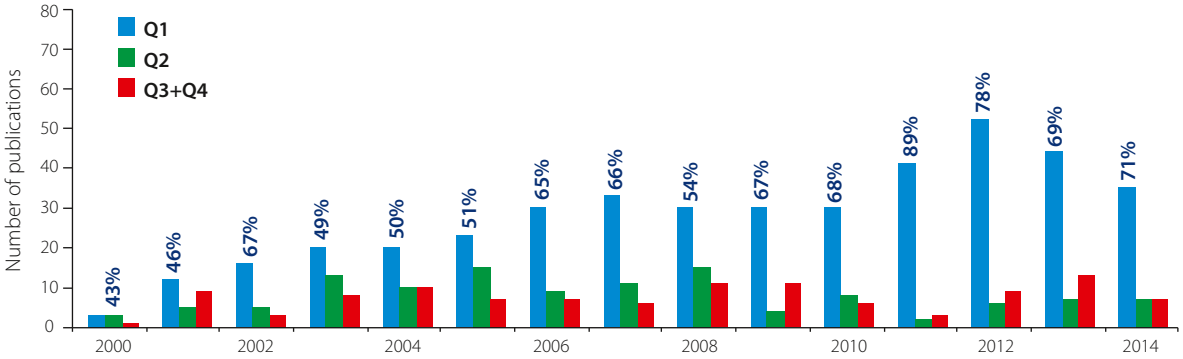
## Cumulative citations, patent applications and spin-offs (2000-2014)
## Hirsch index = 60




