

ANNUAL REPORT 2021

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY IN WARSAW

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Deputy Director for Science
Jacek Jaworski

Deputy Director for Development
Urszula Białek-Wyrzykowska

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DIRECTOR'S NOTE

Another year of the new pandemic-imposed reality is behind us. COVID-19 deeply affected all aspects of our lives as individuals and societies. Still, the year 2021 can be viewed as a success of science that provided us with powerful weapons against the virus. For me and many other scientists, this is gratifying and motivating at the same time. It clearly demonstrates that collective efforts of researchers spending long hours in laboratories all over the world do translate into inventions that serve humanity.

mRNA-based vaccines are one of such breakthrough inventions that herald an upcoming era of RNA-based therapeutics. To develop the latter we need a detailed understanding of the properties and metabolism of various RNA molecules inside cells and in whole organisms. To meet this need is an overarching goal of the research conducted by several laboratories at the IIMCB that focus on RNA biology. In 2021, we officially inaugurated the Laboratory of RNA-Protein Interactions - Dioscuri Centre led by Prof. Gracjan Michlewski. One year earlier the Laboratory of RNA Biology - ERA Chairs Group headed by Prof. Andrzej Dziembowski started its operations. The arrival of these groups has complemented the already existing expertise of other IIMCB laboratories, increasing the Institute's visibility as a center of RNA research. Moreover, the new arrivals have fostered further scientific interactions between IIMCB research groups, leading to additional exciting collaborations and successful collective grant applications. One of them, the Virtual Research Institute grant, worth over 69 million zloty (approx. 15 million euro), will focus on the application of mRNA technology in immuno-oncology to develop mRNA vaccines for cancer treatment. The IIMCB is the leader of this project that is directed by Prof. Andrzej Dziembowski with the participation of Prof. Marcin Nowotny and myself, and includes scientists from three other Polish institutions: the University of Warsaw, Warsaw Medical University, and the Institute of Physical Chemistry of the Polish Academy of Sciences. The basic research discoveries resulting from the project will be commercialized by PORT Polish Center for Technology Development in Wrocław which operates the Virtual Research Institute program.

The IIMCB strives to be an attractive partner for innovative industries. Last year we signed a collaboration agreement with a Polish biotech company OncoArendi Therapeutics to develop small molecule drugs modulating RNA function. Specifically, Prof. Janusz Bujnicki and his team will work together with the company scientists to optimize a bioinformatics platform, which will be used to discover new small molecules. Moreover, in 2021 we established a business-oriented Genome Engineering Unit at the IIMCB that generates transgenic mouse models as well as provides cloning and genotyping services for academic and industry customers.

In 2021, IIMCB scientists reported the results of their studies in 77 publications. Among the most notable reports are studies on the metabolism of RNA poly(A) tails, carried out in yeast and mouse models, or the investigation of cellular mechanisms of oncogenic signaling by the AXL receptor (see section on Best Papers Award in this Annual Report). Despite physical distancing, we have had a vibrant program for remote scientific exchanges. We hosted a range of distinguished speakers from all over the world at Open IIMCB Online Seminars, several representatives of the international postdoc community as speakers of Spotlight Talks initiated by IIMCB postdocs, and, last but not least, many of our scientists presented their findings at the Internal Seminar Series (see section on seminars in this Annual Report).

Two additional highlights of the scientific life at the IIMCB in 2021 were online symposia organized by PhD students and postdocs (see section on scientific meetings). The International Young Scientists Conference on Molecular and Cell Biology 2021 took place on February 25-26 and was attended by 218 people from 10 countries. Keynote lectures were delivered by prominent scientists whereas aspiring researchers presented their studies as short talks or posters, and interacted with experienced mentors. The 1st Women in Science Symposium WiSS (March 4-5, 2021) hosted over 300 participants from nearly 30 countries who took part in lectures, workshops, and mentoring sessions. This meeting aimed at increasing the awareness of young scientists of their own potential and career opportunities with special emphasis on women's role in the scientific world.

These activities were very much in line with the IIMCB's mission to help our staff, both scientific and administrative, develop their careers in a supportive and inclusive environment. In 2021, we undertook further institutional improvements in this area by introducing a Gender Equality Plan as well as a Disputes and Conflicts Resolution Policy. The latter set of regulations unifies several previous procedures in one comprehensive document that describes the role of mediators at the IIMCB and how they may resolve conflicts, as well as the rules preventing mobbing and discrimination. In 2021, we initiated training sessions on cross-cultural interactions and competences. The programs of these sessions were tailored to various groups of our staff. We will continue these activities in 2022 to improve mutual understanding, acceptance, and efficient communication between staff members of different origins and cultural backgrounds.

Finally, the year 2021 brought us one step closer towards our long-standing goal to have a modern and spacious building for the IIMCB. In September 2021, after a two-year long procedure delayed due to the COVID-19 pandemic, IIMCB finally bought a building plot on Flisa Street in Warsaw that will become the future site of the Institute. I would like to express my special thanks to Anna Zolnik, Deputy Director for Operations, who was the driving force of this purchase, supported by my predecessor Prof. Jacek Kuźnicki. We are now looking forward to the next step, namely an architectural design competition for the new building that should take place in 2022.

As every year, at the IIMCB we look into the future with optimism, powered by many ideas that we develop together, through brainstorming, consultations and constructive disputes among various groups of staff. The guiding principle of all our activities is the commitment to excellent science driven by passionate researchers who are supported by professional administration. We remain united and engaged in realizing our ambitious vision to constantly develop the IIMCB as a research center in molecular and cell biology, which successfully translates its findings for the benefit of society.



Marta Miączyńska

DIRECTORS



Marta Miączyńska
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 IIMCB

The International Institute of Molecular and Cell Biology in Warsaw (IIMCB) was established in Poland in 1995 under an international agreement between the government of the Republic of Poland and UNESCO which was formally enacted by the Polish parliament on the 26th of June, 1997. The provisions of these documents have secured the Institute's special status and independence since it started its operations in 1999.

The Institute is directly overseen by the President of the Polish Academy of Sciences (PAS), who supervises the organization and activities of our Institute, as well as nominates members of the International Advisory Board (IAB) and the Institute's Director. An important role is played by the IAB that provides strategic advice on research directions of the Institute, evaluates the scientific output of laboratories, and provides an assessment of candidates for the positions of Laboratory Leaders and Director.

IIMCB is a research institute in the field of life sciences, holding the highest scientific category (A+) based on a parametric evaluation of research entities in Poland that was conducted by the Ministry of Science and Higher Education (current Ministry of Education and Science) in 2017. The main objectives of IIMCB include: basic research in the field of molecular and structural biology and bioinformatics (structure and function of proteins, nucleic acids and their complexes, genomics and epigenomics), cell biology (transport within the cell, signalling pathways) and molecular medicine (mechanisms of neoplasmic growth and ageing, molecular basis of metabolic, neurological and neurodegenerative diseases). The results of IIMCB's studies are disseminated through leading journals, often in the open access system. The second objective of the activities of IIMCB is a practical application of scientific achievements related to civilization diseases and genetically-based diseases, providing biotechnology solutions that can be used in the economy and as research services sold to commercial companies. Moreover, IIMCB is involved in various educational programs as well as popularization activities performed by the Centre for Innovative Bioscience Education (BioCEN). Scientific excellence which we pursue in IIMCB involves the implementation of ambitious research projects and scientific initiatives, and forming partnerships with leading research centers in Poland and abroad.

INTERNATIONAL ADVISORY BOARD

MEMBERS



Walter Chazin

Vanderbilt University, USA
Chair of the Board



Thomas Braun

Max Planck Institute for Heart and Lung Research, Germany



Bernd Bukau

University of Heidelberg, Germany



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Institute of Genetics and Molecular Medicine, France



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Max Planck Institute for Biophysical Chemistry, Germany



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Adam Mickiewicz University, Poland



Caroline Kisker

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Peter Sicinski

Harvard Medical School, USA



Lilianna Solnica-Krezel

Washington University School of Medicine, USA



Anne Spang

University of Basel, Switzerland



Barry Stoddard

Fred Hutchinson Cancer Research Center, USA

PERMANENT ADVISOR



Angelo Azzi

Tufts University, USA

ORGANIZATIONAL STRUCTURE

DIRECTOR

- Aging and Longevity Strategic Project
- Auresine Strategic Project
- Genome Engineering Unit
- Human Resources Unit
- Self-contained position for strategic support
- Self-contained position for veterinary affairs
- Self-contained position for OHS
- Institute's Archives

DEPUTY DIRECTOR FOR DEVELOPMENT

- Biophysics and Structural Biology Facility
- Microscopy and Cytometry Facility
- Zebrafish Core Facility
- Scientific Coordination Unit
- PR Unit
- Self-contained position for commercialisation
- Centre for Innovative Bioscience Education

DEPUTY DIRECTOR FOR SCIENCE

- Laboratory of Structural Biology
- Laboratory of Bioinformatics and Protein Engineering
- Laboratory of RNA Biology – ERA Chairs Group
- Laboratory of Molecular and Cellular Neurobiology
- Laboratory of Neurodegeneration
- Laboratory of Cell Biology
- Laboratory of RNA-Protein Interactions – Dioscuri Centre
- Laboratory of Iron Homeostasis
- Laboratory of Protein Structure
- Laboratory of Protein Metabolism
- Laboratory of Zebrafish Developmental Genomics
- Laboratory of Biomolecular Interactions and Transport AMU/IIMCB
- Grants Office

DEPUTY DIRECTOR FOR OPERATIONS

- IT Unit
- Operations Unit
- Public Procurement Unit

DEPUTY DIRECTOR FOR FINANCE/ CHIEF ACCOUNTANT

- Financial and Accounting Unit

MISSION

We support ambitious scientists of any nationality, driven by passion to pursue frontier research that aims to make a difference for society. We follow the principles of scientific freedom, integrity, and responsibility. We help researchers develop their careers through training and mentoring at all levels, and we encourage collaborations among them. We provide efficient administrative support that enables scientists to focus on their research.

GOALS OF THE IIMCB DEFINED IN THREE MAIN AREAS

SCIENTIFIC QUALITY

- Make important scientific discoveries and report them in high-quality publications
- Strive for scientific excellence in our research, rather than simply collecting points in the parametric evaluation of Polish research institutions
- Be internationally recognized among the best research institutions in Europe

INSTITUTIONAL DEVELOPMENT AND PARTNERSHIPS

- Obtain a larger building and reach a critical mass of ~20 research groups with complementary expertise, supported by professional state-of-the-art core facilities
- Increase internal synergies between research groups
- Build strong national and international networks of academic and industrial partners for intellectual exchange, collaboration, and training
- Improve the visibility of IIMCB, also through enhanced activity in social media

ORGANIZATIONAL CULTURE

- Give every staff member a sense of common mission and shared responsibility
- Ensure transparent internal regulations, including the principles of the equal treatment of all coworkers and stipulations of the HR Excellence in Research Award
- Support the career development of all coworkers
- Provide a clear institutional structure, effective internal procedures, and the division of duties
- Lessen administrative duties for scientists
- Support collegiality at all levels of the Institute
- Foster a professional and friendly work atmosphere and effective internal communication among all staff members
- Care for the common property and areas of the Institute
- Adjust the organization and management of the Institute according to its growth and emerging needs

EU-LIFE

GENERAL INFORMATION

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

EU-LIFE is an alliance of research centers 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 consortium 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.

