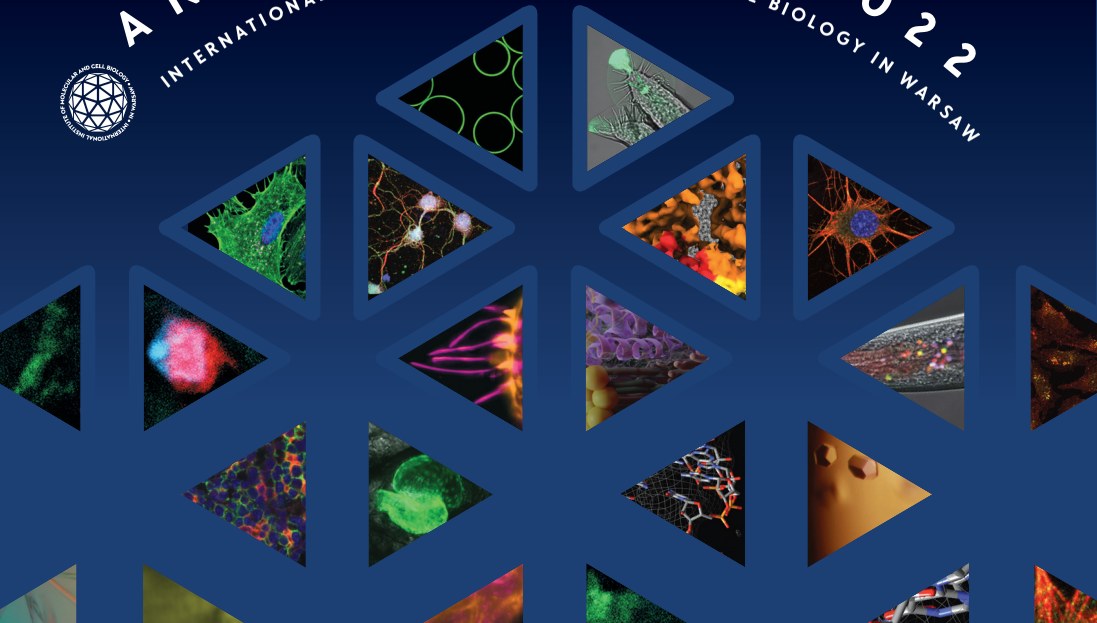
ANNUAL REPORT 2022

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY IN WARSAW



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Deputy Director for Development
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DIRECTOR'S NOTE

The year 2022 brought new developments at the IIMCB that allow us to look ahead with optimism. On the scientific side, we recruited two fantastic Junior Lab Leaders, reported exciting findings, and received accolades for our research activities. On the institutional side, we won a strategic Teaming for Excellence grant from the European Commission and continued improving our internal organization. Finally, our new building came an important step closer to completion as the competition for its architectural concept was concluded.

As a result of an international competition conducted in 2022, two Junior Lab Leaders are joining the IIMCB: Dr. Aleksandra Kotodziejczyk, who is relocating from the Weizmann Institute of Science (Rehovot, Israel) and Dr. Ewelina Matecka-Grajek from Johns Hopkins University (Baltimore, USA). The new groups: the Laboratory of Cellular Genomics headed by Dr. Kotodziejczyk, and the Laboratory of Single-Molecule Biophysics led by Dr. Matecka-Grajek will start their activities in April 2023. They will broaden the scope of IIMCB research in cell and RNA biology, adding single-cell genomics and single-molecule imaging to our technology portfolio.

Among the most notable findings published by IIMCB scientists in 2022 are: the first cryo-EM structure of TnsB transposase that acts on Tn7 transposons, the molecular mechanism underlying the synergistic cooperation of CHN-1 and UFD-2 ubiquitin ligases in substrate ubiquitylation, and a novel function of Adar-mediated A-to-I RNA editing in regulating embryonic patterning and innate immunity (see Section on Best Papers Award). In 2022, IIMCB scientists published 53 articles, several of which were accompanied by press releases on our website and on social media channels. Our scientific exchange was vibrant (see Section on Scientific Events). After a pandemic-imposed break, we could enjoy in-person interactions during retreats organized in outside locations, including a general IIMCB Retreat, a Lab Leaders' and Directors' Retreat, and a PhD Students Report Session. Together with the European Molecular Biology Laboratory (EMBL), an online Info Day was organized to present opportunities for Polish scientists to cooperate with the EMBL.

The research achievements of our scientists were recognized and awarded in 2022. Prof. Marcin Nowotny won a Prize of the Foundation for Polish Science – the most prestigious distinction for a scientist in Poland – for the elucidation of molecular mechanisms of DNA damage recognition and repair. Prof. Andrzej Dziembowski received a Prime Minister's Award for his discovery of novel mechanisms of gene expression regulation through mRNA 3' end modifications and an ERC Advanced Grant for the project "ViveRNA". Prof. Matthias Bochtler and his team obtained an Award of the Minister of Education and Science for their studies on the mechanisms of DNA methylation and demethylation regulating the epigenetic states of genomes. Overall, the quality of research performed at the IIMCB has been once more recognized by the Polish Ministry of Education and Science which provides our core funding. We were again awarded an elite A+ category, a result of the evaluation of all institutions for the years 2017-2021, based on publications, grants and the social impact of scientific findings.

Of strategic importance for the immediate future, we are very proud that our plan of institutional development for years 2023-29 was approved for funding with nearly 15 M euro by the European Commission in the Teaming for Excellence program under Horizon Europe. Our project entitled "RNA and Cell Biology – from Fundamental Research to Therapies" (RACE), scored 14.5 out of 15 points and was ranked first among 31 European projects evaluated in the second stage of the competition. RACE aims to elevate the IIMCB into a world-class Centre of Excellence in RNA and Cell Biology that will combine outstanding science with professional commercialization activities. Within RACE, we will (1) recruit new research groups and broaden collaborations with external partners, (2) train younger generations of researchers for academia and industry, (3) further develop our Core Facilities, (4) establish an internal technology transfer support, and (5) digitalize and upgrade our management and administrative processes. In all these endeavors, we will be supported by two partners: the Medical Research Council, Human Genetics Unit of the University of Edinburgh, UK and Flanders Institute of Biotechnology (VIB) in Belgium.

In 2022, we implemented further organizational improvements. With care for our doctoral students and their education, a dedicated PhD Office was established. In the 2022/23 academic year we launched a 60-hour lecture course entitled *Methodological Advances in Molecular and Structural Biology* for students of the Warsaw-4-PhD Doctoral School. The course was designed and is coordinated by Professors Janusz Bujnicki, Andrzej Dziembowski and Gracjan Michlewski, and managed by Dr. Iwona Pilecka. Over 60 PhD students from the IIMCB and neighboring institutions currently attend it. In 2022, we also organized a Research Data Management and Open Science course for PhD students and all scientists at the IIMCB.

We are continuing to develop the IIMCB Core Facilities so that our scientists and external customers can benefit from specialized expertise, equipment and research services. In 2022 two new units were established: Animal Housing and Preclinical Drug Development. The latter unit offers the production of proteins in different expression systems and their structural analysis, for in-house scientists or biotech companies. Further units will be established in the framework of the RACE project.

Finally, the results of the competition for an architectural concept of our new building were announced during a ceremonial gala on December 16, 2022. It was attended by high-profile guests, representing the Ministry of Education and Science, the Ministry of Finance, Warsaw city authorities, our International Advisory Board and science funding institutions in Poland (see Section on Concept of the IIMCB building). As the next step, a building design will be prepared in 2023, before tendering for a construction company. We are excited about the future of the IIMCB in this attractive and spacious building.

On December 15, 2022 I started my second term of office as director of the IIMCB, a result of competition conducted by the International Advisory Board. I want to thank all IIMCB staff for their support and the hard work that I witnessed during my first term. It has been a privilege to serve the community of the IIMCB, especially in the challenging times of the pandemic and the reprehensible aggression of Russia against Ukraine that sparked enormous solidarity and various means of help provided to the citizens of Ukraine by our staff. My motto has not changed for the second term of my directorship: only by acting together – like cells and tissues in a biological organism – can we succeed as an institution. I am sure that we will make it happen.

Marta Miączyńska
Warsaw, March 2023

DIRECTORS

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Director

Jacek Jaworski
Deputy Director
for Science

Urszula Białek-Wyrzykowska
Deputy Director
for Development

Anna Zolnik
Deputy Director
for Operations

Hanna Iwaniukowicz
Deputy Director
for Finance

ABOUT THE IIMCB

The International Institute of Molecular and Cell Biology in Warsaw was established under an international agreement between the government of the Republic of Poland and UNESCO and a dedicated parliamentary act of 1997, so it has a unique legal status in the Polish scientific system. The IIMCB is directly supervised by the President of the Polish Academy of Sciences, who appoints members of the International Advisory Board and, upon the Board's recommendation, the Institute's director. The Board provides strategic advice on research directions, approves financial plans, conducts competitions for laboratory leaders and regularly evaluates the scientific outputs of laboratories. For years, the Institute has boasted the highest scientific category (A+) in the evaluation of scientific institutions by the Ministry responsible for science, including the latest one in 2022. To strengthen its international position, in January 2020 the IIMCB joined the EU-LIFE alliance of 15 independent research institutes from 15 European countries. This alliance works for excellence in life sciences, attaching great importance to the quality and integrity of science, while at the same time actively participating in shaping European science policy.

The main research directions at the IIMCB are RNA biology and cell biology, both aimed at understanding the fundamentals of human diseases, which are the basis for creating innovative therapeutic and diagnostic methods. The scientific excellence which we pursue involves the implementation of ambitious research projects and scientific initiatives, and forming partnerships with leading research centers in Poland and abroad. To ensure that the results of this research are translated into clinical applications, the IIMCB is open to cooperation with the pharmaceutical and biotechnological industries, including sharing the resources and expertise of our core facilities.
The IIMCB is involved in educating PhD students as one of the nine founders of the Warsaw PhD School in Natural and BioMedical Sciences (Warsaw-4-PhD). The School offers international doctoral students an interdisciplinary educational and research program in physics, chemistry, biology, and medicine. Next year we will celebrate the graduation of the first alumni of our School. Research at the IIMCB is supported by an annual statutory subsidy from the Ministry responsible for science and a budgetary subsidy from the Polish Academy of Sciences. Still, up to 70% of the yearly institutional budget comes from external competitive sources. Since 2000, our scientists have received 319 grants. Many prestigious ones come from European and other foreign sources, such as: the EU Framework Programmes, including European Research Council, EU Structural Funds through the Foundation for Polish Science, European Molecular Biology Organization, Howard Hughes Medical Institute, the Wellcome Trust, European Economic Area and Norway Grants, and the Polish-Swiss Research Programme. In 2023, the IIMCB will start implementing an institutional project entitled *RNA and Cell Biology – from Fundamental Research to Therapies* [RACE] selected for funding in the Teaming for Excellence programme under Horizon Europe. IIMCB researchers at different career stages also benefit from diverse grants from Polish sources: National Science Centre, National Centre for Research and Development, Polish National Agency for Academic Exchange, Polish Science Fund and Ministry of Education and Science.

The well-being of the members of our community is paramount to us, thus the IIMCB follows the rules put forward by the European Commission in the HR Excellence in Research programme, which the IIMCB joined in 2013. The HR Excellence in Research logo is an accreditation that identifies institutions with a stimulating and favorable working environment. The recently adopted Gender Equality Plan includes measures to take it a step further: providing an assuring and conducive work culture based on the respect for the principles of equality and diversity. In doing so, we enable all employees to freely develop their scientific and personal skills.

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Laboratory of Cell Biology
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Laboratory of Protein Metabolism
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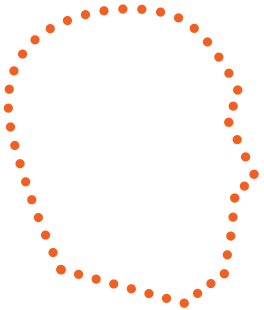


251 Total
155 Female
96 Male
18 Nations
55 Foreigners

12 Lab Leaders
15 Researchers & Senior Researchers
33 Postdoctoral Researchers
12 Research Assistants
16 Research Specialists
49 PhD Students
21 Volunteers
21 Core Facilities Staff
2 BioCEN Staff
5 Technicians
13 Laboratory Support Specialists
48 Administration
4 Others

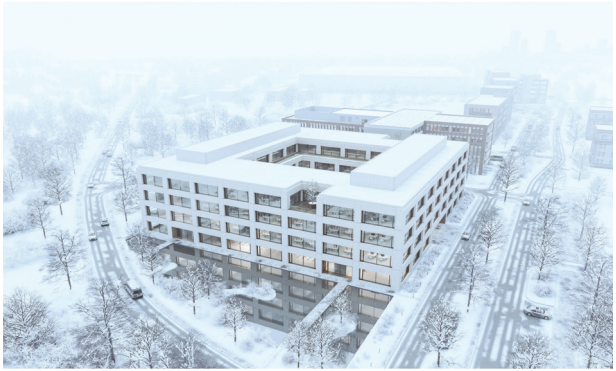
CONCEPT OF THE IIMCB BUILDING

On December 16, 2022, an official announcement of the results of the architectural competition for IIMCB's new building took place. The ceremony received the honorary patronage of the Minister of Education and Science of Poland.

The event was opened by Prof. Marta Miączyńska, who invited high-profile guests to make their addresses, including Mr. Wojciech Murdzek (Secretary of State, Ministry of Education and Science), Dr. Sebastian Skuza (Secretary of State, Ministry of Finance), Ms. Agnieszka Kalinowska-Sottys (President of the Association of Polish Architects), and Dr. Krzysztof Koźmiński (Council of the National Agency for Academic Exchange). Among the participants were members of the International Advisory Board of IIMCB: Prof. Urszula Hibner, Prof. Artur Jarmołowski, and Prof. Lilianna Solnica-Krezel. The event was also attended by representatives of the Polish Academy of Sciences, Foundation for Polish Science, Łukasiewicz Research Network, and City of Warsaw.

The architectural competition was conducted in cooperation with the Association of Polish Architects. The projects were assessed in a two-stage process by a jury that was composed of representatives of the Association of Polish Architects and IIMCB. Fourteen projects were submitted to the first stage, and five outstanding projects proceeded to the second stage. Finally, three awards and two honorable mentions were given.

Atelier Tektura Sp. z o.o., an architectural studio from Warsaw, won the competition. Their project envisages four above-ground floors and one underground floor, with a total building area of over 20,000 m^2 and a usable space of nearly 14,000 m^2. The work of Atelier Tektura was recognized for a functional and rational solution to the room layout. Moreover, flexibility of the project was appreciated, owing to the possibility of modifying the layout and other structural solutions. Justification of the competition results also emphasized the good organization of traffic in the investment area and use of internal parking. The jury honored a simple aesthetic body with a dignified character that reflects the institution's nature.



EU-LIFE

GENERAL INFORMATION

EU-LIFE represents leading research centers in life sciences to support and strengthen European research excellence and be a voice for research and policy in Europe.

EU-LIFE is an alliance of independent research institutes whose mission is to support and strengthen European research excellence. EU-LIFE members include leading research institutes that are internationally renowned for producing excellent research, widely transferring knowledge, and nurturing talent. Since its founding in 2013, EU-LIFE has become a stakeholder in science policy development, participating regularly in the European science policy dialogue. IIMCB became the first Polish member of the EU-LIFE alliance in 2020. As a member of EU-LIFE, IIMCB is working with 14 other institutes toward achieving and maintaining excellence in the life sciences, emphasizing quality and responsible science and highlighting issues that are related to European science policies.

ORGANIZATION

The structure of EU-LIFE includes a Board of Directors, a Strategy Group, several Working Groups and Task Forces, and an EU-LIFE Office.

The Strategy Group focuses on the EU-LIFE organization and strategic actions, such as defining new areas of cooperation and partnership, identifying areas of science policymaking, deciding on EU-LIFE initiatives, and proposing action plans. The Strategy Group is composed of a Board of Directors of member institutes, their main representatives, the EU-LIFE Executive Director, and chairs of Working Groups. The IIMCB representatives in the Strategy Group are **Marta Miączyńska** as the Director of IIMCB and **Urszula Białek-Wyrzykowska** as the main representative.

The Core Facilities Working Group is a forum for discussing challenges that are unique to core facilities. Key activities of this Working Group in 2022 were the publication of an article on recognizing and acknowledging core facilities in research journals, the launch of a series of TechWatch webinars that highlight new research technologies, working on issues that are related to core facility-specific career development, and the propagation of best knowledge and practices in core facility management. In 2023, the Core Facilities Working Group will focus on preparing the next edition of the Core Facilities Working Group Benchmarking Report, research data management (in collaboration with the IT Working Group), and drafting the *Core Facility Life-cycle* document. **Joanna Dodzian** and **Krzysztof Skowronek** are the IIMCB representatives in this Working Group.

The Gender Equality, Diversity, and Inclusion Working Group coordinates gender equality activities, develops indicators for monitoring gender equality issues in EU-LIFE institutes, and shares best practices in this area. In 2022, the Gender Equality, Diversity, and Inclusion Working Group implemented the following topics: (i) development of key indicators, (ii) coordination of EU-LIFE active bystander training, and (iii) submission of a joint grant application [HORIZON-2022-ERA-D3-01-8-2: *Support to the implementation of inclusive gender equality plans*]. The IIMCB representatives in this Working Group are **Agnieszka Faliszewska** and **Katarzyna Fiedorowicz**.

The Grants and Funding Strategies Working Group is a discussion forum for maximizing funding opportunities in EU-LIFE institutes, sharing best practices in pre- and post-award grant management, drafting grant policies and guidelines, and developing grant-related training. In 2022, the Grants and Funding Strategies Working Group's work focused on the following topics: preparing MSCA booklet that covers all areas of support that are offered in EU-LIFE institutes for applicants, preparing a poster by representatives from EU Widening countries to promote institutes and the Horizon Europe Hop On Facility, sharing best practices in applying for competitive funding based on the current exchange of information, and gathering and analyzing statistics on the participation of EU-LIFE institutes in Horizon 2020 (final data) and Horizon Europe (closed calls). The IIMCB representatives in this Working Group are **Dorota Libiszowska** and **Marcin Ogonowski**.

The Technology Transfer Working Group is a platform for sharing best practices in the field of intellectual property and knowledge transfer. The focus of meetings in 2022 included start-up policy, data policy, Proof-of-Concept funding, and rules and procedures when Principal Investigators leave an institute. On October 11, 2022, the Technology Transfer Working Group organized the yearly online pitching event with presentations of seven advanced scientific projects from EU-LIFE research institutions. At this event, all members of the Technology Transfer Working Group introduced the commercial potential of their institutions to 14 representatives of venture capital firms. The EU-LIFE Community Meeting in Heraklion on October 26-27, 2022, was an excellent occasion for the Technology Transfer Working Group to meet and discuss in person. The group members exchanged experiences with information resources and tools that are used at their Technology Transfer Offices. The IIMCB representative in this Working Group is **Iwona Pilecka**.

The IT Working Group is a community of specialists who are dedicated to addressing Information Technology challenges. In 2022, the IT Working Group discussed and tested the Integrated Rule-Oriented Data System as an example of secure data storage. The topics of research data storage and security, big data analysis, network access control, and remote work challenges were also covered. The IIMCB representative in this Working Group is **Paweł Kobylarz**.

The Recruitment and Training Working Group focuses on ensuring continued professional development for researchers at all stages of their careers, supporting partner institutes in the recruitment process by sharing job offers and opportunities, discussing mobility experiences (overcoming administrative barriers), and defining best practices in terms of grants and employment contracts, with a special focus on postdocs. In 2022, the Recruitment and Training Working Group concentrated on sharing best practices in training and recruitment processes in each institution. Recruitment and Training Working Group members recommended training courses that were highly successful in their organizations. The group gathered information on how to most effectively conduct training and how to collect feedback about it. The IIMCB representative in this Working Group is **Aleksandra Janicka**.

eulife

The Science Communication Working Group is a forum for sharing best practices and experiences of people who are responsible for popularization and promotion of science. The main points of interest include media and public relations management, common campaigns in social media, communicating scientific output, and sharing best practices on benchmarking papers. The Science Communication Working Group also designs and implements the EU-LIFE internal and external communication strategy. In 2022, the Science Communication Working Group coordinated the 12-week Twitter campaign DNA 2.0 to which all EU-LIFE members were invited. The storytelling workshops were also organized; as a result, a special booklet with texts prepared by each institute will be published in 2023. In 2022, the Science Communication Working Group also prepared for EU-LIFE's anniversary, planning the implementation of further social media campaigns, videos, and other promotional materials. The IIMCB representatives in this Working Group are **Daria Goś** and **Małgorzata Staszkowska-Wągrodzka**.

Annual EU-LIFE Community Meeting, Heraklion, Greece, October 26-27, 2022

MEMBERS

HORIZON 2020 ERA CHAIRS PROJECT AT IIMCB: MOSaIC

MOLECULAR SIGNALING IN HEALTH AND DISEASE – INTERDISCIPLINARY CENTRE OF EXCELLENCE

EC Project Officer
Cristina Marcone

Implementation period
2018-2023

Project Coordinator
Jacek Kuźnicki

Funding
2,498,887.50 EUR

Project Manager
Dorota Libiszowska

Call
H2020 WIDESPREAD-03-2017

MOSaIC project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 810425

IIMCB aspires to reach a level of excellence that is tantamount to the best European centers and gain recognition among them. The MOSaIC project, funded under the H2020 ERA Chairs scheme, helps us unlock our R&I potential, attain scientific and organizational levels of the best European institutes, and eventually become fully recognized in the European Research Area. Thanks to MOSaIC, we established the ERA Chairs Research Group, headed by an outstanding scientist, Andrzej Dziembowski, and have been introducing structural improvements in science management and Human Resources activities for more efficient support for researchers. MOSaIC has been implemented since November 1, 2018. Below we highlight the project's main achievements since then.

LABORATORY OF RNA BIOLOGY – ERA CHAIRS GROUP

Andrzej Dziembowski, ERA Chairs Group Leader

Andrzej Dziembowski, an internationally renowned Polish scientist in the field of RNA research, won an open international competition for the ERA Chairs Group Leader at IIMCB. On December 1, 2019, he established the Laboratory of RNA Biology – ERA Chairs Group. They study the post-transcriptional regulation of gene expression to answer questions about how processive ribonucleases shape transcriptomes of mammalian cells through RNA degradation and how poly[A] and poly[U] polymerases regulate protein production (see page 22).

AWARDED GRANTS

In total, the ERA Chairs Group has been awarded 8 grants, including the highly prestigious **ERC Advanced Grant**, which was given to a Polish scientist in the life sciences field for the first time. The ViveRNA project focuses on understanding the mechanisms that regulate the stability of both endogenous and therapeutic mRNAs. Prof. Dziembowski also heads a large collaborative grant, the HERO Virtual Research Institute (WIB), which is funded by the Polish Science Fund. This project aims to develop the next generation of mRNA – based cancer immunotherapies.

- **ViveRNA**, **ERC Advanced Grant**, Horizon Europe, European Commission, Andrzej Dziembowski
- **HERO WIB**, Polish Science Fund, Andrzej Dziembowski [Leader], other IIMCB groups involved: Marta Miączyńska and Marcin Nowotny; University of Warsaw, Medical University of Warsaw, and Institute of Physical Chemistry of the Polish Academy of Sciences [Partners]
- **GRIEG**, EEA and Norway Grants/NCN, Andrzej Dziembowski [Coordinator], University of Warsaw and University of Bergen [Partners]
- **MAESTRO**, NCN, Andrzej Dziembowski
- **OPUS**, NCN, Andrzej Dziembowski
- **SONATA**, NCN, Monika Kusio-Kobiałka
- **SONATINA**, NCN, Tomasz Kuliński
- **PASIFIC**, PAS, Ewa Poniecka

DISSEMINATION OF ERA CHAIRS GROUP RESEARCH RESULTS

PUBLICATIONS & PREPRINTS THAT ACKNOWLEDGE MOSaIC

Krawczyk PS, **Gewartowska O**, **Mazur M**, **Orzeł W**, **Matylla-Kulińska K**, **Jeleń S**, Turowski P, Śpiewla T, **Tarkowski B**, Tudek A, **Brouze A**, Wasilewska A, Nowis D, Gołąb J, Kowalska J, Jemielity J, **Dziembowski A**, **Mroczek S**. SARS-CoV-2 mRNA vaccine is re-adenylated in vivo, enhancing antigen production and immune response. *bioRxiv*, 2022; doi: 10.1101/2022.12.01.518149

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LECTURES AND POSTERS AT INTERNATIONAL MEETINGS AND CONFERENCES

Andrzej Dziembowski, lecture: *Oncogenic mechanisms of DIS3 mutations*, Journée Louise Harel on lncRNA, Non canonical ORFs and cancer, February 2023, Paris, France

Andrzej Dziembowski, lecture: *In vivo re-adenylation of mRNA-1273 boosts its efficacy*, 10th International mRNA Health Conference, November 2022, Boston, USA

Andrzej Dziembowski, lecture: *Complex metabolic pathways of mRNA vaccines*, EMBO Members Meeting 2022, October 2022, Heidelberg, Germany

Vladislava Liudkovska, lecture: *Cytoplasmic polyadenylation by TENT-5 regulates the innate immune response in worms*, 23rd *C. elegans* Conference, Genetics Society of America, June 2021, Rockville, USA [online]

Andrzej Dziembowski, introduction lecture, chairman of *RNA Turnover* session, 27th Annual Meeting of the RNA Society, May-June 2022, Boulder, USA

Andrzej Dziembowski, lecture: *TENT5 cytoplasmic non-canonical poly(A) polymerases regulate the innate immune response in animals*, CSHL Eukaryotic mRNA Processing, August 2021, Cold Spring Harbor, USA [online]

Andrzej Dziembowski, lecture: *A tale of tails in yeast and mouse – what we can learn from Direct RNA sequencing on Nanopores*, Institute Pasteur Seminaire du Departement Genomes & Genetique, January 2021, Paris, France [online]

Olga Gewartowska, poster: *Mouse models for in vivo studies of mRNA vaccines metabolism*, 10th International mRNA Health Conference, November 2022, Boston, USA

Paweł Krawczyk, poster: *Direct RNA sequencing with dedicated computational algorithms – a method of choice for quality control and analysis of the metabolism of mRNA therapeutics*, 10th International mRNA Health Conference, November 2022, Boston, USA

Paweł Krawczyk, poster: *Complex life of mRNA-1273 vaccine poly(A) tail in immune cells* and flash talk: *The complex life of mRNA-1273 vaccinepoly(A) tailin immunecells*, EMBL Symposium: The complex life of RNA, October 2022, Heidelberg, Germany

Paweł Krawczyk, lecture: *Direct RNA nanopore sequencing for transcriptome-wide polyadenylation analysis*, NGSymposium in Computational Biology, September 2022, Warsaw, Poland

Michał Brouze, poster: *TENT5-mediated cytoplasmic polyadenylation of mRNAs encoding secreted proteins is essential for both spermatogenesis and oogenesis in mice*, EMBO Workshop RNA localization and local translation, July 2022, Barcelona, Spain

Bartosz Tarkowski, poster: *mRNAs of hypothalamic neuropeptides are polyadenylated in the cytoplasm*, Gordon Research Conference on Hypothalamus, July 2022, Ventura, USA

Natalia Gumińska, poster: *Direct detection of non-adenosine nucleotides within poly(A) tails – a new tool for the analysis of post-transcriptional mRNA tailing*, 27th Annual Meeting of the RNA Society, May-June 2022, Boulder, USA

Karolina Kasztelan, poster: *Identification of proteins involved in the regulation of double-stranded RNA level in the nucleus of human cells*, 27th Annual Meeting of the RNA Society, May-June 2022, Boulder, USA

Vladislava Liudkovska, poster: *TENT5 cytoplasmic non-canonical poly(A) polymerases regulate the innate immune response in animals*, 26th Annual Meeting of the RNA Society, May-June 2021, Seattle, USA [online]

INVITED SPEAKERS AT IIMCB

Radislav Sedláček, Institute of Molecular Genetics, Czech Academy of Sciences, Czech Centre for Phenogenomics, lecture: *Gateway to a comprehensive description of gene functions*

Torben Heick Jensen, Technical University of Denmark, lecture: *Nuclear fates of RNA 3' ends*

TRAINING AND NETWORKING ACTIVITIES

Bartosz Tarkowski, online advanced course: Next generation sequencing bioinformatics

Bartosz Tarkowski, online course: Introduction to RNA-seq and functional interpretation

Natalia Gumińska, online course: Introduction to statistics in R

Natalia Gumińska, online course: An introduction to nanopore direct RNA sequencing

Natalia Gumińska, study course: Data science in business applications

Zuzanna Mackiewicz, EMBO Practical Course: *C. elegans*: from genome editing to imaging, Heidelberg

Olga Gewartowska, PATHBIO course: Morphological mouse phenotyping, Barcelona

Aleksandra Brouze, EMBO Workshop: RNA 3' end formation and the regulation of eukaryotic genomes, Oxford

ERA Chairs Group members, Lab retreat with Grzegorz Kudła's and Joseph Marsh's laboratory members, Poland

AWARDS AND RECOGNITIONS

Andrzej Dziembowski, Prime Minister Award for scientific achievements

Andrzej Dziembowski, Honorary chair position named after Prof. Szybalski at the Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk

Olga Gewartowska, START 2022 scholarship from the Foundation for Polish Science for the best young scientists

Natalia Gumińska, Laureate of the RNA Society Poster Award at 27th Annual Meeting of the RNA Society and recipient of the certificate of recognition for excellence in RNA research

EFFECTIVE SCIENCE MANAGEMENT AND IMPROVED HUMAN RESOURCES ACTIVITIES

MOSaIC SUPPORTED OPEN ACCESS AND ETHICS PRACTICES AT IIMCB

We developed systemic support for IIMCB researchers in Open Access publishing, the preparation of data management plans, and managing ethical issues, including receiving permits for work with Genetically Modified Organisms and Microorganisms.
We introduced an Open Access policy and appointed an Open Access Data Steward. Internal Research Data Management and Research Integrity policies were prepared and are undergoing approval by the IIMCB Directors. In November 2022, we organized training on Research Data Management and Open Science for IIMCB staff. This covered: (i) Introduction to Research Data Management and Open Science, (ii) FAIR Data in the Research Data Lifecycle, and (iii) Data Sharing in Practice.

WARSAW PHD SCHOOL IN NATURAL AND BIOMEDICAL SCIENCES

With MOSaIC's contribution, IIMCB, together with eight other institutes, established the Warsaw PhD School in Natural and BioMedical Sciences [Warsaw-4-PhD]. By December 2022, 18 PhD students were affiliated with IIMCB. They attended various courses according to the School's curriculum and are involved in research in IIMCB laboratories (see page 80).

SCIENTIFIC EXCELLENCE

- Andrzej Dziembowski, ERA Chairs Group Leader
- Laboratory of RNA Biology – ERA Chairs Group
- 36 ERA Chairs Group Members
- 8 awarded grants
- 7 publications with acknowledgments of MOSaIC
- 8 lectures and 8 posters at international conferences
- 9 specialized trainings for ERA Chairs Group Members
- 4 awards and recognitions
- Genome Engineering Unit offering research services

MOSaIC's achievements at a glance

- Open Access Policy and Data Steward
- Training on Research Data Management and Open Science
- 18 IIMCB PhD students at Warsaw-4-PhD doctoral school
- Professional Human Resources Unit and Strategy
  - Recruitment processes
  - Soft skills trainings
  - Support for foreign employees
  - Disputes and Conflicts Resolution Policy
- Gender Equality Plan, Gender Officer, and Gender Equality Working Group
- Events communicating MOSaIC
  - MOSaIC kick-off meeting
  - Opening of ERA Chairs Laboratory
  - International Young Scientists Conference
  - 1st Women in Science Symposium
  - Interview of Prof. Dziembowski at biotechnologia.pl
  - Dr. Gewartowska presentation at EU-LIFE TechWatch Series

ORGANIZATIONAL EXCELLENCE

EXPLOITATION OF RESEARCH OUTCOMES AND RESEARCH SERVICES

Thanks to MOSaIC, IIMCB undertakes activities toward the acquisition of knowledge, mentoring, the protection of discoveries, and the development of research services.

Mentoring

Through SPARK Poland, IIMCB staff have access to various SPARK activities, including:

- Biomedical Innovation and Entrepreneurship Training Course for European Students
- SPARK Europe Innovator Café
- SPARK Europe Webinar Series

The SPARK Poland mentoring program 2020-2022 supported two IIMCB projects:

- *Drug repurposing for depression treatment using novel screening platform*, Jaworski Lab
- *Antibacterial wound dressings based on bacteriolytic enzymes*, Auresine Strategic Project

Protection of discoveries

IIMCB continued to take action toward the legal protection of two discoveries:

- *Recombinant polypeptide for use as a medicine, antiseptic agent, antibacterial agent, anti-inflammatory agent, compositions comprising it and uses thereof* [P.431445, PCT/PL2020/050075]. This invention was commercialized by granting an exclusive license to the licensee that provided the most favorable offer.
- *Peptidoglycan hydrolase, compositions comprising it, uses thereof, and a method of hydrolysis utilizing it* [P.438441, PCT/PL2022/050043].

Research services

Prof. Dziembowski founded and supervises the Genome Engineering Unit (see page 68), providing custom-made transgenic mouse models for external and internal users. In 2022, IIMCB established the Preclinical Drug Development Unit (see page 70) to offer protein production, purification and structural analysis.

HUMAN RESOURCES ACTIVITIES AND STRATEGY

Thanks to MOSaIC, IIMCB employed a professional Human Resources manager who organized the Human Resources Unit according to advanced standards, composed of personnel with required competencies. Soon after, the Human Resources Strategy for IIMCB established a number of support measures for in-house staff at all career stages:

- Comprehensive administrative and formal support for recruitment processes
- Workshops for nearly 250 IIMCB staff members on career development, soft skills, management, and the prevention of discrimination by proactively responding to inappropriate behavior
- Comprehensive system of support for foreign employees
- Disputes and Conflicts Resolution Policy
- Benefits for employees regarding additional holidays, flexible working hours, and work systems

Human Resources also played a central role in developing the Gender Equality Plan for IIMCB in line with best European standards. They established a Working Group on Gender Equality Opportunities, bringing together representatives of all employee groups. The Gender Equality Plan prioritizes activities that foster a working environment where all individuals are treated equally, with respect, and with fairness.

Activities that are planned in the Gender Equality Plan are compatible with the objectives of the Human Resources Strategy. In 2022, the Gender Equality Plan included:

- Guidelines on Balanced Gender Representation in Committees, Councils, Delegations, Teams, and other Advisory Bodies
- Guide Listing all Entitlements of Parents at IIMCB both nationwide and internally
- Recruitment Handbook, a document that compiles a list of recommendations to ensure that the recruitment process for PhD students and scientific and non-scientific staff at IIMCB is conducted fairly, objectively, and with transparency
- *Active Bystander* workshop on how to prevent discrimination by proactively responding to inappropriate behavior [one training workshop for scientists and one training workshop for non-scientific staff]

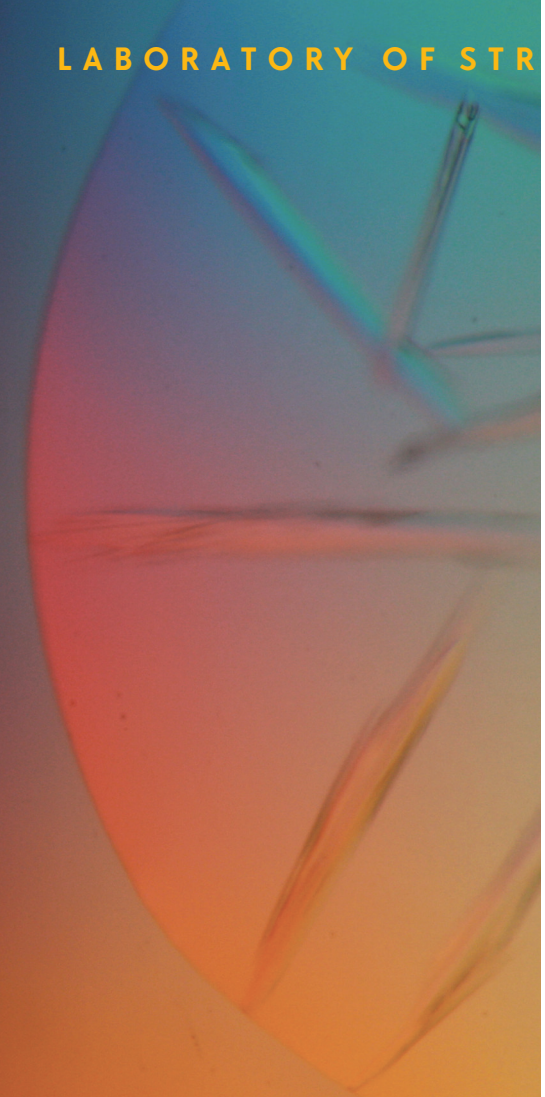
MOSaIC FINAL CONFERENCE

We cordially invite all interested researchers to the Polish RNA Biology Meeting, a conference that marks the culmination of MOSaIC, that will be held at IIMCB on September 28-30, 2023. We guarantee great speakers, the presentation of new discoveries, and three days of sharing experiences. Check the meeting website at pl-rna.iimcb.gov.pl. Let's celebrate MOSaIC's achievements together.

MOSaIC project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 810425

RESEARCH GROUPS

LABORATORY OF STRUCTURAL BIOLOGY



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

PROFESSIONAL EXPERIENCE
2011-Present Professor, Head of Laboratory of Structural Biology, International Institute of Molecular and Cell Biology in Warsaw, Poland and Laboratory of Genome Engineering, Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland
2007-2011 Part-time Director of Structural Biology, Cardiff University, United Kingdom
2001-2010 Head, Joint MPG-PAS Junior Research Group, International Institute of Molecular and Cell Biology in Warsaw, Poland
2000 Patent training, Weickmann & Weickmann
1999-2000 Postdoctoral Fellow, Max Planck Institute of Biochemistry, Martinsried, 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

HONORS, PRIZES AND AWARDS
2022 Team Award of the Minister of Education and Science for significant achievements in scientific activities
2018 TEAM, Foundation for Polish Science
2018 International Academic Partnerships Programme, Polish National Agency for Academic Exchange
2018 DAINA, National Science Centre
2015 HARMONIA, National Science Centre
2014 MAESTRO, National Science Centre
2011 TEAM, Foundation for Polish Science
2005 Professor Stefan 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

DOCTORATES DEFENDED UNDER LAB LEADER'S SUPERVISION
R. Filipek, M. Firczuk, M. Lipka, R. Szczepanowski, M. Kaus-Drobek, M. Sokołowska, G. Chojnowski, H. Korza, M. Wojciechowski, W. Siwek, P. Haniewicz, A.A. Kazrani, K. Mierzejewska, A. Slyvka, M. Kisiała, D. Rafalski, Anna Stroynowska-Czerwińska.

GROUP MEMBERS

Lab Leader
Matthias Bochtler, PhD, Professor

Senior Researcher
Honorata Czapińska, PhD, DSc Habil

Postdoctoral Researcher
Anton Slyvka, PhD

PhD Students
Anna Fedenko, MSc
Igor Helbrecht, MSc (IBB)
Terry Karimi, MSc
Magdalena Klimczak, MSc Eng.
Katarzyna Krakowska, MSc
Abhishek Pateria, MSc Eng.
Dominik Rafalski, MSc Eng. (PhD defense in June 2022)
Anna Stroynowska-Czerwińska, MSc Eng.
(PhD defense in March 2023)

Technician
Julia Pac, MSc (part-time)

Laboratory Support Specialist
Aleksandra Jakielaszek, MSc Eng.

SELECTED PUBLICATIONS

IIMCB Best Papers Award

Stroynowska-Czerwinska AM, Klimczak M, Pastor M, Kazrani AA, Misztal K, Bochtler M. Clustered PHD domains in KMT2/MLL proteins are attracted by H3K4me3 and H3 acetylation-rich active promoters and enhancers. ***Cell Mol Life Sci***, 2023; 80(1):23

Czapińska H, Bochtler M. *The Nt-rule for serine, but not cysteine catalytic triads.* ***Angew Chem Int Ed Engl***, 2022; e202206945

Winiewska Szajewska M, **Czapinska H**, Kaus Drobek M, **Fricke A**, Mieczkowska K, Dadlez M, **Bochtler M**, Poznański J. *Competition between electrostatic interactions and halogen bonding in the protein–ligand system: structural and thermodynamic studies of 5,6-dibromobenzotriazole-hCK2α complexes.* ***Sci Rep***, 2022; 12(1):18964

Niescierowicz K, Pryszcz L, Navarrete C, Tralle E, Sulej A, Abu Nahia K, Kasprzyk ME, Misztal K, Pateria A, Pakuta A, Bochtler M, Winata C. *Adar-mediated A-to-I editing is required for embryonic patterning and innate immune response regulation in zebrafish.* ***Nat Commun***, 2022; 13(1):5520

Ravichandran M, **Rafalski D**, Davies CI, Ortega-Recalde O, Nan X, Glenfield CR, Kotter A, **Misztal K**, Wang AH, **Wojciechowski M, Razew M**, Mayyas IM, Kardailsky O, Schwartz U, Zembrzycki K, Morison IM, Helm M, Weichenhan D, Jurkowska RZ, Krueger F, Plass C, Zacharias M, **Bochtler M**, Hore TA, Jurkowski TP. *Pronounced sequence specificity of the TET enzyme catalytic domain guides its cellular function.* ***Sci Adv***, 2022; 8(36):eabm2427

Pastor M, Czapinska H, Helbrecht I, Krakowska K, Lutz T, Xu S, **Bochtler M.** Crystal structures of the EVEHNH endonuclease VcaM4I in the presence and absence of DNA. ***Nucleic Acids Res***, 2021; 49(3):1708-23

Bochtler M. Distinction between self and non-self in restriction modification: The mysterious case of type III enzymes. ***Structure***, 2021; 29(6):512-514

Bochtler M, Fernandes H. DNA adenine methylation in eukaryotes: Enzymatic mark or a form of DNA damage? ***BioEssays***, 2021; 43(3):e2000243

Xu G-L, **Bochtler M**. Reversal of nucleobase methylation by dioxygenases. ***Nat Chem Biol***, 2020; 16:1160-69

Bochtler M. Arrhenius-law-governed homo- and heteroduplex dissociation. ***Phys Rev E***, 2020; 101, 032405

Fricke T, Smalakyte D, **Lapinski M, Pateria A, Weige C**, Pastor M, **Kolano A, Winata C**, Siksnys V, Tamulaitis G, **Bochtler M**. Targeted RNA Knockdown by a Type III CRISPR-Cas Complex in Zebrafish. ***CRISPR J***, 2020; 3(4):299-313

Tomkuvienė M, Ikasalaitė D, **Slyvka A**, Rukšenaitė A, Ravichandran M, Jurkowski TP, **Bochtler M**, Klimašauskas S. Enzymatic Hydroxylation and Excision of Extended 5-Methylcytosine Analogues. ***J Mol Biol***, 2020; 432(23):6157-67

Kisiala M, Kowalska M, Pastor M, Korza HJ, **Czapinska H, Bochtler M**. Restriction endonucleases that cleave RNA/DNA heteroduplexes bind dsDNA in A-like conformation. ***Nucleic Acids Res***, 2020; 48(12):6954-69

Slyvka A, Zagorskaitė E, **Czapinska H**, Sasnauskas G, **Bochtler M**. Crystal structure of the EcoKMcrA N-terminal domain (NEco): recognition of modified cytosine bases without flipping. ***Nucleic Acids Res***, 2019; 47(22):11943-55

Lutz T, Flodman K, Copelas A, **Czapinska H**, Mabuchi M, Fomenkov A, He X, **Bochtler M**, Xu S. A protein architecture guided screen for modification dependent restriction endonucleases. ***Nucleic Acids Res***, 2019; 47(18):9761-76

Tamulaitiene G, Manakova E, Jovaisaite V, Tamulaitis G, Grazulis S, **Bochtler M**, Siksnys V. Unique mechanism of target recognition by PfoI restriction endonuclease of the CCGG-family. ***Nucleic Acids Res***, 2019; 47(2):997-1010

Kisiala M, Copelas A, **Czapinska H**, Xu S, **Bochtler M**. Crystal structure of the modification-dependent SRA-HNH endonuclease TagI. ***Nucleic Acids Res***, 2018; 46(19):10489-503

Stroynowska-Czerwinska A, Piasecka A, Bochtler M. Specificity of MLL1 and TET3 CXXC domains towards naturally occurring cytosine modifications. ***Biochim Biophys Acta Gene Regul Mech***, 2018 Dec;1861(12):1093-1101.