## Number and average IF of journals with IIMCB's publications 2000-2014

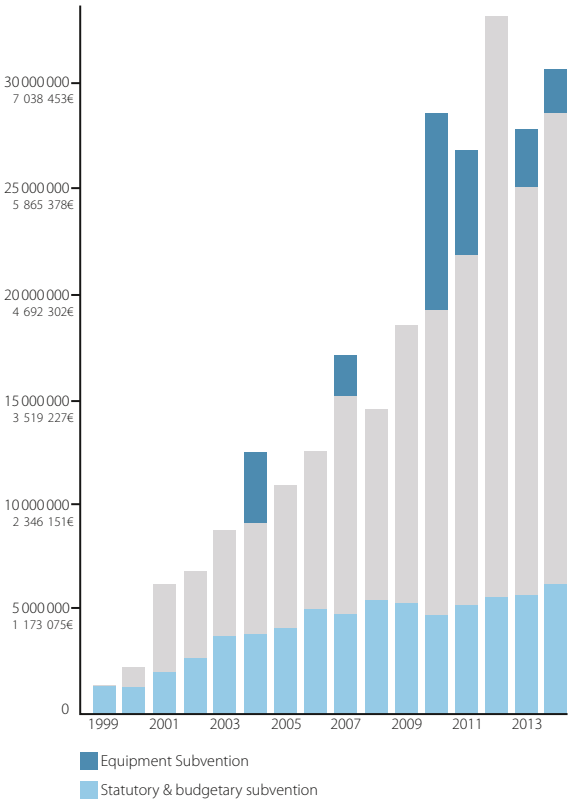



## Number of publications in Quartiles (Q) in Journals Category and % of Q1



# Diversity of Funding IIMCB'2014

## Annual Income in PLN (and Euro)



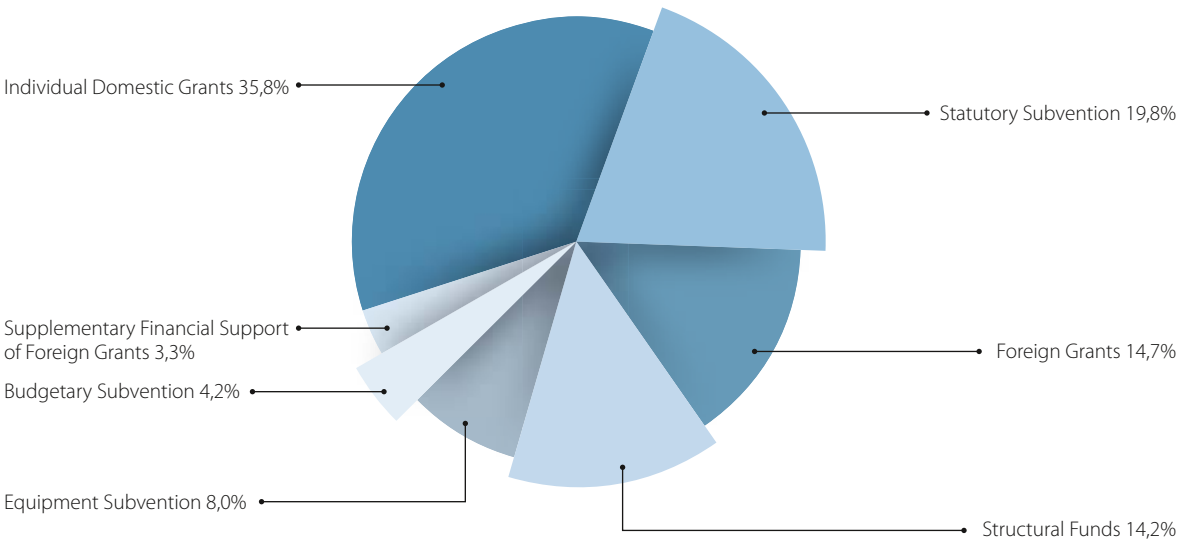

## Profit & loss statement (amounts in PLN)

| | amounts in PLN |
|---|---|
| **A. Net revenue on sales and equivalents*** | 30 270 171 |
| **B. Operational activity costs:** | 30 872 529 |
| Depreciation (equipment) | 850 054 |
| Research materials | 8 507 390 |
| Utilities | 578 446 |
| Services | 3 570 067 |
| Fees and taxes | 767 325 |
| Salaries and wages | 11 959 751 |
| Social and health insurance | 3 049 624 |
| Other operational expenses, in this: | 1 589 873 |
| business trips | 1 032 848 |
| property insurance | 29 274 |
| fellowships | 527 577 |
| others | 174 |
| **C. Other operational income (subventions)** | 618 201 |
| **D. Other operational expenses** | 73 |
| **E. Financial income (interests)** | 181 455 |
| **F. Financial expenses (others)** | 86 786 |
| **Profit on business activity (A-B+C-D+E-F)** | **110 439** |

## Sources of Funding

| | amounts in PLN | amounts in EUR(1) |
|---|---|---|
| Statutory Subvention | 6 055 070 | 1 420 611 |
| Budgetary Subvention | 1 274 000 | 298 900 |
| Individual Domestic Grants | 10 951 024 | 2 569 276 |
| Structural Funds | 4 355 484 | 1 021 862 |
| Supplementary Financial Support of Foreign Grants | 1 017 671 | 238 761 |
| Foreign Grants | 4 484 037 | 1 052 023 |
| Equipment Subvention | 2 446 144 | 573 902 |
| **Total** | **30 583 430** | **7 175 335** |

(1) 1 EUR - 4,2623 @ 31st Dec'2014



# Education

# Educational Activities

IIMCB continues its doctoral program in partnership with other institutions of the Ochota Campus. Currently 43 PhD students are on board within the doctoral programs of the two Warsaw research institutes: Institute of Biochemistry and Biophysics PAS (IBB) and the Nencki Institute of Experimental Biology PAS. The PhD students of IIMCB are self-organized as a group with the representative Dawid Głów. The postdoctoral fellows are similarly self-organized with group representatives Elżbieta Purta and Karolina Górecka and their meetings are devoted to the presentation of personal experience of the young scientists.

Opening of the Academic Year 2014/2015 at Biocentrum Ochota, 17.10.2014.

On October 17th the Opening of the Academic Year 2014/2015 was held at Biocentrum Ochota. The PhD students of IIMCB, IBB and the Nencki Instiute organized the ceremony with the invited keynote speakers, **Prof. Andrzej Udalski** and **Prof. Virginijus Siksnys**. The event started with the awards ceremony for the best PhD dissertations of year 2013. The opening lecture "Extrasolar Planetary Systems" was delivered by Prof. Andrzej Udalski from the Warsaw University Poland. The next lecture "CRISPR - Cas craze: from microbial antiviral defense system to genome editing" was presented by Prof. Virginijus Siksnys from the Vilnius University, Lithuania. There was a poster session with a competition for the best poster. The ceremony was honored by the presence of Prof. Michał Kleiber, the President of the Polish Academy of Sciences and directors of all individual institutes of Biocentrum Ochota. More than 150 PhD students of Biocentrum Ochota participated in the event. Furthermore, PhD students from Institute of Molecular Biology and Genetics (Ukraine) accepted the invitation and joined the Opening. The event was supported by all institutes of Biocentrum Ochota. Moreover, IIMCB supported Prof. Siksnys' visit and VitaInSilica, Bio-Rad, Eppendorf were the sponsors of the awards.