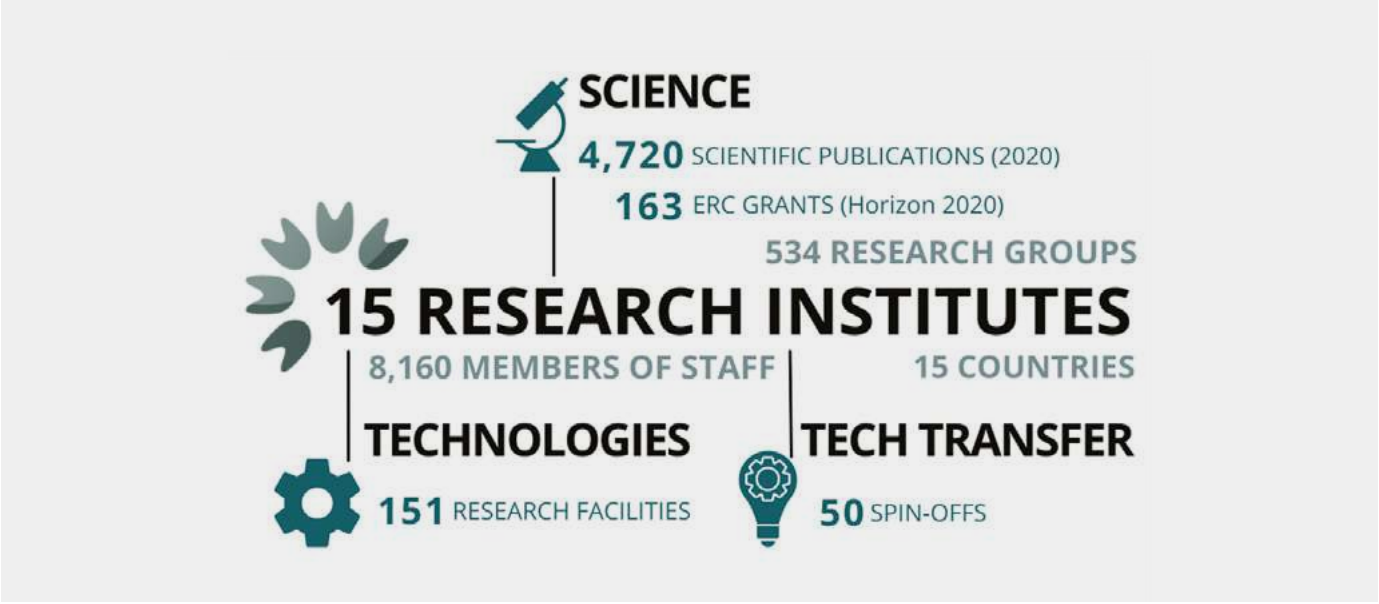
ORGANIZATION

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 group is composed of a Board of Directors of member institutes, their main representative, the EU-LIFE Executive Director, and chairs of Working Groups. The IIMCB representatives in the Strategy Group are Marta Miączyńska as Director of IIMCB and Urszula Białek-Wyrzykowska as the main representative.

The Core Facilities Working Group is a forum for discussing core facility-specific challenges. The main themes of 2021 were the preparation of an EU-LIFE Core Facilities Benchmarking Report that is based on a survey and contains a wide variety of operational parameters across partner institutes, the launch of a series of TechWatch webinars that presented new research technologies, and sharing best practices and expertise in Core Facility management. In 2022, the Working Group will concentrate on ways to secure the recognition and acknowledgment of Core Facilities in research publications and issues that are related to Core Facility-specific career development and research data management (in collaboration with the IT Working Group). The IIMCB representatives in this Working Group are Joanna Dodzian and Krzysztof Skowronek.

The Gender Equality Working Group coordinates gender equality activities, develops indicators for monitoring gender equality issues in EU-LIFE institutes, and shares good practices in this area. In 2021, the Working Group implemented the following topics: (i) three bullying and harassment workshops for Directors and Human Resources Heads, (ii) defining goals and identifying external providers for EU-LIFE active bystander training, and (iii) two pilot sessions of bystander training for group leaders. The IIMCB representative in this Working Group is 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 2021, the Working Group's work covered three distinct areas: sharing best practices in applying for competitive funding, brainstorming about resources and training that the grants office can offer internally to their researchers, and gathering statistics on the participation of EU-LIFE institutes in Horizon Europe. The IIMCB representatives in this Working Group are Dorota Libiszowska and Marcin Ogonowski.

The Technology Transfer Working Group is a platform for sharing experiences and good practices in the field of intellectual property, licensing, and spin-off creation. In October 2021, the Technology Transfer Working Group organized an online pitching event to identify projects with commercialization potential from selected advanced scientific projects of EU-LIFE research institutions. Pitching events offer scientists an opportunity to talk confidentially to venture capitalists and professionals with venture creation experience to gain insights into, and feedback on, key data and adjustments of business plans. The next pitching event is planned for October 2022 in Crete. Since March 2021, the IIMCB representative in this Working Group is Iwona Pilecka.

The IT Working Group is a community of specialists who are dedicated to addressing IT challenges. The main domains of discussion in 2021 were big data analysis, network access control, and explorations of remote work environments. In 2022, the Working Group will discuss and test the Integrated Rule-Oriented Data System (iRODS) as an example of secure data storage. The IIMCB representative in this Working Group is Paweł Kobylarz.

The Recruitment and Training Working Group focuses on the following issues: ensuring continuing professional development for researchers at all stages of their careers, supporting partner institutes in the recruitment process by sharing job offers and opportunities, mobility experiences (overcoming administrative barriers), and defining best practices in terms of grants and employment contracts, with a special focus on postdocs. In 2021, the Working Group concentrated on sharing best practices and collected information from institutions that are affiliated with EU-LIFE. The group began preparing the final database with courses that are organized in EU-LIFE institutes. Based on the database, gaps and synergies will be identified to allow applications for funding common courses in the future. The IIMCB representatives in this Working Group are Agnieszka Faliszewska and Katarzyna Marszałek.

The Science Communication Working Group is a forum for sharing good practices and experiences of people who are responsible for popularization and promotion. The main points of interest include media and public relations management, common strategies in social media, scientific output, and sharing best practices on benchmarking papers. This Working Group also designs and implements the EU-LIFE internal and external communication strategy. In 2021, the Working Group launched a long-term Twitter campaign to which all EU-LIFE members have been introduced. The main topics covered COVID-19, science policy, science for society, and research excellence. The group's activities also focused on strengthening storytelling skills, which resulted in a series of workshops that were organized in 2021. The IIMCB representative in this Working Group is Daria Goś.

www.eu-life.eu

MEMBERS

HORIZON 2020 ERA CHAIRS PROJECT AT IIMCB: MOSaIC

"MOLECULAR SIGNALING IN HEALTH AND DISEASE – INTERDISCIPLINARY CENTRE OF EXCELLENCE"

Project Coordinator
Jacek Kuźnicki

EC Project Officer
Cristina Marcone

Project Manager
Dorota Libiszowska

Implementation period
2018-2023

Funding
2 498 887.50 EUR

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 have established the ERA Chairs Research Group, headed by an outstanding scientist, Andrzej Dziembowski, and have been introducing structural improvements in science management and HR activities for more efficient support for researchers. Until October 31, 2021, MOSaIC has been implemented for three years and we highlight the project's main achievements during this time.

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 the transcriptomes of mammalian cells through RNA degradation and how poly(A) and poly(U) polymerases regulate protein production.

AWARDED GRANTS

- **HERO WIB**, Polish Science Fund, Andrzej Dziembowski (Leader), other IIMCB groups involved: Miączyńska and 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, and 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

DISSEMINATION OF ERA CHAIRS GROUP RESEARCH RESULTS

IIMCB Best Papers Award

PUBLICATIONS THAT ACKNOWLEDGE MOSAIC

Liudkovska V, **Dziembowski A**. Functions and mechanisms of RNA tailing by metazoan terminal nucleotidyltransferases. ***Wiley Interdiscip Rev RNA***, 2021; 12(2):e1622

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

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

Hojka-Osinska A, Chlebowski A, Grochowska J, Owczarek EP, Affek K, Kłosowska-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

SEMINARS AND POSTERS AT INTERNATIONAL MEETINGS AND CONFERENCES

Andrzej Dziembowski, lecture: *A tale of tails in yeast and mouse – what we can learn from Direct RNA sequencing on Nanopores*, Institute Pasteur Séminaire du Département Génomes & Génétique

Andrzej Dziembowski, lecture: *TENT5 cytoplasmic non-canonical poly(A) polymerases regulate the innate immune response in animals*, CSHL EUKARYOTIC mRNA PROCESSING

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

Vladislava Liudkovska, poster: *TENT5 cytoplasmic non-canonical poly(A) polymerases regulate the innate immune response in animals*, 26th Annual Meeting of the RNA Society

RENOWNED SCIENTISTS GAVE OPEN SEMINARS 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*

EFFECTIVE SCIENCE MANAGEMENT AND IMPROVED HR ACTIVITIES

MOSAIC SUPPORTED OPEN ACCESS AND ETHICS PRACTICES AT IIMCB

- Support for IIMCB researchers in Open Access publishing, preparation of data management plans and ethics for their project proposals
- Securing Genetically Modified Organisms and Microorganisms work permits
- Initiation of work on Research Data Management (RDM) internal policy: participation in workshops, creation of RDM working group, conducting a questionnaire on RDM among lab leaders

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 2021, 17 PhD students affiliated with IIMCB enrolled in Warsaw-4-PhD, attended various courses, and worked on their research projects. IIMCB's dedicated staff has provided efficient management of the school's procedures, recruitments and promotional activities.

TOWARD TRANSLATIONAL RESEARCH BY IIMCB WITH SPARK

The SPARK Global network promotes translational research worldwide. IIMCB participated in preparation of the SPARK Poland concept with the Nencki Institute of Experimental Biology (Polish Academy of Sciences) as a leader. One of the first SPARK Poland initiatives was the mentoring program that was launched in January 2020. Two IIMCB projects were admitted to the program:

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

Within the framework of MOSaIC, IIMCB promotes and facilitates access to SPARK Global seminars, trainings and workshops for IIMCB Staff: 24 webinars and 2 training courses.

HR ACTIVITIES AND HR STRATEGY

Thanks to MOSaIC, IIMCB employed a professional HR manager who organized the HR Unit according to advanced standards composed of personnel with required competencies. Soon after, the HR 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, and management
- Comprehensive system of support for foreign employees
- Disputes and Conflicts Resolution Policy

Human Resources also played a central role in developing the Gender Equality Plan for IIMCB in line with best European standards. They have 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.

www.mosaic.iimcb.gov.pl

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



HUMAN RESOURCES STRATEGY FOR RESEARCHERS

The year 2021 was marked by the COVID-19 pandemic. We worked in accordance with rules we established in 2020 to continue ensuring the safety of our employees and simultaneously maintain scientific and administrative activities of the Institute. We consequently performed tasks based on the HR Strategy for IIMCB that was implemented within the Horizon 2020 MOSaIC project, "MOlecular Signaling in Health and Disease – Interdisciplinary Centre of Excellence," in line with Human Resources Strategy for Researchers (HRS4R) guidelines. We also performed tasks that are included in the project, "Integrated Support Programme for Foreigners at IIMCB," granted by the National Agency for Academic Exchange within the Welcome to Poland Program.