Czapinska H, Kowalska M, Zagorskaite E, Manakova E, **Slyvka A**, Xu SY, Siksnys V, Sasnauskas G, **Bochtler M.** Activity and structure of EcoKMcrA. ***Nucleic Acids Res***, 2018; 46(18):9829-41

Slyvka A, Mierzejewska K, Bochtler M. Neilike 1 (NEIL1) excises 5-carboxylcytosine directly and stimulates TDG-mediated 5-formyl and 5-carboxylcytosine excision. ***Sci Rep***, 2017; 7(1):9001

Bochtler M, Kolano A, Xu G-L. DNA demethylation pathways: Additional players and regulators. ***Bioessays***, 2017; 39(1):1-13

Mierzejewska K, Bochtler M, Czapinska H. On the role of steric clashes in methylation control of restriction endonuclease activity. ***Nucleic Acids Res***, 2016; 44(1):485-95

DESCRIPTION OF CURRENT RESEARCH

Our laboratory is focused on chromatin modifications and associated chromatin reader domains, with a particular interest in DNA methylation.

DNA demethylation

DNA demethylation proceeds in three fundamentally different ways. Replication-independent active demethylation occurs by the ten eleven translocation (TET)-catalyzed oxidation of 5-methylcytosines (5mC), which can be replaced by base excision repair. In terminally differentiated cells, active DNA demethylation is the only option. However, in other cells, it is unlikely to be preferred because it involves the formation of single-strand break intermediates that threaten DNA integrity. There are two types of replication-dependent demethylation: active-passive and passive. Active-passive demethylation involves the local suppression of maintenance methylation in the presence of DNMT1 and UHRF1 through the oxidation of 5mC to 5-hydroxymethylcytosine (5hmC) in the parent strand. Passive demethylation involves the suppression of maintenance methylation machinery and is only relevant at a few developmental stages.

- How have TETs evolved? TETs are related to ALKBH proteins and catalyze the oxidation-methylation of methyl groups through the same radical-based mechanism. However, unclear is how the specificity for 5mC has evolved. If our evolutionary hypothesis is correct, then it should be possible to return TETs to an "ancestral" DNA repair function. We have already demonstrated this in some TET paralogs. We are now in the process of clarifying the differences between the convertibility of TET paralogs into DNA repair enzymes and completing this story.

- How do TETs arrive at their targets? We initially described the binding properties of the TET3 CXXC domain toward DNA-containing CpG and its modifications. In 2022, we published our work in collaboration with Dr. Jurkowski (Cardiff) and Dr. Hore (Otago) on TET enzyme sequence preferences and their structural basis (Ravichandran et al., *Sci Adv*, 2022). Our follow-up analysis shows that TETs are sensitive to the DNA sequence and strongly focused on the chromatin context. We are currently attempting to determine the relative importance of chromatin modifications and DNA sequence specificity.

- What is the role of TET-mediated active demethylation in non-terminally differentiated cells? The active-passive pathway, which does not require single-strand break intermediates, is far superior for locus-specific demethylation. However, it is unsuitable for repairing ectopic methylation which results from errors in methylation maintenance machinery at the replisome. We are currently investigating the possible role of TETs as epigenome repair enzymes by analyzing levels of context-independent methylation in wildtype and TET knockout cells in the KG-1 acute myeloid leukemia cell line.

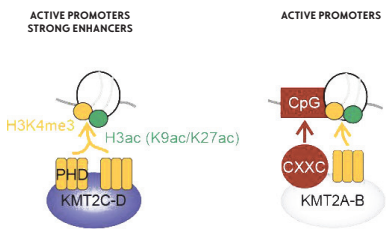
- Can active-passive demethylation be detected in cells? DNA integrity arguments support an important role in active-passive DNA demethylation, but to our knowledge, this mechanism has only been demonstrated *in vitro* with purified DNMT1 and not DNMT1 in the context of the replisome. We are currently attempting to demonstrate the mechanism in cells using direct nanopore sequencing with metabolic labelling (bromodeoxyuridine), which allows us to distinguish daughter and parent strands.

- Passive demethylation relies on the suppression of DNMT1 activity. In collaboration with Prof. Wong (Shanghai), we are looking at the consequences of acute experimental suppression of the activity of DNMT1, UHRF1, and both. We observe transcriptional activation and also see very different responses of particular genes. We expect chromatin marks to play a role, and we are now correlating reactivation data with the chromatin state.

Chromatin-based positive transcriptional memory

In a developmental context, the best-known positive genetic memory system is the Trithorax system, named after body segment identification changes in *Drosophila* mutants. Mammalian equivalents of Trithorax and Trithorax-related are COMPASS-like complexes, which maintain transcription-promoting H3K4me3 and H3K4me1 at promoters and enhancers, respectively.

- How do COMPASS-like complexes, more specifically their catalytic subunits (called KMT2A-D proteins), find their targets? Using CUT&RUN and greenCUT&RUN (novel alternatives to ChIP-sequencing), we showed that clustered PHD domains in KMT2 proteins alone are sufficient to find a subset of active promoters and strong enhancers (Fig.1). PHD domains co-localize with regions that are enriched with H3K4me3 and H3 acetylation. H3 acetylation is essential in the chromatin context because it decreases the interaction with nucleosomal DNA and allows the availability of H3K4me3 for the binding reading domain. We also clarified the division of labor between KMT2A-D proteins. KMT2A and KMT2B are known to be primarily responsible for keeping promoters active, whereas KMT2C and KMT2D are associated with maintaining active enhancers. We have now linked this genetic property to the presence of CXXC domains in KMT2A/B but not KMT2C/D. CXXC domains bind non-methylated CpG (Stroynowska-Czerwinska et al., *BBA Gene Regul Mech*, 2018), which is enriched in CG islands of active promoters but not enhancers. Experimentally, a fusion of the CXXC domain to PHD triplets dramatically changed promoter/enhancer preferences of the constructs (Stroynowska-Czerwinska et al., *Cell Mol Life Sci*, 2023).



Chromatin reader domains of KMT2A-D/MLL1-4

Fig. 1. Chromatin targeting by chromatin reader domains of KMT2A-D proteins. Clustered PHD domains bind H3K4me3 in the presence of H3 acetylation marks (H3K9ac and H3K27ac) and localise the protein to the active promoters and enhancer regions. The CXXC domain has a rigid specificity for non-modified CpG, limiting the KMT2A-B binding to active promoters only.

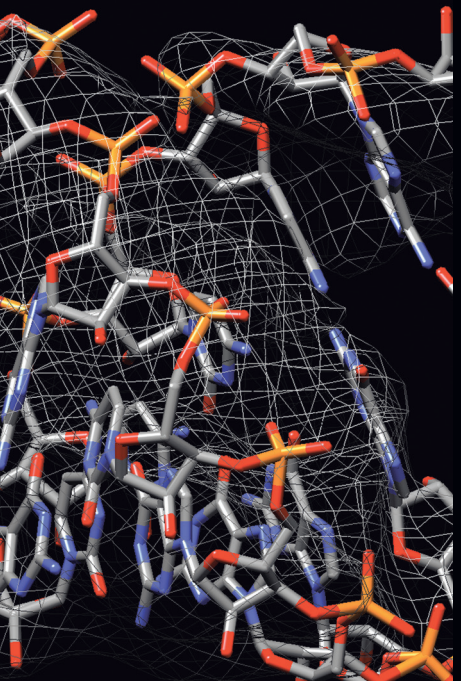
Nucleobase modifications

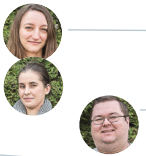
In 2022, we focused on two nucleobase modifications: adenine deamination in RNA and momylation in DNA.

- What is the role of adenine deamination in zebrafish RNA? This work was triggered by an observation by our colleague, Dr. Winata, that zebrafish Adar is abundant in early zebrafish embryos, where it is both maternally deposited and later also expressed from the embryonic genome. This suggested a developmental role for zebrafish Adar. Indeed, Dr. Winata showed that zebrafish Adar is required for antero-posterior and dorso-ventral axes and patterning. Our bioinformatic analysis of the transcriptome revealed ubiquitous editing in the maternal and earliest zygotic transcripts, the majority of which occurred in the 3' untranslated region. Interestingly, transcripts that are involved in gastrulation and dorso-ventral and antero-posterior patterning were found to contain multiple editing sites. Adar deficiency is ultimately lethal. Using RNA sequencing, we showed that zebrafish, similar to mammals, respond to Adar deficiency with strong activation of the innate immune response, which is a likely cause of lethality (Niescierowicz et al., *Nat Commun*, 2022).

- What is the momylation pathway? Momylation (i.e., the addition of carbamoylmethyl to the N6 group of adenine) is one of the (now few) DNA modifications that occur through biochemically poorly understood pathways. Biologically, the Mom modification is used by phage Mu to protect its genome from host endonucleases. Bioinformatics studies by Prof. Bujnicki showed that Mom belongs to GNAT acyltransferases. However, the structure of the carbamoylmethyl modification appeared to be incompatible with simple acyl transfer, and the reaction could not be reconstituted *in vitro*. In collaboration with the Dr. Weigele group at New England Biolabs, we demonstrated that phage utilizes host machinery to catalyze momylation in an alternative way.

LABORATORY OF BIOINFORMATICS AND PROTEIN ENGINEERING





GROUP MEMBERS

Lab Leader
Janusz M. Bujnicki, PhD, Professor

Senior Researchers
Elżbieta Purta, PhD
Filip Stefaniak, PhD

Postdoctoral Researchers
Evgenii Baulin, PhD
Belisa R. H. de Aquino, PhD (until June 2022)
Georgios Kritikos, PhD (until December 2022)
Satyabrata Maiti, PhD
Sunandan Mukherjee, PhD
Angana Ray, PhD
Tales Rocha de Moura, PhD
Tomasz Wirecki, PhD

Research Assistants
Agata Bernat, MSc
Katarzyna Merdas, MSc
Małgorzata Kurkowska, MSc
(until October 2022)

Research Specialists
Radosław Gziński, MSc (until June 2022)
Ytalia Lavalle, BSc (until April 2022)
Ryhor Nikalayeu, MSc (since October 2022)

Junior Research Specialists
Yuyang Cai, MSc
Muhammad Ehsan Soddique, MSc
(since November 2022)
Adriana Fedco, MSc (until March 2022)
Dominik Sordyl, MSc (since November 2022)

PhD Students
Masoud Amiri Farsani, MSc
Nagendar Badepally Goud, MSc
Andrea Cappannini, MSc
Farhang Jaryani, MSc
Seyed Naeim Moafinejad, MSc

Volunteer
Davyd Bohdan, MSc

Technician
Iwona Ptasiewicz (part-time)

Laboratory Support Specialist
Katarzyna Grzelak, MSc

LAB LEADER

Janusz M. Bujnicki, PhD, Professor

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

PROFESSIONAL EXPERIENCE
2002-Present Professor, Head of Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, Poland
2019-Present Scientific Advisor, Łukasiewicz Research Network – PORT Polish Center for Technology Development (25% appointment)
2006-2020 Associate Professor (extraordinarius), Bioinformatics Laboratory, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland
2010-2011 Deputy Director, International Institute of Molecular and Cell Biology in Warsaw (1 year rolling position)
2008 Visiting Professor, University of Tokyo, Japan (sabbatical)
2004-2006 Assistant Professor, Adam Mickiewicz University, Poznań, Poland
2001 Visiting Scientist, National Center for Biotechnology Information, National Institutes of Health, Bethesda, Maryland, USA
1999-2002 Research Scientist, Bioinformatics Laboratory, International Institute of Molecular and Cell Biology in Warsaw, Poland
1998-2000 Senior Research Assistant, Henry Ford Hospital, Detroit, Michigan, USA

SELECTED PROFESSIONAL AFFILIATIONS
2019-2023 Member, Committee for Science Evaluation, Ministry of Education and Science
2020 Member, Advisory Group on Preventing, Counteracting and Combating COVID-19, Ministry of Science and Higher Education
2019-Present Member, University Council of the University of Warsaw (Chairman, 2019-2020)
2018-Present Member, Academia Europaea
2017-Present Member, European Molecular Biology Organization (EMBO)
2016-Present Corresponding Member, Polish Academy of Sciences
2016-2017 Member, Council of the National Science Congress
2015-Present European Commission's Scientific Advice Mechanism (Member, Group of Chief Scientific Advisors, 2015-2020; Expert, 2020-Present)
2014-2018 Member, Scientific Policy Committee, Polish Ministry of Science and Higher Education
2013-Present Executive Editor, Nucleic Acids Research
2013-2016 Member, Scientific Committee of the Innovative Medicines Initiative
2013-2015 Member, Science Europe: Life, Environmental and Geo Sciences (LEGS) Scientific Committee
2011-2016 Member, Polish Young Academy, Polish Academy of Sciences
2007-Present Member, Polish Bioinformatics Society (founding member; Vice-President, 2007-2010; President, 2011-2013)
2007-Present Member, RNA Society
2001-Present Member, International Society for Computational Biology (Senior Member, 2015-Present)

SELECTED AWARDS AND FELLOWSHIPS
2022 Honorary Membership of the Polish Bioinformatics Society
2019 Andre Mischke Young Academy of Europe Prize for Science and Policy
2019 Honorary Award "For Merits for Inventiveness", Prime Minister at the request of the Polish Patent Office
2017 Award for Organizational Achievements, Ministry of Science and Higher Education
2016 Crystal Brussels Sprout Award
2015 Jan Karol Parnas Award of the Polish Biochemical Society
2014 National Science Centre Award for outstanding scientific achievements
2014 Master Award, Foundation for Polish Science
2014 Prime Minister Award for outstanding scientific achievements
2014 Selected as one of "25 leaders for the next 25 years" by Teraz Polska magazine of the Polish Promotional Emblem Foundation
2014 Knight's Cross of the Order of Polonia Restituta
2014 Award in the Science category of the national plebiscite "Poles with Verve"
2013 ERC Proof of Concept Grant
2012 Award for Outstanding Research Achievements, Ministry of Science and Higher Education
2010 ERC Starting Grant (2011-2015)
2009 Scholarship for Outstanding Young Scientists, Minister of Science and Higher Education
2009 Award for Research Achievements, Ministry of Science and Higher Education
2006 Prime Minister Award for habilitation thesis
2006 Young Researcher Award in Structural and Evolutionary Biology, Visegrad Group Academies of Sciences
2003, 2004 START Scholarship for Young Scientists, Foundation for Polish Science
2002-2005 EMBO/HHMI Young Investigator Award
2002 Award for best Polish genetics-related publication in 2002, Polish Genetics Society
2001 Award for best Polish publication on nucleic acid biochemistry in 2000, Polish Biochemical Society and Sigma-Aldrich

DOCTORATES DEFENDED UNDER LAB LEADER'S SUPERVISION
A. Żylicz-Stachula, A. Chmiel, I. Cymerman, A. Czerwoniec, M. Gajda, M. Pawłowski, J. Sasin-Kurowska, J. Kosiński, A. Obarska-Kosińska, S. Pawlak, E. Purta, K. Tkaczuk, Ł. Kościński, M. Rother, W. Potrzebowski, I. Korneta, T. Puton, J. Kasprzak, I. Tuszyńska, Ł. Kozłowski, M. Werner, A. Kamaszewska, A. Philips, K. Milanowska, M. Piętal, D. Matelska, K. Majorek, M. Domagalski, T. Osiński, M. Machnicka, M. Magnus, K. Szczepaniak, M. Zielińska, Astha, I. Foik, D. Toczydłowska-Socha, K. Poleszak.

SELECTED PUBLICATIONS

○ IIMCB Best Papers Award

Luo B, Zhang C, Ling X, **Mukherjee S**, Jia G, Xie J, Jia X, Liu L, **Baulin EF**, Luo Y, Jiang L, Dong H, Wei X, **Bujnicki JM**, Su Z. Cryo-EM reveals dynamics of *Tetrahymena* group I intron self-splicing. ***Nature Catal***, 2023; in press

Jia X, Pan Z, Yuan Y, Luo B, Luo Y, **Mukherjee S**, Jia G, Liu L, Ling X, Yang X, Miao Z, Wei X, **Bujnicki JM**, Zhao K, Su Z. Structural basis of sRNA RsmZ regulation of *Pseudomonas aeruginosa* virulence. ***Cell Res***, 2023; doi: 10.1038/s41422-023-00786-3

Moafinejad SN, **Pandaranadar Jeyeram IPN**, **Jaryani F**, **Shirvanizadeh N**, **Baulin E**, **Bujnicki J**. *1D2DSimScore*: A novel method for comparing contacts in biomacromolecules and their complexes. ***Protein Sci***, 2023; 32(1):e4503

Cappannini A, Mosca K, **Mukherjee S**, Moafinejad SN, Sinden RR, Arluison V, **Bujnicki J**, Wien F. NACDDB: Nucleic Acid Circular Dichroism Database. ***Nucleic Acids Res***, 2023; 51(D1):D226-D231

Boccaletto P, **Stefaniak F**, **Ray A**, **Cappannini A**, **Mukherjee S**, **Purta E**, **Kurkowska M**, **Shirvanizadeh N**, Destefanis E, Groza P, Avşar G, Romitelli A, Pir P, Dassi E, Conticello SG, Aguilo F, **Bujnicki JM**. MODOMICS: a database of RNA modification pathways. 2021 update. ***Nucleic Acids Res***, 2022; 50(D1):D231-D235

Szulc NA, **Mackiewicz Z**, **Bujnicki JM**, **Stefaniak F**. fingeRNAt-A novel tool for high-throughput analysis of nucleic acid-ligand interactions. ***PLoS Comput Biol***, 2022; 18(6):e1009783

Mohammadi-Arani R, Javadi-Zarnaghi F, **Boccaletto P**, **Bujnicki JM**, **Ponce-Salvatierra A**. DNAzymeBuilder, a web application for in situ generation of RNA/DNA-cleaving deoxyribozymes. ***Nucleic Acids Res***, 2022; 50(W1):W261-W265

Stefaniak F, **Bujnicki JM**. AnnapuRNA: A scoring function for predicting RNA-small molecule binding poses. ***PLoS Comput Biol***, 2021; 17(2):e1008309

Ponce-Salvatierra A, **Boccaletto P**, **Bujnicki JM**. DNAmoreDB, a database of DNAzymes. ***Nucleic Acids Res***, 2021; 49(D1):D76-D81

Wirecki TK, **Merdas K**, **Bernat A**, **Boniecki MJ**, **Bujnicki JM**, **Stefaniak F**. RNAProbe: a web server for normalization and analysis of RNA structure probing data. ***Nucleic Acids Res***, 2020; 48(W1):W292-W299

○ Manfredonia I, **Nithin C**, **Ponce-Salvatierra A**, **Ghosh P**, **Wirecki TK**, Marinus T, Ogando NS, Snijder EJ, van Hemert MJ, **Bujnicki JM**, Incarnato D. Genome-wide mapping of SARS-CoV-2 RNA structures identifies therapeutically-relevant elements. ***Nucleic Acids Res***, 2020; 48(22):12436-52

○ **Toczydlowska-Socha D**, **Zielinska M**, **Kurkowska M**, **Astha**, **Almeida CF**, **Stefaniak F**, **Purta E**, **Bujnicki JM**. Human RNA cap1 methyltransferase CMTr1 cooperates with RNA helicase DHX15 to modify RNAs with highly structured 5' termini. ***Philos Trans R Soc Lond B Biol Sci***, 2018; 373(1762): 20180161

○ **Boccaletto P**, **Magnus M**, **Almeida C**, **Zyla A**, **Astha**, **Pluta R**, **Baginski B**, **Jankowska E**, **Dunin-Horkawicz S**, **Wirecki T**, **Boniecki M**, **Stefaniak F**, **Bujnicki JM**. RNArchitecture: a database and a classification system of RNA families, with a focus on structural information. ***Nucleic Acids Res***, 2018; 46(D1):D202-D205

Boniecki MJ, **Lach G**, **Dawson WK**, **Tomala K**, **Lukasz P**, **Soltysinski T**, **Rother KM**, **Bujnicki JM**. SimRNA: a coarse-grained method for RNA folding simulations and 3D structure prediction. ***Nucleic Acids Res***, 2016; 44(7):e63

Ukleja M, Cuellar J, Siwaszek A, **Kasprzak JM**, Czarnocki-Cieciura M, **Bujnicki JM**, Dziembowski A, Valpuesta J. The architecture of the Schizosaccharomyces pombe CCR4-NOT complex. ***Nature Commun***, 2016; 7:10433

Dawson WK, **Bujnicki JM**. Computational modeling of RNA 3D structures and interactions. ***Curr Opin Struct Biol***, 2015; 37:22-8

Stefaniak F, Chudyk E, Bodkin M, **Dawson WK**, **Bujnicki JM**. Modeling of RNA-ligand interactions. ***WIREs Comput Mol Sci***, 2015; 5(6):425-39

○ **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; 43(5):2864-73

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:e151

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

DESCRIPTION OF CURRENT RESEARCH

Our group 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. We are currently focusing on developing software for the structural prediction and modeling of RNA and complexes of RNA with proteins and small-molecule ligands.

To date, we have developed and made publicly available one of the first methods for the automated comparative (template-based) modeling of three-dimensional (3D) RNA structures (ModeRNA; https://iimcb.genesilico.pl/moderna) and a method for *de novo* (template-free) RNA structure modeling (SimRNA; https://genesilico.pl/software/stand-alone/simrna, also available as a web server at https://genesilico.pl/SimRNAweb). We also developed methods for modeling RNA-metal and RNA-ligand complexes and a method for predicting the structure of RNA-protein complexes (https://genesilico.pl/NPDock). Other methods for RNA bioinformatics include a method for the classification of contacts in RNA 3D structures (ClaRNA; https://iimcb.genesilico.pl/clarna) and a method for the flexible superposition of RNA 3D structures and their fragments (SupeRNAlign; https://genesilico.pl/supernalign). We also developed various databases, including a database of RNA modification pathways and enzymes (MODOMICS; https://genesilico.pl/modomics), a database of RNA 3D motifs and their interactions (RNA Bricks; https://iimcb.genesilico.pl/rnabricks), a structural classification of known families of structured non-coding RNAs (RNArchitecture; https://iimcb.genesilico.pl/RNArchitecture), and a database of DNAzymes (https://www.genesilico.pl/DNAmoreDB).

Our experimental research focuses on elucidating sequence-structure-function relationships in bio-macromolecules (currently mainly RNA and RNA-protein complexes, also with small organic molecules) using biophysics, biochemistry, molecular biology, and cell biology techniques. We tightly integrate theoretical and experimental research. We often experimentally test functional and structural predictions for RNAs, proteins, and their complexes that are obtained using computational methods. For structural studies, we combine cryoelectron microscopy, X-ray crystallography, and low-resolution methods, such as small-angle X-ray scattering and structure probing by chemical modification.

RECENT HIGHLIGHTS

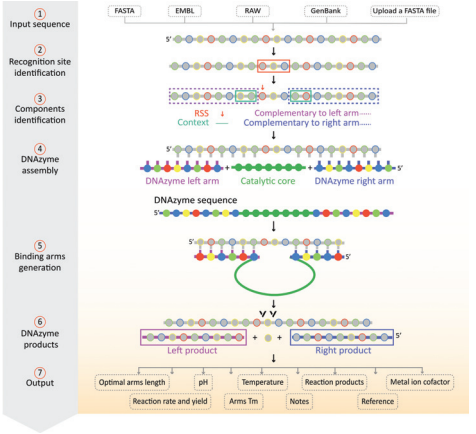
fingeRNAt: A novel tool for the analysis of nucleic acid-ligand interactions

RNA has recently emerged as an attractive target for new drug development. Unfortunately, the supply of computational methods to study RNA and its interactions with small chemical molecules is very limited, and the need to develop new tools is growing. We developed a new computational method, fingeRNAt, to automatically detect and classify non-covalent interactions with RNA. The resulting data can help decipher the nature of interactions and identify main factors that are responsible for the formation of molecular complexes. We experimentally analyzed solute structures of small-molecule RNA complexes to determine the most abundant binding features (i.e., the most common interactions or their hot spots). The results of this analysis may help elucidate binding mechanisms and design new active molecules. We also propose to use the data that are generated by our software as new metrics for quantitative pairwise comparisons of molecular complexes. We showed that it is more reliable than current methods in cases in which interactions are difficult to classify. We showed that results of our program can be used for high-throughput analyses of molecular complexes and the search for functionally active molecules. Our fingeRNAt software is freely available at https://github.com/n-szulc/fingeRNAt.

Publication:
Szulc NA, **Mackiewicz Z**, **Bujnicki JM**, **Stefaniak F**. fingeRNAt: a novel tool for high-throughput analysis of nucleic acid-ligand interactions. ***PLoS Comput Biol***, 2022; 18(6):e1009783

New bioinformatics tools for the analysis of DNAzymes

DNAzymes, also known as deoxyribozymes or DNA enzymes, are single-stranded oligodeoxyribonucleotide molecules that have the ability to catalyze chemical reactions, similar to proteins and ribozymes. Although DNAzymes have not been found in living organisms, they have been synthesized in the laboratory through *in vitro* selection. The selected DNAzyme sequences can catalyze a diverse range of chemical reactions using DNA, RNA, peptides, or small organic compounds as substrates. To provide a comprehensive resource for DNAzyme information, the **DNAmoreDB database** was developed to collect and organize various data types, such as sequences, selection conditions, catalyzed reactions, kinetic parameters, substrates, cofactors, structural information, and literature references. Currently, DNAmoreDB contains information on DNAzymes that catalyze 20 different reactions. The database includes a submission form for new data, a REST-based API system for machine-readable format retrieval, and such search features as keywords and BLASTN. DNAmoreDB is publicly available at https://www.genesilico.pl/DNAmoreDB.



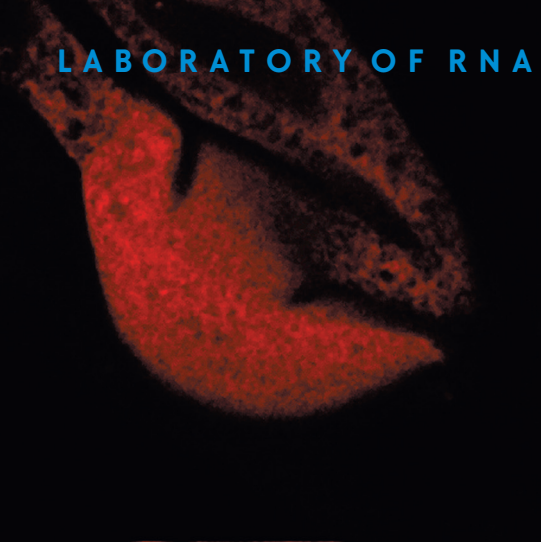
Although DNAzymes are highly attractive, selecting the appropriate DNAzyme to cleave a specific substrate is a complex task that requires expertise and extensive literature research. In collaboration with Dr. Fatemeh Javadi-Zarnaghi and her coworkers at the University of Isfahan, we developed the **DNAzymeBuilder tool** that provides an efficient and automated solution to replace manual DNAzyme design. DNAzymeBuilder uses an internal database that contains information on RNA- and DNA-cleaving DNAzymes, including their best operating reaction conditions, kinetic parameters, the type of cleavage reaction catalyzed, the specific sequence recognised by the DNAzyme, the cleavage site within that sequence, and special design features required for optimal DNAzyme activity. By analyzing this information together with the user's input sequence, DNAzymeBuilder can quickly generate a list of DNAzymes that are able to perform the cleavage reaction, along with such detailed information as expected yield, reaction products, and optimal reaction conditions. DNAzymeBuilder is an invaluable resource to help researchers integrate DNAzymes into their daily research activities and is freely available at https://iimcb.genesilico.pl/DNAzymeBuilder.

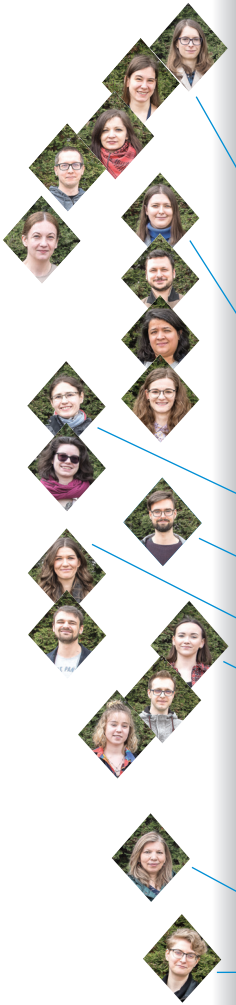
Publications:
Ponce-Salvatierra A, **Boccaletto P**, **Bujnicki JM**. DNAmoreDB, a database of DNAzymes. ***Nucleic Acids Res***, 2021; 49(D1):D76-D81

Mohammadi-Arani R, Javadi-Zarnaghi F, **Boccaletto P**, **Bujnicki JM**, **Ponce-Salvatierra A**. DNAzymeBuilder, a web application for *in situ* generation of RNA/DNA-cleaving deoxyribozymes. ***Nucleic Acids Res***, 2022; 50(W1):W261-W265

Fig. 1. DNAzymeBuilder assembles nucleic acid cleaving DNAzymes for the site-specific cleavage of RNA, DNA, or chimeric substrates and provides detailed information on reaction conditions and products. Original image: https://iimcb.genesilico.pl/DNAzymeBuilder/algorithm

LABORATORY OF RNA BIOLOGY – ERA CHAIRS GROUP





GROUP MEMBERS

Lab Leader
Andrzej Dziembowski, PhD, Professor

Senior Researcher
Seweryn Mroczek, PhD, DSc Habil

Postdoctoral Researchers
Aleksandra Brouze, PhD
Areta Czerwińska, PhD
Natalia Gumińska, PhD
Paweł Krawczyk, PhD
Tomasz Kuliński, PhD
Monika Kusio-Kobiałka, PhD
Ewa Poniecka, PhD
Bartosz Tarkowski, PhD

Research Assistants
Kamila Affek, MSc
Michał Brouze, MSc
Paula Andrea Castañeda Londoño, MSc
Ewelina Patrycja Owczarek, MSc
Karolina Kasztelan, MSc

Research Specialists
Agnieszka Czarnocka-Cieciura, MSc
Sebastian Jeleń, PhD
Karolina Piechna, MSc

Junior Research Specialists
Wiktor Antczak, BSc
Hanna Grzesik, BSc
Karolina Jaworska, MSc

PhD Students
Zuzanna Mackiewicz, MSc
Michał Mazur, MSc

Volunteers
Julia Cieślicka, BSc
Dawid Dzadz, BSc
Julia Gilewska
Jakub Guzek, BSc
Maksym Kuchma, PhD
Vladyslava Liudkovska, PhD
Katarzyna Matylla-Kulińska, PhD
Maria Nizik
Aleksandra Okrasa
Wiktoria Orzeł, MSc
Barbara Popławska
Monika Powójska
Julia Rydz
Marcin Szpila, MSc
Wiktoria Szymanek

Technician
Alina Zielińska, BSc (part-time)

Laboratory Support Specialist
Paula Kwapisz, MSc

LAB LEADER

Andrzej Dziembowski, PhD, Professor

DEGREES
2014 Professor of Biological Sciences, nomination by the President of the Republic of Poland
2009 DSc Habil in Molecular Biology, University of Warsaw, Poland
2002 PhD in Biology, cum laude, Department of Genetics, Faculty of Biology, University of Warsaw, Poland
1998 MSc in Molecular Biology, University of Warsaw, Inter-Faculty Individual Studies in Mathematics and Natural Sciences, Poland

PROFESSIONAL EXPERIENCE
2019-Present Professor, Head of the Laboratory of RNA Biology – ERA Chairs Group, International Institute of Molecular and Cell Biology in Warsaw, Poland
2011-Present Associate Professor, Faculty of Biology, University of Warsaw, Poland
2014-2019 Full Professor, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland
2010-2014 Associate Professor, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland
2008-2010 Assistant Professor, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland
2006-2011 Assistant Professor, Department of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Poland
2002-2006 Postdoctoral Fellow, Centre de Genetique Moleculaire, Centre National de la Recherche Scientifique, Gif sur Yvette, France

MEMBERSHIP IN SCIENTIFIC SOCIETIES, ORGANIZATIONS AND PANELS
2020 Corresponding Member, Polish Academy of Sciences
2018 Member, European Molecular Biology Organization
2004 Member, RNA Society

FELLOWSHIPS AND AWARDS
2023 ViveRNA ERC Advanced Grant
2022 Prime Minister Award for scientific achievements
2022 Honorary chair position named after Prof. Szybalski at the Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk
2021 HERO WIB Project [Lider], Virtual Research Institute, Polish Science Fund
2020 GRIEG, EEA and Norway Grants, and NCN
2018 Prize of the Foundation for Polish Science
2014 Master Award, Foundation for Polish Science
2013 Ideas for Poland Award, Foundation for Polish Science
2013 Knight's Cross Order of Polonia Restituta for scientific achievements, President of Poland
2013 Jakub Karol Parnas Award for the best publication in biochemistry, Polish Biochemical Society
2013 National Science Centre Award for outstanding scientific achievements
2012 ERC Starting Grant [2012-2019]
2010 Member, Polish Young Academy, Polish Academy of Sciences
2010 Prime Minister Award for the habilitation thesis
2009 Scholarship for Outstanding Young Scientists, Minister of Science and Higher Education
2006 EMBO Installation Grant
2002 Postdoctoral fellowship, Foundation for Polish Science
2002 Prime Minister Award for PhD thesis
2001 START Scholarship for Young Scientists, Foundation for Polish Science

DOCTORATES DEFENDED UNDER LAB LEADER'S SUPERVISION
K. Drążkowska, M. Lubas, A. Siwaszek, M. Ukleja, M. Czarnocki-Cieciura, O. Gewartowska, P. Krawczyk, E. Furmańczyk, A. Pyzik, T. Kuliński, V. Liudkovska, D. Cysewski.

SELECTED PUBLICATIONS

IIMCB Best Papers Award

Krawczyk PS, Gewartowska O, Mazur M, Orzeł W, Matylla-Kulińska K, Jeleń S, Turowski P, Spiewla T, **Tarkowski B**, Tudek A, **Brouze A**, Wesołowska A, Nowis D, Gołąb J, Kowalska J, Jemielity J, **Dziembowski A, Mroczek S**. SARS-CoV-2 mRNA vaccine is re-adenylated in vivo, enhancing antigen production and immune response *bioRxiv*, 2022; doi: 10.1101/2022.12.01.518149

Brouze A, Krawczyk PS, Dziembowski A, Mroczek S. *Measuring the tail: Methods for poly(A) tail profiling. Wiley Interdiscip Rev RNA*, 2023; 14(1):e1737

Liudkovska V, Krawczyk PS, Brouze A, Gumińska N, Wegierski T, Cysewski D, **Mackiewicz Z**, Ewbank JJ, Drabikowski K, **Mroczek S, Dziembowski A**. TENT5 cytoplasmic noncanonical poly(A) polymerases regulate the innate immune response in animals. *Sci Adv*, 2022; 8[46]:eadd9468

Guegan F, Rajan KS, Bento F, Pinto-Neves D, Sequeira M, **Gumińska N, Mroczek S, Dziembowski A**, Cohen-Chalamish S, Doniger T, Galili B, Estévez AM, Notredame C, Michaeli S, Figueiredo LM. A long noncoding RNA promotes parasite differentiation in African trypanosomes. *Sci Adv*, 2022; 8[24]:eabn2706

Tudek A, **Krawczyk PS, Mroczek S**, Tomecki R, Turtola M, **Matylla-Kulińska K**, Jensen TH, **Dziembowski A**. Global view on the metabolism of RNA poly(A) tails in yeast Saccharomyces cerevisiae. *Nat Commun*, 2021; 12[1]:4951

Gewartowska O, Aranaz-Novaliches G, **Krawczyk PS, Mroczek S, Kusio-Kobiałka M, Tarkowski B**, Spoutil F, Benada O, Kofroňova O, Szwedziak P, Cysewski D, **Gruchota J, Szpila M**, Chlebowski A, Sedlacek R, Prochazka J, **Dziembowski A**. Cytoplasmic polyadenylation by TENT5A is required for proper bone formation. *Cell Rep*, 2021; 35[3]:109015

Scheer H, de Almeida C, Ferrier E, Simonnot Q, Poirier L, Pflieger D, Sement FM, Koechler S, Piermaria C, **Krawczyk P, Mroczek S**, Chicher J, Kuhn L, **Dziembowski A**, Hammann P, Zuber H, Gagliardi D. The TUTase URT1 connects decapping activators and prevents the accumulation of excessively deadenylated mRNAs to avoid siRNA biogenesis. *Nat Commun*, 2021; 12[1]:1298

Liudkovska V, Dziembowski A. Functions and mechanisms of RNA tailing by metazoan terminal nucleotidyltransferases. *Wiley Interdiscip Rev RNA*, 2021 Jul 22:e1622

Turtola M, Manav MC, Kumar A, Tudek A, **Mroczek S, Krawczyk PS, Dziembowski A**, Schmid M, Passmore LA, Casanal A, Jensen TH. Threelayered control of mRNA poly(A) tail synthesis in Saccharomyces cerevisiae. *Genes Dev*, 2021; 35[17-18]:1290-1303

Hojka-Osinska A, Chlebowski A, Grochowska J, Owczarek EP, Affek K, Ktosowska-Kosicka K, Szczesny RJ, **Dziembowski A**. Landscape of functional interactions of human processive ribonucleases revealed by high-throughput siRNA screenings. *iScience*, 2021; 24[9]:103036

Bilska A, Kusio-Kobiałka M, **Krawczyk PS, Gewartowska O, Tarkowski B**, Kobyłecki K, Nowis D, **Golab J, Gruchota J, Borsuk E, Dziembowski A, Mroczek S.** Immunoglobulin Expression and the Humoral Immune Response Is Regulated by the Non-Canonical poly(A) Polymerase TENT5C. *Nat Commun*, 2020; 11(1):2032

Kuzniewska B, Cysewski D, Wasilewski M, Sakowska P, Milek J, Kulinski TM, Winiarski M, Kozielewicz P, Knapska E, Dadlez M, Chacinska A, **Dziembowski A,** Dziembowska M. Mitochondrial protein biogenesis in the synapse is supported by local translation. *EMBO Rep*, 2020; 21[8]:e48882

Płochińska R, Brzostek A, Słomka M, Dziadek J, Young D, ^**Dziembowski A**. Proteomic and transcriptomic experiments reveal an essential role of RNA degradosome complexes in shaping the transcriptome of *Mycobacterium tuberculosis*. *Nucleic Acids Res*, 2019; 47[11]:5892-905

Warkocki Z, Krawczyk PS, Adamska D, Bijata K, Garcia-Perez JL, ^**Dziembowski A**. Uridylation by TUT4/7 Restricts Retrotransposition of Human LINE-1s. *Cell*, 2018; 174[6]:1537-48.e29

Dhir A, Dhir S, Borowski LS, Jimenez L, Teitell M, Rotig A, Crow YJ, Rice GI, Duffy D, Tamby C, Nojima T, Munnich A, Schiff M, de Almeida CR, Rehwinkel J, ^**Dziembowski A**, Szczesny RJ, Proudfoot NJ. Mitochondrial doublestranded RNA triggers antiviral signalling in humans. *Nature*, 2018; 560[7717]:238-42

Pietras Z, Wojcik MA, Borowski LS, Szewczyk M, Kulinski TM, Cysewski D, Stepien PP, ^**Dziembowski A**, Szczesny RJ. Dedicated surveillance mechanism controls G-quadruplex forming non-coding RNAs in human mitochondria. *Nat Commun*, 2018; 9[1]:2558

^ no IIMCB affiliation

DESCRIPTION OF CURRENT RESEARCH

Stability regulation of endogenous and therapeutic mRNAs *in vivo*

Proteins are synthesized on the basis of messenger RNA (mRNA). Therefore, the stability of mRNA plays a crucial role in regulating gene expression. With the development of mRNA vaccines against COVID-19, which have been administered in billions of doses, we are now witnessing a revolution in biological drugs. mRNA will soon be used not only for vaccination against infectious diseases but also for cancer immunotherapy. mRNA replacement therapies for Mendelian diseases are in active development. There are also intensive efforts to target mRNA to various organs. In the case of mRNA therapeutics, inherent RNA instability is the main limiting factor for broad therapeutic applications.

Our laboratory is interested in how the stability of endogenous and therapeutic mRNAs is regulated. Extremities of both endogenous and therapeutic mRNAs are protected from degradation by the 5'-end 7-methylguanylate-cap structure, which is recognized by the translation initiation factor eIF4e, and by the 3'-end poly(A) tail, which is bound by poly(A) binding proteins (PABPs). Both posttranscriptional additions to mRNA are essential for canonical translation. mRNA decay pathways rely on the shortening of poly(A) tails via the process of deadenylation. Poly(A) tails that are shorter than 20 nucleotides no longer interact with PABPs, leading to the rapid degradation of mRNA. Deadenylation can be counteracted by cytoplasmic polyadenylation, a process that is mainly studied in the context of gametogenesis and in neurons. The impact of non-canonical polyadenylation in somatic cells is less well known, but our recent work suggests that its role has been greatly underestimated in certain cell types (Bilska et al., *Nat Commun*, 2020; Gewartowska et al., *Cell Rep*, 2021; Liudkovska et al., *Sci Adv*, 2022). The removal of poly(A) tails by PAN2/3 and CCR4-NOT deadenylases, their regulation, and other steps of mRNA turnover are now well understood mechanistically. Critical structures have been solved, and reactions have been reconstituted *in vitro*. In contrast, much less is known about the tissue specificity of mRNA decay, which is currently the main focus of our laboratory. To study the regulation of mRNA stability, it is essential to profile the lengths of poly(A) tails. We have implemented direct RNA sequencing (DRS) on the Oxford Nanopore platform (Bilska et al., *Nat Commun*, 2020; Brouze et al., *Wiley Interdiscip Rev RNA*, 2023; Gewartowska et al., *Cell Rep*, 2021; Scheer et al., *Nat Commun*, 2021; Tudek et al., *Nat Commun*, 2021; Turtola et al., *Genes Dev*, 2021; Workman et al., *Nat Methods*, 2019; Fig. 1).