## 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 PAS 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)

## Theses defended

- **Marcin Jaciuk**, PhD thesis: *Structural studies of UvrA - bacterial DNA repair protein*, thesis advisor: M.Nowotny, 10.02.2015, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland
- **Andrzej Nagalski**, PhD thesis: *Transcription factors regulatory network in the mouse thalamic complex*, thesis advisor: M. Wiśniewska, 04.04.2014, Nencki Institute of Experimental Biology PAS, Warsaw, Poland
- **Dorota Matelska**, PhD thesis: *Identification and comparative analysis of selected cis-regulatory motifs of non-coding RNA in bacteria*, thesis advisor: J. Bujnicki, 10.06.2014, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland
- **Łukasz Sadowski**, PhD thesis: *Role of endocytosis in signaling of platelet-derived growth factor*, thesis advisor: M. Miączyńska, 27.10.2014, Nencki Institute of Experimental Biology PAS, Warsaw, Poland
- **Małgorzata Kurkowiak**, PhD thesis: *Analysis of new genes involved in Primary Ciliary Dyskinesia (PCD)*, thesis advisor: M. Witt, 17.11.2014, Institute of Human Genetics PAS, Poznań, Poland
- **Wojciech Siwek**, PhD thesis: *The mechanism of action of N6-methyladenine dependent restriction endonuclease R.DpnI*, thesis advisor: M. Bochtler, 27.11.2014, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland
- **Agnieszka Mamińska**, PhD thesis: *Role of endocytic proteins in regulation of the NF-kB signaling pathway*, thesis advisor: M. Miączyńska, 09.12.2014, Nencki Institute of Experimental Biology PAS, Warsaw, Poland
- **Agnieszka Górnicka**, PhD thesis: *Transport and maturation of mitochondrial intermembrane space proteins*, thesis advisor: A. Chacińska, 11.12.2014, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland


**Prof. Marta Miączyńska with ten laureates of the "Grasz o staż" contest, ceremonial gala, 25.06.2014**

## "Grasz o staż" scholarship program

**IIMCB, as the only scientific institution, took part in the statewide "Grasz o staż" contest** for undergraduate and graduated students**.** IIMCB has financed ten internships in its laboratories.

## IIMCB educational campaign

The International Institute of Molecular and Cell Biology in Warsaw is organizing free workshops that are part of the "Be Healthy as a Fish" educational campaign that is geared toward 5th-6th grade elementary school students. This campaign is being implemented in the 2014/2015 school year. Its inauguration took place in September 2014 during the Festival of Science.

The objective of this program is to teach children basic knowledge about the life of fish and possibilities of their use in studies of certain human diseases. During the workshops, children watch the *Be Healthy as a Fish* educational movie, perform simple biological experiments, observe zebrafish under a microscope, and take part in discussions about the genetic similarities of humans and fish.

Since December 2012, IIMCB has been implementing the FishMed project, supported by the European Union. Within this project, IIMCB opened one of the largest facilities to grow and breed lines of zebrafish as research models in this part of Europe. IIMCB is pioneering research, with zebrafish as a model organism. Using this species as an example, children have the opportunity to learn how important fish are to us and discover what we as a society can gain in the future through the work of biologists.

**Daria Filipek, PR Specialist helping children at the "Be Healthy as a Fish" workshop**

The materials that are used for the campaign were developed by scientists from IIMCB in Warsaw, in cooperation with a methodologist and specialists in the field of scientific education. Since September 2014, IIMCB has conducted 16 workshops, with 327 participants from 16 schools (as of March 31, 2015).

The IIMCB Zebrafish Core Facility actively supports the "Be Healthy as a Fish" workshops, and BioCEN is a partner in this program.


# Centre for Innovative Bioscience Education (BioCEN)

Head: **Dr. Agnieszka Chołuj**

Projects Manager:
Aleksandra Kot-Horodyńska

Coordinators:
Anna Wasążnik (until January 2014)
Nina Trojan (since February 2014)

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 PAS (IBD), the Institute of Biochemistry and Biophysics PAS (IBB), University of Warsaw's Faculty of Biology, and the BioEducation Foundation. IIMCB housed the BioCEN laboratory, office and administration until June 2014. Our educational activities has been suspended since Novemeber 2014. Now the BioCEN laboratory and office are located in 21 Kołłątaj Secondary School in Warsaw (93 Grójecka Street). Since April 2015 IIMCB acts as a BioCEN Strategic Sponsor.

BioCEN also coordinates a second laboratory at the Warsaw University of Life Sciences. In 2014, 2669 young participants attended laboratory workshops and over 80 biology teachers participated in Symposium. In the span of 13 years of its activity BioCEN was visited by 19,000 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.

In 2014 a new topic of laboratory workshop for senior secondary schools: *Protozoa as model organisms* and two new topics for elementary schools: *Acidic or not acidic* and *The secrets of food* were developed.

## Partner in the project „Be Healthy as a Fish"

The International Institute of Molecular and Cell Biology in Warsaw invited 5th-6 th grade elementary school students to take part in free workshops, which are part of the "Be Healthy as a Fish" educational campaign. The objective of the program is to teach children basic knowledge about the life of fish and about possibilities of their use in studies on certain human diseases. We would like to introduce children to modern science in a friendly way. We will focus on the field of biology in a way that complements the children's classroom curriculum and that encourages them to broaden their biology interests in the future.

In its role of a partner in the project, BioCEN took part in co-creating and reviewing the text of a book under the same title as the project, promoted the workshop on its website and in social media, and provided consultations with an educational expert.

## 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 2014, as part of teacher training, the 13 th BioCEN and Nencki Institute Symposium for teachers was organized. One of the speakers during the Symposium was **Prof. Jacek Jaworski**, who delivered a lecture entitled *Reach beyond what the eye can see, or microscopes worth a Nobel prize*.