We managed to accomplish most of these activities thanks to support and cooperation of IIMCB administrative and scientific staff.

KEY ACTIVITIES IN 2021

- Implementation of the **Recruitment Process for Non-Scientific Positions**
- Support for Institute **activities that promote integration and staff development**, including co-organizing an integration meeting
- Assistance with organizing the **1st Women in Science Symposium**
- Organization of **cross-cultural training** for directors, administrative staff, senior researchers, and researchers
- Organization of **training in scientific illustration** for postdoctoral researchers, researchers, and senior researchers
- Preparation of **Disputes and Conflicts Resolution Policy**, which includes the prevention of mobbing, anti-discrimination activities, and the institution of the employee mediator
- Establishment of the **Working Group on Gender Equality Opportunities** and elaboration of **Gender Equality Plan for IIMCB**

RESEARCH GROUPS



LABORATORY OF STRUCTURAL BIOLOGY

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)
Magdalena Klimczak, MSc Eng.
Katarzyna Krakowska, MSc
Michał Pastor, MSc
Abhishek Pateria, MSc Eng.
Dominik Rafalski, MSc Eng.
Anna Stroynowska-Czerwińska, MSc Eng.

Other Co-worker

Terry Karimi, MSc

Technician

Julia Pac, MSc (part-time)

Laboratory Support Specialist

Aleksandra Jakielaszek, MSc Eng.

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

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.

SELECTED PUBLICATIONS

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DESCRIPTION OF CURRENT RESEARCH

Our laboratory works on chromatin modifications. Most of our work focuses on DNA methylation, but we have also worked on other chromatin modifications and associated DNA reader domains.

Replication of DNA methylation

As a consequence of semi-conservative DNA replication, each daughter cell inherits one methylated strand and one non-methylated DNA strand. Traditionally, the reestablishment of DNA methylation in the nascent strand is attributed to parental-strand instruction-guided activity of the maintenance methyltransferase DNMT1. However, the low fidelity of maintenance methylation on naked DNA *in vitro* and presence of histone binding domains in DNMT1 and other replisome-associated proteins (e.g., UHRF1) suggest that methylation is partially conveyed by cross-talk with histone modifications. If so, then methylation should be "transversely" correlated across DNA strands and also "longitudinally" correlated along a single DNA strand. We developed a variation of hairpin-bisulfite sequencing that reduces the error rate of methylation calling. It interrogates four instead of two separate reads and allows us to accurately measure both transverse and longitudinal correlations in DNA methylation. Our data point to strong longitudinal correlations that decay over the length scale of a nucleosome. Interestingly, this decay does not occur to the baseline value of 0. There are even longer-range correlations, presumably because of the higher order chromatin structure. We have been able to measure these longer-range correlations by direct methylation calling in long nanopore reads. We found interesting patterns (e.g., ripples) in the correlation signal that we are not yet able to reliably correlate with features of the chromosome structure. In this year of reporting, we began to focus on the temporal dimension. We do this using pulse labeling experiments with BrdU as the marker, which is detected simultaneously with DNA methylation information by nanopore sequencing, followed by analyses of raw pore current reads by deep neuronal networks.

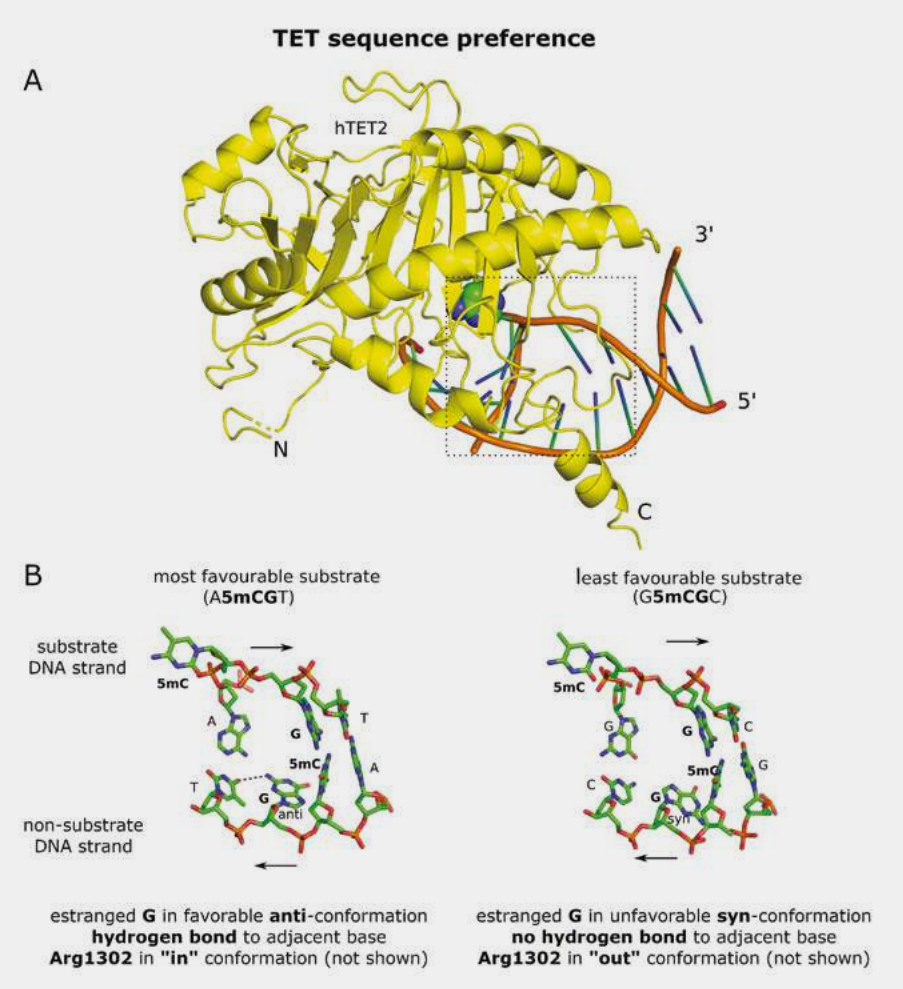


Fig. 1 TET2 activity is influenced by the sequence of DNA bases flanking a CpG site. Crystals of hTET2 were grown with most and least favorable substrates. (A) Overview of the structure. (B) DNA conformation around the CpG for the most and least favorable substrate. TET preferences depend on the compatibility of DNA sequence with TET imposed DNA structure. 5'-preference is purely dependent on intra-strand interactions in the non-substrate strand. For the preferred substrate, the estranged 2'-deoxyguanosine adopts a favorable anti-conformation and engages in a hydrogen bond with the neighboring 2'-deoxynucleotide. For the disfavored substrate, it is forced into an unfavorable syn-conformation, and cannot hydrogen bond with its neighbor. TET sequence preferences are biologically relevant: the most favorable target sequences coincide with the E-box binding motif of pluripotency transcription factors and are most rapidly demethylated in vivo. Figure adapted from our manuscript with the groups of Dr. Jurkowski and Dr. Hore.

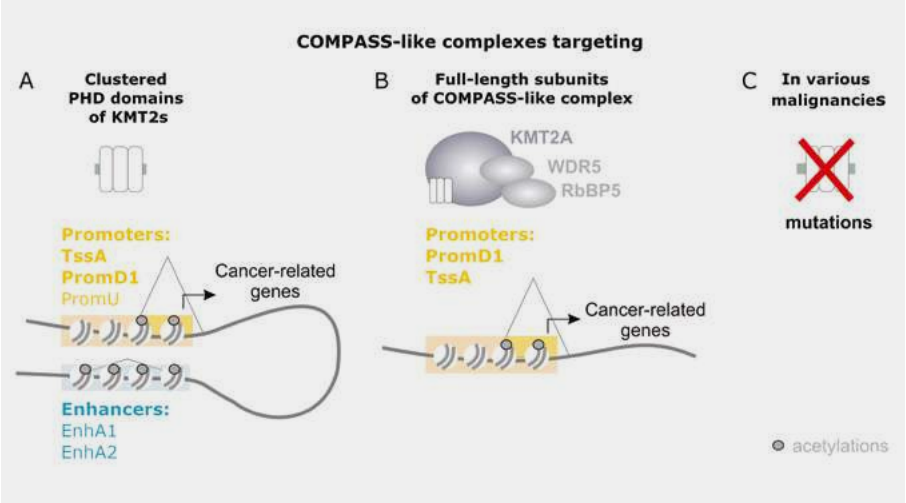


Fig. 2 Clustered PHD domains of KMT2 proteins target the COMPASS-like complexes to active highly acetylated promoter and enhancer regions. (A) Our greenCUT&RUN data indicate that isolated PHD domain clusters localize to active acetylated promoters and enhancers. (B) The localization of PHD domains at promoters is consistent with our CUT&RUN data for full-length KMT2 proteins and other COMPASS-like complex subunits (RbBP5, WDR5). (C) The analysis of the COSMIC database indicates that the mutations of PHD domains within KMT2 proteins are frequently observed in malignancies.

Reversibility of DNA methylation

In eukaryotes, DNA methylation can be erased in the absence of DNA replication. DNA methylation reversal relies on the oxidation of methyl groups to hydroxyl, formyl, or carboxyl groups by TET dioxygenases or related enzymes. In collaboration with Dr. Jurkowski (Cardiff University) and Dr. Hore (Otago University), we discovered that dioxygenases have intrinsic sequence preferences and that the "most favorable" target sequences are demethylated *in vitro* > 100-fold faster than the "least favorable" ones. Intriguingly, the sequence preferences of all TET paralogs are similar. This initially puzzling result could be explained by co-crystal structures with the most and least optimal substrates. TETs impose a non-canonical DNA structure (with a flipped base) that strongly favors some sequence contexts. The 5′-preference can be understood as a consequence of interactions in the non-substrate strand. For preferred sequences, the estranged 2′-deoxyguanosine can engage in hydrogen bond and adopt the favorable anti-conformation of the glycosidic bond. For suboptimal sequences, the hydrogen bond is not possible, and the 2′-deoxyguanosine is forced into an unfavorable syn-conformation (Fig. 1). The 3′-preference is more conventional and attributable to favorable interactions with a conserved arginine residue in TETs. During the previous reporting period, we showed that the observed preference is relevant *in vivo* in embryonic stem cells and in the germline. In 2021, we analyzed published bisulfite single-cell data that were collected for early human embryos. We showed that the sequence preference also has consequences for demethylation rates in the transition from the pronuclear stage to the two-cell stage when active demethylation of the paternal pronucleus by TETs is expected.