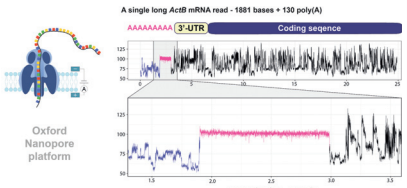


Fig. 1. Direct RNA sequencing on the Oxford Nanopore platform. Example of the raw signal output with marked poly(A) tail

Endogenous mRNAs

By analyzing poly(A) tail length dynamics in yeast strains that are devoid of all relevant nucleases and polymerases, we discovered that the main deadenylase complexes (PAN2/3 and CCR4-NOT) have an unexpectedly large pool of nonoverlapping substrates (Tudek et al., *Nat Commun*, 2021), challenging the widely accepted biphasic deadenylation model, the process of which is initiated by PAN2/3 and completed by CCR4-NOT. In parallel, we studied the role of the TENT5 family of non-canonical cytoplasmic poly(A) polymerases, which had been elusive for many years (Kuchta et al., *Nucleic Acids Res*, 2016; Liudkovska and Dziembowski, *Wiley Interdiscip Rev RNA*, 2021; Mroczek et al., *Nat Commun*, 2017). We showed that TENT5s polyadenylate and stabilize mRNAs that encode secreted proteins, leading to enhanced expression (Bilska et al., *Nat Commun*, 2020; Gewartowska et al., *Cell Rep*, 2021; Liudkovska et al., *Sci Adv*, 2022). In simple metazoa, such as the worm *Caenorhabditis elegans*, there is only one TENT5, which is essential for a proper innate immune response. TENT5 polyadenylates mRNA that encode secreted antibacterial proteins (Liudkovska et al., *Sci Adv*, 2022). There are four TENT5s in mammals (TENT5A-D) that are differentially expressed in tissues and organs (Liudkovska and Dziembowski, *Wiley Interdiscip Rev RNA*, 2021). We generated knockouts of each TENT5 and used DRS to analyze its effects on transcriptomes. Notably, there were molecular and physiological phenotypes in every cell type that expressed TENT5s. TENT5 regulates the expression of immunoglobulins in B cells (Bilska et al., *Nat Commun*, 2020), collagens in osteoblasts (Gewartowska et al., *Cell Rep*, 2021), antimicrobial proteins in macrophages (Liudkovska et al., *Sci Adv*, 2022).
We are currently analyzing how deadenylation and cytoplasmic polyadenylation regulate the stability of endogenous mRNAs is various tissues and cell types.

Therapeutic mRNAs

We implemented nanopore direct RNA sequencing (DRS) to enable the analysis of single therapeutic mRNA molecules, providing *in vivo* information about the sequence and poly(A) tails. We initially focused on the Moderna mRNA-1273 anti-Covid19 vaccine (Krawczyk et al., *bioRxiv*, 2022). We discovered that its metabolism is cell type- and tissue-specific (Krawczyk et al., *bioRxiv*, 2022). In model cell lines that are often used in preclinical studies, mRNA-1273 is swiftly degraded in a process that depends on CCR4-NOT-mediated deadenylation. In contrast, intramuscularly inoculated mRNA-1273 undergoes more complex modifications. Notably, mRNA-1273 molecules are re-adenylated, and their poly(A) tails can be extended over the initial 100 adenosines. Detailed analyses of immune cells that are involved in antigen production revealed that vaccine mRNA in macrophages is very efficiently re-adenylated, and poly(A) tails can reach up to 200 adenosines. In contrast, vaccine mRNA in dendritic cells undergoes slow deadenylation-dependent decay. We further demonstrated that the enhancement of mRNA stability in macrophages is mediated by TENT5 poly(A) polymerases, the expression of which is induced by the vaccine itself (Fig. 2). The lack of TENT5-mediated re-adenylation results in lower antigen production and severely compromises specific immunoglobulin production following vaccination. Our findings revealed an unexpected principle for the high efficacy of mRNA vaccines and opened new possibilities for their improvement.
We are currently performing a more comprehensive analysis of therapeutic mRNAs in different destinations. Moreover, with our collaborators, we aim to translate knowledge that is gained to design better mRNA therapeutics with highly controllable stability in the target destinations.

Other interests

For many years, we studied the mechanisms of mRNA degradation by the primary eukaryotic ribonuclease, the exosome complex. We are currently analyzing the role of selected exoribonucleases using transgenic mouse models (Brouze et al., *bioRxiv*, 2022; Kulinski et al., *under revision*).
The nuclear catalytic subunit of the exosome is frequently mutated in multiple myeloma, a cancer of terminally differentiated B cells. Therefore, we are interested in understanding the role of mutations of *DIS3* in the pathogenesis of multiple myeloma (Kulinski et al., *under revision*).

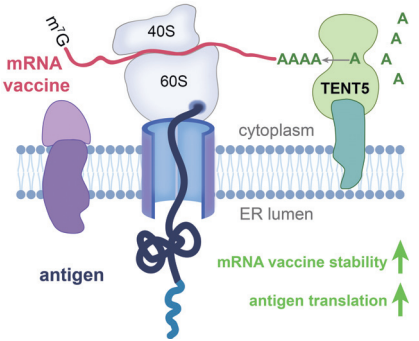
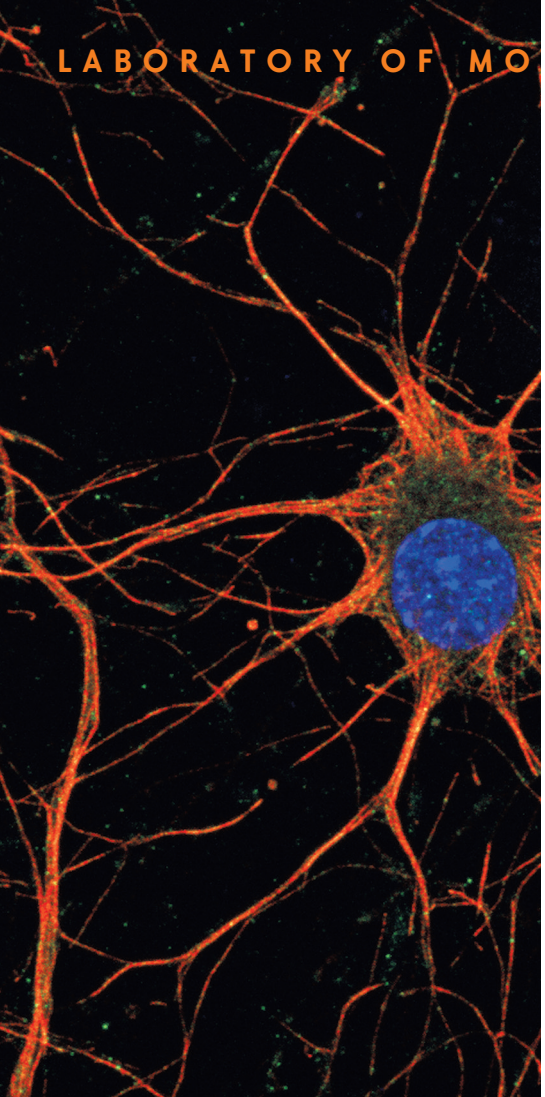


Fig. 2. Model of action of TENT5 poly(A) polymerases, which target transcripts encoding proteins translated on the endoplasmic reticulum (ER), including vaccine mRNAs

LABORATORY OF MOLECULAR AND CELLULAR NEUROBIOLOGY



GROUP MEMBERS

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Małgorzata Urbańska, PhD
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Agnieszka Brzozowska, PhD

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Tomasz Dulski, PhD
Roberto Pagano, PhD
Aleksandra Tempes, PhD

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Katarzyna Machnicka, MSc

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Karolina Bogusz, MSc
Olga Doszyń, MSc
Shiwani Kumari, MSc
Magdalena Młostek, MSc
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Katarzyna Orzoł, MSc

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, University of Warsaw, Poland
2001 PhD in Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1996 MSc in Biology, Department of Genetics, University of Warsaw, Poland

PROFESSIONAL EXPERIENCE
2018-Present Deputy Director for Science, International Institute of Molecular and Cell Biology in Warsaw, Poland
2010-2013 Deputy Director, International Institute of Molecular and Cell Biology in Warsaw, Poland
2005-Present Professor, Head of Laboratory of Molecular and Cellular Neurobiology, International Institute of Molecular and Cell Biology in Warsaw, Poland

RESEARCH TRAINING
2016 Research visit (3 weeks) with Prof. William Harris, Cambridge University, Cambridge, UK
2011 Research visit (2 weeks) with Dr. Carlo Sala, CNR Institute of Neuroscience and 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 Scientifique, Paris, France
1996-2002 PhD student (until 2001) and Postdoctoral Associate (until May 2002) with Prof. L. Kaczmarek, Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1995-1996 Master's degree, Prof. P. Węgleński, Department of Genetics, University of Warsaw, Poland

FELLOWSHIPS AND AWARDS
2020 Prime Minister Award for Scientific Achievements
2009 Division II: Biological and Agricultural Sciences, Polish Academy of Sciences Award for series of publications on "New molecular mechanisms of mTORopathy and epilepsy"
2018 TEAM, Foundation for Polish Science
2014 Master Award, Foundation for Polish Science
2011 Prime Minister Award for habilitation thesis
2009 Division II: Biological and Agricultural Sciences, Polish Academy of Sciences Award for series of publications on MMP9 (together with teams of Prof. Kaczmarek and Dr. Wilczyński)
2002 Prime Minister Award for PhD thesis
2001 START Scholarship for Young Scientists, Foundation for Polish Science

MEMBERSHIP IN SCIENTIFIC SOCIETIES, ORGANIZATIONS, AND PANELS
2023-2026 Member of the Polish Academy of Sciences Division II Council of Provosts (term 2023-2026)
2021-2025 Member, Scientific Council, National Geria-trics, Rheumatology and Rehabilitation Institute in Wasaw
2019 Member, Scientific Council of the Institute of Pharmacology, Polish Academy of Sciences (terms 2019-2022, 2023-2026)
2017 Vice President, Polish Neuroscience Society (term 2017-2019)
2015 Corresponding Member, Warsaw Scientific Society
2015 Member, Scientific Council of the Nencki Institute of Experimental Biology, Polish Academy of Sciences (terms: 2015-2018, 2019-2022, 2023-2026 [Vice Chair of Council])
2011 Member, Neurobiology Committee, Polish Academy of Sciences (terms 2011-2014; 2015-2018; 2019-2022)

DOCTORATES DEFENDED UNDER LAB LEADER'S SUPERVISION
Ł. Świech, A. Malik, M. Perycz, M. Urbańska, A. Skałecka, J. Lipka, A. Urbańska, M. Firkowska, K. Kisielewska, A. Kościelny, A. Tempes, M. Kędra.

SELECTED PUBLICATIONS

○ IIMCB Best Papers Award

Roszkowska M, Krysiak A, Majchrowicz L, Nader K, Beroun A, Michaluk P, Pekala M, **Jaworski J**, Kondrakiewicz L, Pulcian A, Knapska E, Kaczmarek L, Kalita K. SRF depletion in early life contributes to social interaction deficits in the adulthood. ***Cell Mol Life Sci***, 2022; 79(5):278

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Scheper M, Romagnolo A, Besharat ZM, Iyer AM, Moavero R, Hertzberg C, Weschke B, Riney K, Feucht M, Scholl T, Petrak B, Maulisova A, Nabbout R, Jansen AC, Jansen FE, Lagae L, Urbanska M, Ferretti E, **Tempes A**, **Blazejczyk M**, **Jaworski J**, Kwiatkowski DJ, Jozwiak S, Kotulska K, Sadowski K, Borkowska J, Curatolo P, Mills JD, Aronica E, Epistop Consortium Members. miRNAs and isomiRs: Serum-Based Biomarkers for the Development of Intellectual Disability and Autism Spectrum Disorder in Tuberous Sclerosis Complex. ***Biomedicines***, 2022; 10(8):1838

Prentzell MT, Rehbein U, Cadena Sandoval M, De Meulemeester AS, Baumeister R, Brohee L, Berdel B, Bockwoldt M, Carroll B, Chowdhury SR, von Deimling A, Demetriades C, Figlia G; Genomics England Research Consortium, de Araujo MEG, Heberle AM, Heiland I, Holzwarth B, Huber LA, **Jaworski J**, **Kedra M**, Kern K, **Kopach A**, Korolchuk VI, van 't Land-Kuper I, **Macias M**, Nellist M, Palm W, Pusch S, Ramos Pittol JM, Reil M, Reintjes A, Reuter F, Sampson JR, Scheldeman C, Siekierska A, Stefan E, Teleman AA, Thomas LE, Torres-Quesada O, Trump S, West HD, de Witte P, Woltering S, Yordanov TE, **Zmorzynska J**, Opitz CA, Thedieck K. G3BPs tether the TSC complex to lysosomes and suppress mTORC1 signaling. ***Cell***, 2021; 184(3):655-74

○ **Kedra M**, **Banasiak K**, **Kisielewska K**, **Wolinska-Niziol L**, **Jaworski J**, **Zmorzynska J**. TrkB hyperactivity contributes to brain dysconnectivity, epileptogenesis, and anxiety in zebrafish model of Tuberous Sclerosis Complex. ***Proc Natl Acad Sci USA***, 2020; 117(4):2170-9

Tarkowski B, **Kuchcinska K**, **Blazejczyk M**, **Jaworski J**. Pathological mTOR mutations impact cortical development. ***Hum Mol Genet***, 2019; 28(13): 2107-19

Urbanska M, Kazmierska-Grebowska P, Kowalczyk T, Caban B, Nader K, Pijet B, Kalita K, **Gozdz A**, Devijvere H, Lechate B, Jaworski T, Grajkowska W, Sadowski K, Jozwiak S, Kotulska K, Konopacki J, Van Leuven F, van Vlieth E, Aronica E, **Jaworski J**. GSK3 activity alleviates epileptogenesis and limits GluA1 phosphorylation. ***eBioMedicine***, 2019; 39:377-87

○ **Koscielny A**, **Malik AR**, **Liszewska E**, **Zmorzynska J**, **Tempes A**, **Tarkowski B**, **Jaworski J**. Adaptor Complex 2 Controls Dendrite Morphology via mTOR-Dependent Expression of GluA2. ***Mol Neurobiol***, 2018; 55(2):1590-606

○ **Skalecka A**, **Liszewska E**, Bilinski R, Gkogkas C, Khoutorsky A, **Malik AR**, Sonenberg N, **Jaworski J**. mTOR kinase is needed for the development and stabilization of dendritic arbors in newly born olfactory bulb neurons. ***Dev Neurobiol***, 2016; 76(12):1308-27

○ **Malik AR**, **Liszewska E**, **Skalecka A**, **Urbanska M**, Iyer AM, **Swiech LJ**, **Perycz M**, **Parobczak K**, **Pietruszka P**, **Zarebska MM**, **Macias M**, Kotulska K, Borkowska J, Grajkowska W, Tyburczy ME, Jozwiak S, Kwiatkowski DJ, Aronica E, **Jaworski J**. Tuberous sclerosis complex neuropathology requires glutamate-cysteine ligase. ***Acta Neuropathol Commun***, 2015; 3:48

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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, Defilippi P, Akhmanova A, Hoogenraad CC. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. ***Neuron***, 2009; 61:85-100

^**Jaworski J**, Spangler S, Seeburg DP, Hoogenraad CC, Sheng M. Control of dendritic arborization by the phosphoinositide-3'-kinase-Akt-mammalian target of rapamycin pathway. ***J Neurosci***, 2005; 25(49):11300-12

^ no IIMCB affiliation

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 regulating translation (mTOR complex 1 [mTORC1]) or influencing the actin cytoskeleton (mTORC2). The postdoctoral work of Dr. Jaworski showed that the regulation of mTOR-dependent translation contributes to dendritogenesis (Jaworski et al., *J Neurosci*, 2005). This was subsequently confirmed by our recent work in which we identified the GluA2 subunit of glutamate receptors as a protein that is both translated in an mTORC1-dependent manner and vital for dendritogenesis (Koscielny et al., *Mol Neurobiol*, 2018). 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 neuronal functions of mTOR and neuropathology is still poorly understood. Since the inception of our laboratory, we have sought to identify mTOR partners and regulated proteins that are involved in neuronal development and characterize mTOR dysfunction in neuropathology.

To achieve our scientific objectives, we have been primarily using a well-established, relatively simple, and robust model of the dendritogenesis of neurons that are cultured *in vitro*. Using this approach, we performed both proof-of-principle experiments and unbiased screens that clearly demonstrated mTOR functions during neuronal development beyond the canonical control of translation (e.g., regulation of the cytoskeleton and transcription). These experiments also extended our general knowledge of molecular mechanisms downstream of mTOR and new mechanisms that underlie dendritogenesis (Swiech et al., *J Neurosci*, 2011; Urbanska et al., *J Biol Chem*, 2012; Urbanska et al., *Sci Rep*, 2017; Malik et al., *J Biol Chem*, 2013).

Progress toward achieving our research goals allowed us to merge some objectives and refine our main focus toward identification of the cellular compartment-specific regulation and functions of mTOR in developing neurons, with a particular focus on intracellular trafficking events, which were at the center of our research efforts during the last few years (Main Research Objective 1). Notably, both the role of mTOR in intracellular trafficking control and the role of membrane trafficking in neuronal development and disease are still understudied topics. Therefore, focusing on these areas (e.g., the interplay between mTORCs and molecular motors, such as the dynein-dynactin complex and kinesins, and small guanosine triphosphatases of the Rab family and their regulators) creates an opportunity to successfully proceed with our research in otherwise extremely crowded fields of the molecular biology of mTOR and mTOR-related disorders. In 2021, we obtained resources to investigate the role of mTOR in the nucleus of neurons. Within the MAESTRO grant from the National Science Centre, we will study the role of mTOR interactions with the nuclear protein Brahma-related gene-1 (Brg1) in normal and aberrant neuronal activity.

An important part of our work during the last 6 years has been to develop and characterize new approaches to study mTOR functions *in vivo* beyond dendritogenesis (i.e., *in utero* brain electroporation in rodents and transgenic zebrafish) and in clinically relevant material (e.g., patient samples, primary cultures, induced pluripotent stem cells, and organoids). These modern techniques, together with newly identified mTOR-controlled molecular processes, are critically important for our second main objective, namely understanding the molecular pathology of mTORopathies (Main Research Objective 2), which are diseases that are related to mTOR dysregulation (e.g., tuberous sclerosis complex [TSC] and epilepsy). By studying mTOR in the context of the control of dendritic arbor morphology, we identified a significant gap in the literature about this phenomenon. Dendritic arbor morphology is unique for different types of neurons and reflects their precise adjustment to functions they perform within particular neuronal networks.

Although dendrites must remain intact for more than 80% of a neuron's lifespan, little is known about the molecular mechanisms that underlie this phenomenon. To date, very few proteins have been identified to be essential for the stability of mature dendritic arbors. Disturbances in dendritic arbor stability in the mature brain are related to prolonged stress and mood disorders (e.g., depression). At later stages of brain aging, when cognitive decline develops, dendrites may also deteriorate. Intriguingly, recent studies reported changes in mTOR signaling in mood disorders and aging. Thus, our new Main Research Objective 3 seeks to understand molecular mechanisms of dendrite stability and their disruption in mood disorders and the aging brain.

One of the most intriguing results of the past year was obtained in Main Research Objective 1. Studying regulation of the non-canonical interaction of the AP2 adaptor complex with the dynein-dynactin motor, which we previously discovered with the Haucke Laboratory (Kononenko et al., *Nat Comm*, 2017), we realized the potential of mTORC1 to control it. Our data strongly suggest that lower mTORC1 activity, leading to autophagy initiation, results in an increase in the AP2-dynactin interaction, likely at the surface of lysosomes [Tempes, Bogusz, Brzozowska et al., *bioRxiv*, 2022; see also Fig. 1]. We are currently investigating functional consequences of this phenomenon for lysosomal transport and autophagosome-lysosome interactions. Within Main Research Objective 2, results of our long-term collaborative efforts with clinicians within the Epistop and Epimarker projects were published, revealing many important clinical and molecular aspects of the epileptogenesis process that is ongoing in TSC infants [e.g., Scheper et al., *Biomedicines*, 2022; Hulshof et al., *Neurology*, 2022]. Data from the Epimarker project also became a basis for two pending patent applications on predictive markers of epilepsy in TSC patients. Lastly, within Main Research Objective 3, we finalized the functional analysis of genes that are potentially involved in regulating mature neurons' dendritic arbor stability. Our data show that destabilizing agents (e.g., disturbed neuronal activity or inflammation) activate separate transcriptional programs that eventually lead to dendritic arbor simplification. Among deregulated genes, we identified at least a dozen that are critical for dendritic arbor stabilization. We are currently testing their relevance using *in vivo* models.

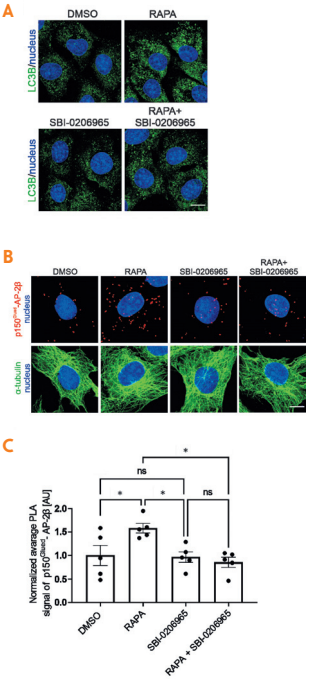
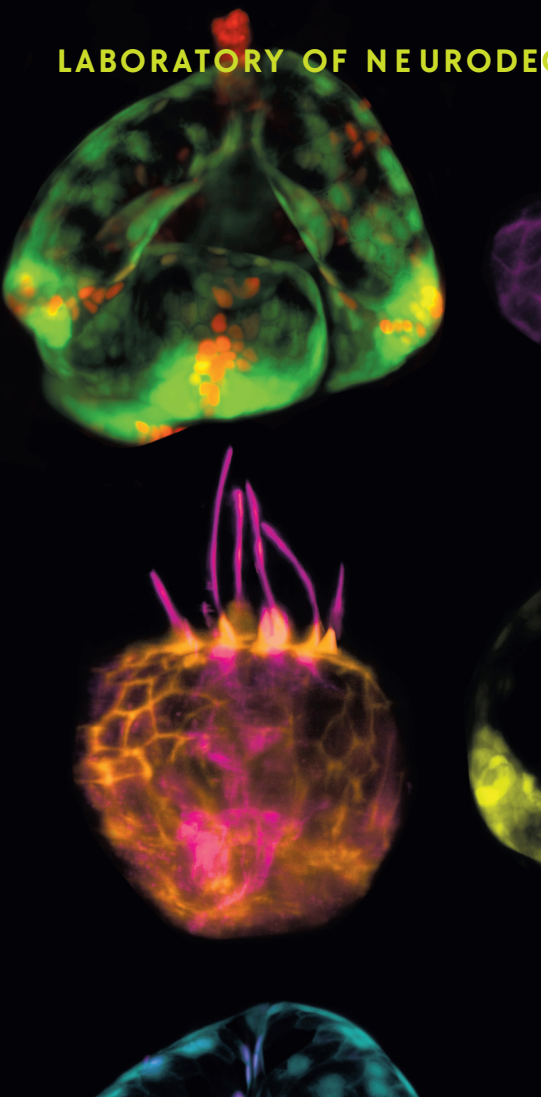


Fig. 1. Autophagy initiation is required for mTOR-dependent regulation of dynactin-AP2 complex formation (**A**) In Rat2 fibroblasts, 2 hours of rapamycin treatment effectively induces autophagy as revealed by LC3 accumulation, and the autophagy initiation inhibitor SBI-0206965 blocks this effect. (**B**) Representative images of Rat2 fibroblasts with PLA p150^{Glued}/ AP -2β signals (red) treated with rapamycin [RAPA] alone or in combination with SBI-0206965. Scale bar = 10 μm. (**C**) Quantification of the number of p150^{Glued}/ AP -2β PLA puncta in cells treated as in B. *p < 0.05 (one-way ANOVA followed by Tukey's multiple-comparison post hoc test). Modified from Tempes, Bogusz, Brzozowska et al. (2022) *BioRxiv*

LABORATORY OF NEURODEGENERATION











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Senior Researchers
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Małgorzata Korzeniowska, PhD
(until March 2022)
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Łukasz Majewski, PhD

Research Assistant
Sofiia Baranykova, MSc (since September 2022)

PhD Students
Razieh Amini, MSc (since March 2022)
Ruchi Prakash Jain, M. Tech.
Rishikesh Kumar Gupta, MSc Tech. (PhD defense in January 2022; until January 2022)
Ewelina Latoszek, MSc Eng.

Undergraduate Students
Dominik Bielecki, BSc (until December 2022)
Samuel Oluwafemi Egbuwalo, BSc
(until July 2022)
Kamil Krzesimowski, BSc (until November 2022)
Marta Piechota, BSc

Volunteers
Nina Gan, MSc Eng. (from IPPH[1], since August 2022)
Justyna Jędrychowska, PhD
(until December 2022)
Jagna Kądziołka (August 2022)
Małgorzata Korzeniowska, PhD
(from MMRI[2], June-August 2022)
Daniel Kozłowski, BSc (since April 2023)
Monika Kwiatkowska, MSc Eng.
(from IBCH[3], since December 2020)
Paula Martín Malle (January-February 2022)
Anna Sarosiak, MSc 9 (from IPPH[1])
Iga Wasilewska, PhD (MMRI[2])
Magdalena Widziołek-Pooranachandran, PhD
(October 2022, from JU[4])

Technician
Monika Matuszczyk (part-time)

Laboratory Support Specialist
Dominika Dubicka-Boroch, MSc

[1] Institute of Physiology and Pathology of Hearing
[2] Mossakowski Medical Research Institute Polish Academy of Sciences
[3] Institute of Bioorganic Chemistry Polish Academy of Sciences
[4] Jagiellonian University

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

PROFESSIONAL EXPERIENCE
2001-Present Professor, Head of Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology in Warsaw, Poland
2001-2018 Director, International Institute of Molecular and Cell Biology in Warsaw, Poland; Feb-Dec 2018 Acting Director, International Institute of Molecular and Cell Biology in Warsaw, Poland
2000-2001 Director, Centre of Excellence Phare Sci-Tech II, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1999-2001 Acting Director, International Institute of Molecular and Cell Biology in Warsaw, Poland; Organizer and Director, Centenarian Program
1996-2002 Head, Laboratory of Calcium Binding Proteins, professor 2002-2014 Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1991-1992 Deputy Scientific Director, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1986-1992 Associate Professor and Head of Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1984-1985 Research Associate, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1980-1981 Postdoctoral Fellow, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1976-1980 PhD Student, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

PROFESSIONAL TRAINING
July 2018 Visiting Professor, Laboratory of H. Burgess, National Institute of Child Health and Human Development, Bethesda, MD, USA
July 2015 Visiting Professor, Laboratory of W. Harris, University of Cambridge, UK
July 2014 Visiting Professor, Laboratory of B.E. Snaar-Jagalska, Leiden University, The Netherlands
1992-1995 Visiting Professor, Laboratory of D. Jacobowitz, National Institute of Mental Health, Bethesda, MD, USA
1981-1984 Visiting Fellow (postdoc), Laboratory of E.D. Korn, National Institute of Heart, Lung and Blood, Bethesda, MD, USA

MEMBERSHIP IN SCIENTIFIC SOCIETIES, ORGANIZATIONS, AND PANELS
2020-Present Ordinary Member, Polish Academy of Sciences
Dec 2020-2022 President of the Council of the National Science Centre
2018-2020 Member, Council of the National Science Centre and Chair of International Commission
2020-Present External expert in biotechnology, Łukasiewcz Research Network – PORT Polish Center for Technology Development
2017-2018 Deputy Chair, Council of Provosts, Division II: Biological and Agricultural Sciences, Polish Academy of Sciences
2016-2021 Member, International Advisory Board, Małopolska Centre of Biotechnology, Jagiellonian University
2011-2014 Member, Science Policy Committee, and Rotating President (Jul-Dec 2012), Ministry of Science and Higher Education
2008-April 2023 Board Member, European Calcium Society; Society member since 1997
2008-2018 Member, Board of Directors, and Rotating President (Jul-Dec 2016, Jul-Dec 2013, Jul-Dec 2010), Biocentrum-Ochota Consortium
2006-2011 Member, Advisory Group, 7FP HEALTH, European Commission
2004-2019 Corresponding Member, Polish Academy of Sciences
2002-Present Honorary Chair and co-founder, BioEducation Foundation
2002-Present Head of Program Board, Centre for Innovative Bioscience Education
1993-2014 Member, Scientific Council, Nencki Institute of Experimental Biology, Polish Academy of Sciences
1996-1998 & 2000-2002 Vice-President, Biotechnology Committee, Polish Academy of Sciences
1989-1991 General Secretary, Polish Biochemical Society; Society member since 1977

HONORS, PRIZES AND AWARDS
2013 Award from the Division II: Biological and Agricultural Sciences, Polish Academy of Sciences for series of works on β-catenin
2013 Crystal Brussels Sprout Award for outstanding achievements in 7FP EU
2011 Konorski Award from the Polish Neuroscience Society and Committee on Neurobiology, Polish Academy of Sciences
2008 Officer's Cross of the Order of Polonia Restituta
2003 Prime Minister Award for Scientific Achievements
2001 Award from the Division II: Biological and Agricultural Sciences, Polish Academy of Sciences for work on calcium binding proteins
1998 Knight's Cross of the Order of Polonia Restituta

DOCTORATES DEFENDED UNDER LAB LEADER'S SUPERVISION
A. Filipek, J. Kordowska, U. Wojda, J. Hetman, M. Palczewska, M. Nowotny, K. Billing-Marczak, Ł. Bojarski, W. Michowski, K. Misztal, M. Figiel, K. Honarnejad, A. Jaworska, K. Gazda, F. Maciąg, J. Jędrychowska, I. Wasilewska, R.K. Gupta.

SELECTED PUBLICATIONS

○ IIMCB Best Papers Award

Latoszek E, **Piechota M**, **Liszewska E**, Hansikova H, Klempir J, Mühlback A, Landwehrmeyer GB, **Kuznicki J**, **Czeredys M**. *Generation of three human iPSC lines from patients with Huntington's disease with different CAG lengths and human control iPSC line from a healthy donor*. ***Stem Cell Res***, 2022; 64:102931

Latoszek E, **Wiweger M**, Ludwiczak J, Dunin-Horkawicz S, **Kuznicki J**, **Czeredys M**. *Siah-1-interacting protein regulates mutated huntingtin protein aggregation in Huntington's disease models*. ***Cell Biosci***, 2022; 12(1):34

Wiweger M, **Majewski L**, Adamek-Urbanska D, **Wasilewska I**, **Kuznicki J**. npc2-Deficient Zebrafish Reproduce Neurological and Inflammatory Symptoms of Niemann-Pick Type C Disease. ***Front Cell Neurosci***, 2021; 15:647860

Dyrda A, **Kuznicki J**, **Majewski L**. Annexin A3: a newly identified player in store operated calcium entry. ***Acta Neurobiol Exp***, 2021; 81:307-13

Jedrychowska J, **Gasanov EV**, **Korzh V**. Kcnb1 plays a role in development of the inner ear. ***Dev Biol***, 2020; 471:65-75

Wasilewska I, **Gupta RK**, Wojtaś B, **Palchevska O**, **Kuźnicki J**. stim2b Knockout Induces Hyperactivity and Susceptibility to Seizures in Zebrafish Larvae. ***Cells***, 2020; 9(5):1285

Soman SK, **Bazała M**, Keatinge M, Bandmann O, **Kuznicki J**. Restriction of mitochondrial calcium overload by mcu inactivation renders neuroprotective effect in Zebrafish models of Parkinson's disease. ***Biol Open***, 2019; 8:bio044347

Maciag F, **Majewski L**, Boguszewski PM, **Gupta RK**, **Wasilewska I**, Wojtas B, **Kuznicki J**. Behavioral and electrophysiological changes in female mice overexpressing ORAI1 in neurons. ***BBA Mol Cell Res***, 2019; 1866(7):1137-50

Gazda K, **Kuznicki J**, **Wegierski T**. Knockdown of amyloid precursor protein increases calcium levels in the endoplasmic reticulum. ***Sci Rep***, 2017; 7:14512

○ **Szewczyk LM**, Brozko N, **Nagalski A**, Rockle I, Werneburg S, Hildebrandt H, **Wisniewska MB**, **Kuznicki J**. ST8SIA2 promotes oligodendrocyte differentiation and the integrity of myelin and axons. ***Glia***, 2017; 65(1):34-49

Majewski L, **Maciag F**, Boguszewski PM, **Wasilewska I**, Wiera G, Wojtowicz T, Mozrzymas J, **Kuznicki J**. Overexpression of STIM1 in neurons in mouse brain improves contextual learning and impairs long-term depression. ***BBA Mol Cell Res***, 2017; 1864(6):1071-87

Misztal K, **Brozko N**, **Nagalski A**, **Szewczyk LM**, **Krolak M**, **Brzozowska K**, **Kuznicki J**, **Wisniewska MB**. TCF7L2 mediates the cellular and behavioral response to chronic lithium treatment in animal models. ***Neuropharmacology***, 2017; 113(Pt A):490-501

Nagaraj S, Laskowska-Kaszub K, Debski KJ, Wojsiat J, Dabrowski M, Gabryelewicz T, **Kuznicki J**, Wojda U. Profile of 6 microRNA in blood plasma distinguish early stage Alzheimer's disease patients from non-demented subjects. ***Oncotarget***, 2017; 8(10):16122-43

Wegierski T, **Gazda K**, **Kuznicki J**. Microscopic analysis of Orai-mediated store-operated calcium entry in cells with experimentally altered levels of amyloid precursor protein. ***Biochem Biophys Res Commun***, 2016; 478(3):1087-92

Gruszczynska-Biegala J, **Kuznicki J**. Native STIM2 and ORAI1 proteins form a calcium-sensitive and thapsigargin-insensitive complex in cortical neurons. ***J Neurochem***, 2013; 126(6):727-38

Jaworska A, Dzbek J, Styczynska M, **Kuznicki J**. Analysis of calcium homeostasis in fresh lymphocytes from patients with sporadic Alzheimer's disease or mild cognitive impairment. ***BBA Mol Cell Res***, 2013; 1833(7):1692-9

Wisniewska MB, **Nagalski A**, Dabrowski M, **Misztal K**, **Kuznicki J**. Novel β-catenin target genes identified in thalamic neurons encode modulators of neuronal excitability. ***BMC Genomics***, 2012; 13:635

Wisniewska MB, **Misztal K**, **Michowski W**, Szczot M, Purta E, Lesniak W, Klejman ME, Dabrowski M, Filipkowski RK, **Nagalski A**, Mozrzymas JW, **Kuznicki J**. LEF1/beta-catenin complex regulates transcription of the Cav3.1 calcium channel gene (Cacna1g) in thalamic neurons of the adult brain. ***J Neurosci***, 2010; 30(14):4957-69

DESCRIPTION OF CURRENT RESEARCH

We are interested in the molecular mechanisms that are involved in neurodegeneration, with a special emphasis on the role of Ca^{2+} homeostasis and signaling. These processes are being studied at the genomic, proteomic, and cellular levels using mostly zebrafish and mice as model organisms. Our projects focus on proteins that are involved in store-operated Ca^{2+} entry (SOCE) and Ca^{2+} homeostasis in mitochondria and the involvement of K^+ channels in the brain ventricular system (BVS) using zebrafish models (for reviews, see Wegierski and Kuznicki, *Cell Calcium*, 2018; Winata and Korzh, *FEBS Lett*, 2018).

Role of STIM proteins in store-operated Ca^{2+} entry in neurons

We previously showed that stromal interaction molecule 1 (STIM1) in neurons is involved in a thapsigargin-induced SOCE-like process, whereas STIM2 is mostly active after the ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid-driven depletion of extracellular Ca^{2+} (Gruszczynska-Biegala et al., *PLoS One*, 2011; Gruszczynska-Biegala and Kuznicki, *J Neurochem*, 2013). We searched for new partners of STIMs other than ORAI channels and found that endogenous STIMs associate with GluA subunits of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (Gruszczynska-Biegala et al., *Front Cell Neurosci*, 2016). Using zebrafish as a model, we study STIM2 functions *in vivo*. We evaluated the expression of Calcium Toolkit genes in the zebrafish brain and established levels of SOCE components (Wasilewska et al., *Genes*, 2019). Deficiency in Stim2a or Stim2b resulted in a significant increase in mobility in zebrafish larvae and affected neuronal activity by increasing the frequency of Ca^{2+} oscillations and altering gene expression (Wasilewska et al., *Cells*, 2020; Gupta et al., *Int J Mol Sci*, 2020). RNA sequencing revealed the upregulation of several genes, including *annexin A3a* (*anxa3a*). We used Ca^{2+} imaging and electrophysiological recordings to determine the effect of Annexin A2, A3, and A6 overexpression on SOCE. The results indicate that Annexin A3 is a positive modulator of SOCE (Dyrda et al., *Acta Neurobiol Exp*, 2021). We recently analyzed the phenotype of (*stim2a;stim2b*)$^{-/-}$ 5 days postfertilization larvae by performing behavioral analysis, histochemistry, and single-cell RNA sequencing (manuscripts in preparation).

Dysregulation of Ca^{2+} homeostasis in neurodegenerative diseases

We characterized transgenic mice that overexpressed key SOCE proteins (STIM1, STIM2, and ORAI1) specifically in brain neurons (Majewski et al., *BBA Mol Cell Res*, 2017; Majewski et al., *Int J Mol Sci*, 2019; Gruszczynska-Biegala et al., *Cells*, 2020; Majewski et al., *Int J Mol Sci*, 2020). Interestingly, a novel sex-dependent role for ORAI1 in neural function was described (Maciag, Majewski et al., *BBA Mol Cell Res*, 2019). Siah-1 interacting protein/S100 binding protein (SIP) and Huntingtin associated protein-1 (HAP1A) were previously found to be dysregulated in Huntington's disease (HD; Czeredys et al., *Front Mol Neurosci*, 2013). In HD pathology, HAP1A was shown to be involved in the regulation of abnormal SOCE (Czeredys et al., *Front Mol Neurosci*, 2018), whereas the role of SIP was found in the regulation of mutant huntingtin aggregation (Latoszek et al., *Cell Biosci*, 2022). We now investigate the role of SOCE, HAP1A, and SIP in the context of medium spiny neuron (MSN) neurodegeneration using YAC128 mice and human induced pluripotent stem cell (hiPSC) lines that are reprogrammed from different onsets of HD and control fibroblasts. hiPSC lines were characterized using different methods (Latoszek et al., *Stem Cell Res*, *2022*) and are now being used to obtain hiPSC-derived MSNs and organoids for further research.
In collaboration with Oliver Bandmann (University of Sheffield), we used a *pink1* mutant (*pink*$^{-/-}$) zebrafish line to study alterations of Ca^{2+} homeostasis (Flinn et al., *Ann Neurol*, 2013; Soman et al., *Eur J Neurosci*, 2017). We generated *mcu* knockout zebrafish, which are viable and fertile (Soman et al., *Biol Open*, 2019). The *pink1*$^{-/-}$/*mcu*$^{-/-}$ double-knockout line exhibited no loss of dopaminergic neurons, suggesting that Ca^{2+} that enters mitochondria via the mitochondrial Ca^{2+} uniporter is involved in pathology of the *pink1* mutant (Soman et al., *Biol Open*, 2019).
Using CRISPR/Cas9 technology, we created *npc2*, *sgsh*, and *ppp3ca* zebrafish mutant lines and used them as models of Niemann-Pick type C disease (*npc2*; Wiweger et al., *Front Cell Neurosci*, 2021), mucopolysaccharidosis type III A, and calcineurin variant associated with epilepsy (*ppp3ca*; Rydzanicz et al., *Eur J Hum Genet*, 2018). We also created several reporter lines under the *elavl3* promoter (e.g., with calcium sensors [CEPIA2mt and CAMPARI2], NFAT or HyPer3) to monitor *in vivo* Ca^{2+} or reactive oxygen species in neurons in wildtype and mutant zebrafish.

Development of hollow organs

Subunits of the voltage-gated K^+ channels Kcnb1 (Kv2.1) and Kcng4 (Kv6.4) are expressed in hollow organs (BVS, ears, and eyes) where they form tetrameric K^+ channels and antagonize each other. Kcnb1 deficiency in zebrafish causes microcephaly, and Kcnb1 gain-of-function causes hydrocephalus, whereas effects of Kcng4b experimental manipulation are opposite (Jedrychowska and Korzh, *Dev Dyn*, 2019). The BVS forms during early neural development (Korzh, *Cell Mol Life Sci*, 2018). Its deficiencies have been linked to several neurodegenerative diseases, including epilepsy. Formation and function of the BVS depend on the ependyma (i.e., cells that line the BVS cavity), circumventricular organs, including the choroid plexus (Garcia-Lecea et al., *Front Neuroanat*, 2017; Korzh and Kondrychyn, *Semin Cell Dev Biol*, 2020), and the subcommissural organ (Yang et al., *Cell Tissue Res*, 2021). To study the role of K^+ channels in the development of hollow organs, we generated a zebrafish mutant of *kcnb1* and three mutants of *kcng4b* with deficiencies in the BVS and ears. To further characterize the role of KCNB1 in development, we demonstrated that it regulates inflation of the ear and formation of otic stones (i.e., otoliths; Jedrychowska et al., *Dev Biol*, 2021).

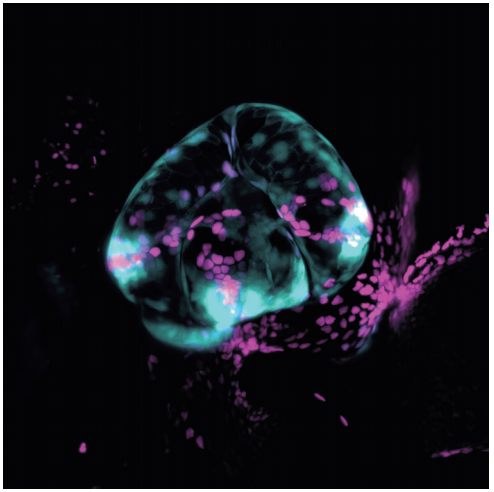
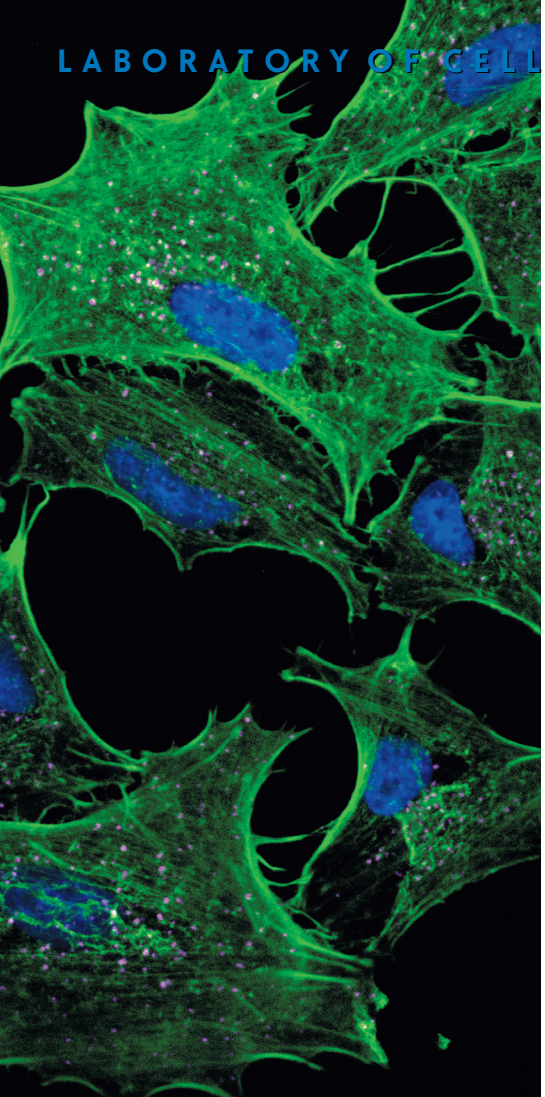


Fig. 1. The 5 days postfertilization zebrafish inner ear expressing Tbx2a transgene (turquoise) in sensory patches. The Wnt signaling marker (TCF, magenta) expressed in endolymphatics (by the lightsheet microscopy).