On 7 June 2014 BioCEN conducted a training section for teachers in the town of Siedlce. During practical workshop sessions, the participants improved their teaching methods in order to be able to provide support to students in their active and independent exploration of science.

## 5th Interactive Knowledge Picnic – 7 June 2014, Rzeszów

It was the first time when BioCEN took part in a large knowledge popularization event organized in the southern part of Poland (in Rzeszów). An extensive program of workshops and shows was presented by more than 260 exhibitors and educators.

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*

## 1st Educational Picnic in Mikołajki – 4 October, Mikołajki

BioCEN was the main organizer of the 1st Educational Picnic, held in the town of Mikołajki at the Hydrobiology Station of the Nencki Institute of Experimental Biology. Groups of students from local schools were present on the site throughout the day, taking part in workshops, demonstrations and experiments presented at eight experiment stations. Moreover, a group of students from senior secondary school participated in professional laboratory workshops on the isolation of proteins and their functions.

## Family laboratory workshops

In 2014 we continued laboratory workshops for younger children, accompanied by their parents or 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. Adults who accompanied the children also took part in carrying out experiments, sharing in the fun and learning. Currently, we offer four different workshops to families who visit our laboratory, including topics such as:

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

## Staff and co-workers

Persons who coordinate and administrate BioCEN are:
Dr. Agnieszka Chołuj, Aleksandra Kot-Horodyńska, Anna Wasążnik, Nina Trojan, Marcin Wiśniewski (as a coordinator at the Warsaw University of Life Sciences).

Animators: Aleksandra Piechnik, Marta Strumiłło, Jacek Patryn, Marek Kulka, Katarzyna Krzyczmonik, Ewa Lewczuk, Kryspin Andrzejewski, Kamil Synoradzki, Maciej Lirski, Andrzej Kozłowski, Paulina Mrozek, Dominika Strzelecka, Katarzyna Łepeta, Artur Franczuk, Marta Kępska, Waldemar Skowron, Róża Pogorzelska, Izabela Szczepańska, Piotr Sytek, Karolina Więcek, Katarzyna Szymańska, Agnieszka Góral, Emilia Maciejewska, Joanna Kalita, Anna Wojtania, Aleksandra Gierach, Marta Zienkiewicz, Michał Spanier.

Co-workers: Piotr Horodyński, Kamil Koper, Emilia Rejmak-Kozicka, Iwona Filipiuk.

# Administration & Staff

# Administration

### Administration Unit (from left to right)

- **Dominika Dubicka-Boroch** Senior Administration and Organization Specialist
- **Daria Filipek** PR Specialist
- **Dorota Makarewicz** Deputy Director for Operations
- **Natalia Raszka** Junior Administration Specialist
- **Agnieszka Gwara** Administration Specialist
- **Robert Banasiak** Junior Maintenance Specialist
- **Tomasz Miętek** Tenders Specialist (not in the picture)
- **Agnieszka Karbowska** Senior Administration Specialist (until April 2015, not in the picture)

### FishMed Project Manager

- **Urszula Białek-Wyrzykowska**

### Scientific Cooperation Unit

- **Katarzyna Nakielska** Scientific Cooperation Specialist
- **Agnieszka Wagner-Ziemka** Senior Scientific Cooperation Specialist
- **Marcin Ogonowski** Scientific Cooperation Specialist
- **Dorota Libiszowska** Senior Scientific Cooperation Specialist
- **Aleksandra Nałęcz-Tolak** Scientific Cooperation Specialist
- **Grażyna Omarska** Head of Scientific Cooperation Unit (until March 2015, not in the picture)

### Human Resources Unit

- **Monika Domańska-Paśko** Junior Human Resources Specialist
- **Beata Tkacz** Senior Human Resources Specialist

### Financial Unit

- **Monika Nowicka** Payroll Specialist
- **Hanna Iwaniukowicz** Deputy Director of Finance / Chief Accountant
- **Renata Knyziak** Accounting Specialist
- **Małgorzata Bytner** Accounting Specialist
- **Agnieszka Kuna** Accounting Specialist