Prevention of ectopic DNA methylation

Ectopic, context-inappropriate DNA methylation can arise from errors of the maintenance methyltransferase DNMT1. However, such methylation could also principally arise from nucleotide or 2′-deoxynucleotide salvage. In our 2021 review article, we argued that nucleotide salvage is likely the only explanation for adenine methylation in mammalian DNA, which has recently attracted much interest. If 6-methyladenine can be introduced to DNA, then this should also be possible for 5mC, at least in circumstances when cell death makes methylated DNA building blocks abundantly available. In the past year, we began testing cellular defenses against this pathway of epigenome degradation in a zebrafish model and *in vitro*. Surprisingly, we found that at least a model polymerase of the B-type (that includes leading and lagging strand Polε and Polδ polymerases) did not act as a safeguard. This finding focused our interest on deaminases and phosphatases that sanitize the 2′-deoxynucleotide pool. Some of these enzymes are poorly characterized, even in cases where localization and other circumstantial evidence make an epigenome protective role likely. In the past reporting year, we examined some enzymes biochemically and determined a cryo-EM structure of one of them. Our structural work was performed in collaboration with the laboratory of Prof. Alex Wlodawer (U.S. National Cancer Institute), which provided a generous beam-time and cryo-EM expertise from which we could learn to establish the technique in our own laboratory.

Positive transcriptional memory

DNA methylation and the Polycomb system mediate negative transcriptional memory. At the other end of the spectrum, positive transcriptional memory is preserved by COMPASS-like complexes, formed by KMT2, WDR5, Ash2L, RbBP5 and DPY30 core subunits, that help maintain H3K4 trimethylation at promoters and H3K4 monomethylation at enhancers. The ways in which COMPASS-like complexes find their targets are not fully understood. We focused on the possible role of PHD reader domains in KMT2 proteins (catalytic subunits of COMPASS-like complexes). The PHD domains of KMT2 proteins are very highly conserved (in some cases more than the catalytic domains) and occur in clusters of three or even four. To our knowledge, this arrangement is unique for KMT2 proteins and not seen for PHD domains in other chromatin reader proteins. Using greenCUT&RUN (a modern alternative to ChIP-seq that requires fewer reads), we mapped targets of clustered PHDs and analyzed binding sites with regard to experimental ENCODE data and the much more extensive, hidden-Markov model (HMM) derived chromatin states and marks. We showed that clustered PHD domains on their own localize to highly acetylated active promoters (TssA, PromD1, PromU) and to a lesser extent to highly acetylated active enhancers (EnhA1, EnhA2). Using CUT&RUN, we further demonstrated strong overlap between targets of isolated clustered PHD domains and full-length KMT2 proteins or other subunits of COMPASS-like complexes (RbBP5, WDR5). Analyses of cancer databases further showed that loss-of-function mutations in KMT2 proteins, particularly enhancer-directed KMT2C and KMT2D, are frequent in a broad spectrum of malignancies. Altogether, our data suggest that clustered PHD domains in KMT2 proteins help establish a positive feedback loop that is required for COMPASS-like complexes to mediate positive transcriptional memory (Fig. 2).

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LABORATORY OF BIOINFORMATICS AND PROTEIN ENGINEERING

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Pietro Boccaletto, MSc
Michał Boniecki, PhD (until October 2021)

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 (100% appointment)
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-Present 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), European Science Advisors Forum
2016-Present Corresponding Member, Polish Academy of Sciences
2016-2017 Member, Council of the National Science Congress
2015-2020 Member, Group of Chief Scientific Advisors, European Commission's Scientific Advice Mechanism
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

2019 André 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's 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

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

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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

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Chojnowski G, **Walen T**, **Bujnicki JM**. RNA Bricks: a database of RNA 3D motifs and their interactions. ***Nucleic Acids Res***, 2014; 42(1):D123-D131

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Matelska D, **Purta E**, **Panek S**, **Boniecki MJ**, **Bujnicki JM**, **Dunin-Horkawicz S.** S6:S18 ribosomal protein complex interacts with a structural motif present in its own mRNA. ***RNA***, 2013; 19(10):1341-48

Puton T, **Kozlowski L**, **Rother KM**, **Bujnicki JM**. CompaRNA: a server for continuous benchmarking of automated methods for RNA secondary structure prediction. ***Nucleic Acids Res***, 2013; 41(7):4307-23

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; http://iimcb.genesilico.pl/moderna/) and a method for de novo (template-free) RNA structure modeling (SimRNA; http://genesilico.pl/software/stand-alone/simrna, also available as a web server at http://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 (http://genesilico.pl/NPDock). Other methods for RNA bioinformatics include a method for the classification of contacts in RNA 3D structures (ClaRNA; http://iimcb.genesilico.pl/clarna/) and a method for the flexible superposition of RNA 3D structures and their fragments (SupeRNAlign; http://genesilico.pl/supernalign/). We also developed various databases, including a database of RNA modification pathways and enzymes (MODOMICS; http://modomics. genesilico.pl), a database of RNA 3D motifs and their interactions (RNA Bricks; http://iimcb.genesilico.pl/rnabricks/), a structural classification of known families of structured non-coding RNAs (RNArchitecture; http://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 cryo-electron microscopy, X-ray crystallography, and low-resolution methods, such as small-angle X-ray scattering (SAXS) and structure probing by chemical modification.

RECENT HIGHLIGHTS

AnnapuRNA: A scoring function for predicting RNA-small molecule binding poses

RNA is considered an attractive target for new small-molecule drugs. The design of active compounds can be facilitated by computational modeling. Most of the available tools that have been developed for these prediction purposes, such as molecular docking and scoring functions, are parametrized for protein targets. The performance of these methods, when applied to RNA-ligand systems, is insufficient.

To overcome problems with the computational modeling of RNA–small-molecule complexes, we developed AnnapuRNA (https://github.com/filipspl/AnnapuRNA), a new knowledge-based scoring function that was designed to evaluate RNA-ligand complex structures that are generated by any computational docking method. We also evaluated three main factors that may influence structure prediction: starting conformation of a ligand, docking program, and scoring function used. We applied the AnnapuRNA method for a *post hoc* study of recently published structures of the FMN riboswitch.

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

MODOMICS: a database of RNA modification pathways. 2021 update

The MODOMICS database (https://iimcb.genesilico.pl/modomics/) has been a manually curated and centralized resource since 2006, storing and distributing comprehensive information about modified ribonucleosides (Fig. 1). It originally only contained data on chemical structures of modified ribonucleosides, their biosynthetic pathways, the location of modified residues in RNA sequences, and RNA-modifying enzymes. Over the years, prompted by the accumulation of new knowledge and new types of data, it has been updated with new information and functionalities.

In the 2021 release of MODOMICS, we created a catalog of RNA modifications that are linked to human diseases (e.g., attributable to mutations of genes that encode modification enzymes). MODOMICS has been linked extensively to the RCSB Protein Data Bank, and sequences of experimentally determined RNA structures with modified residues have been added. This expansion was accompanied by including nucleotide 5'-monophosphate residues. We redesigned the web interface and upgraded the database backend. Additionally, a search engine for chemically similar modified residues was included that can be queried by SMILES codes or by drawing chemical molecules. Finally, previously available datasets of modified residues, biosynthetic pathways, and RNA-modifying enzymes have been updated. Overall, we provide users with a new, enhanced, and restyled tool for research on RNA modification.

Publication:
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***, 2021; 50:D231-D235

www.iimcb.gov.pl/bujnicki-lab
www.genesilico.pl

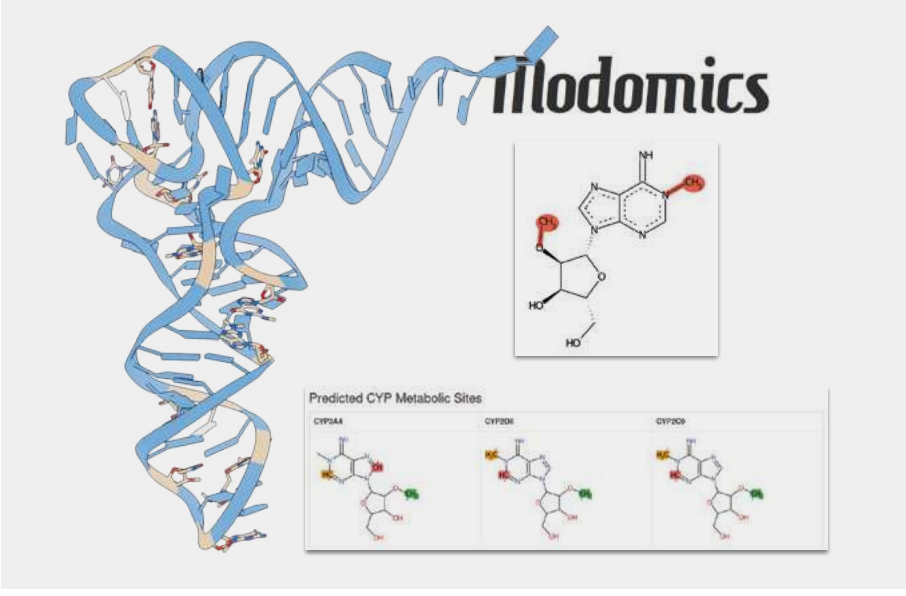


Fig. 1 MODOMICS is a manually curated and centralized resource, storing and distributing comprehensive information about modified ribonucleosides.

Example of an experimentally determined 3D structure of RNA with modified residues colored in orange (yeast phenylalanine tRNA, left).

Chemistry-related information on the modified residues was substantially updated in 2021 release of the database. For each modification molecule the modified groups are highlighted red (a structure of 1,2'-O-dimethyladenosine, upper right); predictions of sites in a molecule that are most liable to metabolism by cytochrome P450 are displayed for each structue (bottom right).



LABORATORY OF RNA BIOLOGY – ERA CHAIRS GROUP

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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 (100% appointment)
2011-Present Associate Professor, Department of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Poland (25% employment)
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 Génétique Moléculaire, 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

2021 HERO WIB Project (Lider), Virtual Research Institute, Polish Science Fund
2020 GRIEG, EEA and Norway Grants, and NCN
2018 Prize for scientific achievements, 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.