LABORATORY OF CELL BIOLOGY

GROUP MEMBERS

Lab Leader
Marta Miączyńska, PhD, Professor

Senior Researchers
Jarosław Cendrowski, PhD
Ewelina Szymańska, PhD
Daria Zdzalik-Bielecka, PhD (until December 2022)

Postdoctoral Researcher
Ranjana Maurya, PhD

Research Assistant
Patrycja Daszczuk, MSc

PhD Students
Malwina Grębowicz-Maciukiewicz, MSc
Marta Chwałek, MSc
Marta Wróbel, MSc

Undergraduate Student
Bartosz Jary

Technician
Monika Matuszczyk (part-time)

Laboratory Support Specialists
Alicja Machaj, MSc
Renata Wyszyńska, MSc

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
1992 MSc in Molecular Biology, Jagiellonian University, Cracow, Poland
1991 BSc in Biological Sciences, University of Wolverhampton, UK

PROFESSIONAL EMPLOYMENT
2018-Present Director, International Institute of Molecular and Cell Biology in Warsaw, Poland
June 2014-Dec 2015 Deputy Director for Science, International Institute of Molecular and Cell Biology in Warsaw, Poland
June 2013-May 2014 Deputy Director, International Institute of Molecular and Cell Biology in Warsaw, Poland
2005-Present Professor, Head of Laboratory of Cell Biology, International Institute of Molecular and Cell Biology in Warsaw, Poland

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

HONORS, PRIZES, AND AWARDS
2021 Prime Minister's Award for outstanding scientific achievements
2021 Member, EMBO Council
2020 Corresponding Member, Polish Academy of Sciences
2019 Member, Academia Europaea
2017 Member, European Molecular Biology Organization (EMBO)
2016-2018 Member, Council of the National Science Centre
2016 TEAM, Foundation for Polish Science
2012 MAESTRO, National Science Centre
2011 Polish-Swiss Research Programme grant
2007 Habilitation Fellowship of L'Oreal Poland for Women in Science
2006-2012 International Senior Research Fellowship, Wellcome Trust, UK
2006-2010 International Research Scholar, Howard Hughes Medical Institute, USA
2006-2010 Partner Group grant, Max Planck Society, Germany
2001-2004 Postdoctoral Fellowship, Max Planck Society, Germany
1999-2000 Long-Term Postdoctoral Fellowship, Human Frontier Science Program Organization
1998-1999 Erwin Schrödinger Postdoctoral Fellowship, Austrian Science Fund
1993-1996 Bertha von Suttner PhD Scholarship, Austrian Ministry of Science
1990-1991 Studentship, Council of the European Communities: TEMPUS Scheme

DOCTORATES DEFENDED UNDER LAB LEADER'S SUPERVISION
M. Olchowik, A. Urbańska, A. Hupałowska, Ł. Sadowski, A. Mamińska, A. Toruń, K. Jastrzębski, M. Maksymowicz, K. Wojciechowska, A. Poświata.

SELECTED PUBLICATIONS

IIMCB Best Papers Award

Wróbel M, Cendrowski J, Szymańska E, Grębowicz-Maciukiewicz M, Budick-Harmelin N, Macias M, Szybińska A, Mazur M, Kolmus K, Goryca K, Dąbrowska M, Paziewska A, Mikula M, **Miączyńska M**. ESCRT-I fuels lysosomal degradation to restrict TFEB/TFE3 signaling via the Rag-mTORC1 pathway. *Life Sci Alliance*, 2022; 5:e202101239

Poświata A, Kozik K, Miączyńska M, Zdzalik-Bielecka D. Endocytic trafficking of GAS6-AXL complexes is associated with sustained AKT activation. *Cell Mol Life Sci*, 2022; 79:316

Wolińska-Nizioł L, Romaniuk K, Wojciechowska K, Surga K, Kamaszewski M, Szudrowicz H, **Miączyńska M**. Tollip-deficient zebrafish display no abnormalities in development, organ morphology or gene expression in response to lipopolysaccharide. *FEBS Open Bio*, 2022; 12:1453-64

Oroń M, Grochowski M, Jaiswar A, Legierska J, **Jastrzębski K**, Nowak-Niezgoda M, Kołos M, Kaźmierczak W, Olesiński T, Lenarcik M, Cybulska M, Mikula M, **Żylicz A, Miączyńska M**, Zettl K, Wiśniewski JR, Walerych D. The molecular network of the proteasome machinery inhibition response is orchestrated by HSP70, revealing vulnerabilities in cancer cells. *Cell Rep*, 2022; 40:111428

Sekar R, Motzler K, Kwon Y, Novikoff A, Jülg J, Najafi B, Wang S, Warnke AL, Seitz S, Hass D, Gancheva S, Kahl S, Yang B, Finan B, Schwarz K, Okun JG, Roden M, Blüher M, Müller TD, Krahmer N, Behrends C, Plettenburg O, **Miaczynska M**, Herzig S, Zeigerer A. Vps37a regulates hepatic glucose production by controlling glucagon receptor localization to endosomes. *Cell Metab*, 2022; 34:1824-1842.e9

Zdzalik-Bielecka D, Kozik K, Poswiata A, Jastrzebski K, Jakubik M, Miaczynska M. Bemcentinib and gilteritinib inhibit cell growth and impair the endo-lysosomal and autophagy systems in an AXL-independent manner. *Mol Cancer Res*, 2022; 20:446-55

Zdzalik-Bielecka D, Poświata A, Kozik K, Jastrzębski K, Schink KO, Brewińska-Olchowik M, Piwocka K, Stenmark H, **Miączyńska M**. The GAS6-AXL signaling pathway triggers actin remodeling that drives membrane ruffling, macropinocytosis, and cancer-cell invasion. *Proc Natl Acad Sci USA*, 2021; 118:e2024596118

Kolmus K, Erdenebat P, Szymańska E, Stewig B, Goryca K, Derezińska-Wołek E, Szumera-Ciećkiewicz A, Brewińska-Olchowik M, Piwocka K, Prochorec-Sobieszek M, Mikula M, **Miaczynska M**. Concurrent depletion of Vps37 proteins evokes ESCRT-I destabilization and profound cellular stress responses. *J Cell Sci*, 2021; 134:jcs.250951

Cendrowski J, Kaczmarek M, Mazur M, Kuzmicz-Kowalska K, Jastrzebski K, Brewinska-Olchowik M, Kominek A, Piwocka K, **Miaczynska M**. Splicing variation of BMP2K balances abundance of COPII assemblies and autophagic degradation in erythroid cells. *eLife*, 2020; 9:e58504

Cendrowski J, Miaczynska M. Splicing variants of an endocytic regulator, BMP2K, differentially control autophagic degradation in erythroid cells. *Autophagy*, 2020; 16:2303-04

Szymańska M, Nowak P, Kolmus K, Cybulska M, Goryca K, Derezińska-Wołek M, Szumera-Ciećkiewicz A, Brewińska-Olchowik M, Grochowska A, Piwocka K, Prochorec-Sobieszek M, Mikula M, **Miączyńska M**. Synthetic lethality between VPS4A and VPS4B triggers an inflammatory response in colorectal cancer. *EMBO Mol Med*, 2020; 12:e10812

Banach-Orłowska M, Wyszyńska R, Pyrzyńska B, **Maksymowicz M**, Gołąb J, **Miączyńska M**. Cholesterol restricts lymphotoxin β receptor triggered NF-κB signaling. *Cell Commun Signal*, 2019; 17:171

Banach-Orłowska M, Jastrzębski K, Cendrowski J, Maksymowicz M, Wojciechowska K, Korostyński M, Moreau D, Gruenberg J, **Miaczynska M**. The topology of lymphotoxin β receptor accumulated upon endolysosomal dysfunction dictates the NF-κB signaling outcome. *J Cell Sci*, 2018; 131:jcs218883

Szymanska E, Budick-Harmelin N, Miaczynska M. Endosomal "sort" of signaling control: The role of ESCRT machinery in regulation of receptor-mediated signaling pathways. *Semin Cell Dev Biol*, 2018; 74:11-20

Mamińska A, Bartosik A, Banach-Orłowska M, Pilecka I, Jastrzębski K, Zdzalik-Bielecka D, Castanon I, Poulain M, Neyen C, **Wolińska-Nizioł L, Toruń A, Szymańska E**, Kowalczyk A, Piwocka K, Simonsen A, Stenmark H, Furthauer M, Gonzalez-Gaitan M, **Miaczynska M**. ESCRT proteins restrict constitutive NF-κB signaling by trafficking cytokine receptors. *Sci Signal*, 2016; 9:ra8

DESCRIPTION OF CURRENT RESEARCH

We study molecular mechanisms of intracellular membrane trafficking and signaling in health and disease. We seek to understand how endosomal compartments contribute to the trafficking and signaling of receptors for growth factors and cytokines and how the dysfunction of endosomes affects cell physiology. We are particularly interested in alterations that occur in signaling and trafficking processes in cancer cells because such changes may represent vulnerabilities of cancer cells to specific therapies. In parallel, we are also interested in trafficking pathways that operate in specific cell types or certain stages of cell differentiation.

In one of our previous projects, we described inflammatory signaling that was induced intracellularly upon endosome dysfunction (Mamińska et al., *Sci Signal*, 2016). As an underlying molecular mechanism, we found that the aberrant endocytic trafficking of cytokine receptors can cause their accumulation on endosomal membranes and the ligand-independent activation of nuclear factor-κB signaling, resulting in a fully engaged inflammatory cellular response. This mechanism occurs upon the dysfunction of components of endosomal sorting complexes required for transport (ESCRT) and is evolutionarily conserved from fly to human cells.

In our molecular oncology projects, we discovered synthetic lethality between two paralogous ATPases of ESCRT machinery, VPS4A and VPS4B (Szymańska et al., *EMBO Mol Med*, 2020). We showed that the VPS4B gene was frequently deleted in many cancer types, including in colorectal cancer, reflected by low VPS4B mRNA and protein levels in colorectal cancer samples from patients. The perturbation of VPS4A protein in tumor cells with the loss or low levels of VPS4B induced the death of cells that were grown *in vitro* and in a tumor xenograft model in mice. Moreover, upon the concomitant depletion of VPS4A and VPS4B proteins, dying cancer cells secreted immunomodulatory molecules that mediated inflammatory and anti-tumor responses. Overall, our results identified a novel pair of druggable targets for personalized oncology, thereby providing a rationale for developing VPS4 inhibitors for the precision treatment of VPS4B-deficient cancers. We also discovered lower gene expression of the ESCRT-I components VPS37A and VPS37B in colorectal cancer (Kolmus et al., *J Cell Sci*, 2021). At the molecular level, we showed that the concurrent depletion of VPS37 proteins evoked destabilization of the ESCRT-I complex and profound cellular stress responses.

Most recently, we revealed that the ESCRT-I complex is also indispensable for the biogenesis and functioning of lysosomes (Wróbel, Cendrowski et al., *Life Sci Alliance*, 2022). These organelles degrade various types of macromolecules that derive from endocytic and autophagic processes that ensure nutrient supply to fuel cellular metabolism. Lysosomes have lately gained much attention because targeting their function emerges as a promising strategy to treat cancer. We uncovered that the lack of ESCRT-I led to lysosome enlargement through inhibition of the degradation of their resident membrane proteins. This effect was accompanied by impairments in the delivery of internalized cholesterol to lysosomes. Using an RNA sequencing approach, we discovered that cells that lacked ESCRT-I activated transcriptional responses to counteract the improper delivery of nutrients that derive from lysosomal degradation. These responses involved the higher expression of genes whose products are known to induce the biosynthesis of cholesterol and *de novo* generation of lysosomes. We further revealed that these transcriptional changes resulted from the activation of TFEB/TFE3 transcription factors that are master regulators of autophagy and lysosome biogenesis. These factors can be activated by multiple cues. Upon ESCRT-I dysfunction, the activity of TFEB/TFE3 transcription factors was specifically induced by inhibition of the Rag GTPase-mTORC1 pathway. Overall, our results identify the ESCRT-I complex as an important regulator of lysosomal homeostasis (Fig. 1). Its function ensures the proper delivery of macromolecular cargo for degradation in lysosomes. This cargo is delivered from endosomes, autophagosomes, and lysosomal membranes. Consequently, ESCRT-I deficiency causes the improper supply of lysosome-derived nutrients, termed "lysosomal nutrient starvation".

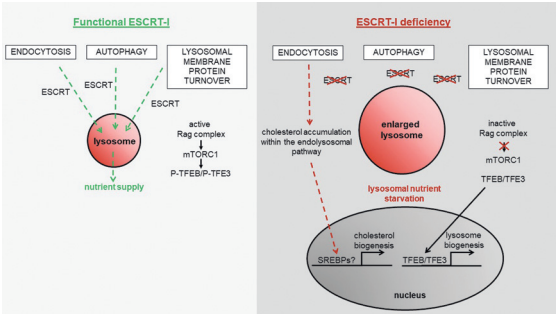


Fig. 1. Model of ESCRT-I function in maintaining lysosomal homeostasis and nutrient supply. [Left] The functional ESCRT-I complex mediates the flow of macromolecular cargo from endocytosis, autophagy, and lysosomal membrane protein turnover to lysosomes for degradation. This allows the proper supply of nutrients for cellular metabolism. [Right] The lack of ESCRT-I inhibits lysosomal membrane protein turnover, resulting in the enlargement of lysosomes. Impairments in cargo delivery to lysosomes cause lysosomal nutrient starvation, manifested by the induction of a starvation-like transcriptional response through the Rag-mTORC1-dependent activation of TFEB/TFE3 transcription factors. Author: Marta Wróbel

In another line of research, we focused on the receptor tyrosine kinase AXL, which is overexpressed in late-stage, metastatic, and drug-resistant cancers of various origins. Although the first AXL inhibitors are in clinical trials, cellular mechanisms of action of AXL remain unknown. By identifying the interactome of AXL, we revealed that the ligand-stimulated AXL receptor induces several actin-dependent processes (Zdzalik-Bielecka et al., *Proc Natl Acad Sci U S A*, 2021). Specifically, AXL activation induced the formation of circular dorsal ruffles and peripheral ruffles at the plasma membrane, macropinocytosis, and focal adhesion turnover. Such increases in membrane ruffling and macropinocytosis result in increases in the invasion and nutrient acquisition of cancer cells, respectively. We also characterized the endocytosis of AXL and discovered that ligand-bound receptors were rapidly internalized via several endocytic pathways, including both clathrin-mediated and clathrin-independent routes (Poświata et al., *Cell Mol Life Sci*, 2022). The majority of the internalized receptor was not degraded but rather recycled back to the plasma membrane, coinciding with the sustained activation of AKT kinase signaling. Furthermore, we studied cellular effects of AXL inhibitors that are at various stages of clinical development (Zdzalik-Bielecka et al., *Mol Cancer Res*, 2022). We found that LDC1267 is a potent and specific inhibitor, whereas bemcentinib and gilteritinib exert off-target effects on cell growth and the endolysosomal and autophagy systems. These findings may help interpret results of ongoing clinical trials of AXL inhibitors.

Finally, while studying the cell type-specific regulation of membrane transport pathways during erythropoiesis, we identified cellular functions of a relatively poorly studied kinase, BMP2K, and its involvement in erythroid differentiation (Cendrowski et al., *eLife*, 2020; Cendrowski et al., *Autophagy*, 2020). We found that BMP2K acts in multiple membrane trafficking processes, including clathrin-mediated endocytosis, autophagy, and the regulation of COPII assemblies that are involved in secretion. Intriguingly, we found that two splicing variants of BMP2K (the longer BMP2K-L variant and shorter BMP2K-S variant) have partly different interactomes and exhibit opposite functions in SEC16A-dependent autophagy and erythroid differentiation. We propose that the BMP2KL/S regulatory system fine-tunes erythroid maturation through an unusual mechanism of two splicing variants of a kinase that play opposing roles in intracellular processes.

LABORATORY OF RNA – PROTEIN INTERACTIONS – DIOSCURI CENTRE







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Magdalena Wołczyk, PhD

Research Assistant
Natalia Stec, MSc (until January 2023)

Research Specialists
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Gracjan Michlewski, PhD, Professor

DEGREES
2021 Professor of Biological Sciences, nomination by the President of the Republic of Poland
2012 DSc Habil in Biochemistry, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland
2005 PhD summa cum laude in Biological Chemistry, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland
2001 MSc in Biotechnology, Adam Mickiewicz University, Poznań, Poland

PROFESSIONAL EXPERIENCE
2023-Present Scientific Board Member, Institute of Biochemistry and Biophysics Polish Academy of Sciences
2021-Present Professor, Head of Laboratory of RNA-Protein Interactions – Dioscuri Centre, International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland
2021-Present Honorary Lecturer, Infection Medicine, University of Edinburgh, Edinburgh, United Kingdom
2021-Present Editorial Board Member of the Communications Biology, Springer Nature Group
2020 Reader, Infection Medicine, University of Edinburgh, United Kingdom
2018-2020 Associate Professor, Zhejiang University-University of Edinburgh Institute, Haining, China
2018-2020 Senior Lecturer, Infection Medicine, University of Edinburgh, Edinburgh, United Kingdom
2011-2017 Medical Career Award Fellow, Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, United Kingdom
2005 -2010 Postdoctoral Fellow, Human Genetics Unit, Medical Research Council, Edinburgh, United Kingdom

HONORS, PRIZES, AND AWARDS
2021-2025 Polish Returns Programme, Polish National Agency for Academic Exchange
2021-2025 Dioscuri Centre for RNA-Protein Interactions in Human Health and Disease, Max Planck Society, Germany and National Science Centre, Poland
2019-2022 Project Grant, UK Government's Biotechnology and Biological Sciences Research Council
2018 Moray Endowment Fund Award, UK
2017-2019 Seed Award in Science, Wellcome Trust, UK
2017 Travel Grant, RNA Society
2011-2015 Career Development Award, Medical Research Council, UK
2010 International Travel Grant, The Royal Society
2008, 2010 Scholarship, Keystone Symposia
2004-2006 Award for Scientific Achievements, Polish Genetic Society
2001 Fellowship Award, Minister of National Education, Poland
2001 Fellowship Award, Adam Mickiewicz University Foundation

DOCTORATES DEFENDED UNDER LAB LEADER'S SUPERVISION
J.S. Nowak, B. Ozkan, G. Heikel, A. Downie Ruiz Velasco, S. Zhu.

SELECTED PUBLICATIONS

Gómez-Tortosa E, Baradaran-Heravi Y, Dillen L, Choudhury NR, Agüero Rabes P, Pérez-Pérez J, Kocoglu C, Sainz MJ, Ruiz González A, Téllez R, Cremades-Jimeno L, Cárdaba B; EU EOD Consortium; Van Broeckhoven C, **Michlewski G**, van der Zee J. TRIM25 mutation (p.C168T), coding for an E3 ubiquitin ligase, is a cause of early-onset autosomal dominant dementia with amyloid load and parkinsonism. ***Alzheimers Dement***, 2022; doi: 10.1002/alz.12913

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^**Michlewski G**, Caceres JF. Antagonistic role of hnRNP A1 and KSRP in the regulation of let-7a biogenesis. ***Nat Struct Mol Biol***, 2010; 17(8):1011-8

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^**Michlewski G**, Sanford JR, Caceres JF. The splicing factor SF2/ASF regulates translation initiation by enhancing phosphorylation of 4E-BP1. ***Mol Cell***, 2008; 30(2):179-89

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DESCRIPTION OF CURRENT RESEARCH

RNA-protein interactions in human health and disease

RNA-binding proteins (RBPs) play a crucial role in regulating gene expression through their interactions with RNA. These proteins play a vital role in maintaining cellular balance and promoting normal development and are involved in many human diseases. Recent advancements in high-throughput proteomics have led to the discovery of new RBPs, but our understanding of their function remains limited. One landmark study by Castello et al. (*Cell*, 2012) identified 860 RBPs in HeLa cells, with over 300 of them being previously unknown to have RNA-binding properties and lacking any recognizable RNA-binding domains. Subsequent studies identified hundreds of novel RBPs in various cells and tissues, but there is still much to learn about what determines their molecular functions and roles in human health and disease.

Functional and structural characteristics of novel RBPs and RNA-protein interactions in the innate immune response to RNA viruses

RNA viruses have caused numerous outbreaks, and SARS-CoV-2, the virus that is responsible for COVID-19, is a major contributor to this trend. Another infamous RNA virus, influenza A virus (IAV), leads to the deaths of up to 500,000 individuals annually worldwide, imposing heavy socioeconomic burdens globally. These are just two examples among many RNA viruses that pose serious threats to human health and economies. Thus, a comprehensive understanding of host-virus interactions at the molecular level is crucial to develop effective strategies to inactivate viruses and prevent future outbreaks.

Our research on RNA viruses and their interactions with host cells has led to the discovery and investigation of a new RBP, the E3 ubiquitin ligase TRIM25 (Choudhury et al., *Cell Rep*, 2014; Choudhury et al., *BMC Biol*, 2017). TRIM25 is a member of a large (> 80 members) family of tripartite motif-containing proteins, most of which have E3 ubiquitin ligase activity. TRIMs share an amino-terminal tripartite domain arrangement (RING–Bbox1/2–coiled coil) but differ in their C-terminal domains, which categorize them into various subtypes. Many TRIMs are either positive or negative regulators of innate immune pathways, the first line of defense against such pathogens as viruses. TRIM25 is increasingly recognized as a crucial factor in the innate immune response to RNA viruses, including influenza A virus, SARS-CoV-2, and dengue virus, and other significant human pathogens. Viruses have developed proteins to deactivate TRIM25, with the nonstructural protein 1 (NS1) that is produced from the 8th segment of influenza A virus being the primary antagonist of the innate immune response.

TRIM25's most well-known function involves activating the pattern-recognition receptor RIG-I, which detects 5'-triphosphate (5'-ppp) on viral RNA and triggers the innate immune response. Upon binding to 5'-ppp-RNA, RIG-I undergoes TRIM25-mediated ubiquitination, which activates a signaling cascade that ultimately leads to the phosphorylation of interferon regulatory factors 3 and 7 and nuclear factor-κB. These factors then translocate to the nucleus and induce the expression of type I interferon. However, the role of TRIM25 in the RIG-I pathway has been questioned by recent reports (Heyman et al., *Immunol Cell Biol*, 2019; Cadena et al., *Cell*, 2019). Our recent publication reported on a new mechanism by which TRIM25 binds to influenza A mRNA and destabilizes it, contributing to antiviral activity (Choudhury et al., *Nucleic Acids Res*, 2022; Fig. 1). We are currently focusing on understanding the mechanism of the TRIM25-mediated destabilization of RNAs and other RBPs that are involved in sensing and regulating innate immune pathways. These findings shed new light on the role of RBPs in the antiviral response and offer potential avenues for developing new antiviral therapies.

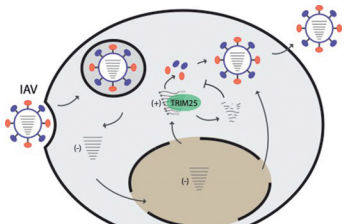


Fig. 1. Model of TRIM25 sensing and inhibiting IAV infection by controlling viral mRNA stability. During IAV infection TRIM25 binds to abundantly produced positive strand RNAs and triggers their degradation, which in turn slows down viral replication.

Regulation of microRNAs through RBPs for the treatment of Parkinson's disease

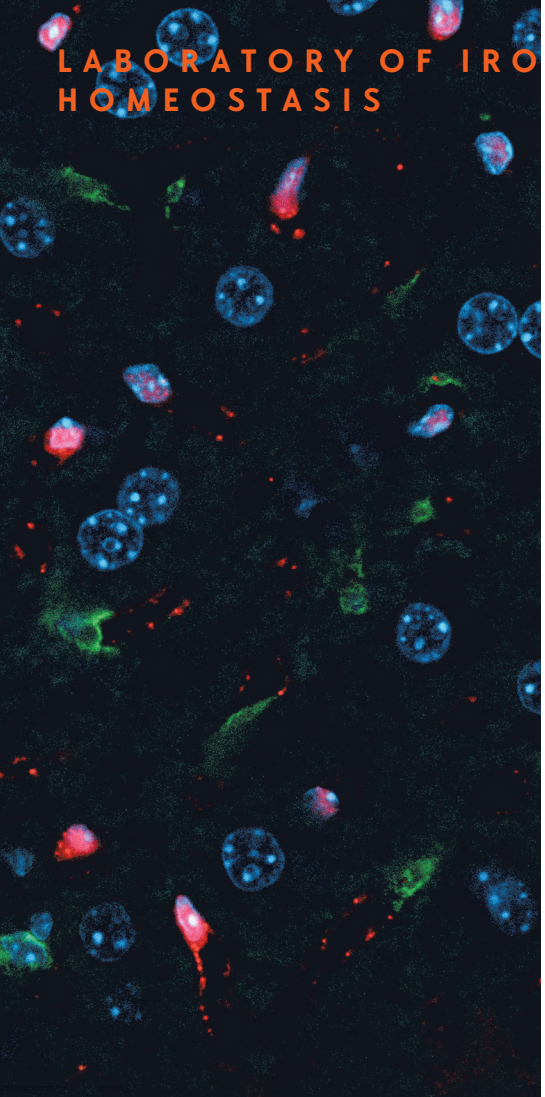
Parkinson's disease (PD) is an incurable neurodegenerative disorder that affects individuals of all ages, but it is most prevalent among the elderly. Over 1% of the population over 60 years of age suffer from it. According to United Nations projections, there will be 2.1 billion people over 60 years of age by 2050, translating to 21 million in this age group who suffer from PD. The main cause of PD is overproduction and accumulation of the protein α-synuclein (α-Syn) in brain cells of affected individuals. This excessive expression and aggregation of α-Syn is a significant contributor to the development of PD. One of the defining characteristics of PD is the loss of dopaminergic neurons in the substantia nigra of the midbrain, leading to dopamine deficiency and causing motor symptoms. Another hallmark of PD is the presence of Lewy bodies, intracellular round inclusions that are composed largely of aggregated α-Syn. Duplications and triplications of the gene that encodes α-Syn (*SNCA*) have been observed in both familial and sporadic forms of PD. A growing body of evidence indicates that reducing α-Syn levels can be beneficial for PD patients, and several clinical trials are underway to examine this possibility using experimental medical treatments and vaccines.

miRNA-7, also known as miR-7, is a cellular RNA that has been found to negatively regulate the production of α-Syn. By binding to α-Syn mRNA, miR-7 stops production of the protein, keeping levels normal in healthy individuals. However, levels of miR-7 and other miRs, such as miR-153, that also negatively impact α-Syn production, are lower in PD, leading to the overproduction and accumulation of α-Syn. We showed that the HuR (ELAVL1) RBP is a naturally occurring inhibitor of miR-7 production (Choudhury et al., *Genes Dev*, 2012). Additionally, evidence suggests that HuR increases in PD, and its binding to the α-Syn mRNA 3'-untranslated region stabilizes the transcript, allowing for an increase in α-Syn production. This implies that disrupting the RNA/HuR complex may have a positive impact on miR-7 and negative effect on α-Syn, offering a potential new approach for PD therapy.

To identify new and evaluate existing RNA-protein interaction inhibitors, we developed a fluorescent on-bead screening platform, RNA Pull-Down Confocal Nanoscanning (RP-CONA; Zhu et al., *Nucleic Acids Res*, 2021). RP-CONA uses fluorescent on-bead screening to identify small molecules that modulate the strength of RNA-protein interactions. Using this platform, we found that quercetin, a naturally occurring molecule, increased cellular miR-7 levels and decreased α-Syn expression in a HuR-dependent manner. However, the exact mechanism by which this effect occurs and its specificity and efficacy in PD-relevant cells have not yet been explored. We are working toward understanding and intervening in RNA regulatory networks that are involved in the etiology of PD, which may provide new avenues for the treatment of PD and other diseases that are associated with disorders of gene expression and RNA metabolism.

Dioscuri Centre of Scientific Excellence. The Programme initiated by the Max Planck Society (MPG), managed jointly with the National Science Centre in Poland and mutually funded by the Polish Ministry of Education and Science (MEiN) and the German Federal Ministry of Education and Research (BMBF)
The project is co-financed by the Polish National Agency for Academic Exchange within Polish Returns Programme

LABORATORY OF IRON HOMEOSTASIS



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DEGREES

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2007 MSc in Biotechnology, Faculty of Biochemistry, Biophysics, and Biotechnology, Jagiellonian University, Cracow, Poland

PROFESSIONAL EXPERIENCE

2017-Present Professor, Head of Laboratory of Iron Homeostasis, International Institute of Molecular and Cell Biology in Warsaw, Poland

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2007-2011 Doctoral research with Prof. Martina Muckenthaler and Prof. Matthias W. Hentze, Molecular Medicine Partnership Unit, European Molecular Biology Laboratory and Heidelberg University, Heidelberg, Germany

2006-2007 Master thesis research with Prof. Józef Dulak and Prof. Alicja Józkowicz, Department of Medical Biotechnology, Jagiellonian University, Cracow, Poland

2006 Undergraduate research during Erasmus fellowship with Dr. Claudine Kieda, Centre De Biophysique Moleculaire, Centre National de la Recherche Scientifique, Orleans, France

2001 Undergraduate research during Erasmus scholarship with Dr. Claudine Kieda, Centre De Biophysique Moleculaire, Centre National de la Recherche Scientifique, Orleans, France

HONORS, PRIZES AND AWARDS

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2020 Scholarship for Outstanding Young Scientists, Minister of Science and Higher Education

2019 OPUS, National Science Centre

2016 POLONEZ, National Science Centre

2014 Independent research grant, University of Heidelberg

2011 Invitation to 61st Lindau Meeting of Nobel Laureates, Lindau, Germany

2015, 2014, 2011, 2010, 2009 Travel Grants to attend and present data at the international conferences in iron biology

2007 Louis-Jeantet PhD Scholarship for young researchers from Eastern Europe to support PhD studies at European Molecular Biology Laboratory

2006 Erasmus Scholarship, Centre National de la Recherche Scientifique, Orleans, France

SELECTED PUBLICATIONS

Slusarczyk P*, Mandal PK*, Zurawska G, Niklewicz M, Chouhan K, Mahadeva R, Jończy A, Macias M, Szybinska A, Cybulska-Lubak M, Krawczyk O, Herman S, Mikula M, Serwa R, Lenartowicz M, **Pokrzywa W, Mleczko-Sanecka K**. Impaired iron recycling from erythrocytes is an early hallmark of aging. *eLife*. 2023; 12:e79196

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Tejchman A, Lamerant-Fayel N, Jacquinet JC, Bielawska-Pohl A, ^**Mleczko-Sanecka K**, Grillon C, Chouaib S, Ugorski M, Kieda C. Tumor hypoxia modulates podoplanin/CCL21 interactions in CCR7+ NK cell recruitment and CCR7+ tumor cell mobilization. *Oncotarget*, 2017; 8(19):31876-87

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DESCRIPTION OF CURRENT RESEARCH

Sufficient iron supplies are critical for vital cellular functions, such as energy production and RNA/DNA processing and repair. However, excessive free iron can cause oxidative damage and lead to organ failure. Thus, the maintenance of iron balance is essential for the proper functioning of organisms. Our laboratory investigates new cell type-specific mechanisms that control iron homeostasis and studies how iron status affects specialized cellular functions. We employ mouse models and primary cell cultures and apply advanced techniques, including flow cytometry, cell sorting, and "omics" approaches. Defining regulatory mechanisms at the level of individual cell types will improve our understanding of both mammalian physiology and diseases that are associated with iron dyshomeostasis.

Aging impairs iron-recycling capacity in the organism

One of the leading themes of our research is the process of iron recycling, the main source of iron for all cells in the body, including the daily production of ~200 billion red blood cells (RBCs). Iron recycling is accomplished primarily by red pulp macrophages (RPMs) via the erythrophagocytosis of senescent RBCs, their degradation in phagolysosomes, and the release of iron via the iron exporter ferroportin to replenish the plasma iron pool. Iron export capacity is regulated by the hormone hepcidin. Nonetheless, little is known about the biology of RPMs and mechanisms that contribute to iron turnover.

Aging affects iron homeostasis, reflected by tissue iron loading and anemia in the elderly. Given that RPMs continuously process iron, we suspected that their cellular functions might be susceptible to age-dependent decline, a possibility that has been unexplored to date. Our recently published paper (Slusarczyk, Mandal et al., *eLife*, 2023) reported that 10- to 11-month-old female mice exhibited iron loading in RPMs, largely attributable to a drop in the iron exporter ferroportin, which diminished their erythrophagocytosis capacity and lysosomal activity (Fig.1). Mechanistically, using readouts from aged RPMs and *in vitro* cultures of RPM-like cells, we showed that the lower erythrophagocytic activity of RPMs was caused by a combination of higher iron levels, lower expression of the heme-catabolizing enzyme heme oxygenase 1 (HO-1), and endoplasmic reticulum stress. Furthermore, we identified a loss of RPMs during aging, which was attributable to the combination of proteotoxic stress and iron-dependent cell death that resembled ferroptosis. We demonstrated that these impairments led to the retention of senescent hemolytic RBCs in the spleen and the formation of undegradable iron- and heme-rich extracellular protein aggregates, likely derived from ferroptotic RPMs. We further showed that feeding mice an iron-deficient diet alleviated iron accumulation in RPMs, enhanced their ability to clear erythrocytes, and reduced RPM damage. Consequently, we found that iron-restricted feeding ameliorated the hemolysis of splenic RBCs and reduced the burden of protein aggregates, mildly increasing serum iron availability in aging mice. In summary, we identified RPM collapse as an early hallmark of aging and demonstrated that dietary iron restriction improved iron turnover efficacy. Future studies should investigate whether RPM dysfunction affects other cells within the splenic microenvironment during aging.

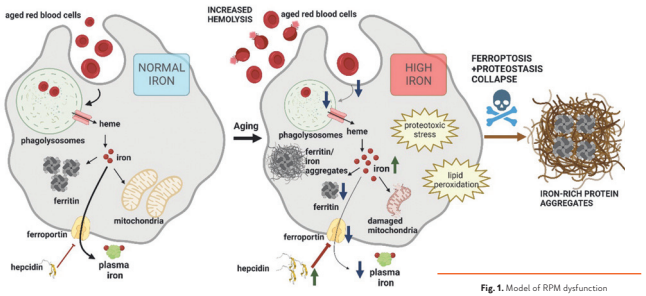


Fig. 1. Model of RPM dysfunction and collapse during aging.

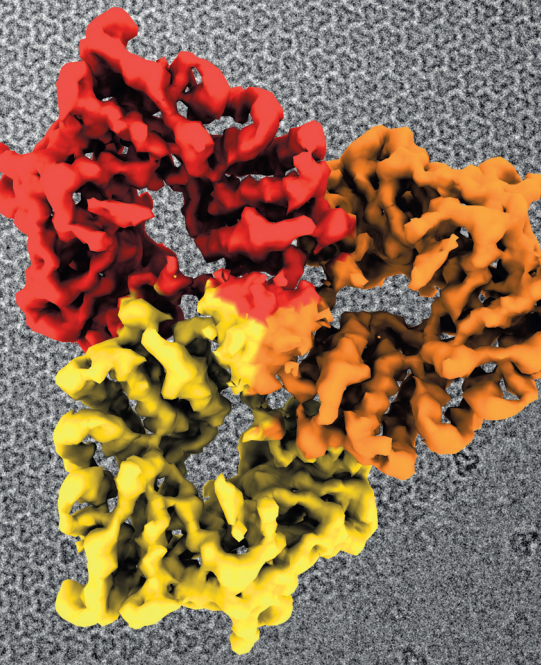
Deciphering iron sensing mechanisms in liver sinusoidal endothelial cells (LSECs)

Hepcidin is a master regulator of body iron homeostasis. Nevertheless, despite growing knowledge of the molecular control of iron balance, the genetic basis for variations in body iron parameters is still not fully understood. To gain insights into signaling pathways and biological processes that affect hepcidin expression, we previously designed and conducted large-scale RNAi screens for novel hepcidin regulators, providing new evidence of hepcidin transcriptional control (Mleczko-Sanecka et al., *Blood*, 2010, 2014; Mleczko-Sanecka et al., *Haematologica*, 2017; Sonnweber et al., *Gut*, 2014; Pasricha et al., *Nat Commun*, 2017). When iron levels in the body increase, iron-sensing mechanisms are engaged to enhance hepcidin production and prevent further dietary iron uptake. Bone morphogenetic protein 6 (BMP6) is a cytokine that is produced by liver sinusoidal endothelial cells (LSECs) and stimulates hepcidin production in hepatocytes in response to an iron challenge. Despite the critical role of BMP6 in iron sensing and the maintenance of iron balance in the body, unclear are the ways in which systemic and liver iron levels translate into alterations of *Bmp6* mRNA levels in LSECs. Using a murine model of aging-triggered liver iron loading, one of our advanced projects identified a new mechanism for *Bmp6* regulation. We found that *Bmp6* can be induced by excessive iron in a manner that is independent of the previously published factor NRF2 and remains under the control of the transcription factor ETS1. Another line of our research identified a novel role for LSECs in the clearance of free hemoglobin, a key component of RBCs. We demonstrated that this previously unknown function of LCESs likely contributes to physiological iron recycling from hemoglobin and plays a role in heme detoxification during hemolysis, coupled with induction of the iron-sensing BMP6-hepcidin axis that restores homeostasis.

Identifying novel mechanisms for regulation of the iron transporter ZIP14

Body iron levels increase above homeostatic levels when the iron challenge persists or when hepcidin responses are dysregulated. This ultimately leads to excessive saturation of the plasma iron-binding protein transferrin and the generation of so-called non-transferrin-bound iron (NTBI). This form of redox-active iron is toxic and currently considered the main contributor to iron-overload disorders, such as hereditary hemochromatosis and some severe anemias [e.g., thalassemias]. Liver hepatocytes are the primary cell type that acquires NTBI. Progressive iron accumulation in hepatocytes leads to impairments in liver function and a higher risk of aggressive hepatocellular carcinoma. Interestingly, the severity of iron loading, particularly in hemochromatosis, differs substantially between patients. The genetic basis of this variation is still not fully understood. One of our ongoing projects seeks to understand the molecular processes that contribute to NTBI uptake in hepatocytes. Specifically, we aim to identify signaling mechanisms that alter the hepatic expression of ZIP14 [which is encoded by *SLC39A14*], a key metal transporter that is responsible for NTBI uptake in the liver. To decipher the regulatory mechanisms of ZIP14, we conducted a small-scale CRISPR screen for candidate transcription factors that may regulate ZIP14 expression. Follow-up secondary analysis of the identified hits revealed that glucocorticosteroids stimulate ZIP14 expression in primary murine hepatocytes and mice.

LABORATORY OF PROTEIN STRUCTURE



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Co-worker
Zara Naz, MSc

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Nicola de Franceschi, PhD

Technician
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Laboratory Support Specialist
Kinga Adamska, MSc

LAB LEADER

Marcin Nowotny, PhD, Professor

DEGREES
2020 Professor of Biological Sciences, nomination by the President of the Republic of Poland
2013 DSc Habil in Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
2002 PhD magna cum laude in Biochemistry, under the supervision of Prof. 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, University of Warsaw, Poland

PROFESSIONAL EMPLOYMENT
2008-Present Professor, Head of the Laboratory of Protein Structure, International Institute of Molecular and Cell Biology in Warsaw, Poland
2016-2018 Deputy Director for Science, International Institute of Molecular and Cell Biology in Warsaw, Poland
2017-2019 Co-founder and Chief Scientific Officer, ProBiostructures, International Institute of Molecular and Cell Biology research service center for pharmaceutical industry

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

MEMBERSHIP AND AWARDS
2022 Prize of the Foundation for Polish Science
2021 Team Award of the Minister of Education and Science for significant achievements in scientific activities
2020 Chair, Scientific Policy Committee, Ministry of Science and Higher Education, Poland
2019 Member, European Molecular Biology Organization
2019 Member, Academia Europea
2018 Member, Scientific Policy Committee, Ministry of Science and Higher Education, Poland
2018 MAESTRO, National Science Centre
2015 Jan Karol Parnas Award for the best Polish biochemical publication (with the group of Prof. Janusz M. Bujnicki)
2013 Knight's Cross of the Order of Polonia Restituta
2012 Prime Minister Award for scientific achievements
2012 Ideas for Poland Award, Foundation for Polish Science
2012 Jan Karol Parnas Award for the best Polish biochemical publication
2012 International Senior Research Fellowship, Wellcome Trust (renewal)
2012 Early Career Scientist Award, Howard Hughes Medical Institute
2011 ERC Starting Grant (2012-2017)
2007 EMBO Installation Grant
2007 International Senior Research Fellowship, Wellcome Trust
2003 Prime Minister Award for PhD thesis
2001, 2002 START Scholarship for Young Scientists, Foundation for Polish Science

DOCTORATES DEFENDED UNDER LAB LEADER'S SUPERVISION
M. Jaciuk, M. Miętus, M. Czarnocki-Cieciura, M. Śmietański, M. Rażew, D. Malik, M. Gapińska.

SELECTED PUBLICATIONS

○ IIMCB Best Papers Award

Hyjek-Składanowska M, Anderson BA, Mykhaylyk V, Orr C, Wagner A, Poznański JT, **Skowronek K**, Seth P, **Nowotny M**. Structures of annexin A2-PS DNA complexes show dominance of hydrophobic interactions in phosphorothioate binding. ***Nucleic Acids Res***, 2023; 51(3):1409-23

○ **Kaczmarska Z***, **Czarnocki-Cieciura M***, **Górecka-Minakowska KM***, Wingo RJ, **Jackiewicz J**, **Zajko W**, Poznański JT, Rawski M, Grant T, Peters JE^, **Nowotny M#**. Structural basis of transposon end recognition explains central features of Tn7 transposition systems. ***Mol Cell***, 2022; 82(14):2618

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Nowacka M, **Nowak E**, **Czarnocki-Cieciura M**, **Jackiewicz J**, Skowronek K, Szczepanowski R, Wohrl BM, **Nowotny M**. Structures of substrate complexes of foamy viral protease-reverse transcriptase. ***J Virol***, 2021; 95(18):e0084821

○ **Hyjek-Składanowska M**, Vickers T, **Napiórkowska A**, Anderson B, Tanowitz M, Crooke ST, Liang XH, Seth PP#, **Nowotny M#**. Origins of the increased affinity of phosphorothioate-modified therapeutic nucleic acids for proteins. ***J Am Chem Soc***, 2020; 142(16):7456-68

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○ **Górecka KM***, Krepl M**, Poznański J, Šponer J, **Nowotny M***. RuvC uses dynamic probing of the Holliday junction substrate to achieve sequence specificity and efficient resolution. ***Nat Commun***, 2019; 10(1):4102

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○ **Razew M**, Warkocki Z, Taube M, Kolondra A, **Czarnocki-Cieciura M**, **Nowak E**, Labedzka-Dmoch K, Kawinska A, Piatkowski J, Golik P, Kozak M, Dziembowski A, **Nowotny M**. Structural analysis of mtEXO mitochondrial RNA degradosome reveals tight coupling of nuclease and helicase components. ***Nat Commun***, 2018; 9(1):97

Figiel M, Krepl M, Poznanski J, Golab A, Šponer J, **Nowotny M**. Coordination between the polymerase and RNase H activity of HIV-1 reverse transcriptase. ***Nucleic Acids Res***, 2017; 45(6):3341-52

○ **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 Rep***, 2015, 10(9):1467-76

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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

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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(2):191-7

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(4):658-70

^**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(7):1005-16

* equal contribution
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DESCRIPTION OF CURRENT RESEARCH

Our laboratory focuses on structural and biochemical studies of nucleic acid-processing enzymes.