# **Staff at IIMCB** (as of 31 March 2015)

## Laboratory of Structural Biology

| | | |
|---|---|---|
| Matthias Bochtler | Head | IIMCB |
| Honorata Czapińska | Vice Head | FNP-TEAM (3/4) |
| Thomas Fricke | FishMed Research Assistant | EU (1/2) |
| Agnieszka Kolano | Postdoctoral Fellow, FishMed | EU |
| Monika Kowalska (Sokołowska) | Postdoctoral Fellow | Ministerial grant- Iuventus Plus |
| Katrzyna Misztal | Postdoctoral Fellow | IIMCB |
| Dario Piano | Postdoctoral Fellow | IIMCB |
| Marek Wojciechowski | Postdoctoral Fellow | IIMCB |
| Asgar Abbas Kazrani | PhD Student | FNP |
| Humberto Fernandes | Postdoctoral Fellow | Volunteer (IBB) |
| Anna Fricke | Postdoctoral Fellow | Volunteer (IBB) |
| Patrycja Haniewicz | PhD Student | FNP-Homing Plus |
| Marlena Kisiała | PhD Student | Volunteer (IBB) |
| Karolina Mierzejewska | PhD Student | FNP |
| Norbert Osiński | PhD Student | FNP |
| Michał Pastor | PhD Student | Volunteer (IBB) |
| Małgorzata Perycz | Postdoctoral Fellow | Volunteer (IBB) |
| Dominik Rafalski | PhD Student | FNP |
| Paulina Okafor | Laboratory-Administrative Partner | FNP-TEAM (1/2) |
| Joanna Krwawicz | Postdoctoral Fellow | Volunteer (IBB) |

## Laboratory of Bioinformatics and Protein Engineering

| | | |
|---|---|---|
| Janusz M. Bujnicki | Head | IIMCB/ERC |
| Michał Boniecki | Postdoctoral Fellow | ERC |
| Grzegorz Chojnowski | Postdoctoral Fellow | ERC/NCN |
| Justyna Czarnecka | Postdoctoral Fellow | ERC |
| Wayne Dawson | Postdoctoral Fellow, FishMed | EU |
| Stanisław Dunin-Horkawicz | Postdoctoral Fellow | ERC/NCN |
| Jarosław Kijek | Postdoctoral Fellow | ERC |
| Maciej Maciejczyk | Postdoctoral Fellow | ERC |
| Martyna Nowacka | Postdoctoral Fellow | NCN |
| Radosław Pluta | Postdoctoral Fellow | NCN |
| Elżbieta Purta | Postdoctoral Fellow | IIMCB |
| Filip Stefaniak | Postdoctoral Fellow | ERC |
| Agata Sulej (Kamaszewska) | Postdoctoral Fellow | ERC |
| Catarina Almeida | PhD Student | NCN |
| Astha | PhD Student | NCN |
| Magdalena Byszewska | PhD Student | NCN |
| Dawid Głów | PhD Student | NCN |
| Elżbieta Jankowska | PhD Student | NCN |
| Magdalena Machnicka (Mika) | PhD Student | NCN |
| Paweł Piątkowski | PhD Student | NCN |
| Krzysztof Szczepaniak | PhD Student | NCN/MNiSW |
| Diana Toczydłowska | PhD Student | FNP, Mistrz Programme |
| Małgorzata Kurkowska (Durawa) | Research Technician | ERC |
| Sylwia Panek | Research Technician | ERC |
| Agnieszka Faliszewska | Laboratory-Administrative Partner | IIMCB |
| Jan Kogut | Computer Administrator/Programmer | IIMCB (1/2) |
| Tomasz Jarzynka | Computer Administrator/Programmer | IIMCB (1/2) |
| Łukasz Munio | Computer Administrator | IIMCB (1/2) |
| Gaja Dreszler | Undergraduate Student | Volunteer |

## Laboratory of Mitochondrial Biogenesis

| | | |
|---|---|---|
| Agnieszka Chacińska | Head | IIMCB |
| Piotr Brągoszewski | Postdoctoral Fellow | NCN Sonata |
| Beata Drabarek | Postdoctoral Fellow | NCN Opus |
| Elżbieta Januszewicz | Postdoctoral Fellow | MNiSW- Ideas Plus |
| Łukasz Samluk | Postdoctoral Fellow | MNiSW- Ideas Plus |
| Anna Sokół | Postdoctoral Fellow, FishMed | EU |

| | | |
|---|---|---|
| Ulrike Topf | Postdoctoral Fellow | NCN Opus |
| Michał Wasilewski | Postdoctoral Fellow | NCN/IIMCB |
| Magdalena Chojnacka | PhD Student | NCN |
| Piotr Chrościcki | PhD Student | NCN |
| Paulina Sakowska | PhD Student | NCN |
| Karthik Mohanraj | PhD Student | NCN |
| Krzysztof Tarasiuk | PhD Student | NCN |
| Lidia Wróbel | PhD Student | NCN |
| Michał Bazała | FishMed Research Asistant | EU (1/2) |
| Aleksandra Matusiak | Laboratory-Administrative Partner | IIMCB |
| Jakub Dominowski | Undergraduate Student | Volunteer |
| Aleksandra Fergin | Undergraduate Student | Volunteer |