SELECTED PUBLICATIONS

IIMCB Best Papers Award

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

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Lubas M, Damgaard CK, Tomecki R, Cysewski D, Jensen TH, ^**Dziembowski A**. Exonuclease hDIS3L2 specifies an exosome-independent 3'-5' degradation pathway of human cytoplasmic mRNA. ***EMBO J***, 2013; 32(13):1855-68

Mroczek S, Krwawicz J, Kutner J, Lazniewski M, Kuciński I, Ginalski K, ^**Dziembowski A**. C16orf57, a gene mutated in poikiloderma with neutropenia, encodes a putative phosphodiesterase responsible for the U6 snRNA 3' end modification. ***Genes Dev***, 2012; 26(17):1911-25

^ no IIMCB affiliation

DESCRIPTION OF CURRENT RESEARCH

Posttranscriptional regulation of gene expression in metazoans

Messenger RNA molecules transmit genetic information from the DNA to the cytoplasm. mRNA stability is thus a critical factor in the control of protein production. In eukaryotes, mRNA extremities are protected from degradation by the 5'-end 7-methylguanylate-cap structure, recognized by the translation initiation factor eIF4e, and by the 3'-end poly(A) tail, bound by poly(A) binding proteins (PABPs). Both additions to mRNA are essential for canonical translation. mRNA decay is regulated by a plethora of factors that act either in *trans*, such as different RNA binding proteins and miRNAs, or in *cis*, such as epigenetic modifications of mRNA (e.g., 6-methyladenosine [m6A]). The coding sequence also significantly impacts mRNA stability, in which nonoptimal codons slow down ribosomes, leading to accelerated decay. All these 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. Conversely, the presence of individual guanosine residues in poly(A) tails can lead to mRNA stabilization by slowing deadenylation. Deadenylation can be counteracted by cytoplasmic polyadenylation, a process that is mainly studied in the context of gametogenesis and neuronal processes. 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 (Gewartowska et al., *Cell Rep*, 2021; Bilska et al., *Nat Commun*, 2020) (Fig. 1, 2).

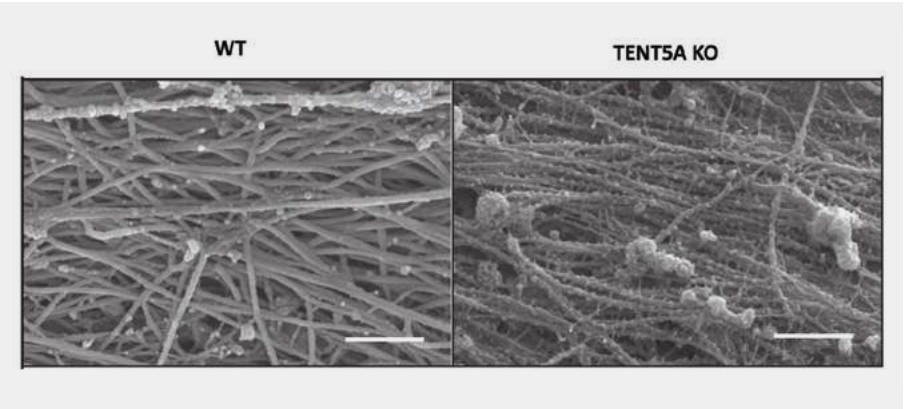


Fig. 1 Lack of TENT5A poly(A) polymerase leads to defects in collagen production and, consequently, bone fragility. It differentiates collagen fiber assembly in the WT from those in Tent5a KO, causing an abnormal and disarranged collagen fiber meshwork visualized by Scanning Electron Microscopy (SEM). Gewartowska et al., Cell Reports, 2021.

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* either from recombinant components or translation-competent extracts. In contrast, much less is known about the tissue specificity of mRNA decay and how it is affected by the subcellular localization of mRNA and the destination of protein products.

The long-term goal of our laboratory is to define principles of mRNA stability regulation in different tissues and cell types. Recently, genome-wide methods of poly(A) tail length profiling and comprehensive genome-wide analyses of mRNA molecules, such as direct RNA sequencing (DRS) on the Oxford Nanopore platform (Workman et al., *Nat Methods*, 2019), have become available. In our research, we successfully implemented DRS for poly(A) tail profiling (Gewartowska et al., *Cell Rep*, 2021; Bilska et al., *Nat Commun*, 2020; Scheer et al., *Nat Commun*, 2021; Tudek et al., *Nat Commun*, 2021; Turtola et al., *Genes Dev*, 2021). This method sequences single molecules of DNA and RNA as they traverse through a protein pore without enzymatic synthesis. Thanks to sophisticated algorithms, the recorded electrical current is base-called into an underlying sequence with remarkably high accuracy in a single-molecule approach. The length of the poly(A) tail is estimated from the raw signal, based on dwell times and the traversing rate of nucleic acid through the pore, thereby allowing the detection of even subtle changes between conditions (Fig. 3).

Our current focus is on the TENT5 family of non-canonical cytoplasmic poly(A) polymerases, which had remained elusive for many years (Mroczek et al., *Nat Commun*, 2017; Kuchta et al., *Nucleic Acids Res, 2016*; Liudkovska et al., *Wiley Interdiscip Rev RNA*, 2021). We and others (Fucci et al., *Cell Rep*, 2020; Manfrini et al., *Cancer Res*, 2020) showed that TENT5s polyadenylate and enhance the expression of mRNAs that encode secreted proteins. There are four TENT5s in mammals (TENT5A-D), which are differentially expressed in tissues and organs (Liudkovska et al., *Wiley Interdiscip Rev RNA*, 2021). We generated knockouts of each TENT5 family member and used DRS to analyze transcriptomes of tissues from wildtype and knockout animals. Notably, molecular and physiological phenotypes were observed for every cell type that expressed TENT5. These cells always have high secretory demand, including immunoglobulins in B cells (Bilska et al., *Nat Commun*, 2020), antimicrobial proteins in macrophages, and collagens in osteoblasts (Gewartowska et al., *Cell Rep*, 2021).

In the future, we plan to enhance the DRS pipeline and combine it with carefully designed transgenic animal models to look globally at mRNA stability and translation regulation.

www.iimcb.gov.pl/dziembowski-lab
www.mosaic.iimcb.gov.pl

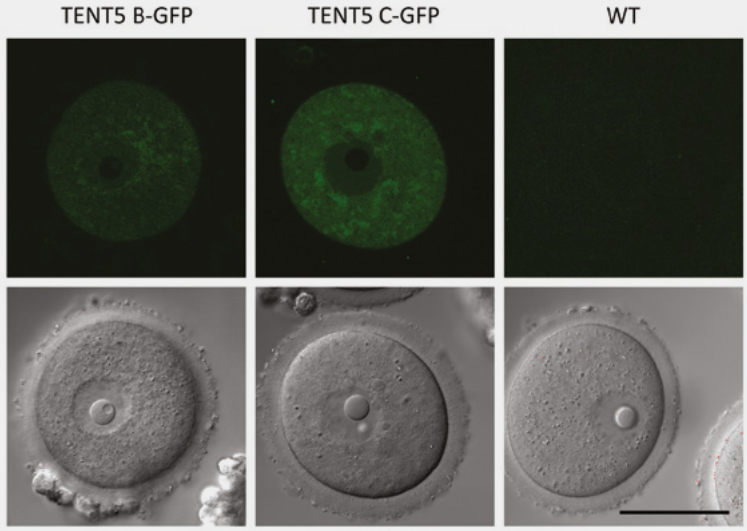


Fig. 2 Expression of TENT5B and TENTBC in mouse oocytes. Oocyes were isolated form knock-in mouse lines with eGFP tagged TENT5B and TENT5C.

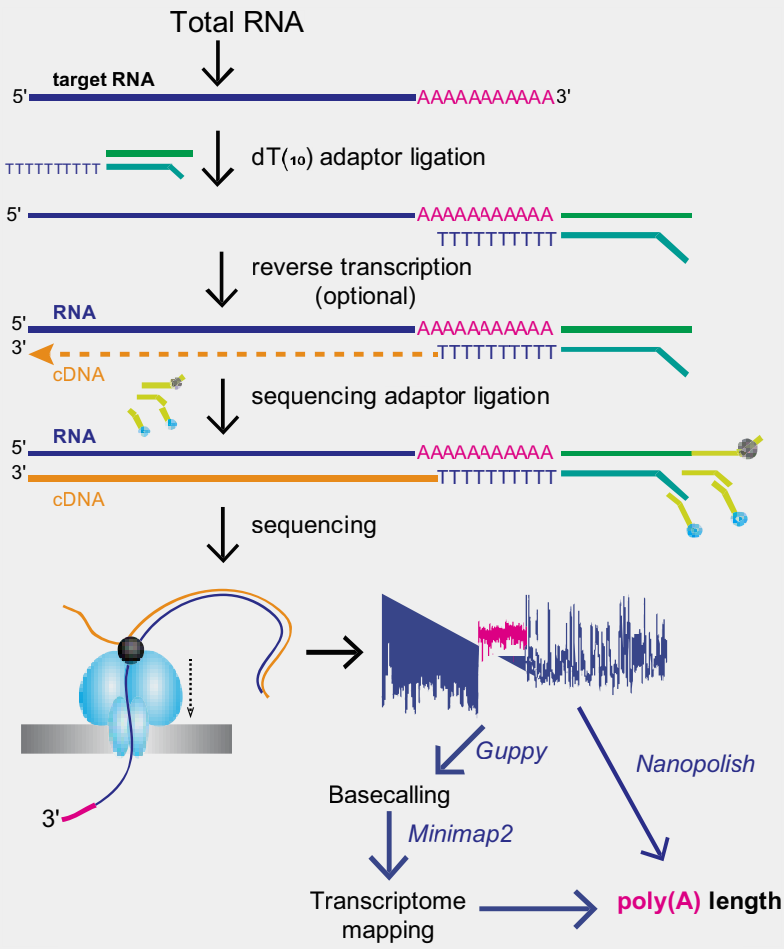


Fig. 3 Schematics of direct RNA sequencing.

The Laboratory of RNA Biology - ERA Chairs Group is supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 810425



LABORATORY OF MOLECULAR AND CELLULAR NEUROBIOLOGY

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Angelika Jocek, MSc

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Jacek Jaworski, PhD, Professor



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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's Award for Scientific Achievements
2020 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

2021-2025 Member, Scientific Council, National Geriatrics, Rheumatology and Rehabilitation Institute in Warsaw
2019 Member, Scientific Council of the Institute of Pharmacology, Polish Academy of Sciences
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
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.