Structures and mechanisms of DNA polymerases involved in abortive infection

Bacteria utilize a wide range of defense strategies to prevent viral infection and virus replication. One of these strategies is abortive infection (Abi), which is an altruistic process of programmed death of the infected cell to prevent the virus from replicating and spreading to other cells. Various Abi systems have been identified that are predicted to work through very different mechanisms. AbiK and Abi-P2 proteins are abortive infection effector proteins that are related to reverse transcriptases (RTs), enzymes that synthesize DNA using RNA or DNA as a template. Previous biochemical studies of AbiK showed that this enzyme can synthesize DNA without any template. Moreover, it does not require a short DNA fragment to start nucleic acid synthesis. Instead, it attaches the first nucleotide of the growing DNA chain to one of its own residues through a mechanism called protein priming.

We determined the first structures of DNA polymerases AbiK and Abi-P2 using X-ray crystallography and cryo-electron microscopy (Figiel, Gapinska, et al., *Nucleic Acids Res*, 2022). Both proteins have two parts: a catalytic RT-like domain and a domain that is unique to Abi proteins, comprising α-helices and serving to stabilize the nascent DNA chain. The structures revealed that AbiK and Abi-P2 form hexamers and trimers, respectively, which is unprecedented for DNA polymerases. The structure of wildtype AbiK contains a single-stranded DNA chain whose 5' end is covalently linked to the priming residue, whereas its 3' portion is accommodated in the central channel of the enzyme. Additionally, a cryo-electron microscopy structure was determined for an AbiK variant that contains a substitution of the protein-priming residue and thus does not contain any covalently bound DNA. Comparisons of the structures with and without DNA showed that protein priming by AbiK involves a change in the arrangement of a mobile part of the protein that harbors the amino acid residue that is involved in priming.

Structural studies of the enhanced binding affinity of therapeutic nucleic acids to proteins

The phosphorothioate (PS) backbone is the most widely used modification in therapeutic nucleic acids, including antisense oligonucleotides (ASOs). This modification involves the replacement of one of the two oxygen atoms in the repeating phosphate groups of the DNA with sulfur. Phosphorothioate-modified nucleic acids show improved properties, such as metabolic stability from nuclease-mediated degradation. One of the hallmarks of these compounds is an enhancement of interactions with cellular proteins. This property facilitates the cellular uptake of nucleic acid drugs and their cellular retention but may also contribute to cytotoxic properties of the drug molecule. Molecular mechanisms of interactions between PS nucleic acids and proteins have not been fully established.

To better understand how PS ASOs interact with cellular proteins, we solved two crystal structures of PS ASO bound to annexin A2 (AnxA2), a calcium-binding protein that was previously implicated in the release of PS ASOs from endo-lysosomal compartments. In our work, we found that interactions between the sulfur atom of the PS linkage and protein surface were mediated by lysine and arginine side chains and had mainly a hydrophobic character, suggesting that the hydrophobic nature of sulfur contributes to the association of PS ASOs with cellular proteins (Hyjek-Składanowska et al., *Nucleic Acids Res*, 2022). We confirmed the importance of contacts that were observed in the crystal structures by performing mutational studies and binding assays. Importantly, high crystal quality allowed us to use a unique experimental setup at the Diamond synchrotron to perform long-wavelength X-ray diffraction experiments. These experiments led to the precise localization of sulfur atoms in the structure and allowed us to establish which of the oxygen atoms were replaced with sulfur in the DNA that bound to the protein. Interestingly, these results showed that stereoisomer preference at a given PS group in the DNA oligonucleotide is determined by the hydrophobic environment around the PS linkage that comes from both the protein and adjacent structural features within the DNA drug, such as methyl groups on cytosine nucleobases.

Overall, our results provide valuable insights into the general mechanism of the enhanced binding of PS ASOs to cellular proteins and indicate that hydrophobic interactions between PS linkages and lysine and arginine residues account for the association of ASOs with classes of proteins that are not known to bind natural DNA. These studies will be helpful for designing a new generation of DNA-based drugs that are stable and less toxic. Our studies were performed in cooperation with Ionis Pharmaceuticals (Carlsbad, California, USA; a leading company in RNA-targeted therapeutics) and Diamond synchrotron (Didcot, Oxfordshire, UK).

Structural basis of Tn7 transposon end recognition

Transposons, also known as "jumping genes", are DNA fragments that can move inside or between genomes in a process called transposition. In bacteria, transposons are involved in the transfer of antibiotic resistance and virulence genes. Bacterial Tn7 elements are among the best-studied and most widespread DNA transposons. Tn7 mobility is mediated by five element-encoded proteins. Translocation occurs via a cut-and-paste mechanism that is executed by the heteromeric transposase TnsA-TnsB, which is recruited to the target by the AAA+ adenosine triphosphatase TnsC that interacts with one of the two target selectors, TnsD or TnsE. TnsD directs the element to the conserved chromosomal attTn7 site, whereas TnsE allows transposition to conjugal plasmids. CRISPR-associated transposon elements that use CRISPR-Cas systems for RNA-guided DNA transposition are all related to Tn7 and encode TnsB-like transposases. They may provide a more precise tool for next-generation gene editing.

In collaboration with the Joe Peters group (Cornell University), we study the structure and mechanism of prototypic *E. coli* Tn7 TnsB. We used cryo-electron microscopy to determine the structure of a complex of TnsB with double-stranded DNA substrates that corresponded to the right end of the transposon at 2.7 Å resolution (Kaczmarska, Czarnocki-Cieciura, Górecka-Minakowska, et al., *Mol Cell*, 2022; Fig. 1). The structure shows that TnsB adopts a beads-on-a-string architecture. When it interacts with repeating binding sites, the DNA-binding and catalytic domains are arranged in a tiled and intertwined fashion. TnsB forms few base-specific contacts with DNA, resulting in binding preference rather than strict specificity. The formation of an array of TnsB molecules that bind to the multiple weakly conserved sites at the appropriate spacing converts this preference into specific end recognition. We proposed a model of the TnsB strand-transfer complex that helps understand the mechanism by which Tn7 TnsB interprets subtle differences in the spacing of diverged binding sites and explains key features of Tn7 transposition systems.

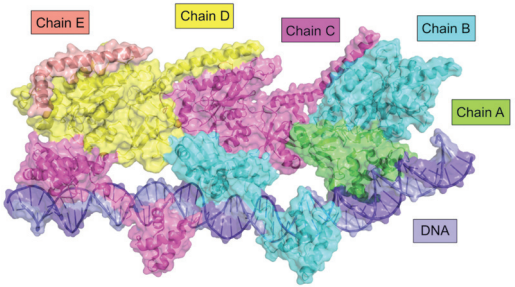
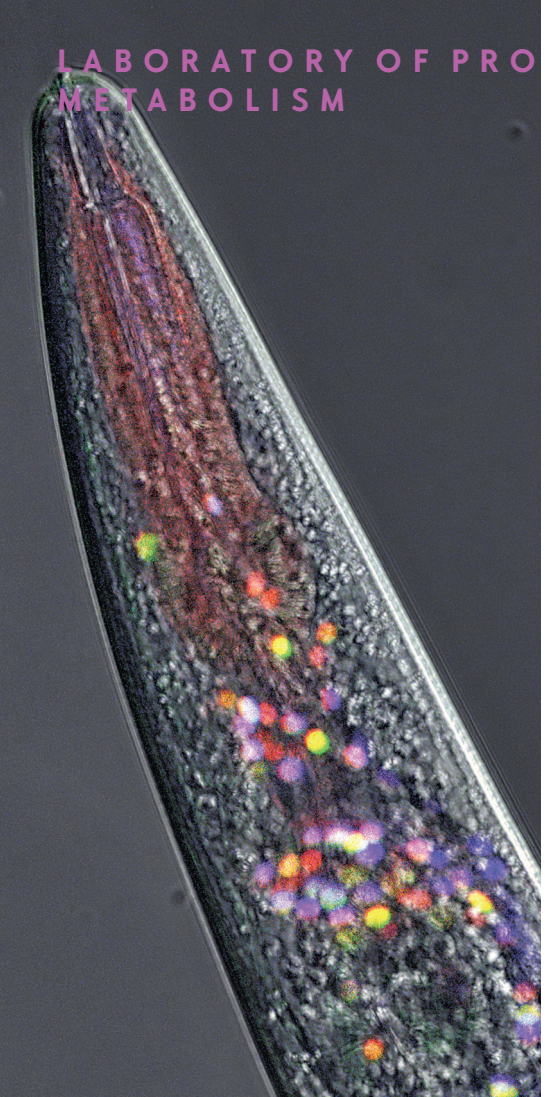


Fig. 1. Structure of E. coli TnsB bound to the fragment of the right transposon end. The TnsB chains and DNA are colored and labeled.

LABORATORY OF PROTEIN METABOLISM



LAB LEADER

Wojciech Pokrzywa, PhD, DSc Habil

DEGREES
2020 DSc Habil in Biological Sciences, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland
2009 PhD in Biological Engineering and Agronomic Sciences, Institute of Life Sciences, Molecular Physiology Group, Catholic University of Louvain, Belgium
2006 Master of Advanced Science in Biological Engineering and Agronomic Sciences, Catholic University of Louvain, Belgium
2004 MSc in Microbiology, University of Wrocław, Poland

RESEARCH EXPERIENCE
2017-Present Professor, Head of Laboratory of Protein Metabolism, International Institute of Molecular and Cell Biology in Warsaw, Poland
2009-2017 Postdoctoral fellow, Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Germany
2004-2008 PhD studies, Institute of Life Sciences, Molecular Physiology Group, Catholic University of Louvain, Belgium

HONORS, PRIZES, AND AWARDS
2022 SONATA BIS, National Science Centre
2020 GRIEG, EEA and Norway Grants, and National Science Centre
2018 FIRST TEAM, Foundation for Polish Science
2018 EMBO Installation Grant
2017 OPUS, National Science Centre
2005 PhD Fellowship, FNRS-Fund for Scientific Research, Belgium

PREPRINTS
Thapa P, **Banasiak K**, Serwa R, **Pokrzywa W**. SAH and C. elegans Longevity: Insights from an AHCY Deficiency Model. *Research Square*, 2022; doi: 10.21203/rs.3.rs-2855835/v1

Szulc NA, **Piechota M**, **Thapa P**, **Pokrzywa W**. Lysine-deficient proteome can be regulated through non-canonical ubiquitination and ubiquitin-independent proteasomal degradation. ***bioRxiv***, 2023; doi: 10.1101/2023.01.18.524605

Banasiak K, Szczepańska A, Kołodziejska K, Ibrahim AT, **Pokrzywa W***, Turek M. Pheromone-dependent olfaction bidirectionally regulates muscle extracellular vesicles formation. ***bioRxiv***, 2022; doi: 10.1101/2022.12.22.521669

Szulc NA, **Stefaniak F**, **Piechota M**, **Cappannini A**, **Bujnicki JM**, **Pokrzywa W**. DEGRONOPEDIA – a web server for proteome-wide inspection of degrons. ***bioRxiv***, 2022; doi: 10.1101/2022.05.19.492622

Piechota M, **Biriczova L**, **Kowalski K**, **Szulc NA**, **Pokrzywa W**. CHIP ubiquitin ligase is involved in the nucleolar stress management. ***bioRxiv***, 2022; doi: 10.1101/2022.05.17.492288

GROUP MEMBERS

Lab Leader
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Postdoctoral Researchers
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Małgorzata Piechota, PhD

Research Specialists
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Patryk Marcinkowski, MSc
Marta Niklewicz, MSc

PhD Students
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Aniruddha Das, MSc
Anwesha Sarkar, MSc
Natalia Szulc, MSc
Pankaj Thapa, MSc

Technician
Krzysztof Franczak (part-time)

Laboratory Support Specialist
Anna Grabowska, PhD (part-time)

SELECTED PUBLICATIONS

○ IIMCB Best Papers Award

Dubey AA, Krygier M, **Szulc NA**, Rutkowska K, Kosińska J, Pollak A, Rydzanicz M, Kmieć T, Mazurkiewicz-Bełdzińska M, **Pokrzywa W***, Płoski R. A novel de novo FEM1C variant is linked to neurodevelopmental disorder with absent speech, pyramidal signs, and limb ataxia. *Hum Mol Genet*, 2023; 32(7):1152-61

○ **Das A**, **Thapa P**, Santiago U, **Shanmugam N**, **Banasiak K**, Dąbrowska K, Nolte H, **Szulc NA**, Gathungu RM, Cysewski D, Kriger M, Dadlez M, Nowotny M, Camacho CJ, Hoppe T, **Pokrzywa W**. A heterotypic assembly mechanism regulates CHIP E3 ligase activity. *EMBO J*, 2022; 41(15):e109566

Banasiak K, Turek M, **Pokrzywa W.** Preparation of *Caenorhabditis elegans* for Scoring of Muscle-derived Exophers. *Bio-protocol*, 2022; 10.21769/BioProtoc.4586

Pekec T, Lewandowski J, Komur AA, Sobańska D, Guo Y, Świtońska-Kurkowska K, Małecki JM, **Dubey AA**, **Pokrzywa W**, Frankowski M, Figiel M, Ciosk R. Ferritin-mediated iron detoxification promotes hypothermia survival in Caenorhabditis elegans and murine neurons. *Nat Comm*, 2022; 13(1):4883

Balaji V, Müller L, Lorenz R, Kevei É, Zhang WH, Santiago U, Gebauer J, Llamas E, Vilchez D, Camacho CJ, **Pokrzywa W**, Hoppe T. (2022). A Dimer-Monomer Switch Controls CHIP-Dependent Substrate Ubiquitylation and Processing. *Mol Cell*, 2022; 82(17):3239-3254.e11

Turek M, **Banasiak K**, **Piechota M**, **Shanmugam N**, **Macias M**, Śliwińska MA, **Niklewicz M**, **Kowalski K**, Nowak N, Chacinska A, **Pokrzywa W**. Muscle derived exophers promote reproductive fitness. *EMBO Rep*, 2021; 22(8):e52071

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Albert M-C, Brinkmann K, **Pokrzywa W**, Gunther SD, Kronke M, Hoppe T, Kashkar H. CHIP ubiquitylates NOXA and induces its lysosomal degradation in response to DNA damage. *Cell Death & Disease*, 2020; 11(9):740

Fatima A, Irmak D, Noormohammadi A, Rinschen MM, **Das A**, Leidecker O, Schindler C, Sanchez-Gaya V, Wagle P, **Pokrzywa W**, Hoppe T, Rada-Iglesias A, Vilchez D. The ubiquitin-conjugating enzyme UBE2K determines neurogenic potential through histone H3 in human embryonic stem cells. *Commun Biol*, 2020; 3(1):262

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Koyuncu S, Saez I, Lee HJ, Gutierrez-Garcia R, **Pokrzywa W**, Fatima A, Hoppe T, Vilchez D. The ubiquitin ligase UBR5 suppresses proteostasis collapse in pluripotent stem cells from Huntington's disease patients. *Nat Comm*, 2018; 9(1):2886

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Kevei E, **Pokrzywa W***, Hoppe T. Repair or destruction-an intimate liaison between ubiquitin ligases and molecular chaperones in proteostasis. *FEBS Lett*, 2017; 591(17):2616-35

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DESCRIPTION OF CURRENT RESEARCH

We focus on mechanisms of protein metabolism and maintenance of the balance between the synthesis and degradation of proteins. We explore the regulation of translation, the ubiquitin-proteasome system (UPS), the chaperone network, and muscular exophers in proteostasis. Sometimes we get also intrigued by topics beyond this list. We use a combination of biochemical, microscopic, molecular genetics, and bioinformatics techniques, supported by mammalian cell assays and the nematode *C. elegans*.

WE FOCUS MAINLY ON THE FOLLOWING PROJECTS:

Cellular adaptation to cold

To counteract cold, organisms developed various responses, ranging from cold avoidance to adaptation. The latter strategy is used by hibernating animals, which, in extreme cases, can survive subzero temperatures for many days. We focus on deciphering mechanisms that alter the abundance and types of cellular messenger RNAs and proteins because these kinds of molecules are critical for live-or-die decisions of the cell. We are also investigating the role of protein quality control networks and the ubiquitin system during *C. elegans* recovery from cold stress. In addition, we conduct drug screens to identify molecules that support the ability of *C. elegans* to survive cold stress.

Regulation of exophergenesis

In our previous work, we showed that body wall muscles of *C. elegans* release exophers that can transport muscle-synthesized yolk proteins to support offspring development, increasing their odds of development and survival. However, we do not know how exophergenesis is regulated in response to external factors that impact animal development and reproduction. *C. elegans* exhibits a range of social behaviors that are primarily governed by various pheromones. Pheromone and sensory neuron-based communication between animals modulates animal growth, generation time, and maternal provisioning, and we explore this system to determine the influence of social cues on exophergenesis. We previously found that exophers are differentially modulated by sex-specific ascarosides (i.e., pheromones that are used in communication between individuals) and found that sensory neurons and the G-protein coupled receptor 173 regulate exophergenesis in response to environmental stimuli and pheromones. We also found that AQR/PQR/URX neurons, which are directly exposed to pseudocoelomic fluid and monitor the worm's body interior, restrict muscle exopher production (Fig. 1). To our knowledge, our study was the first to report how animal communication influences somatic extracellular vesicle production. We currently explore this model to identify the molecular mechanism of exophergenesis at the muscle level.

Regulation of lysine-deficient proteome through non-canonical ubiquitination

An extensive lysine-less region (i.e., lysine desert) in the yeast E3 ligase Slx5 was shown to counteract its ubiquitin-dependent turnover. We conducted bioinformatic screens among prokaryotes and eukaryotes to describe the scope and conservation of this phenomenon. We found that lysine deserts are widespread among bacteria using pupylation-dependent proteasomal degradation, an analog of the UPS. In eukaryotes, lysine deserts appear with increasing organismal complexity, and the most evolutionarily conserved are enriched in UPS members. Using VHL and SOCS1 E3 ligases, which elongated their lysine desert during the course of evolution, we established that they are non-lysine ubiquitinated, which does not influence their stability and can be subjected to proteasome turnover regardless of ubiquitination. Our data suggest that a combination of non-lysine ubiquitination and ubiquitin-independent degradation may control the function and fate of the lysine-deficient proteome because the presence of lysine deserts does not correlate with half-life. We currently study the regulation of other lysine-depleted receptors of cullin-RING ligases.

DEGRONOPEDIA: a web server for the proteome-wide inspection of degrons

A degradation-targeting degron comprises a nearby ubiquitin-modified residue and an intrinsically disordered region that interacts with the proteasome. Degron signaling has been studied over recent decades, but there are no resources for the systematic screening of degron sites to facilitate studies on their biological significance, such as targeted protein degradation approaches. To bridge this gap, we are developing DEGRONOPEDIA (https://degronopedia.com/), a web server that allows the exploration of degron motifs in proteomes of several model organisms and maps these data to lysine, cysteine, threonine, and serine residues that can undergo ubiquitination and to intrinsically disordered regions that are proximal to them, both in sequence and structure. The server provides the evolutionary context of degrons and reports post-translational modifications and pathogenic mutations within the degron and its flanking regions as these can modulate the degron's accessibility. DEGRONOPEDIA allows analyses of custom sequences/structures to examine them for degron motifs. We also implemented machine learning to predict the stability of protein N- and C-termini, facilitating the identification of substrates of N-/C-degron pathways. This project also concerns the experimental validation of predicted degrons in a cellular context. We are continually implementing new features of DEGRONOPEDIA based on the feedback from users and expanding the database of degron motifs because our tool aims to stimulate research on degron signaling.

E3 ligase complexes in the regulation of lipid metabolism

The cooperation of E3 ligases (i.e., essential components of the UPS that recognize damaged and unwanted proteins) can lead to the formation of alternative ubiquitination structures that aid in directing substrate specificity. CHIP and its worm ortholog CHN-1 are E3 ubiquitin ligases that link the chaperone system with the UPS. CHN-1 can cooperate with UFD-2, another E3 ligase, to accelerate ubiquitin chain formation; however, the basis for the high processivity of this E3 set has remained obscure. We study the molecular mechanism and function of the CHN-1–UFD-2 complex in *C. elegans*. Our data show that UFD-2 binding promotes cooperation between CHN-1 and ubiquitin-conjugating E2 enzymes by stabilizing the CHN-1 U-box dimer. However, the HSP70/HSP-1 chaperone outcompetes UFD-2 for CHN-1 binding, thereby promoting a shift to the autoinhibited CHN-1 state by acting on a conserved residue in its U-box domain. The interaction with UFD-2 enables CHN-1 to efficiently ubiquitylate and regulate *S*-adenosylhomocysteinase, a key enzyme in the *S*-adenosylmethionine regeneration cycle, which is essential for *S*-adenosylmethionine-dependent methylation (Fig. 2). Our results define the molecular mechanism that underlies the synergistic cooperation of CHN-1 and UFD-2 in substrate ubiquitination. We currently investigate new substrates of the CHN-1-UFD-2 complex that are involved in lipid metabolism.

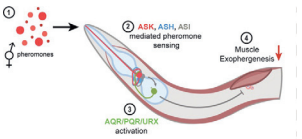


Fig. 1. Model of the olfaction-dependent regulation of muscle extracellular vesicle formation. ASK, ASH, and ASI neurons sense hermaphrodite pheromones, leading to muscle exophergenesis downregulation through AQR/PQR/URX activation. ASK, AWB, and ADL neurons sense male pheromones, leading to muscle exophergenesis upregulation through signaling that derives from in utero accumulating embryos.

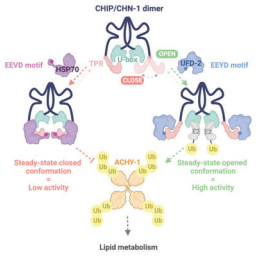
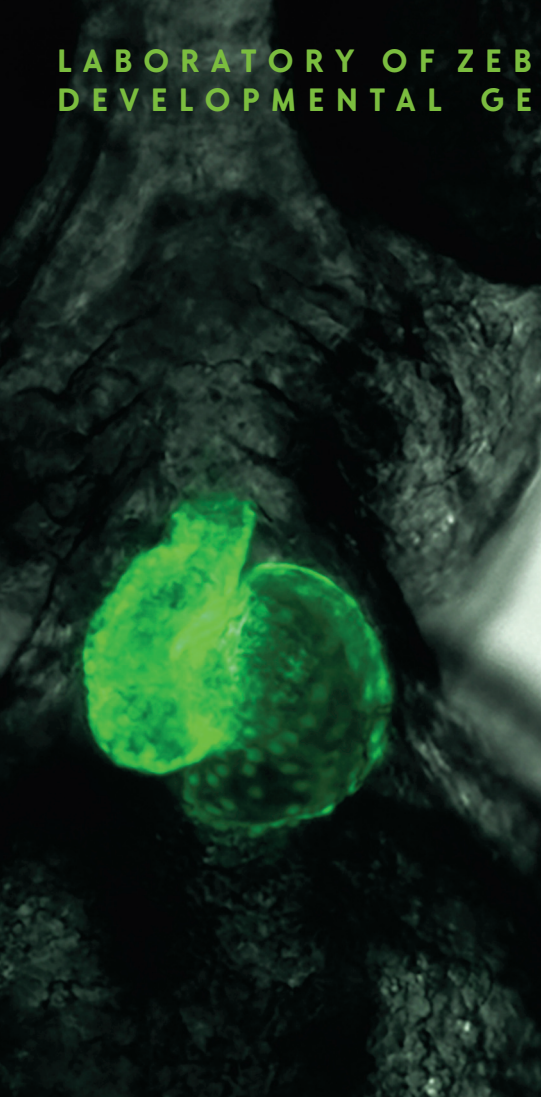


Fig. 2. Model of the regulation of CHIP quality control ubiquitin ligase activity and its substrate selectivity. The E3 ligase UFD-2 stimulates the ubiquitination activity of CHIP/CHN-1. UFD-2 binding promotes the dimerization of CHIP/CHN-1 U-box domains and E2 enzyme discharging capacity. HSP70/HSP-1, by latching the U-box and TPR domains, stabilizes the autoinhibitory state of CHIP/CHN-1, thus limiting its interactions with E2s and UFD-2. Assembly with UFD-2 enables CHIP/CHN-1 to regulate lipid metabolism via S-adenosyl-homocysteinase (AHCY-1) ubiquitination.

LABORATORY OF ZEBRAFISH DEVELOPMENTAL GENOMICS



LAB LEADER

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DEGREES
2021 DSc Habil in Biological Sciences, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
2009 PhD in Biology, Department of Biological Sciences, National University of Singapore
2004 BSc (Honors) in Biology, Department of Biological Sciences, National University of Singapore

PROFESSIONAL EXPERIENCE
2020-Present Professor, Head, Laboratory of Zebrafish Developmental Genomics, International Institute of Molecular and Cell Biology in Warsaw, Poland
2014-2019 Professor, Head, Laboratory of Zebrafish Developmental Genomics, Max Planck/International Institute of Molecular and Cell Biology in Warsaw Research Group, Poland
2013-2014 Research Associate, Genome Institute of Singapore (with a research visit in 2013 to 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 Prof. Gong Zhiyuan and Prof. Vladimir Korzh, Department of Biological Sciences, National University of Singapore

HONORS, PRIZES AND AWARDS
2016 FIRST TEAM, Foundation for Polish Science
2016 OPUS (as a partner), National Science Centre
2014 OPUS, National Science Centre
2000-2004 ASEAN Undergraduate Scholarship
2003 Science Faculty Dean's List, National University of Singapore

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Eugeniusz Tralle, MSc Eng.

Trainee
Maria Rosaria Coppola, MSc

Technician
Julia Kędzierska, MSc (part-time)

Laboratory Support Specialist
Agnieszka Konkol, MSc Eng.

SELECTED PUBLICATIONS

○ IIMCB Best Papers Award

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○ **Pawlak M**, **Kedzierska KZ**, **Migdal M**, **Abu Nahia K**, Ramilowski JA, Bugajski L, Hashimoto K, **Marconi A**, Piwocka K, Carninci P, **Winata CL**. Dynamics of cardiomyocyte transcriptome and chromatin landscape demarcates key events of heart development. ***Genome Res***, 2019; 29(3):506-19

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Piven OO, **Winata CL**. The canonical way to make a heart: β-catenin and plakoglobin in heart development and remodeling. ***Exp Biol Med (Maywood)***, 2017; 242(18):1735-45

Aksoy I, Utami KH, **Winata CL**, Hillmer AM, Rouam SL, Briault S, Davila S, Stanton LW, Cacheux V. Personalized genome sequencing coupled with iPSC technology identifies GTDC1 as a gene involved in neurodevelopmental disorders. ***Hum Mol Genet***, 2017; 26(2):367-82

* equal contribution

DESCRIPTION OF CURRENT RESEARCH

Intricate embryonic patterning is achieved through highly precise regulatory mechanisms that ensure controlled gene expression in the correct time and space. Our research aims to elucidate the mechanism by which gene expression is regulated by transcription factors (TFs) and the epigenetic landscape in the development of complex embryonic patterns and structures. We also seek to understand how the disruption of this mechanism leads to human congenital malformations.

ELUCIDATING THE GENOME-WIDE REGULATORY LANDSCAPE OF HEART DEVELOPMENT

Although key genetic factors that regulate the development and function of the heart have been established, the ways in which these factors are regulated and interact with each other and with epigenetic factors to orchestrate different phases of heart development are less understood. We investigate two distinct cell types of the heart: cardiomyocytes (CMs) and cardiac pacemaker cells.

I. Discovery and functional analyses of enhancers in heart development and disease

Large-scale genomics analyses have enabled the discovery of a large number of functional non-coding DNA elements, including thousands of putative enhancers. Nonetheless, an enormous task remains to validate and determine the functions of these enhancers, specifically within the context of human health and disease. In our earlier study, we characterized the dynamics of the transcriptional regulatory landscape during heart development by combining transcriptome profiling (RNA-sequencing) and an assay for chromatin accessibility (ATAC-sequencing) at several key stages of heart development. Our analyses revealed genetic regulatory hubs that drive crucial events of heart development (Pawlak et al., *Genome Res*, 2019).

Among regions with dynamic chromatin accessibility in development were highly conserved noncoding elements that represent putative heart enhancers. We combine both experimental and computational approaches for the discovery and biological validation of these enhancers in the zebrafish model system. We apply various computational and mathematical modeling strategies, including the *in silico* TF footprinting analysis of ATAC-sequencing data and mathematical modeling of the cardiac transcriptional regulatory network based on genomic and epigenomic data. Ultimately, we seek to characterize the contribution of the dynamic transcriptional regulatory landscape to heart development and to identify novel elements (both genic and nongenic) that are associated with congenital heart disease.

II. Genomic dissection of pacemaker development

Pacemaker cells are specialized heart muscle cells that ensure rhythmic contractions of the heart. Once specified, pacemaker progenitor cells further develop low-conductance properties through the expression of gap junction proteins that are distinct from CMs. Defects of the pacemaker could lead to various forms of life-threatening cardiac arrhythmia. However, important questions remain about the molecular mechanisms that regulate their development and how this translates into proper functioning of the pacemaker. To study how the pacemaker develops and functions, we use zebrafish as a model organism because of its similarity to humans with regard to heart physiology and genetics. The bulk transcriptome profiling of isolated pacemaker cells revealed the expression of genes that define the sinoatrial and atrioventricular nodes, including *isl1* and *hcn4* (Minhas et al., *BMC Genomics*, 2021; Abu Nahia et al., *Cell Mol Life Sci*, 2021). We found that the zebrafish pacemaker regions express partially overlapping genes that encode ion channels and connexins, which likely underlie the distinct functions between the two pacemakers. Our results establish that the zebrafish pacemaker possess molecular and physiological hallmarks of mammalian pacemakers in terms of automaticity, low conductance properties, and the conserved expression of developmental genes and markers. Moreover, a number of pacemaker-overexpressed genes have human homologs that are implicated in various forms of congenital heart disease, underscoring the relevance of our transcriptomics resource to studying human heart conditions. To better characterize the heterogeneity of cell types that constitute the pacemaker region and assign molecular identities to each specific cell population, we are currently focusing our analyses at the single-cell level. Detailed knowledge of distinct cell types that constitute the pacemaker and a thorough understanding of their nature are essential for understanding their role in heart development and function. Ultimately, we aim to establish zebrafish as a model of pacemaker dysfunction, identify novel genetic elements that are involved in pacemaker-related human diseases, and generate new mutant lines for functional studies of these factors.

DEVELOPMENTAL CONTROL THROUGH THE POSTTRANSCRIPTIONAL REGULATION OF MATERNAL mRNA EXPRESSION

I. Translational control by cytoplasmic polyadenylation

Maternal mRNAs are initially deposited in the immature oocyte in a translationally dormant state, with a very short poly(A) tail. Two major waves of cytoplasmic polyadenylation occur during oocyte maturation and upon fertilization, resulting in the translational activation of distinct subpopulations of maternal mRNAs. We previously discovered that cytoplasmically polyadenylated maternal mRNAs are increasingly associated with polysomes and that the translational regulation of maternal mRNAs by cytoplasmic polyadenylation is required for the progression of embryonic development by ensuring the activation and clearance of key factors that determine zygotic genome activation (Winata et al., *Development*, 2018). Current work in our laboratory focuses on studying cytoplasmic polyadenylation element binding proteins that are known to regulate cytoplasmic polyadenylation and translation and developing methods and tools for poly(A) tail measurements by long-read RNA sequencing on the Oxford Nanopore platform.

II. RNA editing of maternal mRNAs

RNA editing refers to the posttranscriptional modification of RNA sequences, the most common form of which is A-to-I conversion that occurs through the deamination of adenosine (A) at the C6 position, converting it to an inosine (I). In humans, the misregulation of A-to-I RNA editing in somatic tissues can lead to neurological and metabolic disorders, autoimmune diseases, and cancer. In collaboration with the Bochtler laboratory (IIMCB), we established bioinformatics tools for the discovery of RNA editing events in transcriptomics data and applied it to discover A-to-I editing events during early zebrafish embryogenesis. Our study revealed pervasive editing in maternal and the earliest zygotic transcripts, the majority of which occurred in the 3'-untranslated region. Interestingly, transcripts that are implicated in gastrulation and dorso-ventral and antero-posterior patterning were found to contain multiple editing sites.

Our functional analyses of Adar, the zebrafish ortholog of mammalian ADAR1, further established its maternal role in establishing the antero-posterior and dorso-ventral axes and patterning, and its zygotic role in maintaining innate immune response regulation (Fig. 1; Niescierowicz et al., *Nat. Commun*, 2022).

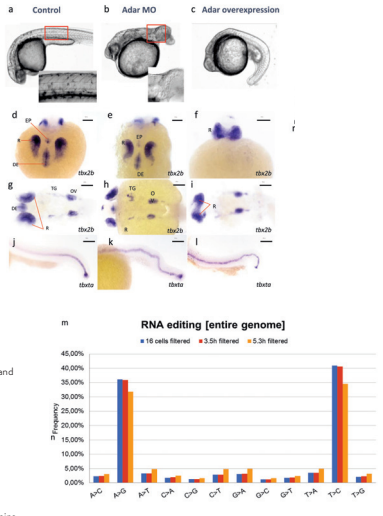
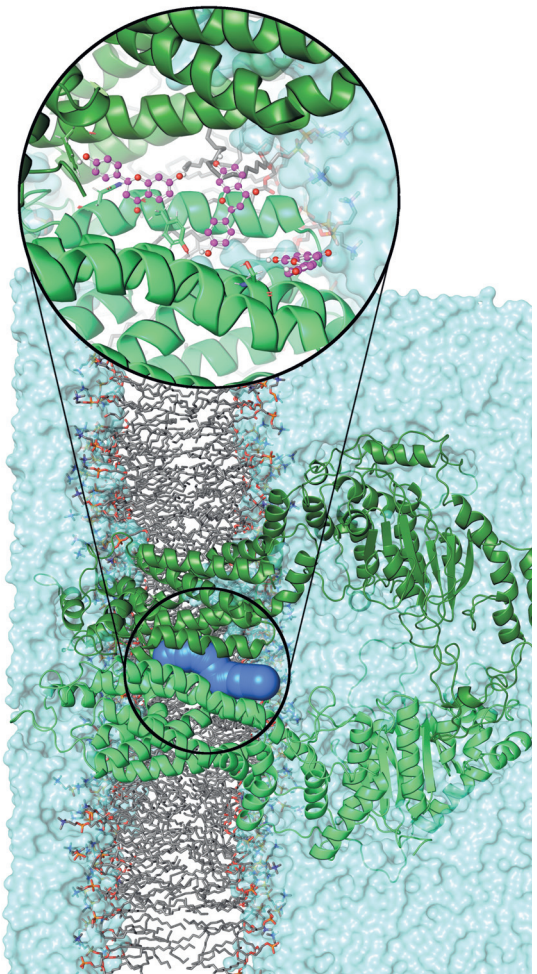
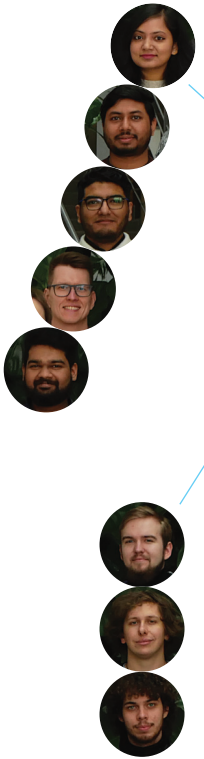


Fig. 1. Role of ADAR-mediated RNA editing in embryonic patterning. (A-C) Phenotypic defects at 24 hours postfertilization caused by adar knockdown and overexpression. Adar morpholino (MO)-injected embryos develop an abnormal phenotype in the posterior part, with a disturbed body axis, shortened tail, and crooked and disorganized notochord. The MO phenotype can be fully rescued with wildtype mRNA injection. (D-I) tbx2b expression marking diencephalon (DE), epiphysis (EP), trigeminal ganglion (TG), and otic vesicle (OV) in Adar MO knockdown and adar mRNA overexpression. (J-L) Expression of tbxta marks the notochord. (M) Mismatches between RNA and DNA sequencing data. The RNA libraries were not strand selective. Therefore, mismatches were read as their complement [i.e., T>C instead of A>G, or C>T as G>A] in roughly half of all cases.

LABORATORY OF BIOMOLECULAR INTERACTIONS AND TRANSPORT AMU/IIMCB IN POZNAŃ





LAB LEADER

Jan Brezovsky, PhD

DEGREES
2011 PhD in Environmental Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic
2007 MSc in Biophysics, Faculty of Science, Masaryk University, Brno, Czech Republic

PROFESSIONAL EXPERIENCE
2016-Present Professor, Head of the joint laboratory, International Institute of Molecular and Cell Biology in Warsaw and Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznań [AMU], Poland
2016 Assistant Professor, Department of Experimental Biology, Masaryk University, Brno, Czech Republic
2015-2016 Postdoctoral Researcher, International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic
2014 Research visit to the group of Prof. Rebecca Wade, Heidelberg Institute of Theoretical Science, Germany
2012-2016 Leader of Research Team, Loschmidt Laboratories, Faculty of Science, Masaryk University, Czech Republic
2009-2011 Research Assistant, Loschmidt Laboratories, Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
2007-2008 Research Assistant, National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic

HONORS, PRIZES AND AWARDS
2020 Member of the FWO Review College
2018 SONATA BIS, National Science Centre
2017 OPUS, National Science Centre
2016 GACR grant, Czech Science Foundation
2015-2016 Member, National Node Committee of European Life-Science Infrastructure for Biological Information, Czech Republic [ELIXIR-CZ]
2011 Dean's prize for outstanding PhD research, Masaryk University, Brno, Czech Republic
2007 Research grant from Masaryk University, Brno, Czech Republic

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Katarzyna Voelkel, MSc

SELECTED PUBLICATIONS

Brezovsky J#, **Thirunavukarasu AS**, **Surpeta B**, **Sequeiros-Borja CE**, **Mandal N**, **Kumar Sarkar D**, **Dongmo Foumthuim CJ**, **Agrawal N**. TransportTools: a library for high-throughput analyses of internal voids in biomolecules and ligand transport through them. ***Bioinformatics***, 2022; 38[6]:1752-53

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Sequeiros-Borja CE, Surpeta B, Brezovsky J#. Recent advances in user-friendly computational tools to engineer protein function. ***Brief Bioinform***, 2021; 22[3]:bbaa150

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Daniel L, Buryska T, Prokop Z, Damborsky J, ^**Brezovsky J#**. Mechanism-based discovery of novel substrates of haloalkane dehalogenases using in silico screening. ***J Chem Inf Model***, 2015; 55[1]:54-62

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Bendl J, Stourac J, Salanda O, Pavelka A, Wieben ED, Zendulka J, ^**Brezovsky J#**, Damborsky J#. PredictSNP: robust and accurate consensus classifier for prediction of diseaserelated mutations. ***PLoS Comput Biol***, 2014; 10[1]:e1003440

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DESCRIPTION OF CURRENT RESEARCH

Research in our laboratory is oriented toward answering fundamental questions about the mechanism of action of various proteins that have biomedical and biotechnological importance. We focus on mechanisms that enable the migration of ligands to and from functional sites that are deeply buried within protein structures. We also explore implications of such processes for functions of living cells. To achieve these goals, we develop new computational protocols and tools and apply them to the analysis of biomedically and biotechnologically relevant proteins.

At any moment, living systems contain thousands of small organic molecules. To exert their function, numerous molecules need to arrive at their sites of action, mostly represented by grooves and protrusions on protein surfaces or by internal cavities. The transport of these molecules around the cell and beyond is mainly governed by tunnels that form inside protein structures. They secure the transport of ions and small molecules between different regions, connecting inner protein cavities with a surface, two different cavities, or even different cellular environments in the case of transmembrane proteins. The presence of very sophisticated transport regulation markedly contributes to the coexistence of individual chemical species within a single compartment or whole cell, avoiding overly disruptive interference. Tunnels are often equipped with additional dynamic elements, called gates, that confer the time-dependent control of transport processes.

Tunnels have been described for enzymes from many catalytic and structural classes. Recent studies estimate that tunnels are present in over 50% of enzymes. The anatomy, physicochemical properties, and dynamics of tunnels determine exchange rates of substrates, products, and other ligands between active sites and the bulk solvent. In many enzymes, several tunnels are present, forming non-trivial networks where individual tunnels can be selective for particular ligands. The engineering of residues that form tunnels can produce mutant enzymes with marked alterations in catalytic properties. The biological relevance of tunnels is further highlighted by the fact that many enzymes that are known to contain tunnels were linked to the development of various diseases, and inhibitors that bind these tunnels became viable drugs. In contrast to ion channels, mechanisms that are employed to balance selectivity and efficiency are unknown for the majority of enzymes with buried active sites. One of the reasons is that various enzymes' tunnels must transport substrate, products, and also often water molecules, each having different physicochemical properties, thus implying that different mechanisms are engaged in their selectivity filters. Further challenges arise from difficulties in identifying transient tunnels, especially those that exist preferentially in closed conformations, rendering them hidden in static protein structures.

These limitations give rise to the frequent omission of transient tunnels from analyses and hence a bias toward permanent or mostly open tunnels only. Even scarcer are studies that focus on putative tunnels that are not functional in their present form but can become activated by mutations in their weak spots. The creation of such new tunnels was shown to cause unforeseen consequences, leading to notable improvements or deficiencies in catalysis. Altogether, these critical limitations result in very few types of selectivity filters that are probed by mutagenesis and hamper the discovery and thorough validation of structure-function relationships that concern enzyme tunnels. Such an understanding will lay the foundation for the construction and optimization of new enzyme tunnels in designed enzymes and the engineering of inhibitors with high residence times in targeted active sites for drug development, thereby revealing the effects of distal mutations in tunnels on the development of pathology.

TO FILL GAPS IN OUR KNOWLEDGE OF LIGAND TRANSPORT PHENOMENA, WE ARE CURRENTLY FOCUSING ON THE FOLLOWING:

Developing tools for the efficient analysis of ligand transport processes

The primary goal of this project is to enable large-scale studies of properties and dynamics of functionally relevant transport tunnels. To this end, we developed the divide-and-conquer approach for the analysis of transport tunnels in large structural ensembles. This method combines utilization of the CAVER3 tool for tunnel calculation with our in-house TransportTools library to accelerate tunnel calculation and reduce hardware resources that are required to analyze long molecular dynamics simulations in detail. By slicing a molecular dynamics trajectory into smaller pieces and performing a tunnel analysis of these pieces by CAVER3, the runtime and resources are considerably reduced. Subsequently, the TransportTools library joins individual parts, resulting in an overall view of the tunnel network for the entire trajectory without a loss of accuracy or the level of obtained molecular details while simultaneously considerably reducing the runtime and RAM required for tunnel analysis.

Understanding selectivity mechanisms in the ABCG transporter

We apply our developed methods to broaden our detailed understanding of functional aspects of various proteins. We have investigated molecular bases of the unusual selectivity of an adenosine triphosphate-binding cassette transporter from the G subfamily (ABCG46) from the plant *M. truncatula* that is able to highly selectively translocate phenylpropanoids across the cell membrane, in which we have discovered an unusually narrow transient access tunnel that leads to the central cavity of this protein and investigated its utilization for the transport of four structurally similar phenylpropanoids (Fig. 1). This path forms an initial filter that is responsible for the selective translocation of phenylpropanoids and limits interference among individual chemical compounds. We also identified the vital role of a remote residue in maintaining the stability of this tunnel by deconvoluting the experimentally observed effects of mutations. These findings exposed novel molecular mechanisms that regulate the transport of phenylpropanoids, which is critical for plant defense against various pathogens.