### Laboratory of Molecular and Cell Neurobiology

| | | |
|---|---|---|
| Jacek Jaworski | Head | IIMCB |
| Magdalena Błażejczyk | Postdoctoral Fellow | EU/IIMCB |
| Agata Góźdź | Postdoctoral Fellow, FishMed | EU (1/2) |
| Aleksandra Janusz-Kamińska | Postdoctoral Fellow | NCN |
| Justyna Jezierska | Postdoctoral Fellow, FishMed | EU |
| Ewa Liszewska | Postdoctoral Fellow | NCN |
| Matylda Macias | Postdoctoral Fellow | NCN |
| Anna Malik | Postdoctoral Fellow | NCN/FNP Pomost |
| Bartosz Tarkowski | Postdoctoral Fellow | NCN |
| Lidia Wolińska-Nizioł | FishMed Research Assistant | EU (1/2) |
| Joanna Lipka | Junior Researcher | Volunteer |
| Agnieszka Kolka | Junior Researcher | NCN/IIMCB |
| Alicja Kościelny (Janiszewska) | Junior Researcher | IIMCB |
| Marcelina Pieprzyk | Junior Researcher/Laboratory-Administrative Partner | IIMCB/NCN |
| Aleksandra Tempes (Piechnik) | Junior Researcher | EU |
| Agnieszka Skałecka | Junior Researcher | NCN/IIMCB |
| Katarzyna Świtoń | Junior Researcher | NCN/IIMCB |
| Anna Urbańska | Junior Researcher | IIMCB |
| Małgorzata Urbańska | Junior Researcher | Volunteer |
| Klaudia Jączyńska | Undergraduate Student | Volunteer |

### Laboratory of Neurodegeneration

| | | |
|---|---|---|
| Jacek Kuźnicki | Head | IIMCB |
| Łukasz Majewski | Vice Head | NCN |
| Tomasz Węgierski | Senior Scientist | IIMCB |
| Joanna Gruszczyńska-Biegała | Senior Postdoctoral Fellow | IIMCB/NCN |
| Magdalena Czeredys | Postdoctoral Fellow | NCBiR |
| Smijin Karthully Soman | Postdoctoral Fellow, FishMed | EU |
| Michał Bazała | FishMed Research Assistant | EU (1/2) |
| Kinga Gazda | PhD Student | NCN |
| Anna Jaworska | PhD Student | FNP |
| Aleksandra Kurek | MSc Student | Volunteer |
| Filip Maciąg | MSc Student | Volunteer |
| Maria Śladowska | MSc Student | NCN |

### Laboratory of Cell Biology

| | | |
|---|---|---|
| Marta Miączyńska | Head | IIMCB/Polish-Swiss Res. Program |
| Anna Bartosik | Postdoctoral Fellow, FishMed | EU |
| Noga Budick-Harmelin | Postdoctoral Fellow | Polish-Swiss Res. Program |
| Jarosław Cendrowski | Postdoctoral Fellow | NCN |
| Agnieszka Mamińska | Postdoctoral Fellow | Polish-Swiss Res. Program |
| Ewelina Szymańska | Postdoctoral Fellow | FNP POMOST |
| Daria Zdżalik | Postdoctoral Fellow | NCN |
| Lidia Wolińska-Nizioł | FishMed Research Assistant | EU (1/2) |
| Kamil Jastrzębski | PhD Student | IIMCB |
| Katarzyna Kuźmicz | Undergraduate Student | Volunteer |
| Richard Welten | Undergraduate Student | Volunteer |
| Agata Mieżaniec | Trainee | Polish-Swiss Res. Program |
| Rafał Sejdak | Trainee | Polish-Swiss Res. Program |
| Paulina Okafor | Laboratory-Administrative Partner | Polish-Swiss Res. Program (1/2) |

### Laboratory of Protein Structure

| | | |
|---|---|---|
| Marcin Nowotny | Head | Wellcome Trust/UE |
| Małgorzta Figiel | Postdoctoral Fellow | EU |
| Vinnet Gaur | Postdoctoral Fellow | Wellcome Trust |
| Karolina Górecka | Postdoctoral Fellow | Wellcome Trust |
| Elżbieta Nowak | Postdoctoral Fellow | EU |
| Agnieszka Topolska-Woś | Postdoctoral Fellow | HHMI |
| Marcin Jaciuk | Postdoctoral Fellow | ERC |
| Deepshikha Malik | PhD Student | ERC |
| Michał Rażew | PhD Student | NCN |
| Mirosław Śmietański | PhD Student | HHMI |
| Agnieszka Gołąb | Research Technician | NCBiR |
| Jakub Gruchota | Research Technician | HHMI |
| Weronika Komorowska | Research Technician | NCBiR |
| Marzena Nowacka | Research Technician | EU |
| Justyna Studnicka | Research Technician | Wellcome Trust |
| Paweł Kustosz | Laboratory-Administrative Partner | EU |

### Zebrafish Developmental Genomic Laboratory

| | | |
|---|---|---|
| Cecilia Winata | Head, FishMed | EU |
| Katarzyna Nieścierowicz | Postdoctoral Fellow, FishMed | EU |
| Michał Pawlak | Postdoctoral Fellow, FishMed | EU |
| Leszek Pryszcz | Postdoctoral Fellow | IIMCB |
| Sreedevi Sugunan | FishMed Research Assistant | EU (1/2) |
| Maciej Łapiński | Research Assistant | NCN |