SELECTED PUBLICATIONS

IIMCB Best Papers Award

Koscielny A, **Liszewska E**, **Machnicka K**, **Wezyk M**, Kotulska K, **Jaworski J**. mTOR controls endoplasmic reticulum-Golgi apparatus trafficking of VSVg in specific cell types. ***Cell Mol Biol Lett***, 2021; 26(1):18

Liszewska E, Majchrowicz L, Krogulec E, Kotulska K, Kaczmarek L, Kalita K, Dobrzyń A, **Jaworski J**. Establishment of two hiPSC lines (IIMCBi001-A and IIMCBi002-A) from dermal fibroblasts of healthy donors and characterization of their cell cycle. ***Stem Cell Res***, 2021; 52:102225

Prentzell MT, Rehbein U, Cadena Sandoval M, De Meulemeester AS, Baumeister R, Brohée 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

Tempes A, **Weslawski J**, **Brzozowska A**, **Jaworski J**. Role of dynein-dynactin complex, kinesins, motor adaptors and their phosphorylation in dendritogenesis. ***J Neurochem***, 2020; 155:10-28

Ogórek B, Hamieh L, Hulshof HM, Lasseter K, Klonowska K, Kuijf H, Moavero R, Hertzberg C, Weschke B, Riney K, Feucht M, Scholl T, Krsek P, Nabbout R, Jansen AC, Benova B, Aronica E, Lagae L, Curatolo P, Borkowska J, Sadowski K, Domańska-Pakieła D, Janson S, Kozlowski P, Urbanska M, **Jaworski J**, Jozwiak S, Jansen FE, Kotulska K; EPISTOP Consortium members, Kwiatkowski DJ. TSC2 pathogenic variants are predictive of severe clinical manifestations in TSC infants: results of the EPISTOP study. ***Genet Med***, 2020; 22(9):1489-97

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

Firkowska M, **Macias M**, **Jaworski J**. ESCRT Proteins Control the Dendritic Morphology of Developing and Mature Hippocampal Neurons. ***Mol Neurobiol***, 2019; 56(7):4866-79

Rojek KO, Krzemień J, Doleżyczek H, Boguszewski PM, Kaczmarek L, Konopka W, Rylski M, **Jaworski J**, Holmgren L, Prószyński TJ. Amot and Yap1 regulate neuronal dendritic tree complexity and locomotor coordination in mice. ***PLoS Biol***, 2019; 17(5): e3000253

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

Kononenko NL, Classen GA, Kuijpers M, Puchkov D, Maritzen T, **Tempes A, Malik AR, Skalecka A,** Bera S, **Jaworski J**, Haucke V. Retrograde transport of TrkB-containing autophagosomes via the adaptor AP-2 mediates neuronal complexity and prevents neurodegenretaion. ***Nat Commun***, 2017; 8: 14819

Blazejczyk M, Macias M, Korostynski M, Firkowska M, Piechota M, **Skalecka A, Tempes A, Koscielny A,** Urbanska M, Przewlocki R, **Jaworski J.** Kainic Acid Induces mTORC1-Dependent Expression of Elmo1 in Hippocampal Neurons. ***Mol Neurobiol***, 2017; 54(4):2562-78

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

Urbanska M, **Gozdz A**, **Swiech LJ**, **Jaworski J**. Mammalian target of rapamycin complex 1 (mTORC1) and 2 (mTORC2) control the dendritic arbor morphology of hippocampal neurons. ***J Biol Chem***, 2012; 287(36):30240-56

Perycz M, **Urbanska AS**, **Krawczyk PS**, **Parobczak K**, **Jaworski J**. Zipcode binding protein 1 regulates the development of dendritic arbors in hippocampal neurons. ***J Neurosci***, 2011; 31(14):5271-85

Swiech L, **Blazejczyk M**, **Urbanska M**, **Pietruszka P**, Dortland BR, **Malik AR**, Wulf PS, Hoogenraad CC, **Jaworski J**. CLIP-170 and IQGAP1 cooperatively regulate dendrite morphology. ***J Neurosci***, 2011; 31(12):4555-68

Jaworski J, Kapitein LC, Montenegro Gouveia S, Dortland BR, Wulf PS, Grigoriev I, Camera P, Spangler SA, Di Stefano P, Demmers J, Krugers H, 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

^**Jaworski J**, Mioduszewska B, Sanchez-Capelo A, Figiel I, Habas A, Gozdz A, Proszynki T, Hetman H, Mallet J, Kaczmarek L. Inducible cAMP early repressor, an endogenous antagonist of cAMP responsive element-binding protein, evokes neuronal apoptosis *in vitro*. ***J Neurosci***, 2003; 23(11):4519-26

^**Jaworski J**, Biederman IW, Lapinska J, Szklarczyk A, Figiel I, Konopka D, Nowicka D, Filipkowski RK, Hetman M, Kowalczyk A, Kaczmarek L. Neuronal excitation-driven and AP-1-dependent activation of timp1 gene expression in rodent hippocampus. ***J Biol Chem***, 1999; 274(40): 28106-12

^ 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). My postdoctoral work 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 hone 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 6 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 Center, 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 the molecular mechanisms of dendrite stability and their disruption in mood disorders and the aging brain.

In 2021, despite the COVID-19 pandemic, we continued our work toward our research goals. One of the most intriguing results the past year was obtained in Main Research Objective 1. mTORC1 has been repeatedly described to localize to the surface of lysosomes where it is activated. Less often, mTOR was also reported to localize on the endoplasmic reticulum and Golgi apparatus membranes that are responsible for transporting newly synthesized proteins to the cell membrane and extracellular space. Remaining unknown, however, is whether mTOR plays a role in cargo passage through the secretory pathway. Using the retention using selective hooks (RUSH) approach and live imaging of cells, (Koscielny et al., *Cell Mol Biol Lett*, 2021) showed that mTOR inhibition enhances the transport of model cargo (e.g., vesicular stomatitis virus G protein [VSVg]) through the secretory pathway in certain cell types, such as highly secretory neuron-like PC12 cells, and primary human fibroblasts (Fig. 1). Paradoxically, in TSC1-deficient cells with high mTORC1 activity but low mTORC2 levels, VSVg transport was enhanced. These data suggest the involvement of mTOR in the regulation of endoplasmic reticulum-to-Golgi apparatus cargo transport in some cells and that disturbances in exocytosis are an additional cellular process improperly regulated in TSC. Further research is needed to pinpoint the molecular mechanisms that are responsible for the contribution of mTOR to endoplasmic reticulum–Golgi apparatus transport.

www.iimcb.gov.pl/jaworski-lab

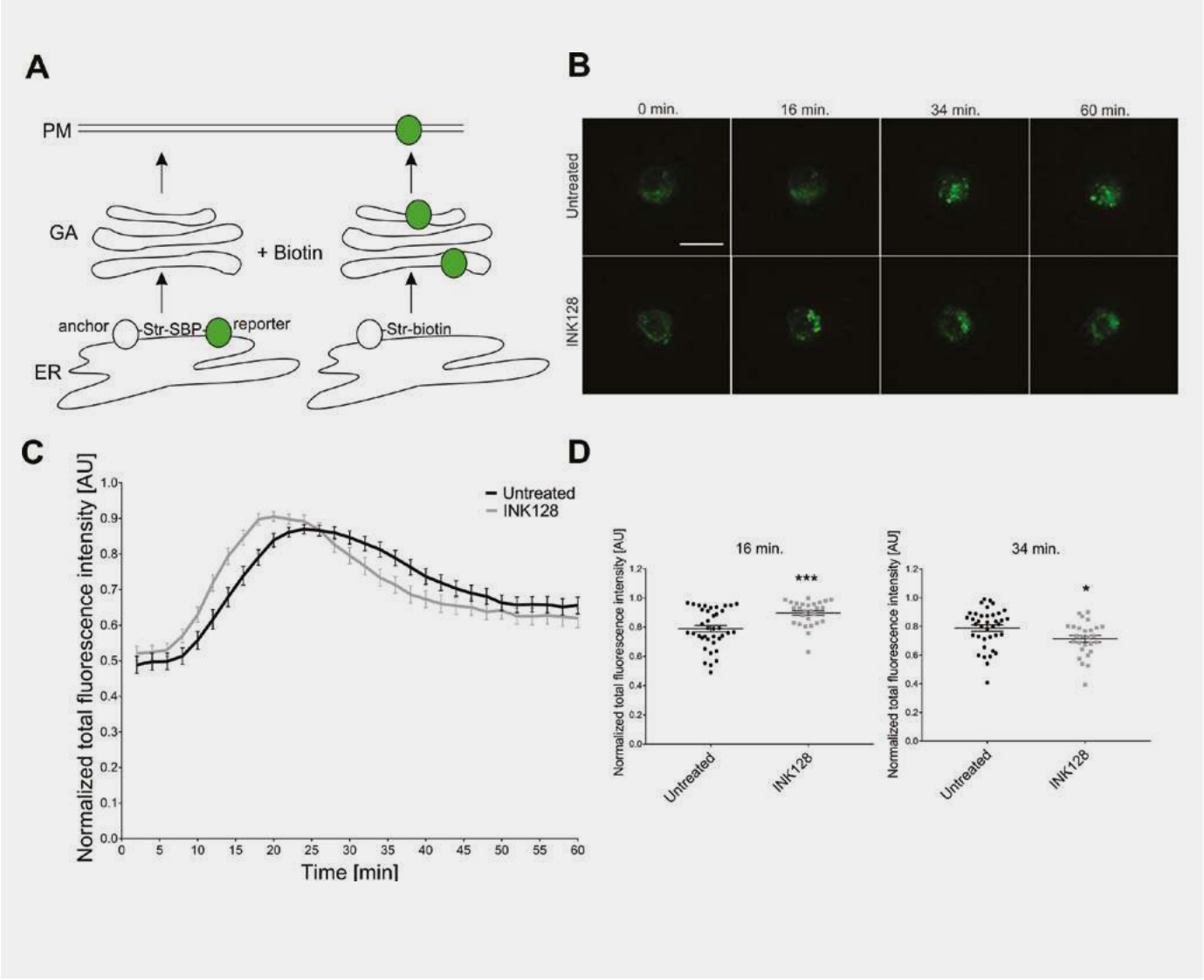


Fig. 1 Inhibition of mTOR affects ER-to-GA VSVg trafficking in selected cell lines. A. Schematic of the RUSH method. Initially, the "anchor" protein traps the reporter protein in the endoplasmic reticulum due to the interaction of Streptavidin (Str) with the sterptavidin-binding protein (SBP). Upon addition of biotin, the reporter protein is released and enters the secretory pathway. **B.** Representative time-lapse spinning-disc microscopy images of living PC12 cells that were transfected with RUSH VSVg probe and treated with mTOR inhibitor INK128 (300 nM, 30 min) or untreated (control). Trafficking of VSVg-EGFP was visualized for 60 min after the addition of biotin (time 0). Scale bar = 20 µm. **C.** The graph depicts VSVg-EGFP fluorescence intensity in the Golgi apparatus region at each time point, normalized to the maximum value. **D.** Comparison of VSVg-EGFP fluorescence intensity in the Golgi apparatus region at 16 min (left panel) and 34 min (right panel). **p < 0.05, ***p < 0.001* (Mann-Whitney test). Presented data (panels B-D) come from Fig. 2 of Koscielny et al. (2021).



LABORATORY OF NEURODEGENERATION

GROUP MEMBERS

Lab Leader

Jacek Kuźnicki, PhD, Professor

Senior Researchers

Magdalena Czeredys, PhD
Małgorzata Korzeniowska, PhD
Vladimir Korzh, PhD
Łukasz Majewski, PhD
Tomasz Węgierski, PhD (until February 2021)

Postdoctoral Researchers

Agnieszka Dyrda, PhD (until April 2021)
Evgeny Gasanov, PhD (until March 2021)

PhD Students

Razieh Amini, MSc (since March 2022)
Rishikesh Kumar Gupta, MSc Tech.
Justyna Jędrychowska, MSc (PhD defense in March 2021)
Ewelina Latoszek, MSc Eng.
Ruchi Prakash Jain, MSc (since December 2021)
Iga Wasilewska, MSc (PhD defense in April 2021)

Undergraduate Students

Dominik Bielecki, BSc (since November 2021)
Samuel Oluwafemi Egbuwalo, BSc (since November 2021)
Marta Piechota, BSc (since November 2021)

Volunteers

Justyna Jędrychowska, PhD (from IPPH[1], since March 2021)
Monika Kwiatkowska, MSc Eng. (from IBCH[2], since December 2020)
Paula Martín Malle (Erasmus, October-December 2021)
Anna Sarosiak, MSc (from IPPH[1], since March 2021)
Iga Wasilewska, PhD (MMRI[3], since September 2021)

Technician

Monika Matuszczyk (part-time)

Laboratory Support Specialist

Dominika Dubicka-Boroch, MSc

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

LAB LEADER

Jacek Kuźnicki, PhD, Professor



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-Present Board Member, European Calcium Society
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

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
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.