Uncovering minimal required tunnels for water permeability into enzyme active sites

Water molecules are the most abundant and the smallest molecules that can be transported through molecular tunnels, potentially representing the most common ligand-transport events in the biomolecular world. Water molecules are also essential co-substrates of hydrolytic enzymes and disruptive factors in many reactions that are catalyzed by enzymes in general. Analyzing massive simulations of three hydrolytic enzymes, we found that water molecules can pass through unexpectedly narrow regions of tunnels, far below their geometrical dimensions. These narrow permeable regions were mostly unique by providing multiple hydrogen bonds to the migrating water molecules, making such a surprising process feasible. Markedly, such events were responsible for transporting approximately 20% of water molecules between active sites of those enzymes and the bulk solvent.

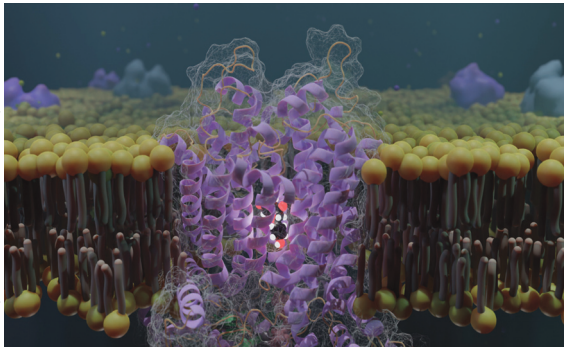

Fig. 1. ATP-binding cassette plant transporter, ABCG46, translocating phenylpropanoid liquiritigenin, across a lipidic membrane. ABCH46 structure is shown as purple cartoon, the liquiritigenin as spheres, and lipids are yellow spheres and brown tails. A number of these transporters can be found across the membrane, indicated by background colored surfaces.

www.iimcb.gov.pl/en/brezovsky-lab

STRATEGIC PROJECT

STUDY ON AGING AND LONGEVITY

Małgorzata Mossakowska, PhD, DSc Habil
Project Coordinator

Research on aging and longevity at IIMCB was launched in 1999 by a pilot study of Polish centenarians (PolStu99). Data that were generated in the PolStu99 project formed the basis for a further research project, *Genetic and Environmental Factors of Longevity of Polish Centenarians* (PolStu2001), commissioned by the Committee for Scientific Research.

The PolSenior project *Ageing of the Polish population – medical, psychological, sociological and economic aspects*, conducted in 2007-2012 and coordinated by IIMCB, was the largest gerontology research initiative in Poland and one of the largest in Europe. Within the framework of the PolSenior project, a bank of biological samples and a database that includes all information from questionnaires and biochemical and genetic analyses were created. Over 100 articles have been published from this effort. The results of the PolSenior project served as the basis for recommendations on public health and social policies for the elderly population that should be developed at both national and local levels. In 2022, several papers reported further analyses of PolSenior data, including one on polypharmacy (Nauman-Podczaska et al., *Int J Environ Res Public Health*, 2022), the prevalence of anticitrullinated protein antibodies (Chudek et al., *Int J Environ Res Public Health*, 2022), and the influence of the ankle-brachial index (Krolczyk et al., *Heart Vessels*, 2022) and abnormalities in physical examination of the arterial system (Krolczyk et al., *Aging Clin Exp Res*, 2022) on mortality.

The PolSenior2 project *Health status and its socio-economic covariates in the older population in Poland* was conducted in 2016-2020, coordinated by the Medical University of Gdańsk. Its methodology was based on the previous PolSenior study. Strategic recommendations for central and local authorities (Rekomendacje Strategiczne dla Rządu i Samorządów, GUMed, 2022) have been published, presented in Parliament and widely disseminated. Małgorzata Mossakowska was one of the co-authors of these recommendations. Papers based on PolSenior2 results that describe the prevalence and risk factors of untreated thyroid dysfunction (Kocelak et al., *PLoS One*, 2022), frailty (Piotrowicz et al., *Aging Clin Exp Res*, 2023), and the obesity paradox in very old adults (Puzianowska-Kuznicka et al., *Nutrients*, 2022) have also been published.

As a result of our cooperation with the Polish Association Supporting People with Inflammatory Bowel Disease (*J-elita*), European Federation of Crohn's and Ulcerative Colitis Associations (EFCCA), and Jagiellonian University Medical College, a new study on the costs that are associated with inflammatory bowel disease was conducted. A comparison of patient-reported indirect costs (Holko et al., *Inflamm Bowel Dis*, 2022) and out-of-pocket expenses (Holko et al., *Eur J Health Econ*, 2022) across 12 European countries revealed a high variability of costs between countries.

BIOPHYSICS AND BIOANALYTICS FACILITY

Krzysztof Skowronek, PhD, DSc Habil
Head

Roman Szczepanowski, PhD
Deputy Head

Agnieszka Ktosińska-Żołądek, MSc
(part-time)
Laboratory Support Specialist

The Biophysics and Bioanalytics Facilty (BBF) consists of four functional sections. **The Molecular Biophysics section** is equipped with analytical instruments for in-depth analyses of proteins and nucleic acids using various methods. Interactions between molecules are studied using several complementary techniques: microcalorimetry (differential scanning calorimetry and isothermal titration calorimetry), surface plasmon resonance, and analytical ultracentrifugation (Beckman Coulter ProteomeLab XL-I). The size of macromolecular complexes is measured by size exclusion chromatography with a multiangle light-scattering (SEC-MALS) detector and analytical ultracentrifugation. The Molecular Biophysics section is also equipped with a wide selection of multiwell plate readers (luminometer, spectrometer, and spectrofluorometer), a spectrofluorometer (with stop-flow), a circular dichroism spectropolarimeter, and a Fourier transform infrared spectrometer. We also offer access to an ultra-performance liquid chromatography system that is equipped with ultraviolet/visible and fluorescence detectors and an assortment of reverse-phase and size exclusion chromatography columns for precise qualitative and quantitative analyses of proteins, nucleic acids, and small molecules.

The Mass Spectrometry section has a liquid chromatography-electrospray ionization (LC-ESI)-Ion Trap mass spectrometer (amaZon speed ETD, Bruker). In addition to prompt standard proteomics analyses (i.e., protein identification and protein integrity assays) for internal users, which are vital for crystallography projects, the Mass Spectrometry section provides non-standard analyses of molecules other than proteins, particularly analyses of RNA samples and nucleosides.

The Genomics section is equipped with an Illumina NextSeq 500 Next Generation Sequencing (NGS) instrument and provides instrumentation for complete sample preparation for sequencing. This includes systems for precise DNA/RNA/chromatin shearing and size selection (Covaris M220, BioRuptor Pico, and BluePippin) and systems for nucleic acid quality and quantity measurements (TapeStation 2200, NanoDrop 3300, and Quantus). The Genomics section also offers a platform for data analysis and storage. The NGS system is used for transcriptome and genome methylation sequencing in model organisms, including zebrafish, mice, and rats.

The General Use Equipment section gathers all other equipment that is administered by the BBF and available to all IIMCB scientists. This section encompasses preparative centrifuges and ultracentrifuges, a vacuum drying system, gel documentation systems (camera-based and scanners), and a cell disruption system.

The BBF provides flexible assistance with methodological principles, experimental design, initial training, and procedures that are needed for specific experiments, data analysis, and final interpretation, serving as a link between scientists and state-of-the-art technology. IIMCB cooperates with rapidly developing biotech companies in Poland. Within the framework of bilateral agreements, the BBF collaborated with biotech companies, including Adamed, Captor Therapeutics, Celon Pharma, Molecure (formerly OncoArendi Therapeutics), Selvita, and Polfa.

The BBF is also available to scientists from other institutions. We have conducted research in collaboration with scientists from the University of Gdańsk, University of Warsaw, Medical University of Lublin, Nencki Institute of Experimental Biology (Polish Academy of Sciences), Institute of Biochemistry and Biophysics (Polish Academy of Sciences), Mossakowski Medical Research Institute (Polish Academy of Sciences), University of Wrocław, Adam Mickiewicz University, Małopolska Centre of Biotechnology of Jagiellonian University, and University of Veterinary Medicine (Vienna, Austria).

BBF staff are also responsible for conducting courses for new employees of IIMCB in the field of laboratory work safety, including work with Genetically Modified Organisms and Microorganisms, and Good Laboratory Practice.

Members of the BBF are among the founding members of the Association of Resources for Biophysical Research in Europe (ARBRE) and Core Technologies for Life Sciences (CTLS; https://ctls-org.eu/) networks. Roman Szczepanowski serves as administrator of the ARBRE webpage, and Krzysztof Skowronek is a co-leader of the Community Engagement Working Group of CTLS.

PUBLICATIONS in 2022

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MICROSCOPY AND CYTOMETRY FACILITY

Tomasz Węgierski, PhD
Head

Matylda Macias, PhD
Senior Specialist

Katarzyna Misztal, PhD
Senior Specialist

Aleksandra Szybińska, MSc
Specialist

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(part-time)
Laboratory Support Specialist

The IIMCB Microscopy and Cytometry Facility (MCF) was created in 2021 to provide specialized services for researchers who work in the field of cell biology. The main focus of the MCF is cell sorting by flow cytometry and the imaging of biological samples using advanced light and electron microscopes. The MCF staff consists of experienced scientists who have broad knowledge of cell biology applications and their equipment. The current services that are provided by the facility can be divided into three groups.

The **cell sorting service** is provided using two high-end cell sorters (Becton Dickinson FACSAria II and Beckman Coulter CytoFLEX SRT [purchased in 2022]). The FACSAria II is equipped with three lasers (violet, blue, and red) and nine fluorescence detectors. With four nozzle sizes, a broad range of samples can be processed with high throughput. Up to four populations can be sorted simultaneously. The CytoFLEX SRT has four lasers (violet, blue, yellow, and red) and detects up to 15 different fluorescence signals. It offers the possibility of sorting into tubes (four distinct populations simultaneously), into plates (including 384 format), and onto microscope slides. With one nozzle size and an automated setup procedure, the sorting experiment is quick and easy to run. Cell sorting on both machines is offered as a full service for occasional customers and users and as equipment access for researchers who are experienced in flow cytometry.

The facility provides **access to a broad range of fluorescence light microscopes**. Most of our microscopes allow optical sectioning, such as confocal (point-scanning or spinning-disk), two-photon, lightsheet, and total internal reflection fluorescence (TIRF), to facilitate high-contrast fluorescence imaging. The facility's newest acquisition is Opera Phenix, a high-content screening system from PerkinElmer for the large-scale imaging of cells (e.g., in RNAi-based microscopy screens) in widefield or confocal mode. Our equipment also includes a Zeiss LSM800 confocal microscope with a high-resolution Airyscan detector, a Zeiss LSM710 NLO dual confocal/multiphoton microscope for the live imaging of cells and tissues, an Andor Revolutions XD system for real-time spinning-disk confocal microscopy and TIRF imaging, a Zeiss Lightsheet Z.1 single-plane illumination microscope for the imaging of fluorescently labeled zebrafish larvae, an Olympus CellR/ScanR imaging station for intracellular calcium measurements, and a Nikon 80i Eclipse microscope with a scanning stage for the mosaic imaging of histochemically or fluorescently stained tissue sections. Two- and three-dimensional image analysis is possible using dedicated software, such as Imaris (Bitplane) and Harmony (PerkinElmer). Full imaging service is possible on Opera Phenix.

The **electron microscopy service** offers analyses of cells, tissues, and virus particles with a FEI Tecnai T12 transmission electron microscope. For the conventional transmission electron microscopy of cells and tissue samples, we use a Leica EM tissue processor. This enables resin processing under constant temperature while avoiding exposure to toxic substances. After saturation with resin, tissue and cell specimens are pretrimmed with a Leica EM TRIM2, which prepares for the next step of processing. Samples are then cut for semi- and ultra-thin sections using a Leica EM UC7 ultramicrotome, and then sections are placed on electron microscopy grids. Material that is prepared this way can be imaged with our electron microscope.

The MCF operates in either full-service mode or access mode, depending on equipment, application, and the customer. In access mode, our staff offers initial training for users and assistance with experimental design, data analysis, and final data interpretation. The MCF is open to IIMCB researchers and external customers from academia and industry. The MCF staff has cooperated with researchers from the University of Warsaw, University of Wrocław, Mossakowski Medical Research Institute (Polish Academy of Sciences), Institute of Biochemistry and Biophysics (Polish Academy of Sciences), and Medical University of Warsaw. The MCF staff has joined various European organizations and initiatives, such as Core Technologies for Life Science (ctls-org.eu), European Light Microscopy Initiative (elmi. embl.org), and Quarep-LiMi (quarep.org).

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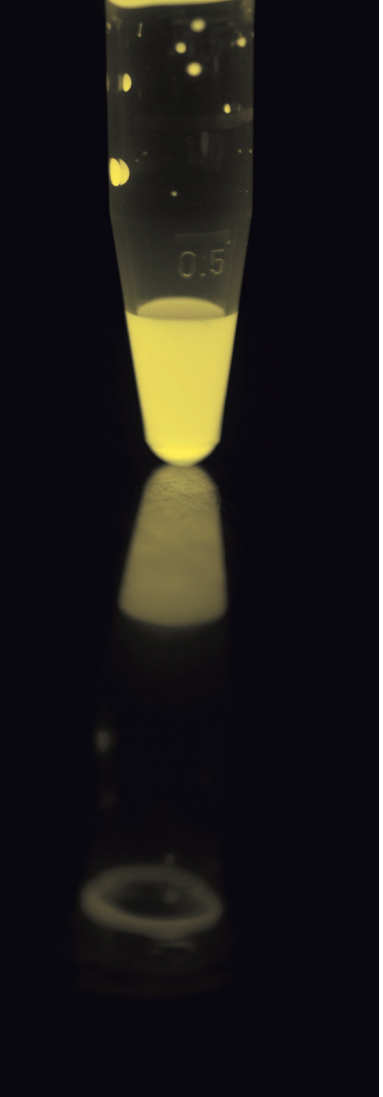

GENOME ENGINEERING UNIT

Olga Gewartowska, PhD
Head

Katarzyna Prokop, MSc
Senior Specialist

Marcin Szpila, Msc (UW)
Senior Specialist

Katarzyna Sałas, MSc
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Weronika Dudzińska, MSc
Junior Specialist

Patrycja Kędzierska, MSc
Junior Specialist

Kacper Sienkan
Junior Specialist

Agnieszka Ktosińska-Żołądek, MSc
(part-time)
Laboratory Support Specialist

The Genome Engineering Unit (GEU) has two main branches of activities: embryology, including the generation of mouse models using CRISPR/Cas9 methodology, and molecular biology, particularly plasmid cloning and genotyping. All services are available to both IIMCB internal users and external clients.

Transgenic mouse generation and assisted reproduction technologies

The GEU seeks to provide high-quality, cutting-edge services for new mouse line generation and other embryological services. The CRISPR/Cas method allows the efficient and targeted mutagenesis of genes. We have generated various types of mutant mice, including indel knockouts, knockouts by the integration of cassettes that contain stop codons, models that harbor the N- and C-terminal insertion of tags (fluorescent proteins, 3xHA, FLAG, CRE-expressing mice), conditional (LoxP) knockouts, and mice that express large (up to 11 kb) transgenes from the ROSA26 locus. We recently implemented FKBP-dTag tagging, which allows timed-controlled protein depletion. This approach allows for studies of essential genes and improves animal welfare by reducing the risk of developing harmful phenotypes.

In contrast to many transgenic facilities, the GEU provides "all-in-one" packages for new mouse line generation, from mutagenesis strategy design to F1 pups, and charges only when the model is successfully generated. To date, we have generated several dozen different mouse lines with a ~95% success rate. The typical project duration is 6-8 months. Around 20 novel mouse lines are generated annually using CRISPR/Cas9 technology under licenses to patents from ERS Genomics.

The GEU can also provide various embryological services, such as *in vitro* fertilization, embryo transfer, rederivation, vasectomy, and embryo or sperm cryopreservation.

All mice maintain specific-pathogen-free (SPF) health status. Embryos are transferred to surrogate mothers under sterile conditions. The animals' health status is verified quarterly by an external provider. All experiments are approved by the Ethics Committee for Animal Testing. We strictly follow the 3R (Reduce, Refine, Replace) principles of animal welfare and always seek to improve the efficiency of our methods to reduce the number of animals that are needed to establish new mouse lines. We comply with advanced pain management protocols to minimize pain during the procedures.

Genotyping

The GEU routinely provides transgenic mouse genotyping services using standard polymerase chain reaction, restriction fragment length analysis, sequencing, and high-resolution melting (HRM) analysis. The current capacity of our genotyping service is 500 samples per week.

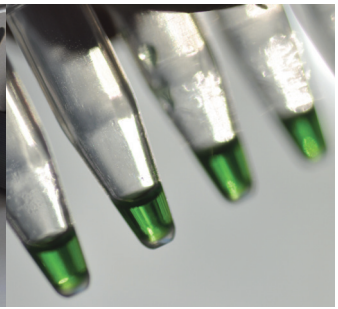
Cloning service

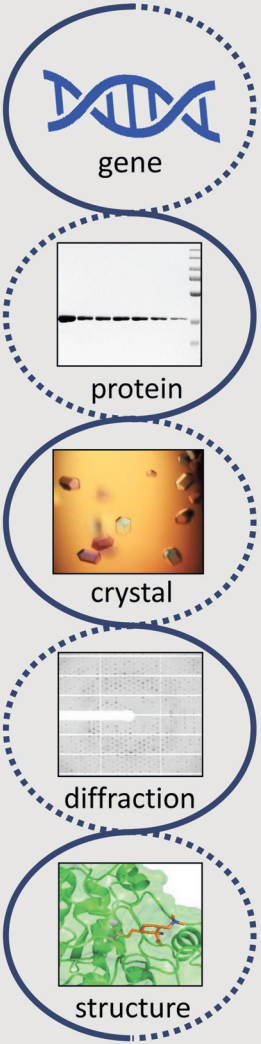
The expertise of our team provides scientific and technical assistance for researchers to choose the most suitable cloning strategy and prepare ready-to-use vectors. Most plasmids are cloned using sequence and ligation-independent cloning (SLIC) methodology, which allows quick and efficient multi-fragment DNA assembly. The cloning service currently runs at a capacity of 450 plasmids per year. Custom constructs are prepared based on a broad range of backbone vectors that are designed for protein purification in bacterial or insect cells, lentiviral and retroviral production, cell transfection, including primary cell lines, etc. A maximum of five inserts can currently be integrated simultaneously, with a total upper size limit of 20 kb. In addition to standard plasmids, we can also prepare constructs based on a linear cloning system (pJazz vectors), which may be useful for cloning potentially toxic and AT- or GC-rich inserts. Recently, Illumina-based plasmid sequencing has been incorporated to partially replace standard Sanger sequencing. The typical project timeline is 3-4 weeks.

Funding

Development of the transgenic mouse service was possible thanks to support from the Foundation for Polish Science through the TEAM-TECH Core Facility grant (2018-2021).

Currently, GEU personnel are supported by the MOSaIC project, which received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 810425.





PRECLINICAL DRUG DEVELOPMENT UNIT

Elżbieta Nowak, PhD, DSc Habil
Head

Marcin Nowotny, PhD, Professor
Scientific Advisor

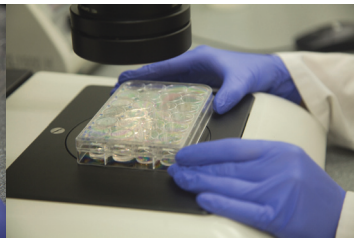
Agnieszka Ktosińska-Żotądek, MSc
(part-time)
Laboratory Support Specialist

The Preclinical Drug Development Unit (PDU) was established in August 2022. The goal of the PDU is to provide services and consultancy in the field of structural biology, with an emphasis on supporting drug discovery projects. This venture comprises a complete range of X-ray crystallography research, from gene to structure, enriched by biophysical and biochemical characterizations of target-ligand interactions.

X-ray crystallography is a well- established and routine method that the team has applied to numerous and R&D and scientific projects. A remarkable advantage is unique expertise in the crystallization of protein-nucleic acid complexes. The team applies state-of-the-art approaches in structural biology that need to be used in the development of nucleic acid-based drug discovery projects.
The PDU continues IIMCB's long-standing cooperation with leading pharmaceutical companies in Poland (e.g., Celon Pharma, Molecure [formerly OncoArendi Therapeutics], and Selvita). Structural biology, biophysics, and biochemistry have supported drug discovery efforts for such diseases as cancer, asthma, and depression.
We are open to cooperating in drug discovery projects and other scientific endeavors in the biotech and pharmaceutical industries and academia.

SERVICES OF THE PRECLINICAL DRUG DEVELOPMENT UNIT

- **Protein production in different expression systems**, including bacterial, mammalian, and insect cells. Based on our experience, we can produce proteins for general use, such as proteases and specific proteins that are used for structural and functional studies.
- **Protein purification** using chromatographic methods.
- **Macromolecular X-ray crystallography of proteins and protein-ligand complexes** (structure-based drug design, hit-to-lead, and structure-based lead optimization).
- **Biophysical and biochemical characterization of target-ligand interactions, enzymatic assay design and optimization**.



https://pdu.iimcb.gov.pl

ANIMAL HOUSING FACILITY

Łukasz Majewski, PhD
Head

Damian Komorowski
Specialist

Monika Matuszczyk (part-time)
Technician

The Animal Housing Facility (AHF) at IIMCB is committed to ensuring the highest standards of humane care for the welfare of animals that are used in research, with the understanding that this commitment is critical to the success of our scientific projects.

In the Animal Housing Facility mice are bred under specific-pathogen-free conditions. Our animal rooms have restricted access and are equipped with individually ventilated cage systems and mobile biosafety changing stations with a superior ventilation system. Our experienced staff perform all aspects of animal husbandry and assist researchers with specialized procedures and protocol development.
IIMCB is registered with the Ministry of Education and Science as an experimental unit that is authorized to conduct animal experiments (registry no. 0051) and a breeding unit that is authorized to breed rodents (registry no. 052). IIMCB is under supervision of the District Veterinary Inspectorate in Warsaw and under authority of the 2nd Local Ethics Committee for Animal Testing in Warsaw.

IIMCB is authorized to operate a Genetic Engineering Facility where the closed use of genetically modified organisms that belong to Risk Category I can be conducted (Decision of the Minister of Environment no. 103/2020). IIMCB is also registered with the Register of Genetic Engineering Facilities (no. 04-27/2020).
All research and breeding activities at the Animal Housing Facility are performed in compliance with Polish and European legislation on the protection of animals that are used for scientific and educational purposes, including the Polish Act of 15 January 2015, European/International guidelines on animal welfare, Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes, and guidelines of the Federation of European Laboratory Animal Science Associations.
All personnel who are involved in animal research at IIMCB are committed to the highest standards of humane animal care, thereby maximizing the reliability of animal research to contribute to health solutions.

https://ahf.iimcb.gov.pl ahf-team@iimcb.gov.pl

ZEBRAFISH CORE FACILITY

The Zebrafish Core Facility (ZCF) has been operational since 2012. It is a breeding and research facility that holds permission granted by the District Veterinary Inspectorate in Warsaw (registry no. 14656251). This number also appears in the UE register of the General Veterinary Inspectorate. Additionally, as a unit of IIMCB, the ZCF is listed on two Ministry of Education and Science registers, the list of experimental units authorized to conduct animal research (registry no. 0051) and the list of breeding units approved to breed zebrafish (registry no. 064). The ZCF was established to introduce a novel vertebrate model, Danio rerio, into IIMCB research. As the first such organization in Poland, the ZCF joined the prestigious European Zebrafish Society (EZS), and it is listed in the database of the Zebrafish Information Network (ZFIN). Since 2019, the ZCF has been included in IIMCB's Research Infrastructure of Molecules and Cells (IN-MOLCELL) within the Polish Roadmap for Research Infrastructures. Moreover, in 2020, the ZCF joined the EU-LIFE alliance's Core Facility Working Group, actively participating in several initiatives and discussions of specific core facility challenges and sharing best practices and expertise in core facility management.

Zebrafish (Danio rerio) is a small-bodied tropical, freshwater fish that was first identified in South Asia. Zebrafish have quickly become a popular candidate as a model for biomedical research due to several key features, including their high genetic similarity to humans, short generation time, and transparent embryos that are easily accessible for genetic manipulations. Moreover, the wide sharing of techniques and collections of mutant/transgenic animals, together with low maintenance costs, make zebrafish an attractive worldwide alternative to mammalian *in vivo* models that can be used to follow the Reduce, Refine, and Replace (3Rs) principles of animal research. **We are proud to have the largest collection of zebrafish in Poland, consisting of both wildtype and genetically modified lines**, including mutants and transgenics.

Zebrafish lines expressing modified genes that are involved in transcription regulation, heart development, neuropathology, and neurodegenerative diseases are part of the ZCF collection. Thanks to our continuous initiatives, IIMCB researchers are able to utilize zebrafish in cutting-edge studies of genetics, developmental biology, and molecular mechanisms of human diseases. The ZCF provides services to IIMCB researchers and external users, including research groups from the University of Warsaw, Medical University of Warsaw, Mossakowski Medical Research Centre (Polish Academy of Sciences), Warsaw University of Life Sciences, Institute of Bioorganic Chemistry (Polish Academy of Sciences) in Poznań, and Adam Mickiewicz University in Poznań. In addition, due to our international reputation and scientific partnerships, we export zebrafish lines to scientific institutes in Europe and beyond.

Maintaining such a substantial number of zebrafish would not be possible without a suitable infrastructure. Our fish are currently housed in 1,210 tanks (eight independent, fully automated aquatic systems). To facilitate the daily work of researchers, the ZCF is also equipped with incubators, microscopes, and microinjection devices and offers a section dedicated to behavioral testing. The space is equipped with automated systems for observing and tracking zebrafish larvae and adults. Additionally, the ZCF performs sperm freezing and in vitro fertilization to preserve genetic lines. Diagnostic and health services for zebrafish are provided by an in-house veterinarian (an expert in the aquatic field and tropical fish diseases) in cooperation with an external zebrafish diagnostic laboratory, allowing us to continuously monitor the health status of the fish colony and uphold the highest standards of animal welfare.

All research and breeding activities at the ZCF adhere to fundamental ethical principles: the Directive/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, and the Polish Act of 15 January 2015 on the protection of animals used for scientific or educational purposes (Journal of Laws of 2015, item 266, as amended). In addition, we comply with the European and Polish guidelines established by the Federation of European Laboratory Animal Science Associations (FELASA) and the Polish Laboratory Animal Science Association (PolLASA). Scientists who use zebrafish for research purposes are required by legislation to obtain certification to work with this animal model. IIMCB is under the authority of the 2nd Local Ethics Committee for Animal Experiments in Warsaw and has an Animal Welfare Committee that oversees animal welfare compliance. The establishment of such a commission and its responsibilities are defined by the Polish Act of 15 January 2015 on the protection of animals used for scientific or educational purposes (Journal of Laws of 2015, item 266, as amended).

The ZCF team comprises seven members, including the head of the facility, five animal caretakers, and a technician. Zebrafish Core Facility personnel provide training courses to new facility users, including practical elements of handling, husbandry, breeding, fin clipping, microinjections, and behavioral testing.

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Animal welfare matters:
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SCIENTIFIC REPRESENTATION

SENIOR RESEARCHERS COUNCIL

The Senior Researchers Council comprises a group of Researchers and Senior Researchers that was established in 2019 and currently consists of 16 members. In 2022, the group was represented by Jarosław Cendrowski and Daria Zdżalik-Bielecka. In December, the new representatives, Filip Stefaniak and Seweryn Mroczek, were elected in a voting procedure.

The key responsibilities of Senior Researchers at IIMCB, in addition to designing and performing scientific projects, are to preserve the expertise of IIMCB, educate younger members of laboratories, and assist Lab Leaders in organizational matters. Our goal is to obtain scientific results and gain experience in small-scale laboratory management to be better prepared to start our own research groups or become professional Core Facility staff.

Researchers and Senior Researchers are actively engaged in educating PhD students. They also contribute to teaching master's students and are involved in academic teaching at the University of Warsaw. Enhancing cooperation between IIMCB and other academic institutions is one of the most important goals of the Senior Researchers Council. Their scientific expertise is attractive to those who plan to attend the Warsaw PhD School in Natural and BioMedical Sciences at IIMCB.

PUBLICATIONS

- Senior Researchers Council members co-authored 25 publications and 5 preprints in high-quality journals, including *Molecular Cell*, *Nucleic Acids Research*, *Science Advances*, *Cell & Bioscience*, and *Wiley Interdisciplinary Reviews: RNA*. Many of them contributed to these publications as first or corresponding authors. The review by Brouze A, Krawczyk PS, Dziembowski A, Mroczek S. Measuring the tail: Methods for poly(A) tail profiling. Wiley Interdiscip Rev RNA. 2023; 14(1):e1737. doi: 10.1002/wrna.1737 (Epub 2022 May 26) was among the top downloaded papers in 2022.

GRANTS

- Many Researchers and Senior Researchers are engaged in their own research projects. In 2022, two new grants were initiated: *Deciphering the role of Nrf2 in the pathophysiology of phosphomannomutase 2 deficiency* (funded by Czech Science Foundation) and *Molecular genetic causes and biochemical consequences of congenital disorders of glycosylation* (funded by Czech Health Research Council), both with Magdalena Czeredys as a collaborator.

AWARDS

- Seweryn Mroczek received distinction from the Committee of Molecular Cell Biology of the Polish Academy of Sciences in the Prof. Krzyżosiak and Prof. Bassalik competitions for the publication *Global view on the metabolism of RNA poly(A) tails in yeast Saccharomyces cerevisiae* (Tudek A, Krawczyk PS, Mroczek S, Tomecki R, Turtola M, Matylla-Kulińska K, Jensen TH, Dziembowski A. *Nat Commun* 2021; 12[1]:4951). doi: 10.1038/s41467-021-25251-w)

HABILITATIONS

- Vladimir Korzh, Doctor of Science (habilitation), *Molecular and genetic analyses of brain ventricular system in zebrafish*, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland, April 2022

LECTURES

- Honorata Czapińska, *Sequencing methods: data analysis*, lecture in the course *Methodological Advances in Molecular and Structural Biology* for PhD students of the Warsaw-4-PhD doctoral school
- Ewelina Szymańska, workshop *In a search of the Achilles heel of cancer cells* for secondary school students as a popularization of IIMCB scientific activity, 26th Festival of Science in Warsaw

IIMCB INTERNAL LECTURES

- Jarosław Cendrowski, *The role of ESCRT-I in crosstalk between endolysosomal trafficking and cell metabolism*
- Honorata Czapińska, *Nt-rule for serine catalytic triads*
- Mariusz Czarnocki-Cieciura, *Structural characterisation of bacterial Tn7 transposase*
- Magdalena Czeredys, *Role of CacyBP/SIP in the regulation of mutant huntingtin aggregation in Huntington's disease*
- Vladimir Korzh, *SCO and Reissner's fiber*
- Ewa Liszewska, *Modeling Tuberous Sclerosis Complex with human induced pluripotent stem cells*

CONFERENCES

- Jarosław Cendrowski presented a poster at the EMBO Workshop *The endoplasmic reticulum: The master regulator of membrane trafficking*, October 2022, Lucca, Italy
- Mariusz Czarnocki-Cieciura gave an oral presentation at the *iNEXT-Discovery 2nd Annual Scientific Meeting*, August 2022, Warsaw, Poland, and the *Joint Meeting of PSRS Members and SOLARIS Centre Users*, September 2022, Kraków, Poland
- Magdalena Czeredys presented a poster at the *Tissue Engineering and Regenerative Medicine International Society Conference*, June-July 2022, Kraków, Poland
- Vladimir Korzh gave a lecture at the 5th *Polish Zebrafish Society Workshop*, June-July 2022, Lublin, Poland
- Ewelina Szymańska presented a poster at the *ESCRT Biology Meeting*, May 2022, Madison, USA, and the EMBO Workshop *Cancer cell signaling: Linking molecular knowledge to cancer therapy*, September 2022, Cavtat, Croatia
- Justyna Zmorzyńska presented a poster at the 6th *Zebrafish PI Meeting*, March-April 2022, Dresden, Germany

PEER-REVIEW

- Vladimir Korzh: *Neuroscience Letters*, *Cell Death and Differentiation*, *Comparative and Structural Biotechnology*
- Filip Stefaniak: *Computational and Structural Biotechnology Journal*, *Briefings in Bioinformatics*, *Nucleic Acids Research*
- Ewelina Szymańska: *BMC Biology*, *BMC Genomics*

EDITOR AND EDITORIAL BOARD MEMBER

- Vladimir Korzh, Editorial Board: *Scientific Reports*, *Zebrafish*
- Seweryn Mroczek, Review Editor: *Frontiers in Genetics* (Transcription and Post-Transcriptional Events)

SUPERVISOR OR AUXILIARY SUPERVISOR OF PHD STUDENTS

- Jarosław Cendrowski for Marta Wróbel
- Honorata Czapińska for Anna Fedenko
- Magdalena Czeredys for Ewelina Latoszek
- Vladimir Korzh for Ruchi Jain and Razieh Amini
- Ewa Liszewska for Lena Majchrowicz
- Seweryn Mroczek for Aleksandra Bilska and Wiktoria Orzeł
- Elżbieta Nowak for Marzena Nowacka (defended in December 2022)
- Filip Stefaniak for Pietro Boccaletto
- Ewelina Szymańska for Malwina Grębowicz-Maciukiewicz
- Justyna Zmorzyńska for Olga Doszyń
- Daria Zdżalik–Bielecka for Agata Poświata (defended in October 2022) and Marta Chwałek

SUPERVISOR OF MSC STUDENTS

- Jarosław Cendrowski for Bartosz Jary
- Magda Czeredys for Samuel Oluwafemi Egbuwalo and Marta Piechota
- Seweryn Mroczek for Julia Cieślicka, Dawid Dadz, Julia Gilewska, Maria Niżik, Barbara Popławska, and Monika Powójska
- Seweryn Mroczek, tutoring Wiktoria Szymanek at Inter-Faculty Individual Studies in Mathematics and Natural Sciences

REVIEWER OF GRANT APPLICATIONS

- Seweryn Mroczek: National Science Centre

OTHER ACHIEVEMENTS

- Magdalena Czeredys, scholarship from Polish Academy of Sciences for a short-term study visit at Yale Stem Cell Center, November 2022, New Haven, USA
- Magdalena Czeredys, scholarship to attend the course *Genetic Engineering of Mammalian Stem Cells*, organized by Wellcome Trust, November 2022, Cambridge, UK

POSTDOCTORAL COUNCIL

The Postdoctoral Council represents all postdoctoral researchers at IIMCB. In 2022, the Council representatives were Natalia Gumińska and Angana Ray. The main focus of the Council is to provide support and resources for the career and personal development of postdoctoral researchers at IIMCB. The Council plans and organizes workshops and courses, shares information about career opportunities, and encourages networking activities. The Council also aims to facilitate communication among postdocs in different groups at IIMCB and strengthen scientific interactions and collaborations with researchers across Poland and abroad.

PUBLICATIONS

In 2022, postdoctoral researchers at IIMCB co-authored 18 publications and 5 preprints, including first authorship, in renowned journals, including *Nature Communications*, *Nucleic Acids Research*, and *Science Advances*, among others. Several preprints were also deposited in *bioRxiv*. Especially active members of our community were Evgenii Baulin, Natalia Gumińska, Paweł Krawczyk, Ivan Trus, Roberto Pagano, and Magdalena Wołczyk, who published at least two scientific papers.

CONFERENCE TALKS

Members of our postdoctoral researcher community delivered more than 10 conference presentations in 2022. They represented IIMCB in scientific events internationally, including *Nanopore Community Meeting*, *27th Annual Meeting of the RNA Society*, *10th International mRNA Health Conference*, and *18th Annual Meeting of Oligonucleotide Therapeutics Society*, and nationally, including *Symposium of Polish Bioinformatics Society*. Evgenii Baulin and Paweł Krawczyk were particularly active, delivering at least four talks.

INVITED TALKS

In 2022, many postdoctoral researchers were invited to be speakers at various conferences. Paweł Krawczyk was an invited speaker at the *NGSymposium in Computational Biology*, Warsaw, Poland, delivering the talk, *Direct RNA nanopore sequencing for transcriptome-wide polyadenylation analysis*. Natalia Gumińska was an invited speaker at the *2nd Annual International Congress on Euglenoids*, delivering the talk, *Investigating the repertoire of nuclear-derived circular extrachromosomal DNAs in Euglena gracilis*.

POSTERS

Postdoctoral researchers at IIMCB also presented several posters at many national and international conferences. Aneta Jończy presented a poster, *The role of glucocorticosteroids in the regulation of Zip14 expression in the liver*, at the *European Iron Club Meeting 2022*, Oxford, United Kingdom. Malwina Hyjek-Składanowska presented a poster, *Origins of the increased affinity of phosphorothioate modified therapeutic nucleic acids for proteins*, at the *XXIV International Round Table on Nucleosides, Nucleotides, and Nucleic Acids*, Stockholm, Sweden. Paweł Krawczyk presented a poster, *Direct RNA sequencing with dedicated computational algorithms: a method of choice for quality control and analysis of the metabolism of mRNA therapeutics*, at the *10th International mRNA Health Conference*, Boston, USA, and a poster, *Complex life of mRNA-1273 vaccine poly[A] tail in immune cells*, at *EMBL*

Symposium: The Complex Life of RNA, Heidelberg, Germany. Our Institute was strongly represented at the 27th *Annual Meeting of the RNA Society* in Boulder, USA, where Natalia Gumińska, Magdalena Wołczyk, and Ivan Trus presented posters.

GRANTS

In 2022, two postdocs received their own research grants. Evgenii Baulin was awarded an EMBO postdoctoral fellowship. Małgorzata Figiel was awarded an OPUS grant (*Structural studies of herpesvirus proteins involved in DNA replication*) from the Polish National Science Centre.

AWARDS

As a co-author of the published article *Global view on the metabolism of RNA poly(A) tails in yeast Saccharomyces cerevisiae* (Tudek et al., *Nat Commun*, 2021), Paweł Krawczyk was recognized with distinctions by the Molecular Cell Biology Committee of the Polish Academy of Sciences in the Prof. Kazimierz Bassalik Award for the best work in microbiology performed in Polish laboratories and the Prof. Włodzimierz Krzyżosiak Award for the best experimental work in the field of nucleic acid research carried out in a Polish laboratory in 2021. Natalia Gumińska received an award from the RNA Society for the poster, *Direct detection of non-adenosine nucleotides within poly(A) tails: a new tool for the analysis of post-transcriptional mRNA tailing*, at the 27th *Annual Meeting of the RNA Society*, Boulder, USA.

SPOTLIGHT TALKS

The idea of Spotlight Talks is to present scientific projects in an understandable way for people from outside the field. The speakers are mostly postdocs and senior researchers who are involved in life science research and have experience in science communication and industry. The talks are usually 15 minutes long, followed by discussions. This initiative is open to the public and regularly advertised via Facebook/LinkedIn/Twitter. In 2022, 14 meetings were organized. The speakers are of different nationalities and come from scientific centers in Poland and abroad. Following the recommendation of the International Advisory Board, we are expanding Spotlight Talks to include career counseling and grantsmanship. We were able to attract recruiters and private sector employees who are ready to bring this form of development closer to fruition.

INPUT ON MENTORSHIP PROGRAM

The Directors and Lab Leaders introduced us to the idea of a mentorship program. We have been in communication with Lab Leaders about establishing such a program, involving initial brainstorming among postdocs and communicating our concerns and expectations to Lab Leaders.

OTHER ACTIVITIES

We strive to improve communication among postdocs. From our official email account for the Postdoctoral Council, we send periodic e-mails to welcome new postdocs, make everyone aware of current developments, and share the latest information. We also encourage postdocs to contact us about their grievances, concerns, and suggestions so that postdoc representatives can raise these issues with relevant authorities.

PHD STUDENTS COUNCIL

The IIMCB PhD Students Council serves as the voice for the entire PhD student body and actively works with IIMCB authorities to promote a better learning environment. The Council aims to stay informed about and play an active role in important developments that may impact PhD students. Its members play an advisory role in PhD-related issues at the institute level. The Council also has a social function and offers all PhD students the opportunity to get to know each other by organizing regular lunches, workshops, and other informal meetings.

In 2022, the Council representatives were Olga Doszyń and Jacek Szymański, replaced in October 2022 by Shiwani Kumari and Marta Chwałek.

PhD students at IIMCB attend four different doctoral schools:

- Warsaw PhD School in Natural and BioMedical Sciences (Warsaw-4-PhD)
- School of Molecular Biology (Institute of Biochemistry and Biophysics, PAS)
- PhD studies at the Nencki Institute of Experimental Biology, PAS
- Postgraduate School of Molecular Medicine (Medical University of Warsaw)

IIMCB representatives in these schools are Zuzanna Mackiewicz (Warsaw-4-PhD), Magdalena Klimczak (Institute of Biochemistry and Biophysics), Jan Węstawski (Nencki Institute of Experimental Biology), and Maciej Migdał (Medical University of Warsaw).

In 2022, 33 PhD students co-authored 19 publications and 9 preprints.

REPORTING SESSION 2022

The yearly reporting session of PhD students took place on June 2022 in the form of an away trip. The event was attended by 45 students, including 41 on-site and 4 remotely via the Zoom platform and had the opportunity to present their work and report their scientific progress. Three students received best presentation awards (by popular vote): Anwesha Sarkar (Laboratory of Protein Metabolism), Zuzanna Mackiewicz (Laboratory of RNA Biology – ERA Chairs Group), and Masoud Amiri Farsani (Laboratory of Bioinformatics and Protein Engineering).