### Department of Molecular Biology

| | | |
|---|---|---|
| Maciej Żylicz | Head | IIMCB |
| Maciej Olszewski | Postdoctoral Fellow, FishMed | EU |
| Milena Wiech | Postdoctoral Fellow | IIMCB/NCN |
| Magdalena Pruszko | FishMed Research Assistant | EU/NCN (1/2) |
| Marcin Herok | PhD Student | IBD/NCN |
| Marta Małuszek | PhD Student | Maternity Leave |
| Zuzanna Tracz-Gaszewska | PhD Student | FNP/NCN |
| Grażyna Orleańska | Laboratory-Administrative Partner | IIMCB (1/2) |
| Julia Zdzieszyńska | Undergraduate Student | Volunteer |
| | | |
| Ewa Paluch | Former Head, Laboratory of Cell Cortex Mechanics MPG/PAS | Free Leave |

### Core Facility

| | | |
|---|---|---|
| Alicja Żylicz | Head | IIMCB |
| Roman Szczepanowski | Vice Head | IIMCB |
| Katrzyna Misztal | Senior Staff Scientist | IIMCB |
| Krzysztof Skowronek | Senior Staff Scientist | IIMCB |
| Tomasz Węgierski | Senior Staff Scientist | IIMCB |
| Piotr Brągoszewski | Radiation Safety Officer | NCN Sonata |

### Zebrafish Core Facility

| | | |
|---|---|---|
| Małgorzata Wiweger | Head, FishMed | EU |
| Piotr Korzeniowski | Veterinarian, FishMed | EU(1/2) |
| Maciej Mańk | Technician, FishMed | EU/IIMCB |
| Krzysztof Surga | Technician, FishMed | EU/IIMCB |
| Olga Chojnacka | Technician | IIMCB |
| Maciej Ochnio | Technician | IIMCB |
| Róża Chmielewska | PhD Student | Volunteer |
| Wioleta Dudka-Ruszkowska | PhD Student | Volunteer |

### Technology Transfer Unit (Biotech-IP)

| | | |
|---|---|---|
| Magdalena Powierża | Head, FishMed | EU |
| Leszek Lipiński | Senior Expert, FishMed | EU (1/2) |
| Adam Sobczak | Project Manager, FishMed | EU (1/2) |
| Hubert Ludwiczak | Specialist, FishMed | EU (1/2) |
| Piotr Potepa | Specialist, FishMed | EU |

### Aurezyna Project

| | | |
|---|---|---|
| Izabela Sabała | Head | NCBR |
| Elżbieta Jagielska | Postdoctoral Fellow | NCBR |
| Maja Grabowska | PhD Student | NCBR |
| Paweł Mitkowski | Research Asssistant | NCBR |

### PolSenior Project

| | | |
|---|---|---|
| Małgorzata Mossakowska | Head | IIMCB |
| Aleksandra Szybalska | Project Assistant | NCBR |
| Przemysław Ślusarczyk | IT Specialist | NCBR (1/2) |

### Bestcilia Project

| | | |
|---|---|---|
| Zuzanna Bukowy-Bieryłło | Organization and Promotion Specialist | EU |

### Research Support Service

| | | |
|---|---|---|
| Wanda Gocal | Technician | IIMCB (DMB) |
| Elżbieta Grzelak | Technician | IIMCB (LN/LMB) |
| Monika Matuszczyk | Technician | IIMCB (LCB/LMCN) |
| Agnieszka Olszewska | Technician | IIMCB (LSB/ZCF/LZDG) |
| Iwona Ptasiewicz | Technician | IIMCB (LPS/LBPE) |
| Alina Zielińska | Technician | IIMCB (LMCN) |

### Centre for Innovative Bioscience Education (BioCEN)

| | | |
|---|---|---|
| Agnieszka Chołuj | Head | IIMCB |

### Directors

| | | |
|---|---|---|
| Jacek Kuźnicki | Director | IIMCB |
| Marta Miączyńska | Deputy Director for Science | IIMCB |
| Michał Witt | Deputy Director for Development | IIMCB (1/4)/EU (1/4) |
| Dorota Makarewicz | Deputy Director for Operations | IIMCB |
| Hanna Iwaniukowicz | Deputy Director for Finance | IIMCB |

### Financial Unit

| | | |
|---|---|---|
| Monika Nowicka | Payroll Specialist | IIMCB |
| Małgorzata Bytner | Accounting Specialist | IIMCB |
| Renata Knyziak | Accounting Specialist | IIMCB |
| Agnieszka Kuna | Accounting Specialist | IIMCB/Structural Funds |

### Human Resources Unit

| | | |
|---|---|---|
| Beata Tkacz | Senior Human Resources Specialist | IIMCB |
| Monika Domańska-Paśko | Junior Human Resources Specialist | IIMCB |