SELECTED PUBLICATIONS

IIMCB Best Papers Award

Latoszek E, **Czeredys M**. Molecular Components of Store-Operated Calcium Channels in the Regulation of Neural Stem Cell Physiology, Neurogenesis, and the Pathology of Huntington's Disease. ***Front Cell Dev Biol***, 2021; 9:657337

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

Jha U, Kondrychyn I, **Korzh V**, Thirumalai V. High behavioural variability mediated by altered neuronal excitability in auts2 mutant zebrafish. ***eNeuro***, 2021; 8(5):ENEURO.0493-20.2021

Yang S, Emelyanov A, You MS, Sin M, **Korzh V**. Camel regulates development of the brain ventricular system. ***Cell Tissue Res***, 2021; 383(2):835-52

Majewski L, **Maciag P**, Boguszewski PM, **Kuźnicki J**. Transgenic mice overexpressing human STIM2 and ORAI1 in neurons exhibit changes in behavior and calcium homeostasis but show no signs of neurodegeneration. ***Int J Mol Sci***, 2020; 21(3):842

Gruszczynska-Biegala J, Strucinska K, **Maciag F**, **Majewski L**, Sladowska M, **Kuznicki J**. STIM Protein-NMDA2 Receptor Interaction Decreases NMDA-Dependent Calcium Levels in Cortical Neurons. ***Cells***, 2020; 9(1):160

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

Gupta RK, **Wasilewska I**, **Palchevska O**, **Kuźnicki J**. Knockout of stim2a Increases Calcium Oscillations in Neurons and Induces Hyperactive-Like Phenotype in Zebrafish Larvae. ***Int J Mol Sci***, 2020; 21(17):6198

Dey K, **Bazala MA**, **Kuznicki J**. Targeting mitochondrial calcium pathways as a potential treatment against Parkinson's disease. ***Cell Calcium***, 2020; 89:102216, review

Gupta RK, **Kuznicki J**. Biological and Medical Importance of Cellular Heterogeneity Deciphered by Single-Cell RNA Sequencing. ***Cells***, 2020; 9(8):E1751, review

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

Majewski L, Wojtas B, **Maciąg F**, **Kuznicki J**. Changes in Calcium Homeostasis and Gene Expression Implicated in Epilepsy in Hippocampi of Mice Overexpressing ORAI1. ***Int J Mol Sci***, 2019; 20(22):5539

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(10): bio044347

Jędrychowska J, **Korzh V**. Kv2.1 voltage-gated potassium channels in developmental perspective. ***Dev Dyn***, 2019; 248(12):1180-94

Wasilewska I, **Gupta RK**, **Palchevska O**, **Kuznicki J**. Identification of Zebrafish Calcium Toolkit Genes and their Expression in the Brain. ***Genes (Basel)***, 2019; 10(3):230

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

Rydzanicz M, Wachowska M, Cook EC, Lisowski P, Kuzniewska B, Szymanska K, Diecke S, Prigione A, Szczaluba K, **Szybinska A**, Koppolu A, Murcia Pienkowski V, Kosinska J, **Wiweger M**, Kostrzewa G, Brzozowska M, Domanska-Pakiela D, Jurkiewicz E, Stawinski P, Gromadka A, Zielenkiewicz P, Demkow U, Dziembowska M, **Kuznicki J**, Creamer TP, Ploski R. Novel calcineurin A (PPP3CA) variant associated with epilepsy, constitutive enzyme activation and downregulation of protein expression. ***Eur J Hum Genet***, 2019; 27:61-9

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Wegierski T, **Kuznicki J**. Neuronal calcium signaling via store-operated channels in health and disease. ***Cell Calcium***, 2018; 74:102-11

Hadar A, Milanesi E, Walczak M, Puzianowska-Kuznicka M, **Kuznicki J**, Squassina A, Niola P, Chillotti C, Attems J, Gozes I, Gurwitz D. SIRT1, miR-132 and miR-212 link human longevity to Alzheimer's Disease. ***Sci Rep***, 2018; 8(1):8465

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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), Ca^{2+} homeostasis in mitochondria, the involvement of K^+ channels in the brain ventricular system, and the *in vivo* analysis of Ca^{2+} homeostasis in neurons using zebrafish models. For recent reviews, see Wegierski and Kuznicki, *Cell Calcium*, 2018 and 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 EGTA-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 AMPA receptors (Gruszczynska-Biegala et al., *Front Cell Neurosci*, 2016). STIM proteins also associate with NMDA receptors *in vitro*. The results suggest cross-talk between STIM proteins and receptors that affect Ca^{2+} influx through these channels (Gruszczynska-Biegala et al., *Cells*, 2020). 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). We generated *stim2a*$^{-/-}$, *stim2b*$^{-/-}$, and *stim2a*$^{-/-}$/*stim2b*$^{-/-}$ knockout zebrafish lines and analyzed them using *in vivo* Ca^{2+} imaging in brain neurons, RNA sequencing, and behavioral tests. 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). Using single-cell RNA-seq, we recently analyzed clusters of brain cells of neuronal origin at 5 days postfertilization in *stim2a*$^{-/-}$/*stim2b*$^{-/-}$ zebrafish larvae. This analysis allowed us to comprehensively identify novel gene markers of brain cells in zebrafish and identify effects of dysregulated calcium homeostasis on these markers. RNA sequencing revealed the upregulation of several genes, including *annexin A3a* (*anxa3a*), which was the most upregulated (five-fold increase) in *stim2b*$^{-/-}$ and *stim2a*$^{-/-}$/*stim2b*$^{-/-}$ fish compared with wildtype fish. This observation and previous findings (Nishiura et al., *Exp Mol Pathol.*, 2014) prompted us to study the role of AnxA3 in SOCE. We used Ca^{2+} imaging and electrophysiological recordings to determine the effect of Annexins A2, A3, and A6 overexpression on SOCE and Ca^{2+} currents in cultured cells. Annexin A3 increased SOCE amplitude and potentiated calcium release-activated calcium currents (I_{CRAC}). These results suggest that Annexin A3 is a positive modulator of SOCE (Dyrda et al., *Acta Neurobiol Exp*, 2021).

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

We have been testing the hypothesis that brain dysfunction during aging is induced by changes in Ca^{2+} homeostasis, which may predispose the brain to sporadic Alzheimer's disease pathologies. Transgenic mice that overexpressed key SOCE proteins (STIM1, STIM2, and ORAI1) specifically in brain neurons under the Thy1 promoter were generated. Characterization of the STIM1 line (Majewski et al., *BBA Mol Cell Res*, 2017; Gruszczynska-Biegała et al., *Cells*, 2020), STIM2/ORAI1 line (Majewski et al., *Int J Mol Sci*, 2020), and ORAI1 line (Maciag, Majewski et al., *BBA Mol Cell Res*, 2019; Majewski et al., *Int J Mol Sci*, 2019) has been reported. Strikingly, aged transgenic ORAI1 mice developed spontaneous seizure-like events that were observed only in females, suggesting a novel, sex-dependent role of ORAI1 in neural function (Maciag, Majewski et al., *BBA Mol Cell Res*, 2019).

We investigated whether SIP (Siah-1 interacting protein/S100 binding protein) facilitates HTT ubiquitination and degradation at the protein level and its effect on aggregation of the pathogenic form of HTT (mutant HTT [mHTT]). We used two different models: (*i*) YAC128 mice that overexpress full-length mHTT and exhibit a delay in mHTT aggregation and (*ii*) a cellular Huntington's disease model in which overexpressed exon 1 mHTT has a strong propensity to aggregate. Our results suggest that wildtype SIP supports the ubiquitination of exon 1 wildtype HTT and mHTT, downregulates their protein levels, and consequently inhibits mHTT aggregation. We found that SIP dimers are elevated in the striatum in YAC128 mice. We propose that an increase in SIP dimerization is responsible for mHTT aggregation during the development of Huntington's disease pathology. SIP mutants that form stable dimers exhibit only residual activity toward HTT ubiquitination and lesser protection of exon 1 mHTT against aggregation (Latoszek et al., *Cell & Bioscience*, 2022). The role of Huntingtin-associated protein-1 (HAP1A) was previously shown to be involved in the regulation of abnormal SOCE in Huntington's disease pathology (Czeredys et al., *Front Mol Neurosci*, 2018). We are now investigating the role of SOCE and HAP1A in the context of medium spiny neuron neurodegeneration using different models of Huntington's disease, including induced pluripotent stem cell (iPSC) lines that are reprogrammed from different onsets of Huntington's disease and control fibroblasts. iPSC lines are currently being characterized using different methods.

A loss-of-function mutation of *PINK1* causes early-onset Parkinson's disease in humans. In collaboration with Oliver Bandmann (The 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). We expressed a mitochondrial Ca^{2+} probe (CEPIA2mt) under a pan-neuronal promoter (*elavl3*) to visualize Ca^{2+} levels in the mitochondrial matrix in zebrafish.

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 (*sgsh*; Wiweger et al., *in preparation*), and calcineurin variant associated with epilepsy (*ppp3ca*; Rydzanicz et al., *Eur J Hum Genet*, 2018). We now analyze Ca^{2+} homeostasis and its impact on the progression of neurodegeneration in these models. For this purpose, we also created several reporter lines under the *elavl3* promoter (e.g., with calcium sensors [G-CEPIAer, GEM-CEPIAer, and CAMPARI2], NFAT or HyPer3). We use these lines 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 potassium channels Kcnb1 (Kv2.1) and Kcng4 (Kv6.4) are expressed in several hollow organs (e.g., brain ventricular system, ears, and eyes) where they form tetrameric K^+ channels and antagonize each other's activity. Kcnb1 deficiency in zebrafish causes microcephaly, and Kcnb1 gain-of-function causes hydrocephalus. Kcng4 acts in the opposite manner (Shen et al., *Development*, 2016). Deficiencies in the brain ventricular system cause epilepsy in humans (Jedrychowska and Korzh, *Dev Dyn*, 2019). Formation of the brain ventricular system occurs during early neural development in vertebrates (Korzh, *Cell Mol Life Sci*, 2018). Deficiencies in the brain ventricular system have been linked to several neurodegenerative diseases. Formation of the brain ventricular system depends on the ependyma (i.e., cells that line the brain ventricular system cavity), circumventricular organs, including the choroid plexus (Garcia-Lecea et al., *Front Neuroanat*, 2017; Korzh and Kondrychyn, *Semin Cell Dev Biol*, 2019), and the subcomissural organ (Yang et al., *Cell Tissue Res*, 2020). To study the role of K^+ channels in the development of hollow organs, we generated a zebrafish mutant of *kcnb1* and two mutants of *kcng4b* with deficiencies in the brain ventricular system 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*, 2020).