AWARDS AND SCHOLARSHIPS

- Agnieszka Bolembach and Jacek Szymański received the RNA Society Poster Award at the 27th *Annual Meeting of the RNA Society*, Boulder, USA
- Marta Gapińska received the Minister of Education and Science Award (a team award) for significant achievements in scientific activities
- Katarzyna Krakowska was awarded third place for her flash talk at the 24th *Heart of Europe Bio-Crystallography Meeting*, Lipno – Dolní Vltavice, Czech Republic
- Ewelina Latoszek was awarded a travel grant from Boehringer Ingelheim Fonds for participating in the *Brain Organoids course* [*CAJAL Advanced Neuroscience Training Programme*], Bordeaux, France
- Ewelina Latoszek was awarded a 1-month research visit grant within the iWarsaw4PhD project, funded by the NAWA STER program, to visit the In-Hyun Park Laboratory, Department of Genetics, Yale School of Medicine, USA
- Natalia Szulc was awarded 1st place in the contest for the best MSc thesis in bioinformatics (defended in 2021), organized by the Polish Bioinformatics Society
- Natalia Szulc was awarded a 1-month research visit grant within the iWarsaw4PhD project, funded by the NAWA STER program, to visit the Valenzano Laboratory, Leibniz Institute on Aging, Fritz Lipmann Institute, Jena, Germany
- Natalia Szulc received the Fulbright Junior Research Award for a research visit at the Dana-Farber Cancer Institute, Boston, USA
- Natalia Szulc was awarded a Social Responsibility of Science (Społeczna Odpowiedzialność Nauki) grant from the Ministry of Education and Science for developing an educational computer game about protein degradation processes in the cell

PARTICIPATION IN CONFERENCES

- Karim Abu Nahia presented a poster and gave a flash talk at the 17th *International Zebrafish Conference*, June 2022, Montreal, Canada
- Karolina Bogusz presented a poster at the *EMBO Meeting Nuclear Structure and Dynamics*, October 2022, Montpellier, France
- Agnieszka Bolembach, Karolina Kasztelan, and Jacek Szymański presented a poster at the 27th *Annual Meeting of the RNA Society*, May-June 2022, Boulder, USA
- Olga Doszyń presented a poster at the 3rd *Italian Zebrafish Meeting*, February 2022, Naples, Italy
- Malwina Grębowicz-Maciukiewicz presented a poster at the *EMBO|FEBS Lecture Course on Molecular mechanisms in signal transduction and cancer*, August 2022, Spetses, Greece
- Farhang Jaryani gave an oral presentation and presented a poster at the *Symposium of Polish Bioinformatics Society*, September 2022, Warsaw, Poland
- Katarzyna Krakowska and Marta Gapińska gave an oral presentation at the 24th *Heart of Europe Bio-Crystallography Meeting*, September 2022, Dolní Vltavice, Czech Republic
- Nishita Mandal gave an oral presentation at the *PRACE-LAB Summit*, October 2022, Poznań, Poland, and the *FORUM Conference*, September 2022, Poznań, Poland; presented a poster at the 46th *FEBS Congress*, July 2022, Lisbon, Portugal, and at *Advances in Protein Folding, Evolution, and Design*, April 2022, Bayreuth, Germany
- Maciej Migdał presented a poster at the *EMBL Conference Transcription and Chromatin*, August 2022, Heidelberg, Germany
- Seyed N. Moafinejad gave an oral presentation at the *Symposium of Polish Bioinformatics Society*, September 2022, Warsaw, Poland
- Dheeraj Kumar Sarkar presented a poster at *Advances in Protein Folding, Evolution, and Design*, April 2022, Bayreuth, Germany
- Carlos Sequeiros-Borja gave an oral presentation at *BioInformatics in Toruń*, June 2022, Toruń, Poland, and the *Symposium of Polish Bioinformatics Society*, September 2022, Warsaw, Poland
- Aravind Selvaram Thirunavukarasu presented a poster at *Advances in Protein Folding, Evolution, and Design*, April 2022, Bayreuth, Germany, and the 10th *International Congress on Biocatalysis*, August-September 2022, Hamburg, Germany
- Bartłomiej Surpeta presented a poster and gave a flash talk at the *Meeting of International Society of Quantum Biology and Pharmacology*, July 2022, Innsbruck, Austria, and presented a poster at the 2nd *European Conference on Computational Biology*, September 2022, Barcelona, Spain
- Natalia Szulc gave an oral presentation at the *EMBO Workshop Protein Termini: From Mechanisms to Biological Impact*, June 2022, Bergen, Norway, and the *Dana-Farber Targeted Protein Degradation Short Talks*, June 2022 [online]; presented a poster at the *EMBO Workshop Ubiquitin and Ubiquitin-like Proteins in Health and Disease*, September 2022, Cavtat, Croatia
- Eugeniusz Tralle presented a poster at the 17th *International Zebrafish Conference*, June 2022, Montreal, Canada
- Gabriela Żurawska and Patryk Ślusarczyk gave an oral presentation at the *European Iron Club*, July 2022, Oxford, UK

OTHER ACTIVITIES

- Agnieszka Bolembach and Jacek Szymański completed a scientific internship at the Rappsilber Laboratory, Institute of Biotechnology, Technische Universität Berlin, November-December 2022, Berlin, Germany
- Zuzanna Mackiewicz participated in the *EMBO Practical Course C. elegans: From Genome Editing to Imaging*, July 2022, Heidelberg, Germany
- Natalia Szulc participated in the virtual *EMBL-EBI Training: Introduction to Multiomics Data Integration and Visualization*

DOCTORATES IN 2022

Dominik Cysewski, *Local translation in the synapse proteomic analysis*, thesis advisor: A. Dziembowski

- **Marta Gapińska**, *Structure and mechanism of action of bacterial reverse transcriptases involved in antiphage defense*, thesis advisor: M. Nowotny
- **Magdalena Kędra**, *Characterization of molecular, neuroanatomical and behavioural changes of zebrafish Tuberous Sclerosis Complex model tsc2vu242/vu242*, thesis advisor: J. Jaworski

Marlena Kisiała, *Structural studies of AvaII and TaqI restriction endonucleases*, thesis advisor: M. Bochtler

Paweł Mitkowski, *Structural and biochemical characterization of catalytic (M23) and substrate-binding (SH3b) domains found in peptidoglycan hydrolases*, thesis advisor: I. Sabała

- **Marzena Nowacka**, *Biochemical and structural studies of foamyviral reverse transcriptases*, thesis advisor: E. Nowak
- **Agata Poświata**, *The interactome of AXL receptor provides insights into its biological roles and intracellular trafficking*, thesis advisor: M. Miączyńska

Dominik Rafalski, *Vertebrate and invertebrate Tet dioxygenases*, thesis advisor: M. Bochtler

Kumar Gupta Rishikesh, *Role of Stim2a protein in the neuroprotection in Danio rerio*, thesis advisor: J. Kuźnicki

Karolina Wojciechowska, *ESCRT-I depletion sensitizes cancer cells to TRAIL-induced apoptosis through accumulated TRAILR2*, thesis advisor: M. Miączyńska

- **Alicja Wysocka**, *Biochemical characterization of selected peptidoglycan hydrolases of the M23 family and their use in antimicrobial wound dressings*, thesis advisor: I. Sabała

○ *promotion with honors*

WARSAW PHD SCHOOL IN NATURAL AND BIOMEDICAL SCIENCES

Warsaw-4-PhD

Warsaw PhD School in Natural and BioMedical Sciences (Warsaw-4-PhD) began its operations in the 2019/2020 academic year. The School is an organized form of education for PhD students who are preparing to obtain their degrees in four disciplines: biology, chemistry, physics, and medical sciences. The School is formed by nine institutions:

- Nencki Institute of Experimental Biology, Polish Academy of Sciences (Nencki Institute) – leading institution
- International Institute of Molecular and Cell Biology in Warsaw (IIMCB)
- Institute of Organic Chemistry, Polish Academy of Sciences (IOC PAS)
- Institute of Physical Chemistry, Polish Academy of Sciences (IPC PAS)
- Institute of Physics, Polish Academy of Sciences (IP PAS)
- Center for Theoretical Physics, Polish Academy of Sciences (CTP PAS)
- Institute of High Pressure Physics, Polish Academy of Sciences (IHPP PAS *Unipress*)
- Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw (MSCI)
- Institute of Psychiatry and Neurology (IPIN)

Admission to Warsaw-4-PhD is preceded by an open international competition in which the leading criterion is the candidate's excellence and predisposition to conduct groundbreaking research. Enrollment occurs three times annually, and candidates commence their education in either the winter or summer semester. Doctoral students, under the guidance of their supervisors, implement individual research plans and develop their research and soft skills. Progress of the implementation of research that is described in the individual research plan is subject to a midterm evaluation that is conducted at the midpoint of the period of education. In October 2022, the first four PhD students successfully passed their midterm evaluation. Education in Warsaw-4-PhD ends with the submission of a dissertation. The next step is to obtain a doctoral degree in a separate procedure that is conducted outside the scope of the School.
IIMCB offers their PhD students the opportunity to work in a vibrant, inclusive, and diverse international community, where their research and social needs are fully met. Believing that personalized academic mentoring is the key to scientific success, we support our doctoral students in their journey to the PhD. We encourage PhD students to participate in international activities, ranging from research visits and conferences to workshops and training, by financing their trips. PhD students who choose to apply for competitive funding are fully supported by our administrative staff at every step. Additionally, IIMCB provides PhD students with access to a private medical package, subsidized opportunities to improve their professional qualifications and knowledge enrichment, and social benefits that are on par with IIMCB employees. We know that students' voices matter. Our appreciation of our PhD students' opinions is demonstrated by regular meetings of PhD Students Council representatives with IIMCB directors and International Advisory Board members. IIMCB endeavors to address the concerns of doctoral students and support their initiatives.

In the 2022/2023 academic year, IIMCB introduced into the Warsaw-4-PhD curriculum an original course, *Methodological Advances in Molecular and Structural Biology*. Each of the two semesters of the course consists of 15 lectures that are given by internal and external experts. By the end of 2022, IIMCB scientists delivered the following lectures:

- Andrzej Dziembowski – *Origin of molecular biology, plasmids, restriction enzymes, ligases. Modern cloning techniques and Introduction to experimental design of wet-lab work with proteins and nucleic acids*
- Janusz Bujnicki – *Introduction to scientific methodology and reasoning*
- Olga Gewartowska – *Principles for mouse line generation using CRISPR/Cas9 method*
- Aleksandra Kołodziejczyk – *Single cell genomics*
- Honorata Czapińska – *Sequencing methods: data analysis*
- Matthias Bochtler – *RNASeq*
- Paweł Krawczyk – *Single molecule DNA and RNA sequencing and sequencing: data analysis*

Warsaw-4-PhD is the largest institute-based doctoral school in Poland. It is committed to internationalization. Our activities to date and plans for the future were recognized and awarded with 2,186,700 PLN of funding by the Polish National Agency for Academic Exchange within the framework of the STER program. The project, *Internationalisation of the Warsaw Doctoral School in Natural and BioMedical Sciences (iWarsaw4PhD)*, started in January, 2022 and is being implemented for three years. Main project tasks and responsible institutes are the following:

- Warsaw-4-PhD promotional movie – IIMCB
- Extension of the Warsaw-4-PhD website – Nencki Institute
- Creation of the Warsaw-4-PhD international campaign in Social Media – IIMCB
- Development of the alumni platform – Nencki Institute
- Competitive 1-month research visits of PhD students to foreign laboratories, designed to support the acquisition of scientific knowledge and research experience – IPC PAS
- Scientific events in Warsaw: summer and winter schools with international lecturers, an advanced lecture series with international experts, and spotlight talks – IP PAS

Disciplines and scientific institutions

https://warsaw4phd.eu/en/

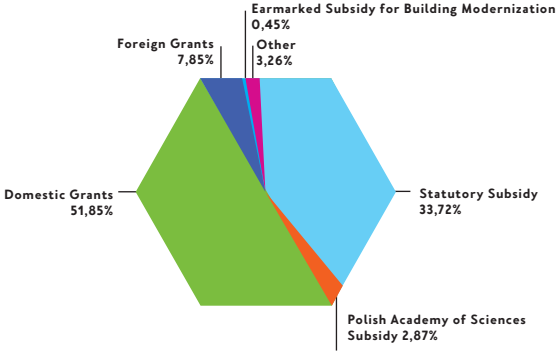
FACTS & FIGURES

DIVERSITY OF FUNDING

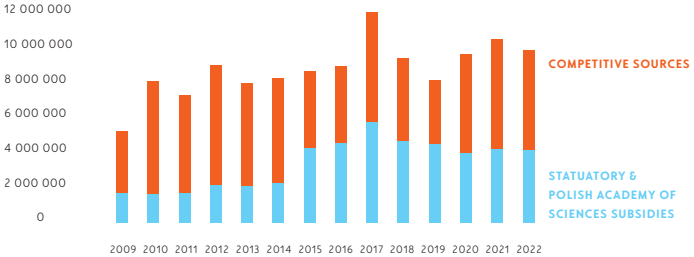
SOURCES OF FUNDING IN 2022

	PLN	EUR*
Statutory Subsidy	14,942,181	3,186,034
Polish Academy of Sciences Subsidy	1,274,000	271,648
Domestic Grants	22,976,587	4,899,163
Foreign Grants	3,479,265	741,863
Earmarked Subsidy for Building Modernization	199,551	42,549
Other	1,445,490	308,213
Total	**44,317,073**	**9,449,471**

* 1 EUR – 4,6899 PLN @ 31st Dec'2022



ANNUAL INCOME 2009–2022 (EUR)

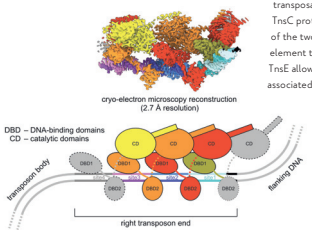


BEST PAPERS AWARDS 2022

The best papers that were affiliated with IIMCB in 2022 were chosen in the yearly Competition *Best Papers Awards*. Any experimental work of a scientific nature with no subject restriction could be submitted to this Competition. The best publications were selected by the Jury consisting of all Laboratory Leaders, based on content and significance and not bibliometric data. Laboratory Leaders were not allowed to vote for papers from their own laboratory. In 2022, the following papers were awarded:

1st PLACE

Kaczmarska Z*, **Czarnocki-Cieciura M***, **Górecka-Minakowska KM***, Wingo RJ, **Jackiewicz J**, **Zajko W**, Poznański JT, Rawski M, Grant T, Peters JE#, **Nowotny M#**. Structural basis of transposon end recognition explains central features of Tn7 transposition systems. ***Mol Cell***, 2022; 82(14):2618-32.e7. doi: 10.1016/j.molcel.2022.05.005



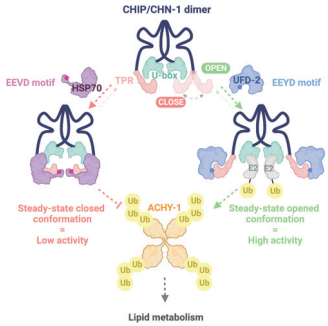
Transposons, also called "jumping genes", are DNA fragments that can move within or between genomes in a process called transposition. In bacteria, transposons are involved in the transmission of antibiotic resistance and virulence genes. Bacterial Tn7 elements are among the best-studied and most widespread DNA transposons. Tn7 mobility is mediated by five element-encoded proteins. Transposition occurs via a cut-and-paste mechanism that is executed by a heteromeric transposase, TnsA-TnsB, which is recruited to the target DNA by TnsC protein, which is an AAA+ ATPase. TnsC interacts with one of the two target selectors, TnsD or TnsE. TnsD directs the element to the conserved chromosomal *att*Tn7 site, whereas TnsE allows transposition to conjugal plasmids. CRISPR-associated transposon (CAST) elements that use element-encoded CRISPR-Cas systems for RNA-guided DNA transposition are related to Tn7 and encode TnsB-like transposases. They may provide new tools for next-generation gene editing. Scientists from the Laboratory of Protein Structure, led by **Marcin Nowotny**, in collaboration with the Joe Peters group from Cornell University, studied the structure and mechanism of prototypic *E. coli* Tn7 TnsB. They used cryoelectron microscopy (cryo-EM) to determine the structure of a complex of TnsB with double-stranded DNA that corresponded to the right end of the transposon at 2.7 Å resolution. The structure shows that multiple TnsB chains, which adopt a beads-on-a-string architecture, interact with repeating binding sites in the DNA. Upon this interaction the DNA-binding and catalytic domains of TnsB chains are arranged in a tiled and intertwined fashion. TnsB forms few base-specific contacts with DNA that lead to binding preference rather than strict specificity. The formation of an array of TnsB molecules that bind to multiple weakly conserved sites at appropriate spacing converts this preference into specific end recognition. These scientists also proposed a model of the TnsB strand-transfer complex that aims to understand late steps of the Tn7 TnsB reaction. Collectively, these results help explain how subtle differences in the spacing of binding sites are used for specific transposon end recognition and define central features of Tn7 transposition systems.

*these authors contributed equally
#corresponding authors
in bold authors affiliated with IIMCB

2nd PLACE

Das A, **Thapa P**, Santiago U, **Shanmugam N**, **Banasiak K**, Dąbrowska K, Nolte H, **Szulc NA**, Gathungu RM, Cysewski D, Krüger M, Dadlez M, **Nowotny M**, Camacho CJ, Hoppe T, **Pokrzywa W***. A heterotypic assembly mechanism regulates CHIP E3 ligase activity. ***EMBO J***, 2022; 41(15):e109566. doi: 10.15252/embj.2021109566

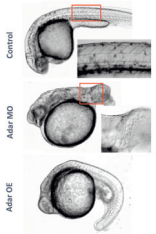
The fate of eukaryotic proteins is supervised by the chaperone network and the ubiquitin-proteasome system (UPS). CHIP (C-terminus of Hsc70-interacting protein) is an important quality control E3 ubiquitin ligase that links the chaperone system with the UPS to degrade damaged proteins. It also mediates chaperone-independent ubiquitylation and can interact with other E3s. However, the regulation of CHIP processivity and substrate selectivity in response to chaperone and E3 binding has remained unclear. Scientists from the Laboratory of Protein Metabolism, led by **Wojciech Pokrzywa**, performed a structural-functional analysis of the complex that was formed by CHIP and UFD-2, another E3 ubiquitin ligase, guided by the idea that they form a highly processive ubiquitylation system alternative to the CHIP/chaperone axis. The data showed that UFD-2 binding promotes structural gain of function in CHIP. The researchers demonstrated that the heat shock protein Hsp70 outcompetes UFD-2 for CHIP binding and negatively regulates activity of the complex by stabilizing the auto-inhibited state of CHIP. Using the nematode *Caenorhabditis elegans*, the scientists discovered that an interaction with UFD-2 enables CHIP to regulate S-adenosylhomocysteinase, an enzyme that is crucial for cellular methylation. The results obtained by the Pokrzywa group open new horizons in research on the cooperation of ubiquitin ligases in gaining high activity and substrate selectivity. In addition, the revealed CHIP processivity switching mechanism has potential application in targeted protein degradation approaches.



3rd PLACE

Niescierowicz K*, **Pryszcz L***, **Navarrete C***, **Tralle E***, **Sulej A***, **Abu Nahia K**, **Kasprzyk ME**, **Misztal K**, **Pateria A**, **Pakula A**, **Bochtler M#**, **Winata C#**. Adar-mediated A-to-I editing is required for embryonic patterning and innate immune response regulation in zebrafish. ***Nat Commun***, 2022; 13(1):5520. doi: 10.1038/s41467-022-33260-6

Adenosine-to-inosine (A-to-I) editing is necessary for regulating the innate immune system in humans and other mammals and it is implicated in human diseases, including autoimmune conditions. The enzyme adenosine deaminase acting on RNA (Adar) is responsible for catalyzing such editing, which entails the deamination of adenosine (A) at the C6 position, giving rise to an inosine (I). Researchers from the **Laboratory of Zebrafish Developmental Genomics** and **Laboratory of Structural Biology, led by Cecilia Winata** and **Matthias Bochtler**, respectively, investigated the role of Adar in zebrafish, where it is highly expressed in the earliest stages of embryogenesis. Genome-wide editing discovery by combined analyses of the parental genome and embryonic transcriptome uncovered prevalent A-to-I editing in maternal and the earliest zygotic transcripts, the majority of which occurred in the 3'-untranslated region. Transcripts that are known to play a role in gastrulation and embryonic patterning were found to contain multiple editing sites, suggesting that Adar may exert its function through them. Through Adar loss- and gain-of-function experiments, the researchers demonstrated that maternal Adar function is essential for proper embryonic patterning along the antero-posterior and dorso-ventral axes, and this function depends on an intact deaminase domain. Analyses of *adar* zygotic mutants revealed the distinct zygotic function of Adar in regulating the innate immune response, a role that is conserved in mammals. Collectively, the study established a novel function of Adar-mediated A-to-I editing in regulating embryonic patterning and revealed the conservation of zygotic Adar function between zebrafish and mammals.



PUBLICATIONS IN 2022

List of papers with IIMCB-affiliated first and/or corresponding author/s

No	Authors	Title	Journal	5-Year IF	Journal Category	Quartile in Category
1	**Kaczmarska Z**, **Czarnocki-Cieciura M**, **Górecka-Minakowska KM**, Wingo RJ, **Jackiewicz J**, **Zajko W**, Poznański JT, Rawski M, Grant T, Peters JE, **Nowotny M**.	Structural basis of transposon end recognition explains central features of Tn7 transposition systems.	Mol Cell. 2022; 82(14):2618-2632.e7 doi: 10.1016/j.molcel.2022.05.005	20.747	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
2	**Niesierowicz K**, **Pryszcz L**, **Nevarrete C**, **Trella E**, **Sulej A**, **Abu Nahia K**, **Kasprzyk ME**, **Misztal K**, **Peteria A**, **Paluta A**, **Bochtler M**, **Winata C**.	Adar-mediated A-to-I editing is required for embryonic patterning and innate immune response regulation in zebrafish.	Nat Commun. 2022; 13(1):5520 doi: 10.1038/s41467-022-33260-6	17.764	MULTIDISCIPLINARY SCIENCES	1
3	**Boccaletto P**, **Stefaniak F**, **Ray A**, **Cappannini A**, **Mukherjee S**, **Purta E**, **Kurkowska M**, **Shirvanizadeh N**, Destefanis E, Groza P, Avşar G, Romitelli A, Pir P, Dassi E, Conticello SG, Aguilo F, **Bujnicki JM**.	MODOMICS: a database of RNA modification pathways. 2021 update	Nucleic Acids Res. 2022; 50(D1):D231-D235 doi: 10.1093/nar/gkab1083	17.210	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
4	Choudhury NR, **Trus I**, Heikel G, **Wolczyk M**, **Szymanski J**, **Bolembach A**, Dos Santos Pinto RM, Smith N, Trubitsyna M, Gaunt E, Digard P, **Michlewski G**.	TRIM25 inhibits influenza A virus infection, destabilizes viral mRNA, but is redundant for activating the RIG-I pathway.	Nucleic Acids Res. 2022; 50(12):7097-7114 doi: 10.1093/nar/gkac512	17.210	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
5	**Figiel M**, **Gagińska M**, **Czarnocki-Cieciura M**, **Zajko W**, **Sroka M**, **Skowronek K**, **Nowotny M**.	Mechanism of protein-primed template-independent DNA synthesis by Abi polymerases.	Nucleic Acids Res. 2022; 50(17):10026-10040 doi: 10.1093/nar/gkac77	17.210	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
6	Mohammadi-Arani R, Javadi-Zarnaghi F, **Boccaletto P**, **Bujnicki JM**, **Ponce-Salvatierra A**.	DNAzymeBuilder, a web application for in situ generation of RNA/DNA-cleaving deoxyribozymes.	Nucleic Acids Res. 2022; 50(W1):W261-W265 doi: 10.1093/nar/gkac269	17.210	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
7	**Liudkovska V**, **Krawczyk PS**, **Brouze A**, **Gumińska N**, **Wegierski T**, Cysewski D, **Mackiewicz Z**, Ewbank JJ, Drabikowski K, **Mroczek S**, **Dziembowski A**.	TENT5 cytoplasmic noncanonical poly(A) polymerases regulate the innate immune response in animals.	Sci Adv. 2022; 8(46):eadd9468 doi: 10.1126/sciadv.add9468	16.090	MULTIDISCIPLINARY SCIENCES	1
8	Ravichandran M, **Rafalski D**, Davies CI, Ortega-Recalde O, Nan X, Olanfield CR, Kotter A, **Misztal K**, Wang AH, **Wojciechowski M**, **Rażew M**, Mayyas IM, Kardalisky O, Schwartz U, Zembrzycki K, Morison IM, Helm M, Weichenhan D, Jurkowska RZ, Krueger F, Plass C, Zacharias M, **Bochtler M**, Hore TA, Jurkowski TP.	Pronounced sequence specificity of the TET enzyme catalytic domain guides its cellular function.	Sci Adv. 2022; 8(36):eabm2427 doi: 10.1126/sciadv.abm2427	16.090	MULTIDISCIPLINARY SCIENCES	1
9	**Czapińska H**, **Bochtler M**.	The Nε-rule for serine, but not cysteine catalytic triads.	Angew Chem Int Ed Engl. 2022; 19:e202206945 doi: 10.1002/anie.202206945	15.311	CHEMISTRY, MULTIDISCIPLINARY	1
10	**Surpeta B**, Grulich M, Palyzová A, Marešová H, **Brezovsky J**.	Common Dynamic Determinants Govern Quorum Quenching Activity in N-Terminal Serine Hydrolases.	ACS Catal. 2022; 12:6359−6374 doi: 10.1021/acscatal.2c00569	14.413	CHEMISTRY, PHYSICAL	1
11	**Das A**, **Thapa P**, Santiago U, **Shanmugam N**, **Banasiak K**, Dąbrowska K, Nolte H, **Szulc NA**, Gathungu RM, Cysewski D, Krüger M, Dadlez M, **Nowotny M**, Camacho CJ, Hoppe T, **Pokrzywa W**.	A heterotypic assembly mechanism regulates CHIP E3 ligase activity.	EMBO J. 2022; 41(15):e109566. doi: 10.15252/embj.2021109566	14.050	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
12	**Podwista A**, **Kozik K**, **Mączyńska M**, **Zdżalik-Bielecka D**.	Endocytic trafficking of GAS6-AXL complexes is associated with sustained AKT activation.	Cell Mol Life Sci. 2022; 79(6):316 doi: 10.1007/s00018-022-04312-3	10.001	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
13	**Brezovsky J**, **Thirunavukarasu AS**, **Surpeta B**, **Sequeiros-Borja CE**, **Mandal N**, **Kumar Sarkar D**, **Dongmo Foumthuim CJ**, **Agrawal N**.	TransportTools: a library for high-throughput analyses of internal voids in biomolecules and ligand transport through them.	Bioinformatics. 2022; 38(6):1752-1753 doi: 10.1093/bioinformatics/btab872	8.778	BIOCHEMICAL RESEARCH METHODS	1
14	**Wysocka A**, **Łętniak Ł**, **Jagielska E**, **Sabała I**.	Electrostatic Interaction with the Bacterial Cell Envelope Tunes the Lytic Activity of Two Novel Peptidoglycan Hydrolases.	Microbiol Spectr. 2022; 10(3):e0045522 doi: 10.1128/spectrum.00455-22	8.113	MICROBIOLOGY	1
15	**Latoszek E**, **Wiwegar M**, Ludwiczak J, Dunin-Horkawicz S, **Kuznicki J**, **Czaradys M**.	Siah-1-interacting protein regulates mutated huntingtin protein aggregation in Huntington's disease models.	Cell Biosci. 2022; 12(1):34 doi: 10.1186/s13578-022-00755-0	8.108	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
16	**Zdzalik-Bielecka D**, **Kozik K**, **Poswiata A**, **Jastrzebski K**, **Jakubik M**, **Miaczynska M**.	Bemcentinib and gilteritinib inhibit cell growth and impair the endo-lysosomal and autophagy systems in an AXL-independent manner.	Mol Cancer Res. 2022; 20(3):446-455 doi: 10.1158/1541-7786.MCR-21-0444	6.750	CELL BIOLOGY	1
17	**Szulc NA**, **Mackiewicz Z**, **Bujnicki JM**, **Stefaniak F**.	fingeRNAt-A novel tool for high-throughput analysis of nucleic acid-ligand interactions.	PLoS Comput Biol. 2022; 18(6):e1009783 doi: 10.1371/journal.pcbi.1009783	5.916	MATHEMATICAL & COMPUTATIONAL BIOLOGY	1
18	De Ridder 2021 et al. and EPISTOP CONSORTIUM including **Jeworski J**, **Tempes A**, **Urbańska M**.	Evolution of electroencephalogram in infants with tuberous sclerosis complex and neurodevelopmental outcome: a prospective cohort study.	Dev Med Child Neurol. 2022; 64(4):495-501 doi: 10.1111/dmcn.15073	5.671	PEDIATRICS	1
19	**Wróbel M**, **Candrowski J**, **Szymańska E**, **Grębowicz-Maciądziewicz M**, **Budick-Harmelin N**, **Macias M**, **Szyłińska A**, **Mazur M**, **Kolmus K**, Goryca K, Dąbrowska M, Paziewska A, Mikula M, **Mączyńska M**.	ESCRT-I fuels lysosomal degradation to restrict TFEB/TFE3 signaling via the Rag-mTORC1 pathway.	Life Sci Alliance. 2022; 5(7):e202101239 doi: 10.26508/lsa.202101239	5.654	BIOLOGY	1
20	Uszczynska-Ratajczak B, Sugunan S, **Kwiatkowska M**, **Migdal M**, Carbonell-Sala S, Sokol A, **Winata CL**, Chacinska A.	Profiling subcellular localization of nuclear-encoded mitochondrial gene products in zebrafish.	Life Sci Alliance. 2022; 6(1):e202201514 doi: 10.26508/lsa.202201514	5.654	BIOLOGY	1
21	**Wolińska-Nizioł L**, **Romaniuk K**, **Wojciechowska K**, **Surga K**, Kamaszewski M, Szudrowicz H, **Mączyńska M**.	Tollip-deficient zebrafish display no abnormalities in development, organ morphology or gene expression in response to lipopolysaccharide.	FEBS Open Bio. 2022; 12(8):1453-1464 doi: 10.1002/2211-5463.13423	2.676	BIOCHEMISTRY & MOLECULAR BIOLOGY	4
22	**Latoszek E**, **Piechota M**, **Liszewska E**, Hansikova H, Klempir J, Mühlback A, Landwehrmeyer GB, **Kuznicki J**, **Czaradys M**.	Generation of three human iPSC lines from patients with Huntington's disease with different CAG lengths and human control iPSC line from a healthy donor.	Stem Cell Res. 2022; 64:102931 doi: 10.1016/j.scr.2022.102931	2.138	CELL BIOLOGY	4
23	**Korsh V**, **Gasanov EV**.	Genetics of Atavism.	Russ J Dev Biol. 2022; 53(3):221–230 doi: 10.1134/S1062360422030043	0.696	DEVELOPMENTAL BIOLOGY in SCIE edition	4

List of papers without IIMCB-affiliated first and/or corresponding author/s

No	Authors	Title	Journal	5-Year IF	Journal Category	Quartile in Category
1	Baranasic D et al. including **Łupiński M**, **Winata C**	Multiomic atlas with functional stratification and developmental dynamics of zebrafish cis-regulatory elements.	Nat Genet. 2022; 54[7]:1037-1050 doi: 10.1038/s41588-022-01089-w	39.320	GENETICS & HEREDITY	1
2	Sekar R, Motzler K, Kwon Y, Novikoff A, Jülg J, Najafi B, Wang S, Warnke AL, Seitz S, Hass D, Gancheva S, Kahl S, Yang B, Finan B, Schwarz K, Okun JG, Roden M, Blüher M, Müller TD, Krahmer N, Behrends C, Plettenburg O, **Mieczynska M**, Herzig S, Zeigerer A.	Vps37a regulates hepatic glucose production by controlling glucagon receptor localization to endosomes.	Cell Metab. 2022; 34[11]:1824-1842.e9 doi: 10.1016/j.cmet.2022.09.022	35.104	CELL BIOLOGY	1
3	Balaji V, Müller L, Lorenz R, Kevei É, Zhang WH, Santiago U, Gebauer J, Llamas E, Vilchez D, Camacho CJ, **Pokrzywa W**, Hoppe T.	A Dimer-Monomer Switch Controls CHIP-Dependent Substrate Ubiquitylation and Processing.	Mol Cell. 2022; 82[17]:3239-3254.e11 doi: 10.1016/j.molcel.2022.08.003	20.747	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
4	Pekec T, Lewandowski J, Komur AA, Sobańska D, Guo Y, Świtońska-Kurkowska K, Małecki JM, **Dubey AA**, **Pokrzywa W**, Frankowski M, Figiel M, Ciosk R.	Ferritin-mediated iron detoxification promotes hypothermia survival in *Caenorhabditis elegans* and murine neurons.	Nat Commun. 2022; 13[1]:4883 doi: 10.1038/s41467-022-32500-z	17.764	MULTIDISCIPLINARY SCIENCES	1
5	Bondarchuk TV, Shalak VF, Lozhko DM, Fatalska A, **Szczepanowski RH**, **Liudkovska V**, Tsuvariev OY, Dadlez M, El'skaya AV, Negrutski BS.	Quaternary organization of the human eEF1B complex reveals unique multi-GEF domain assembly.	Nucleic Acids Res. 2022; 50[16]:9490-9504 doi: 10.1093/nar/gkac685	17.210	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
6	Guegan F, Rajan KS, Benito F, Pinto-Neves D, Sequeira M, **Gumińska N**, **Mrocek S**, **Dziembowski A**, Cohen-Chalamish S, Doniger T, Galili B, Estévez AM, Notredame C, Michaeli S, Figueiredo LM.	A long noncoding RNA promotes parasite differentiation in African trypanosomes.	Sci Adv. 2022; 8[24]:eabn2706 doi: 10.1126/sciadv.abn2706	16.090	MULTIDISCIPLINARY SCIENCES	1
7	Hulshof HM, Kuijf HJ, Kotulska K, Curatolo P, Weschke B, Riney K, Krsek P, Feucht M, Nabbout R, Lagae L, Jansen A, Otte WM, Lequin MH, Sijko K, Benvenuto A, Hertzberg C, Benova B, Scholl T, De Ridder J, Aronica EMA, Kwiatkowski DJ, Jozwiak S, Jurkiewicz E, Braun K, Jansen FE; EPISTOP consortium including **Jaworski J**, **Tempes A**, **Urbańska M**.	Association of Early MRI Characteristics With Subsequent Epilepsy and Neurodevelopmental Outcomes in Children With Tuberous Sclerosis Complex.	Neurology. 2022; 98[12]:e1216-e1225 doi: 10.1212/WNL.0000000000020027	11.786	CLINICAL NEUROLOGY	1
8	Oroń M, Grochowski M, Jaiswar A, Legierska J, **Jastrzębski K**, Nowak-Niezgoda M, Kołos M, Kaźmierczak W, Okeściński T, Lenarcik M, Cybulska M, Mikula M, **Żyłicz A**, **Mięczyńska M**, Zettl K, Wiśniewski JR, Walerych D.	The molecular network of the proteasome machinery inhibition response is orchestrated by HSPTO, revealing vulnerabilities in cancer cells.	Cell Rep. 2022; 40[13]:111428 doi: 10.1016/j.celrep.2022.111428	10.990	CELL BIOLOGY	1
9	Roszkowska M, Krysiak A, Majchrowicz L, Nader K, Beroun A, Michaluk P, Pekala M, **Jaworski J**, Kondrakiewicz L, Puścian A, Knapska E, Kaczmarek L, Kalita K.	SRF depletion in early life contributes to social interaction deficits in the adulthood.	Cell Mol Life Sci. 2022; 79[5]:278 doi: 10.1007/s00018-022-04291-5	10.001	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
10	Udenze D, **Trus I**, Berube N, Karniychuk U.	CpG content in the Zika virus genome affects infection phenotypes in the adult brain and fetal lymph nodes.	Front Immunol. 2022; 13:943481 doi: 10.3389/fimmu.2022.943481	8.877	IMMUNOLOGY	1
11	Makarova K, Zawada K, **Wiweger M**.	Benchtop X-band electron paramagnetic resonance detection of melanin and Nitroxyl spin probe in zebrafish.	Free Radic Biol Med. 2022; 183:69-74 doi: 10.1016/j.freeradbiomed.2022.03.015	8.176	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
12	Bartoszewski S, Dawidziuk M, Kasica N, Durak R, Jurek M, Podwysocka A, Guilbride DL, Podlasz P, **Winata CL**, Gawlinski P.	A Zebrafish/Drosophila Dual System Model for Investigating Human Microcephaly.	Cells. 2022; 11[17]:2727 doi: 10.3390/cells11172727	7.677	CELL BIOLOGY	2
13	Puzianowska-Kuznicka M, Kurylowicz A, Wierucki L, Owczarek AJ, Jagiello K, **Mossakowska M**, Zdrojewski T, Chudek J.	Obesity in Caucasian Seniors on the Rise: Is It Truly Harmful? Results of the PolSenior2 Study.	Nutrients. 2022; 14[21]:4621 doi: 10.3390/nu14214621	7.185	NUTRITION & DIETETICS	1
14	Pawlik B, Grabia S, Smyczyńska U, Fendler W, Dróżdż I, **Liszewska E**, **Jaworski J**, Kotulska K, Jóźwiak S, Mlynarski W, Trelińska J.	MicroRNA Expression Profile in TSC Cell Lines and the Impact of mTOR Inhibitor.	Int J Mol Sci. 2022; 23[22]:14493 doi: 10.3390/ijms232214493	6.628	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
15	Ouma J, Antczak M, Adamiak RW, **Bujnicki JM**, Chen SJ, Ding F, **Ghosh P**, Li J, **Mukherjee S**, **Nithin C**, Pachulska-Wieczorek K, **Ponce-Salvatierra A**, Popenda M, Sarzynska J, **Wirecki T**, Zhang D, Zhang S, Zok T, Westhof E, Miao Z, Szachniuk M, Rybarczyk A.	Computational Pipeline for Reference-Free Comparative Analysis of RNA 3D Structures Applied to SARS-CoV-2 UTR Models.	Int J Mol Sci. 2022; 23[17]:9630 doi: 10.3390/ijms23179630	6.628	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
16	Kuzniewska B, Rejmak K, Nowacka A, Ziółkowska M, Milek J, Magnowska M, **Gruchota J**, **Gewartowska O**, Borsuk E, Salamian A, **Dziembowski A**, Radwanska K, Dziembowska M.	Disrupting interaction between miR-132 and Mmp9 3'UTR improves synaptic plasticity and memory in mice.	Front Mol Neurosci. 2022; 15:924534 doi: 10.3389/fnmol.2022.924534	6.187	NEUROSCIENCES	1

No	Authors	Title	Journal	5-Year IF	Journal Category	Quartile in Category
17	Rosario R, Stewart HL, Choudhury NR, **Michlewski G**, Charlet-Berguerand N, Anderson RA.	Evidence for a fragile X messenger ribonucleoprotein 1 [FMR1] mRNA gain-of-function toxicity mechanism contributing to the pathogenesis of fragile X-associated premature ovarian insufficiency.	FASEB J. 2022; 36[11]:e22612 doi: 10.1096/fj.202200468RR	6.103	BIOCHEMISTRY & MOLECULAR BIOLOGY	2
18	Salerno-Kochan A, Horn A, **Ghosh P**, **Nithin C**, Kościelniak A, Meindl A, Strauss D, Krutyhołowa R, Rossbach O, **Bujnicki JM**, Gaik M, Medenbach J, Glatt S.	Molecular insights into RNA recognition and gene regulation by the TRIM-NHL protein Mei-P26.	Life Sci Alliance. 2022; 5[8]:e202201418 doi: 10.26508/lsa.202201418	5.654	BIOLOGY	1
19	Wojtyniak P, Boratynska-Jasinska A, Serwach K, Gruszczynska-Biegala J, Zablocka B, **Jaworski J**, Kawalec M.	Mitofusin 2 Integrates Mitochondrial Network Remodelling, Mitophagy and Renewal of Respiratory Chain Proteins in Neurons after Oxygen and Glucose Deprivation.	Mol Neurobiol. 2022; 59[10]:6502-6518 doi: 10.1007/s12035-022-02981-6	5.576	NEUROSCIENCES	2
20	Winiewska-Szajewska M, **Czapinska H**, Kaus-Drobek M, **Fricke A**, Mieczkowska K, Dadlez M, **Bochtler M**, Poznański J.	Competition between electrostatic interactions and halogen bonding in the protein–ligand system: structural and thermodynamic studies of 5,6-dibromobenzotriazole-hCK2α complexes.	Sci Rep. 2022; 12[1]:18964 doi: 10.1038/s41598-022-23611-0	5.516	MULTIDISCIPLINARY SCIENCES	2
21	Lehka L, Wojton D, Topolewska M, Chumak V, **Majewski Ł**, Rędowicz MJ.	Loss of Unconventional Myosin VI Affects cAMP/PKA Signaling in Hindlimb Skeletal Muscle in an Age-Dependent Manner.	Front Physiol. 2022; 13:933963 doi: 10.3389/fphys.2022.933963	5.316	PHYSIOLOGY	1
22	Labedzka-Dmoch K, **Rzasew M**, **Gapinska M**, Piatkowski J, Kolondra A, Salmonowicz H, Wenda JM, **Nowotny M**, Golik P.	The Pet127 protein is a mitochondrial 5'-to-3' exoribonuclease from the PD-(D/E)XK superfamily involved in RNA maturation and intron degradation in yeasts.	RNA. 2022; 28[5]:711-728 doi: 10.1261/rna.079083.121	5.274	BIOCHEMISTRY & MOLECULAR BIOLOGY	2
23	Scheper M, Romagnolo A, Besharat ZM, Iyer AM, Moavero R, Hertzberg C, Weschke B, Riney K, Feucht M, Scholl T, Petrak B, Maulisova A, Nabbout R, Jansen AC, Jansen FE, Lagae L, Urbanska M, Ferretti E, **Tempes A**, **Blaszczyk M**, **Jaworski J**, Kwiatkowski DJ, Jozwiak S, Kotulska K, Sadowski K, Borkowska J, Curatolo P, Mills JD, Aronica E, Epistop Consortium Members.	miRNAs and isomiRs: Serum-Based Biomarkers for the Development of Intellectual Disability and Autism Spectrum Disorder in Tuberous Sclerosis Complex.	Biomedicines. 2022; 10[8]:1838 doi: 10.3390/biomedicines10081838	5.225	BIOCHEMISTRY & MOLECULAR BIOLOGY	2
24	Neumann-Podczaska A, Tobis S, Antimisiaris D, **Mossakowska M**, Puzianowska-Kuznicka M, Chudek J, Wieruzki L, Marks P, Wizner B, Sobieszczanska M, Niemir ZI, Kaczmarek B, Wieczorowska-Tobis K.	Polypharmacy in Polish Older Adult Population-A Cross-Sectional Study: Results of the PolSenior Project.	Int J Environ Res Public Health. 2022; 19[3]:1030 doi: 10.3390/ijerph19031030	4.799	PUBLIC, ENVIRONMENTAL & OCCUPATIONAL HEALTH	1
25	Chudek A, Kotyla P, **Mossakowska M**, Grodzicki T, Zdrojewski T, Olszanecka-Glinianowicz M, Chudek J, Owczarek AJ.	The Prevalence of Anticitrullinated Protein Antibodies in Older Poles–Results from a Population-Based PolSenior Study.	Int J Environ Res Public Health. 2022; 19: 14216 doi: 10.3390/ijerph192114216	4.799	PUBLIC, ENVIRONMENTAL & OCCUPATIONAL HEALTH	1
26	Hadar A, Voinsky I, Parkhomenko O, Puzianowska-Kuznicka M, **Kuźnicki J**, Gozes I, Gurwitz D.	Higher ATM expression in lymphoblastoid cell lines from centenarian compared with younger women.	Drug Dev Res. 2022; 83[6]:1419-1424 doi: 10.1002/ddr.21972	4.264	CHEMISTRY, MEDICINAL	2
27	Królczyk J, Piotrowicz K, Skalska A, **Mossakowska M**, Grodzicki T, Gąsowski J.	Mortality of older persons with and without abnormalities in the physical examination of arterial system.	Aging Clin Exp Res. 2022; 34[11]:2897-2904 doi: 10.1007/s40520-022-02232-7	4.075	GERIATRICS & GERONTOLOGY	2
28	Kocełak P, **Mossakowska M**, Puzianowska-Kuźnicka M, Sworczak K, Wyszomirski A, Handzlik G, Stefański A, Zdrojewski T, Chudek J.	Prevalence and risk factors of untreated thyroid dysfunctions in the older Caucasian adults: Results of PolSenior 2 survey.	PLoS One. 2022; 17[8]:e0272045 doi: 10.1371/journal.pone.0272045	4.069	MULTIDISCIPLINARY SCIENCES	2
29	Królczyk J, Skalska A, Piotrowicz K, **Mossakowska M**, Grodzicki T, Gąsowski J.	Disparate effects of ankle-brachial index on mortality in the 'very old' and 'younger old' populations-the PolSenior survey.	Heart Vessels. 2022; 37[4]:665-672 doi: 10.1007/s00380-021-01949-1	1.715	CARDIAC & CARDIOVASCULAR SYSTEMS	4
30	Corona A, **Wycisk K**, Talarico C, Manelfi C, Milia J, Cannalire R, Esposito F, Gribbon P, Zaliani A, Iaconis D, Beccari AR, Summa V, **Nowotny M**, Tramontano E.	Natural Compounds Inhibit SARS-CoV-2 nsp13 Unwinding and ATPase Enzyme Activities.	ACS Pharmacol Transl Sci. 2022; 5[4]:226-239 doi: 10.1021/acsptsci.1c00253	1.310	CHEMISTRY, MEDICINAL	1
31	Sieradzan AK, Czaplewski C, Krupa P, Mozolewska MA, Karczyńska AS, Lipska AG, Lubecka EA, Gołaś E, **Wirecki T**, Makowski M, Ołdziej S, Liwo A.	Modeling the Structure, Dynamics, and Transformations of Proteins with the UNRES Force Field.	Part of the Methods in Molecular Biology book series, 2022; 2376:399-416 doi: 10.1007/978-1-0716-17168_23	N/A	N/A	N/A