### FishMed Project Manager

| | | |
|---|---|---|
| Urszula Białek-Wyrzykowska | FishMed Project Manager | IIMCB (1/2) |

### Scientific Cooperation Unit

| | | |
|---|---|---|
| Dorota Wasiak-Libiszowska | Senior Scientific Cooperation Specialist, Rotational Head | IIMCB/EU |
| Agnieszka Wagner-Ziemka | Senior Scientific Cooperation Specialist | IIMCB |
| Katarzyna Nakielska | Scientific Cooperation Specialist | IIMCB |
| Aleksandra Nałęcz-Tolak | Scientific Cooperation Specialist | IIMCB/EU (3/4) |
| Marcin Ogonowski | Scientific Cooperation Specialist | IIMCB/Structural Funds/EU |

### Administration Unit

| | | |
|---|---|---|
| Dominika Dubicka-Boroch | Senior Administration and Organization Specialist | IIMCB |
| Agnieszka Gwara | Administration Specialist | IIMCB |
| Natalia Raszka | Junior Administration Specialist | IIMCB |
| Tomasz Miętek | Tender Specialist | IIMCB |
| Robert Banasiak | Junior Maintenance Specialist | IIMCB |
| Andrzej Majewski | Technical Support | IIMCB |
| Dudzin Magdalena | HS Specialist | IIMCB (1/4) |

### PR Unit

| | | |
|---|---|---|
| Daria Filipek | PR Specialist, FishMed | EU |

# Important Dates in the Institute's History

**August 2014** IIMCB on the Polish Roadmap for Research Infrastructures as a Consortium Leader with the project entitled "Research Infrastructure of Molecules and Cells (IN-MOL-CELL)"

**July 2014** The Laboratory of Zebrafish Developmental Genomics, IIMCB/Max Planck Research Group commences its activities with Dr. Cecilia Winata as a Lab Leader

**Oct. 2013** IIMCB received the '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

**Oct. 2013** Receipt of the A+ category by IIMCB within the parametric evaluation of Polish academic institutions

**April 2013** Introduction of zebrafish as an animal model for research, start of FishMed

**March 2012** Agreement between Max Planck Society (MPG) and IIMCB on continuation of of MPG/IIMCB Research Groups

**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

**Feb. 2006** Twin MPG-PAS 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

**Jan. 2003** Status of the Centre of Excellence in Molecular Bio-Medicine is granted by the European Commission within 5th Framework Programme

**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. 2001** The MPG-PAS Junior Research Group commences its activities with Dr. M. Bochtler as a Lab Leader

**April 2000** An agreement between the Max Planck Society (MPG) and the Polish Academy of Sciences (PAS) to launch a Joint MPG-PAS Junior Research Group

**Oct. 1999** Prof. M. Żylicz is appointed as Chair of the Department of Molecular Biology

**July 1999** Dr. J. Dastych is appointed as a first Lab Leader at IIMCB

**Jan. 1999** The Institute commences its independent activities; Prof. J. Kuźnicki appointed as Acting Director

**May 1998** Prof. A. Azzi is nominated as the Director of IIMCB

**June 1997** Polish Parliament passes a bill to found the Institute

**June 1996** The Molecular and Cell Biology Department is created by PAS with Prof. M.J. Nałęcz as the Head

**May 1995** An agreement between Poland and UNESCO to establish the Institute

**Oct. 1994** Presidium of Polish Academy of Sciences (PAS) votes to support the Institute

**June 1994** State Committee for Scientific Research (KBN) accepts the activities aimed at establishing the Institute

**Sep. 1991** The proposal to create the Institute was published in the UNESCO Bulletin of MCBN

# Contents

# FISHING FOR MEDICINES AND THEIR TARGETS USING ZEBRAFISH MODELS OF HUMAN DISEASES

FISH MED 2016

# International FishMed Conference on Zebrafish Research

March 18-19, 2016, Warsaw, Poland

**Confirmed speakers:**

**Markus Affolter**, University of Basel, Switzerland
**Shawn Burgess**, National Institutes of Health, USA
**Jose Luis Gomez-Skarmeta**, Pablo de Olavide University, Spain
**Suresh Jesuthasan**, Institute of Molecular and Cell Biology, Singapore
**Koichi Kawakami**, National Institute of Genetics, Japan
**Graham Lieschke**, Monash University, Australia
**Ferenc Mueller**, University of Birmingham, UK
**John Postlethwait**, University of Oregon, USA
**Tatjana Sauka-Spengler**, University of Oxford, UK
**Lilianna Solnica-Krezel**, Washington University School of Medicine, USA
**Joachim Wittbrodt**, University of Heidelberg, Germany

The conference is organized by the International Institute of Molecular and Cell Biology in Warsaw.

FISH MED

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY IN WARSAW

SEVENTH FRAMEWORK PROGRAMME

Conference supported by the European Commission under FP7 GA No 316125 and by the Ministry of Science and Higher Education