GRANTS RUNNING IN 2022

59 grants with total awarded funding
147,687,234 PLN

WIB ŁUKASIEWICZ – PORT Virtual Research Institute

1 project | 28,398,800 PLN

- **Virtual Research Institute**; *Horizon for Excellence in messenger RNA applications in immunoOncology* [HERO]; [UoF/01-WIB-1/2020-011] in partnership with the University of Warsaw, the Medical University of Warsaw, the Institute of Physical Chemistry of the Polish Academy of Sciences; 28,398,800 PLN for the IIMCB [total grant budget: 69,160,450 PLN]; 2022-2027; **A. Dziembowski** [Leader], **M. Miączyńska**, **M. Nowotny**

NATIONAL SCIENCE CENTRE POLAND

38 projects | 74,782,415 PLN

DIOSCURI
- *The Dioscuri Centre for RNA-Protein Interactions in Human Health and Disease* [2019/02/H/NZ1/000020); 6,642,000 PLN; 2021-2025; **G. Michlewski**

MAESTRO
- *The role of mTOR-Brg1 interaction in normal and aberrant neuronal activity* [2020/38/A/NZ3/00447]; 4,092,140 PLN; 2021-2026; **J. Jaworski**
- *Structural and mechanistic studies of bacterial DNA repair* [2017/26/A/NZ1/01098]; 4,228,500 PLN; 2018-2023; **M. Nowotny**
- *Integrative modeling and structure determination of macromolecular complexes comprising RNA and proteins* [2017/26/A NZ1/01083]; 3,500,000 PLN; 2018-2023; **J.M. Bujnicki**
- *Oncogenic mechanisms of DIS3 mutations* [2016/22/A/NZ4/00380]; 3,490,750 PLN; 2017-2022; **A. Dziembowski**

SONATA BIS
- *Adaptation of Proteins to Evade Premature Degradation by the Ubiquitin-Proteasome System* [2021/42/E/NZ1/00190]; 3,686,840 PLN; 2022-2027; **W. Pokrzywa**
- *Identifying unique adaptive responses of red pulp macrophages to iron deficiency* [2020/38/E/NZ4/00511]; 3,613,374 PLN; 2021-2026; **K. Mleczko-Sanecka**

GRIEG (EEA and Norway Grants)
- *Cellular adaptation to cold* [2019/34/H/NZ3/00691]; 3,834,426 PLN; 2021-2024; **W. Pokrzywa**; Partner: University of Oslo, Norway
- *The impact of cytoplasmic polyadenylation on local translation in neurons* [2019/34/H/NZ3/00733]; 1,935,625 PLN; 2020-2024; **A. Dziembowski**; Partner: University of Bergen, Norway; University of Warsaw, Poland

DAINA: POLISH-LITHUANIAN FUNDING INITIATIVE
- *CRISPR tools for the study of embryonic development in zebrafish* [2017/27/L/NZ2/03234]; 1,634,500 PLN; 2018-2023; **M. Bochtler**; Partner: Vilnius University, Lithuania

OPUS
- *Structural and mechanistic studies of (+)RNA virus replication* [2021/41/B/NZ1/03620]; 2,684,000 PLN; 2022-2026; **M. Nowotny**
- *Building a genomic atlas of human inner ear malformations: focus on novel genes and functional non-coding regions* [2021/41/B/NZ5/04390]; 845,460 PLN for the IIMCB [total grant budget: 2,986,560 PLN]; 2022-2026; **V. Korzh**; coordinated by the Institute of Physiology and Pathology of Hearing
- *AXL receptor signaling in cancer cell growth and drug resistance* [2020/39/B/NZ3/03429]; 2,482,764 PLN; 2021-2025; **M. Miączyńska**
- *Rac1 contribution to brain connectivity impairments and neuropsychiatric disorders in Tuberous Sclerosis Complex* [2020/37/B/NZ3/02345]; 2,251,260 PLN; 2021-2025; **J. Zmorzyńska**
- *Identification of novel vulnerabilities of VPS4B-deficient cancers cells* [2020/37/B/NZ3/02991]; 1,878,854 PLN; 2021-2025; **E. Szymańska**
- *Experimental analysis of molecular determinants involved in epilepsy* [2020/39/B/NZ3/02729]; 1,780,590 PLN; 2021-2025; **V. Korzh**
- *Unraveling the influence of posttranscriptional modifications on RNA 3D structure formation and its dynamics, with the integrated use of theoretical and experimental approaches* [2020/37/B/NZ2/02456]; 1,650,000 PLN; 2021-2024; **J.M. Bujnicki**
- *The new methodology for better understanding of ligand-RNA interactions* [2020/39/B/NZ2/03127]; 671,000 PLN; 2021-2024; **F. Stefaniak**
- *Reconstructing cardiovascular cell lineage evolution, one cell at a time* [2019/35/B/NZ2/02548]; 2,631,552 PLN; 2020-2024; **C.L. Winata**
- *Linking abnormal Ca2+ signaling and the unfolded protein response with Huntington's disease pathology in both YAC128 mouse model and iPSCderived neurons from HD patients* [2019/33/B/NZ3/02889]; 1,857,550 PLN; 2020-2024; **M. Czeredys**
- *Analysis of the role of cytoplasmic polyadenylation in the regulation of the innate immune response* [2019/33/B/NZ2/01773]; 2,324,800 PLN; 2020-2023; **A. Dziembowski**
- *Mechanism of RNA ligation in maturation of transfer RNAs* [2019/33/B/NZ1/02839]; 1,985,200 PLN; 2020-2023; **M. Nowotny**
- *Approaching integrative genomics to identify molecular drivers of congenital heart disease* [2018/29/B/NZZ/01010]; 1,880,050 PLN; 2019-2022; **C.L. Winata**
- *Deciphering novel mechanisms that control iron sensing and iron accumulation in the liver* [2018/31/B/NZ4/03676]; 1,778,635 PLN; 2019-2022; **K. Mleczko-Sanecka**
- *Role of TBC1D5 phosphorylation in neurodevelopment and TSC-related cell pathology* [2017/27/B/NZ3/01358]; 1,795,700 PLN; 2018-2022; **J. Jaworski**
- *Enabling routine and reliable analysis of transport tunnels in proteins* [2017/25/B/NZ1/01307]; 1,375,050 PLN; 2018-2022; **J. Brezovsky**
- *Exploring Baltic Sea cyanobacteria for small-molecule inhibitors of microRNA function* [2017/25/B/NZ9/00202]; 27,000 PLN for the IIMCB [total grant budget: 1,410,100 PLN]; 2018-2022; **F. Stefaniak** [partner]; Coordinator: University of Warmia and Mazury in Olsztyn
- *Biochemical and structural studies of retroviral reverse transcriptases evolution* [2016/21/B/NZ1/02757]; 1,145,000 PLN; 2017-2022; **E. Nowak**

POLISH RETURNS (research component funded by NCN)
- *Regulation of microRNAs for the treatment and understanding the etiology of Parkinson's disease* [2021/01/1/NZ1/00001]; 200,000 PLN; 2021-2022; **G. Michlewski**

SONATA
- *A framework for de novo modeling of RNA structures using restraints derived from experimental data* [2021/43/D/NZ1/03360]; 691,252 PLN; 2022-2025; **S. Mukherjee**
- *3D Structure determination of key regulatory regions at the 5' and 3' termini of pathogenic Flaviviruses RNA* [2020/39/D/NZ6/02528]; 895,358 PLN; 2021-2024; **T. Rocha de Moura**
- *Discovery and characterization of RNA structure motifs conserved in positive-sense single-stranded RNA viruses and in other functional RNAs* [2020/39/D/NZZ/02837]; 825,330 PLN; 2021-2024; **T. Wirecki**
- *Elucidating the role of TENT5C-mediated polyadenylation in erythropoiesis* [2019/35/D/NZ3/04253]; 1,482,000 PLN; 2020-2024; **M. Kusio-Kobiałka**
- *Bridging the gap: DNA catalysis explained* [2018/31/D/NZZ/01883]; 1,247,150 PLN; 2019-2022; **M.A. Ponce Salvatierra**
- *Role of Tollip protein in embryonic development and protein homeostasis in the model of zebrafish (Danio rerio)* [2016/21/D/NZ4/00494]; 583,750 PLN; 2017-2022; **Ł. Wolińska-Nizioł**

SONATINA
- *How dysfunction in the nuclear, RNA degrading enzyme DIS3 leads to mitotic defects creating a possible therapeutic strategy for Multiple Myeloma* [2019/32/C/NZ2/00558]; 832,059 PLN; 2019-2022; **T. Kuliński**

PRELUDIUM
- *Living on the edge: evolutionary adaptation of substrate-recruiting subunits of the cullin-RING ubiquitin ligase complexes to avoid premature degradation* [2021/41/N/NZ1/03473]; 190,770 PLN; 2022-2025; **N. Szulc**
- *Deciphering the molecular mechanism of activity switch of the ubiquitin ligase CHIP* [2021/41/N/NZ1/03086]; 132,126 PLN; 2022-2024; **P. Thapa**

FNP Foundation for Polish Science

5 projects | 20,600,306 PLN

- SG OP 4.4. **TEAM** *Molecular mechanism of dendritic arbor stability and its relation to mood disorders* [POIR.04.04.00-00-5CBE/17-00); 3,515,735 PLN; 2018-2023; **J. Jaworski**
- SG OP 4.4. **TEAM** *The interplay between epigenomics and DNA repair* [POIR.04.04.00-00-5D81/17-00]; 3,491,914 PLN; 2018-2023; **M. Bochtler**
- SG OP 4.4. **TEAM** *Structural and biochemical studies of the mechanism of LINE-1 retrotransposition and hepadnaviral replication* [POIR.04.04.00-00-20E7/16-00]; 6,442,834 PLN; 2017-2022 ; **M. Nowotny**
- SG OP 4.4. **TEAM** *Functional interactions of human proteins involved in posttranscriptional regulatory mechanism* [POIR.04.04.00-00-1A72/16-00]; 5,150,000 PLN; 2016-2022; **A. Dziembowski**
- SG OP 4.4. **FIRST TEAM** *The regulation of methionine metabolism by the ubiquitin-proteasome system: CHIPed supervision of the methylation potential* [POIR.04.04.00-00-5EAB/18-00); 1,999,823 PLN; 2018-2022; **W. Pokrzywa**

4 projects | 13,388,022 PLN

- **ERA Chairs** MOSaIC *Molecular Signaling in Health and Disease – Interdisciplinary Centre of Excellence* [810425]; 2,498,887.50 EUR; 2018-2023; **J. Kuźnicki**
- **INFRAIA** iNEXT-Discovery *Infrastructure for transnational access and discovery in structural biology* [871037]; 47,500 EUR for the IIMCB [total grant budget: 9,987,756.50 EUR]; 2020-2024; **M. Nowotny**
- **ITN-MSCA** ROPES *ROles of ePitranscriptomic in diseaSES* [956810]; 227 478,6 EUR for the IIMCB [total grant budget: 3,095,829 EUR]; 2020-2024; **J.M. Bujnicki**
- **EIC – Transition Grant** INCYPRO *A key technology to enable the broad application of proteins in diagnostics and therapeutics* [101057978]; 201,250 EUR for the IIMCB [total grant budget: 2,498,750 EUR]; 2022-2025; **J.M. Bujnicki**

NCBR National Centre for Research and Development

1 project | 3,088,120 PLN

- **STRATEGMED** EPIMARKER *Application of novel diagnostic and therapeutical methods in epilepsy and neurodevelopmental abnormalities in children based on the clinical and cellular model of mTOR dependent epilepsy* [306306]; 3,088,120 PLN for the IIMCB [total grant budget: 16,847,247 PLN]; 2017-2022; **J. Jaworski** [partner]; Coordinator: Medical University of Warsaw

PAN POLISH ACADEMY OF SCIENCES

1 project | 855,871 PLN

- **PASIFIC** *Targeted single-cell gene expression analysis of mRNA vaccine response* [847639]; 855,871 PLN; 2022-2024; **E. Poniecka**

NAWA POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE

6 projects | 4,950,100 PLN

- **STER Programme** *Internationalisation of the Warsaw Doctoral School in Natural and BioMedical Sciences* [BPI/STE/2021/1/00034/U/00001]; coordinated by the Nencki Institute of Experimental Biology; 142,000 PLN for the IIMCB [total grant budget: 1,968,030 PLN]; 2022-2024; **U. Białek-Wyrzykowska**
- **Polish Returns Programme** *Regulation of microRNAs for the treatment and understanding the etiology of Parkinson's disease* [PPN/PPO/2020/1/00006/U/00001]; 2,070,000 PLN; 2021-2025; **G. Michlewski**
- **Seal of Excellence Programme** [PPN/SEL/2020/1/00003/U/00001]; 264,000 PLN; 2021-2023; **A. Ray**
- **Polish-German Exchange Programme** *Regulation of mitochondrial calcium homeostasis by TMBIM5* [PPN/BDE/2020/1/00006/U/00001]; 19,900 PLN; 2021-2022; **J. Kuźnicki**
- **Welcome to Poland Programme** *Integrated support programme for foreigners at IIMCB* [PPI/WTP/2019/1/00054/U/00001]; 454,200 PLN; 2019-2022; **K. Fiedorowicz**
- **International Academic Partnerships** *Molecular basis of enzyme specificity and applications* [PPI/APM/2018/1/00034/U/001); 2,000,000 PLN; 2018-2022; **M. Bochtler**, **I. Sabała**

EMBO

3 projects | 1,623,600 PLN

- **EMBO Postdoctoral Fellowship**; *Exploring RNA folds and remote evolutionary relationships with an improved structural similarity search method* [ALTF 525-2022]; 96,000 EUR; 2022-2024; **E. Baulin**
- **EMBO Bridging Fund**; *Regulation of muscle-derived exophers* [3917]; 4,800 EUR; 2022-2023; **W. Pokrzywa**
- **EMBO Installation Grant** *Identification of signals coordinating the proteolytic quality control networks* [3916] plus **EMBO Small Grant;** 250,000 EUR + 10,000 EUR; 2018-2023; **W. Pokrzywa**

SCIENTIFIC EVENTS

EMBL Info Day
Cooperation with EMBL: Opportunities for Polish scientists
June 9, 2022

This event was dedicated to Polish scientists at all career stages interested in learning about European Molecular Biology Laboratory (EMBL) programs and activities, and ways to get involved with the EMBL. The Info Day was intended to explore new synergies between EMBL and the Polish scientific community and to encourage cooperation on joint projects. The remote sessions covered the opportunities for Polish scientists at EMBL, i.e. scientific collaboration in terms of research infrastructure, services, training, education, and research. There was an introduction to the new scientific programme *Molecules to Ecosystems* for 2022-2026, which will enable EMBL to build on its expertise in molecular biology, using advanced data sciences and theoretical approaches, to expand into new areas including planetary biology, human ecosystems, infection biology, and microbial ecosystems in order to deliver research relevant to pressing societal challenges.

Ministry of Education and Science Republic of Poland

EMBL

iNEXT-Discovery 2nd Annual Scientific Meeting
August 29-30, 2022

iNEXT-Discovery is a consortium that enables access to structural biology research infrastructures for all European researchers. The aim of this second consortium meeting was to present the latest discoveries in structural biology, highlighting the development of new technologies and applications used to conduct experiments in this field. The program included presentations from internal consortium members and external experts.
Keynote Speakers: **Brenda Schulman** (Max Planck Institute of Biochemistry, Germany) and **Leonid Sazanov** (Institute of Science & Technology, Austria).
The meeting was in hybrid format – partners and regional structural biologists were present on-site in Warsaw, while structural biologists from around the world participated remotely.
Organizers: IIMCB and its partners: iNEXT-Discovery and Helmholtz Centre for Materials and Energy (Berlin, Germany). Marcin Nowotny, Head of Laboratory of Protein Structure at IIMCB, coordinated the event.

iNEXT DISCOVERY

HZB Helmholtz Zentrum Berlin

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 871037

IIMCB Retreat
September 12-13, 2022

- Special lecture by **Professor Susan Gasser**, Swiss Institute for Experimental Cancer, Switzerland, *Targeted Histone degradation responds to the DNA damage checkpoint*
- **Director's report** on the IIMCB activities in 2022 and future plans
- Poster session and **competition for the best poster**

Winners of the best poster competition

- **1st Prize**

Karim Abu Nahia, Laboratory of Zebrafish Developmental Genomics
Understanding the embryonic zebrafish heart at single-cell RNA-seq resolution

- **2nd Prize**

Paweł Krawczyk, Laboratory of RNA Biology - ERA Chairs Group
Re-adenylation of mRNA-1273 vaccine in macrophages enhances immune response

Zuzanna Mackiewicz, Laboratory of RNA Biology - ERA Chairs Group
Hunting for the function of the excretory gland cell and NSPC proteins in worms

- **3rd Prize**

Wiktor Antczak, Laboratory of RNA Biology - ERA Chairs Group
Inducible protein degradation in knock-in mouse cells using dTAG/FKBP system

Małgorzata Figiel, Laboratory of Protein Structure
Mechanism of protein-primed template-independent DNA synthesis by Abi polymerases

Natalia Gumińska, Laboratory of RNA Biology - ERA Chairs Group
Ninetails: detection of non-adenosine nucleotides in poly(A) tails based on Oxford Nanopore direct RNA sequencing data

OPEN IIMCB SEMINARS

Sara Szymkuć (Institute of Organic Chemistry PAS, Poland & Allchemy Inc., Highland, USA) *Synthetic connectivity and emergence in the network of prebiotic chemistry*. 13.01.2022

Bożena Kamińska-Kaczmarek (Nencki Institute PAS, Poland) *Employing single-cell omics and nanotechnology to improve therapy of malignant brain tumors*. 20.01.2022

Christos Gkogkas (Biomedical Research Institute, Foundation for Research & Technology Hellas, Greece) *Regulation of protein synthesis in brain health and disease*. 27.01.2022

Eva Maria Novoa (Centre for Genomic Regulation, Spain) *Decoding the epitranscriptome at single molecule resolution*. 10.02.2022

Nazim Bouatta (Harvard Medical School, USA) *Predicting protein structures: from AlphaFold2 to single sequence prediction*. 03.03.2022

Juan Bonifacino (NIH, Bethesda, USA) *Unraveling the function of the adaptor complex AP-4 in protein sorting and neurological disease*. 10.03.2022

Karolina Szczepanowska (IMol PAS, Poland) *Safeguarding a beautiful beast. The quality control and repair of respiratory Complex I*. 17.03.2022

Sumantra Chatterjee (NYU Grossman School of Medicine, USA) *Disrupted regulatory networks and cellular interactions in Hirschsprung disease: Lessons for complex disorders*. 24.03.2022

Mustafa Sahin (Boston Children's Hospital, USA) *Neuronal connectivity in TSC as a model for neurodevelopmental disorders*. 31.03.2022

Agnieszka Dobrzyń (Nencki Institute PAS, Poland) *Thinking Big – from basic science to biotechnology-driven novel approaches to treat diabetes*. 14.04.2022

Mikołaj Stabicki (Broad Institute of MIT and Harvard University, USA) *Novel mechanisms of small molecule-induced protein degradation*. 21.04.2022

Richard I. Morimoto (Northwestern University, USA) *Proteostasis and the challenge of maintaining a stable proteome in aging and diseases*. 19.05.2022

Robert Vacha (CEITEC, Czech Republic) *How proteins or nanoparticles can enter cells?* 26.05.2022

Monika Piwecka (Institute of Bioorganic Chemistry PAS, Poland) *Illuminating non-coding RNA functions and RNA-protein interactions in the mammalian brain*. 02.06.2022

INTERNAL SEMINAR SERIES

Guoliang Xu (Institute of Biochemistry and Cell Biology, China) *TET and TDG enzymes in DNA demethylation and beyond*. 09.06.2022

Alicja Józkowicz (Jagiellonian University, Poland) *Good seed and good soil: a recipe for the youth of the hematopoietic system*. 23.06.2022

Adolfo Poma Bernaola (Łódź University of Technology, Poland) *Modelling large conformational changes of protein complexes by GōMartini approach*. 30.06.2022

Frank Wien (Synchrotron SOLEIL, France) *DISCO SRCD for structure determination of proteins and nucleic acids*. 22.09.2022

Karli Montague-Cardoso (Nature Publishing Group, Springer Nature) *Life as a professional editor and tips for writing your manuscripts*. 06.10.2022

Aleksandra Pękowska (Dioscuri Centre of Chromatin Biology and Epigenomics, Nencki Institute PAS, Poland) *Towards evolutionary and functional genomics of primate astrocytes*. 13.10.2022

Dirk Grimm (University of Heidelberg, Germany) *The fast and the curious – high-throughput in vivo interrogation of synthetic virus libraries*. 27.10.2022

Matthias Mann (Max Planck Institute of Biochemistry, Germany) *Ultra-high sensitive MS-based proteomics: from bench to bedside*. 03.11.2022

Adam Ktosin (Nencki Institute PAS, Poland) *Regulation of gene expression through biomolecular condensation*. 17.11.2022

Nicola de Franceschi (IMol PAS, Poland) *Pulling, bending, squeezing and splitting: in vitro reconstitution of proteins acting on membranes*. 01.12.2022

Vincent Giguère (McGill University, Canada) *Non-canonical nuclear function of mTOR in gene transcription*. 08.12.2022

David Kwiatkowski (Brigham and Women's Hospital and Harvard Medical School, USA) *Tuberous Sclerosis Complex – insights into pathogenesis and therapy*. 15.12.2022

Michał Brouze (Dziembowski Lab) *The role of cytoplasmic polyadenylation by TENT5 family in gametogenesis*. 14.01.2022

Małgorzata Piechota (Pokrzywa Lab) *CHIP ubiquitin ligase is involved in the nucleolar stress management*. 21.01.2022

Eugeniusz Tralle (Winata Lab) *Tracing the cell lineage evolution of the second heart field*. 21.01.2022

Małgorzata Urbańska (Jaworski Lab) *Trifluoperezine, an antipsychotic drug, inhibits growth of cells derived from SEGA and cortical tubers cultured in vitro*. 11.03.2022

Michał Pastor (Bochtler Lab) *Structural studies of EVE-HNH family of modification specific endonucleases*. 11.03.2022

Patryk Ślusarczyk (Mleczko-Sanecka Lab) *Impaired iron recycling from erythrocytes is an early iron-dependent hallmark of aging*. 18.03.2022

Carlos E. Sequeiros-Borja (Brezovsky Lab) *Chain effect of a single mutation: An ABCG transporter tale*. 18.03.2022

Bartosz Tarkowski (Dziembowski Lab) *mRNAs of hypothalamic neuropeptides are polyadenylated in the cytoplasm*. 25.03.2022

Katarzyna Banasiak (Pokrzywa Lab) *Pheromone signaling regulates exopheresis*. 25.03.2022

Maciej Migdał (Winata Lab) *Modeling transcription factor activity based on next generation sequencing data*. 01.04.2022

Katarzyna Krakowska (Bochtler Lab) *BisI family enzymes – modification specific restriction endonucleases*. 01.04.2022

Jarosław Cendrowski (Miączyńska Lab) *The role of ESCRT-I in crosstalk between endolysosomal trafficking and cell metabolism*. 08.04.2022

Karolina Kasztelan (Dziembowski Lab) *Genome-wide siRNA screen reveals Nuclear Pore Complex components as regulators of double-stranded RNA level in the nucleus of human cells*. 08.04.2022

Almudena Ponce-Salvatierra (Bujnicki Lab) *Bridging the gap: DNA catalysis explained*. 22.04.2022

Juan Zeng (Jaworski Lab) *Molecular mechanism of dendritic arbor stability and its relation to mood disorders*. 22.04.2022

Jacek Jaworski (Jaworski Lab) *An endless search for noncanonical mTOR functions*. 13.05.2022

Mariusz Czarnocki-Cieciura (Nowotny Lab) *Structural characterisation of bacterial Tn7 transposase*. 20.05.2022

Anton Slyvka (Bochtler Lab) *Human dCTP deaminase CDADC1*. 20.05.2022

Łukasz Majewski (Kuźnicki Lab) *Store Operated Calcium Entry (SOCE) and its implication in neuronal activity*. 27.05.2022

Pratik Kumar Mandal (Mleczko-Sanecka Lab) *Identifying unique adaptive responses of red pulp macrophages (RPMs) to iron deficiency*. 14.10.2022

Eugene Baulin (Bujnicki Lab) *Improved RNA structural similarity search method*. 14.10.2022

Joanna Dodzian (Zebrafish Core Facility) *Zebrafish Core Facility – service & infrastructure*. 21.10.2022

Olga Gewartowska (Genome Engineering Unit) *Ways Genome Engineering Unit can help facilitate your research*. 21.10.2022

Honorata Czapińska (Bochtler Lab) *Nε-rule for serine catalytic triads*. 28.10.2022

Malwina Hyjek-Składanowska (Nowotny Lab) *Origins of increased affinity of phosphorothioate oligonucleotides to proteins*. 28.10.2022

Krzysztof Skowronek (Biophysics and Bioanalytics Core Facility) *How Biophysics and Bioanalytics Facility can help in your research*. 04.11.2022

Tomasz Węgierski (Microscopy & Cytometry Core Facility) *Microscopy and Cytometry Facility – service & equipment*. 04.11.2022

Natalia Gumińska (Dziembowski Lab) *Detecting non-adenosine residues in poly(A) tails in an Oxford Nanopore direct RNA sequencing data with machine learning*. 18.11.2022

Vladimir Korzh (Kuźnicki Lab) *Evolution of brain ventricular system*. 18.11.2022

Abhishek Dubey (Pokrzywa Lab) *5-Fluorouracil enhances cold survival by inducing alternative protein turnover pathways*. 25.11.2022

Magdalena Mlostek (Jaworski Lab) *mTORC1 and mTORC2 regulate the development of human iPSC-derived neurons*. 25.11.2022

Ewelina Latoszek (Kuźnicki Lab) *Brain organoids – derivation, characterization and transplantation. Experience from the Brain Organoid course, CAJAL Advanced Neuroscience Training Programme*. 02.12.2022

Costantino Parisi (Winata Lab) *Validation of the cardiac enhancer which drives trabecula-specific expression*. 02.12.2022

Dheeraj Kumar Sarkar (Brezovsky Lab) *On the role of initial seeding of high throughput molecular dynamics for effective and accurate simulation of ligand transport processes in enzymes with buried active sites*. 09.12.2022

Andrea Cappannini (Bujnicki Lab) *NACDDB: Nucleic Acid Circular Dichroism Database*. 09.12.2022

SPOTLIGHT TALKS

Syeda Lubna (Birla Institute of Technology and Science, India) *Computational approaches to understand the effects of substitutions on Influenza viral proteins in the Indian population – leading to altered host-pathogen interactions and viral pathogenicity*. 16.02.2022

Abhishek Sau (Texas A&M University, USA) *Mapping Traffic through Nuclear Pores using 3D Super-Resolution Microscopy*. 02.03.2022

Sanchita Mukherjee (Rigel Bioenviron Solutions Private Limited, India) *The happy secret to entrepreneurial journey from academia*. 16.03.2022

Anna Karnkowska (Institute of Evolutionary Biology, University of Warsaw, Poland) *The other eukaryotes: lessons learned from the non-model microbial eukaryotes*. 29.03.2022

Paul Tapas (Johns Hopkins University, USA) *Vectorial folding of telomere overhang promotes higher accessibility*. 05.04.2022

Nirmal Sampathkumar (University of Oxford, UK) *How to determine stable reference genes to use by qPCR in the absence of RNAseq?* 19.04.2022

Amina Mirsakiyeva (Carl Zeiss SMT, Germany) *Academia vs Industry: how to choose?* 27.04.2022

Anna Bajur (King's College London, UK) *CD20-dependent actin remodelling controls initial steps of B cell activation*. 11.05.2022

Logan Mulroney (Italian Institute of Technology, Italy) *Detecting RNA modifications from nanopore ionic current signals*. 25.05.2022

Tomasz Włodarski (University College London, UK) *A computational microscope to study co-translational protein folding*. 29.06.2022

Sohini Sarkar (Western New Mexico University, USA) *Advances in Vibrational Stark Shift Spectroscopy for Measuring Interfacial Electric Fields*. 28.09.2022

Shamasree Ghosh (Umea University, Sweden) *Understanding the differential effect of apolipoprotein E isoforms on the aggregation of amyloid-β*. 26.10.2022

Ananya Rakshit (University of Colorado, USA) *Transcription factor manipulation results in altered Zn^{2+} dynamics in the mamalian cell cycle*. 09.11.2022

Tanaya Bose (Weizmann Institute of Science, Israel) *Prebiotic peptide bond formation by the proto-ribosome: a missing link between RNA and protein dominated world*. 23.11.2022

CENTRE FOR INNOVATIVE BIOSCIENCE EDUCATION

Head
Mikołaj Cup, MSc (since October 2022)
Patrycja Dołowy, PhD (until September 2022)

Project Manager
Agnieszka Muśnicka MSc (since March 2023)
Katarzyna Tomaszewska, MSc (until December 2022)

Laboratory Manager
Paweł Morga, MSc (since August 2022)
Aleksandra Olszańska, BEng (until July 2022)

Communications and Promotion Specialist
Jan Malinowski, MSc (since January 2022)

Volunteer Representatives
Aleksandra Maciejczuk
Małgorzata Pełka
Stanisław Szleszkowski

Educators
Julia Gilewska
Aleksandra Kowalczyk
Miłosz Majka
Małgorzata Malczewska
Maksymilian Nowak
Małgorzata Orłowska
Jakub Tomaszewski

The Centre for Innovative Bioscience Education (BioCEN) was established in 2002 by IIMCB, Nencki Institute of Experimental Biology, Polish Academy of Sciences (Nencki Institute); Institute of Biochemistry and Biophysics, Polish Academy of Sciences (IBB); and Science Festival in Warsaw.
BioCEN exists to bridge the gap between the scientific community and society by providing educational activities that popularize modern experimental biology among the broader community. We use innovative educational methods to provide hands-on experience in topics of interest. Our workshops, presentations, and educational materials are based on sound scientific findings and the expertise of our collaborators. BioCEN receives financial support from IIMCB, which has been BioCEN's Strategic Sponsor since 2015. In addition, BioCEN is subsidized by Nencki Institute, IBB, University of Warsaw Faculty of Biology, and BioEducation Foundation.

The year 2022 was marked by the 20th anniversary of BioCEN and the outbreak of war in Ukraine. Our activities continued to be influenced by the global coronavirus pandemic; hence, we still conducted many activities remotely within the framework of the BioCEN online laboratory and Zoom platform. In June 2021, we began to conduct outdoor workshops in city parks and school yards. BioCEN also held workshops in various schools in Warsaw and outside the city, including schools in small towns and villages. Many of these activities have been permanently incorporated into the general framework of BioCEN and continued throughout 2022. Since the outbreak of war, we have taken steps to support refugee students from Ukraine. We introduced workshops that are linguistically accessible to them and employed teachers (refugees from Ukraine) to conduct workshops together with our team.

Last year was also a year of summaries. For 20 years, we have conducted workshops for over 80,000 students. We run approximately 400 workshops annually. In 2022, we conducted outdoor workshops at schools and science festivals and indoor laboratory workshops in the University of Warsaw Faculty of Biology and Warsaw University of Life Sciences Institute of Biology. Over 2,000 people benefited from our lectures and symposia for teachers.

ACTIVITIES

A total of 7,084 students participated in laboratory and outdoor workshops in 2022, and our materials reached ~8,000 recipients. Moreover, various BioCEN online activities accumulated over 126,000 views.

Our online courses that are organized for biology teachers are building a connection between educators and scientists so they can feel how both groups are important in the scientific community. We strongly encourage teachers to implement practical scientific research protocols in their schools. We equip teachers with classroom activities and affordable experimental kits that can be used in school settings. During the pandemic, we created many additional materials that can be helpful in both home learning environments and schools.

LABORATORY WORKSHOPS

BioCEN workshops cover various areas of life sciences and basics of medicine, with a focus on practical and experimental approaches. Our goal is to cover several scientifically and educationally important topics, such as molecular and cell biology, histology, immunology, biochemistry, biotechnology, microbiology, biophysics, plant physiology, bionics/bioengineering, environmental sciences, and medical sciences. We encourage participating students to express their creativity while working individually on real-life experiments. In 2021, we introduced new workshops to fit the pandemic circumstances, three of which were implemented into permanent offerings of BioCEN in 2022: *CSI: BioCEN*, *Sixth Sense*, and *Out of this World: An introduction to Astrobiology*. Throughout 2022, BioCEN held workshops at the University of Warsaw, the Warsaw University of Life Sciences, and schools in six voivodeships. Materials and syllabi for *CSI: BioCEN* and *Sixth Sense* workshops were created within the framework of BioCEN, co-funded by the project of the Education Department of the Capital City of Warsaw *I Am Experimenting in a Science Laboratory! Biology Laboratory Workshops for Students of Warsaw Elementary Schools and High Schools*. Materials and syllabus for the *Out of this World: An introduction to Astrobiology* were funded by the Polish Academy of Sciences.

ONLINE EVENTS

The BioCEN online laboratory is a remote space that was created especially for educational purposes (biocen.edu.pl/laboratorium-on-line). Interactive materials were made accessible as free downloads in the form of video demonstrations, including virtual reality, mini-scripts, experimental protocols, and podcasts. In 2022, these materials had approximately 8,000 views. The platform is dedicated to youths and adults and includes interactive materials that are simultaneously published on Facebook where they accumulated 126,000 views.

Within the framework of the BioCEN online laboratory, we offered 1.5-hour classes that occurred in real-time via the Zoom platform in groups of 5-25 participants. Classes were conducted in two forms: (1) interactive seminars for people 14+ years old, including *What are Today's Methods of Curing Cancer?*, *Why Do We study Euglenas?*, *The Immune System: The Power Behind the Throne of Health and Illness*,

How Medicines Are Developed, *Obesity and Health*, *Vaccinations*, *The Beginnings of Eukaryotes*, and *The First Multicellular Organisms*, and (2) online science demonstrations with elements of theory for high schools, including *In Vitro Cell Cultures*, *Synergy, and Redox: The Quintessence of Life Scientific Experiment: It Isn't Difficult!*, *Melatonin: Polluted with Light*, *The Microcosm: The World of Bacteria*, and *The Brain: The Orchestra Between the Ears*, and for elementary schools, including *Scientific Experiment: It's Not Difficult!*, *The World of Chemical Reactions*, and *The Microcosm: The World of Bacteria*. Since 2021, 168 classes have been held.

students and graduates of BioCEN workshops. Among the guests of the conference were Prof. Jerzy Duszyński (President of the Polish Academy of Sciences and founder of BioCEN), Prof. Jacek Kuźnicki (founder of BioCEN, Chairman of BioCEN Council), Prof. Marta Miączyńska (Director of IIMCB), Dr. Urszula Białek-Wyrzykowska (Deputy Director for Development of IIMCB), Dr. Patrycja Dotowy (outgoing Head of BioCEN), Mikołaj Cup (new Head of BioCEN), Dr. Joanna Lilipop (former Head of BioCEN), and Dr. Agnieszka Chołuj (former Head of BioCEN). Popular science lectures were delivered by Prof. Paweł Golik (Chairman of the Council for the Promotion of

The activities accompanying the Anniversary Science Festival were promoting the event, as well as the idea of science education, in the media and on the web. The main channels for those activities were internet portals, social media and community-generated broadcasting services such as Youtube and Vimeo. The event created a platform for sharing scientific stories and memories of BioCEN's activities, including the story of Prof. Magdalena Fikus. There were also videos with insights and reflections regarding the mission of BioCEN (including Prof. Jacek Kuźnicki, the former Director of IIMCB and Prof. Agnieszka Dobrzyń, the Director of the Nencki Institute). The materials available online reached 29,373 recipients.

From October 2021 to December 2022, BioCEN has organized online meetings in the form of interviews with invited scientists, hosted by Patrycja Dotowy. Teachers and students choose the topic, and BioCEN proposes the scientist. In 2022, among the invited guests were an astrobiologist, a human DNA researcher, a neurologist, a brain researcher, an oncologist, an arachnologist, a nematologist, a biotechnologist, and an ichthyologist.

20th ANNIVERSARY CONFERENCE

For its 20th anniversary, BioCEN, together with founding institutes and the Council for the Promotion of the Public Understanding of Science (Polish Academy of Sciences), organized a conference on October 8, 2022, at IIMCB. The event was attended by 69 people, including representatives of the world of science, popularization, and education, as well as the Public Understanding of Science, Polish Academy of Sciences; evolutionary genetics), Dr. Takao Ischikawa (Faculty of Biology of the University of Warsaw; promising research on genetic diseases), Dr. Jarosław Bryk (University of Huddersfield, Great Britain, originator of BioCEN; applications of molecular biology and research with Nobel Prize winner Prof. Svante Pääbo), and Małgorzata Malczewska (BioCEN team; archaea isolated from undersea thermal springs). Wiktor Niedzicki (science journalist), Wawrzyniec Kofta (teacher from Władysław IV High School in Warsaw), Halina Podgórska (teacher from K. Hoffmanowa High School in Warsaw), and students from these schools took the floor. The scientific demonstration was conducted by Mikołaj Cup, Jakub Janiec, and Stanisław Szleszkowski from the BioCEN team. In addition to popular science lectures, speeches, and a show, the conference program included a "scientific" cake.

WORKSHOPS FOR REFUGEES

In collaboration with the Council for the Promotion of the Public Understanding of Science (Polish Academy of Sciences) and Ukrainian House in Warsaw within the project *Vaccine Means Health*, BioCEN conducted a workshop on vaccines for refugee children. The scripts were released in four languages: English, Polish, Russian, and Ukrainian. After the war and refugee crisis broke out, we hired Ukrainian- and Russian-speaking teachers and animators to provide our workshops for groups of war refugees in schools and in the laboratory at the University of Warsaw.

SCIENCE CAFE FOR HIGH SCHOOL STUDENTS

BioCEN, together with KARROT Cafe, created classes that were dedicated to high school seniors. Mikołaj Cup and Stanisław Szleszkowski from our team taught students how to pass the graduation exam in biology in Polish and English simultaneously. We provided these workshops for refugee teenagers free of charge. Two refugee teenagers took advantage of this opportunity. Meetings occurred each week between March and June 2022.

PROFESSIONAL TRAINING FOR TEACHERS AND EDUCATORS

One of our main goals is to improve teaching skills of science educators who work at all levels of education. In 2022, BioCEN, together with the Nencki Institute, organized an annual event, the 21st Symposium for Teachers of Biology, which occurred on December. Participation was available both live (32 participants) and online on the Zoom platform (163 participants). This annual symposium has become one of our most important recurring events. During the 2022 meeting, biology teachers from all over Poland had the opportunity to receive up-to-date information on frontline discoveries and become more familiar with cutting-edge studies, such as those that are related to the Nobel Prize in Physiology and Medicine and the Nobel Prize in Chemistry. The symposium program included a presentation by Mikołaj Cup on how to combine reporting of cutting-edge research with high school syllabi.

EXPERIMENTAL KITS AND OTHER SCIENTIFIC TOOLS

We provide alternatives for those who are unable to attend our workshops. BioCEN produces laboratory kits that are commercially available on our website (biocen.edu.pl/en/experimental-kits). All kits come with the necessary chemicals, dishes, tubes, theoretical summaries, instructions, and protocols that are needed by students to perform experiments either at school or at home. Over 50 such kits were distributed to schools and private customers in 2022.

The following experimental kits are available:

- *We Are Studying DNA*
- *The Sweet World of Enzymes*
- *Photosynthetic Dyes*
- *A Necklace with Your Own DNA*

We also emphasize the notion of "learning while playing" as we produced the high-quality BioCEN educational board game *Re-action! Lost Experiment*.

OTHER ACTIVITIES

BIOCAST PODCASTS

BioCAST podcasts are provided by the Centre for Innovative BioScience Education, based on casual talks on biology and science in general, and prepared according to Mikołaj Cup's and Jan Malinowski's idea, both from the BioCEN team. The third season aired in 2022 had five episodes that focused on common myths and misconceptions about scientific achievements. BioCAST is posted on BioCEN's website and other websites, including Spotify, Anchor, Apple Podcasts, Google Podcasts, Overcast, Amazon Music, Mixcloud, iHeartRadio, Castbox, Pocket Casts, RadioPublic, Castro, and Stitcher. BioCAST already has ~2,500 regular subscribers.

In 2022, the podcasts reached more than 10,700 listeners. Our greatest pride regarding BioCAST is the 5/5 audience rating on Spotify, Apple Podcasts, and Google Podcasts.

BioCEN participated in science popularization events in 2022, including the 10th Intercollegiate Biotechnology Symposium *Symbioza* in May, *Eksploracje* Science Picnic in Rzeszów in May, *The Night of Museums* in Kępno in May, 5th School Science Picnic in Józefosław in September, The School Science Day at Hoffmanowa High School in Warsaw in September, and 26th Festival of Science in Warsaw (in collaboration with IIMCB) in September. Together with the March for Science Foundation, BioCEN has continued a run event, *BioCEN Is Vaccinated*, as part of the *March Pro Vaccination* action.

AWARDS AND HONORABLE MENTIONS

BioCEN, together with GD Events, received an honorable mention in the *Nature* category in the Warsaw edition of the *Stoneczniki 2022* competition for the most developmental initiative for children. This trophy is especially important for our team because the award is based on popular vote.

BIOCEN ANIMATORS AND CO-WORKERS

Important members of the BioCEN team include animators and coworkers, without whom our educational activities would not be possible. In 2022, the following individuals collaborated with BioCEN: animators (Dasza Babiuk, Łukasz Biesiadecki, Julia Brzykcy, Julia Gilewska, Rafał Jabłuszewski, Jakub Janiec, Marcin Kleibert, Aleksandra Kowalczyk, Roman Krasowski, Katarzyna Krzyżewska, Mikołaj Kuska, Mitosz Majka, Małgorzata Malczewska, Paweł Morga, Maksymilian Nowak, Małgorzata Orłowska, Malina Pełka, Małgorzata Pełka, Jakub Tomaszewski, Gustaw Zientarski), senior trainers (Aleksandra Maciejczuk, Stanisław Szleszkowski), laboratory support personnel (Tamara Aleksandrzak-Piekarczyk, Izabela Kern-Zdanowicz, Anna Osinka), designer (Paulina Czyż), public relations expert (Jan Malinowski), and Management Board members (Patrycja Dotowy, Maciej Kotliński).

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Senior Specialists
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Agata Szulim

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Paweł Kobylarz

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Piotr Świstowski (part-time)

Senior Specialist
Jakub Skaruz

Specialists
Łukasz Munio
Michał Taperek

Junior Specialists
Kacper Kolasiński
Krzysztof Pakuła

CONTENTS

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