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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'Oréal 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

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SELECTED PUBLICATIONS

IIMCB Best Papers Award

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DESCRIPTION OF CURRENT RESEARCH

We study molecular mechanisms that integrate intracellular signal transduction and membrane trafficking in endocytosis. 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. In our current projects, we investigate alterations that occur in signaling and trafficking processes in cancer cells. 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.

Endocytosis was initially viewed simply as a mechanism of signal termination through the downregulation and degradation of surface receptors. However, more recent data indicate that endosomal compartments and their resident proteins play an important role in transmitting intracellular signals by transporting ligand-receptor complexes and affecting their activity inside the cell (Pilecka et al., *Eur J Cell Biol*, 2007; Sadowski et al., *Exp Cell Res*, 2009; Miaczynska and Bar-Sagi, *Curr Opin Cell Biol*, 2010; Hupalowska and Miaczynska, *Traffic*, 2012; Miaczynska, *Cold Spring Harb Perspect Biol*, 2013; Cendrowski et al., *Cytokine Growth Factor Rev*, 2016; Szymanska et al., *Semin Cell Dev Biol*, 2018; Budick-Harmelin and Miaczynska, *Prog Mol Subcell Biol,* 2018).

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.

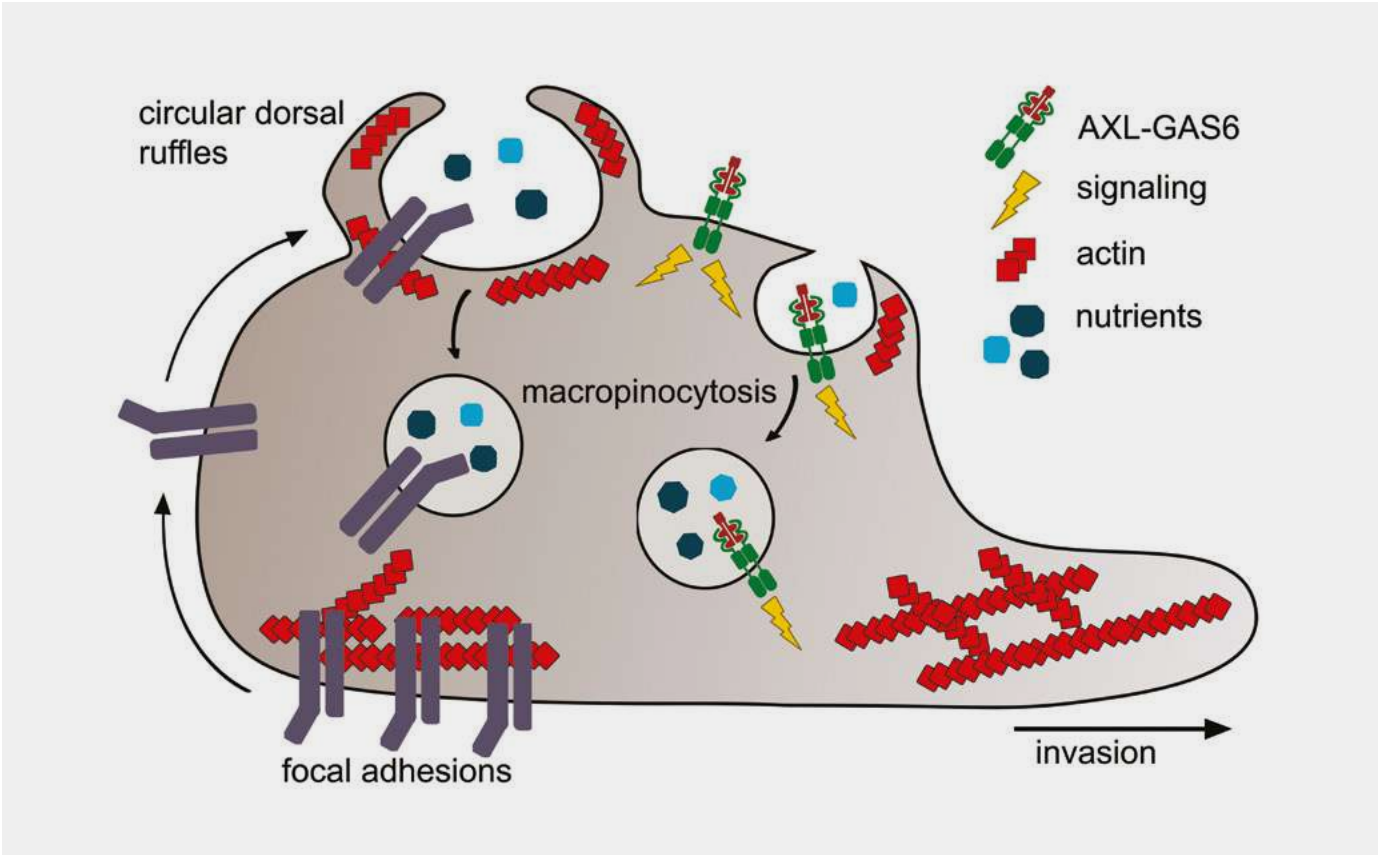
In our molecular oncology projects, we discovered synthetic lethality between two paralogous ATPases of ESCRT machinery, VPS4A and VPS4B (Szymańska et al., *EMBO Mol Med*, 2020). We showed that the *VPS4B* gene was frequently deleted in many cancer types, including in colorectal cancer, reflected by 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 intracellular processes that are activated by ligand-stimulated AXL receptor (Zdżalik-Bielecka et al., *Proc Natl Acad Sci U S A*, 2021). This receptor tyrosine kinase is overexpressed in late-stage, metastatic, or drug-resistant cancers of various origins. Although the first AXL inhibitors are in clinical trials, the cellular mechanisms of action of AXL have remained unknown. We used proximity biotinylation to identify proteins that interact with AXL. Among them, proteins that are involved in actin remodeling represented the main category. We further demonstrated that AXL activation induces several actin-dependent processes, such as 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 (Fig. 1). Overall, we discovered that AXL signaling induces multiple actin rearrangement processes that remodel the morphology and physiology of cells. We also studied cellular effects of AXL inhibitors that are at various stages of clinical development (Zdżalik-Bielecka et al., *Mol Cancer Res*, 2022). We compared cellular phenotypes of the CRISPR-Cas9-induced depletion of AXL and its pharmacological inhibition with bemcentinib, LDC1267, and gilteritinib in glioblastoma cells. 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 interpreting the 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 BMP2K-L/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.

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Fig. 1 AXL-mediated signaling induces actin remodeling that results in membrane ruffling, macropinocytosis and focal adhesion disassembly, which altogether support cancer cell invasion. Signaling initiated by the AXL receptor bound to its ligand GAS6 triggers membrane ruffling, in particular the formation of circular dorsal ruffles. This drives internalization of the ligand-receptor complex and uptake of extracellular nutrients via macropinocytosis. In parallel, circular dorsal ruffles contribute to the disassembly of focal adhesions, thus preparing cancer cells for migration and invasion. Author: Agata Poświata.





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2020 Reader, Infection Medicine, University of Edinburgh, United Kingdom
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2017 Travel Grant, RNA Society
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PREPRINTS

Choudhury NR, Heikel G, **Trus I**, Pinto RM, Trubitsyna M, Gaunt ER, Digard P, **Michlewski G**. TRIM25 inhibits Influenza A infection by destabilising its mRNA and is redundant for the RIG-I pathway. ***bioRxiv***, doi: 10.1101/2021.09.13.460052

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DESCRIPTION OF CURRENT RESEARCH

RNA-protein interactions in human health and disease

RNA-binding proteins (RBPs) are key molecules that control gene expression signaling through RNA-protein interactions. Consequently, they contribute to cellular homeostasis, normal development, and most human diseases. Importantly, new RBPs are being discovered by high-throughput proteomics, but we still have a limited understanding of their function. A pioneering study identified 860 RBPs in HeLa cells (Castello et al., *Cell*, 2012). Remarkably, more than 300 of these proteins were not known for their RNA-binding properties and bear no identifiable RNA-binding domains. Several other studies identified hundreds of novel RBPs in various cells and tissues. Despite the extensive cataloging of novel RBPs, we have a limited understanding of what dictates their molecular functions and RNA-related 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 several epidemics in the 21st century. SARS-CoV-2 virus, which causes COVID-19, has already taken the lives of 2.8 million people and erased USD$10 trillion from the global economy. Another well-known RNA virus, influenza A virus, kills up to 500,000 people annually and causes a significant global socioeconomic burden. These are only two examples of many RNA viruses that cause severe problems for human health and the economy. Thus, a detailed molecular understanding of host-virus interactions is imperative to learn how best to inactivate the virus and prevent major disruptions. Finally, viruses have been used to uncover some of the most important cellular processes, such as mRNA splicing, capping, polyadenylation, and RNA interference, and they continue to provide insights into molecular phenomena that can improve our understanding of the basic biology of living organisms.

We recently discovered and started characterizing a novel RNA binding protein, the E3 ubiquitin ligase TRIM25 (Choudhury et al., *Cell Rep*, 2014; Choudhury et al., *BMC Biology*, 2017). TRIM25 belongs to a large family of tripartite motif-containing proteins (more than 80), most of which have E3 ubiquitin ligase activity. All TRIMs have in common an amino-terminal tripartite domain arrangement (RING–Bbox1/2–coiled coil), but they differ in their C-terminal domains, which categorize them into several subtypes. Many TIRIMs are positive or negative regulators of innate immune pathways (i.e., a first-line defense against such pathogens as viruses). Importantly, TRIM25 is emerging as a key factor in the innate immune response to RNA viruses (including influenza A virus, severe acute

respiratory syndrome, Sindbis virus, and dengue virus, among many others). Despite the essential involvement of TRIM25 in viral RNA-induced innate immune pathways, its RNA-binding functions are still poorly understood.

The most widely reported TRIM25 function involves activation of the pattern-recognition receptor RIG-I, which senses 5'-triphosphate (5'-ppp) moieties on viral RNAs and activates the innate immune response. Upon binding to 5'-ppp-RNA, RIG-I undergoes TRIM25-mediated K63 ubiquitination. This activates a signaling cascade that culminates in the phosphorylation of interferon regulatory factor 3 (IRF-3), IRF-7, and nuclear factor-κB, which translocate to the nucleus and induce type I interferon expression. Importantly, the purported role of TRIM25 in the RIG-I pathway has been recently challenged by numerous reports. We discovered that TRIM25 is an RBP that regulates the stability of host RNA (Choudhury et al., *Cell Rep*, 2014). We also revealed that TRIM25 binds RNA through a novel RNA-binding domain that resides in the PRY/SPRY region and showed that RNA binding appears to be crucial for its E3 ubiquitin ligase activity (Choudhury et al., *BMC Biol*, 2017; Fig. 1). Despite this, the direct binding of TRIM25 to viral RNAs, its 3D structure in complex with host or viral RNAs, and its detailed function in cell biology and innate immunity have not yet been described. Additionally, the RNA-binding potential of other TRIMs with PRY/SPRY domains has not yet been explored.

Within the same theme, we recently co-authored a study that identified global changes in RBP composition and affinity in response to Sindbis virus infection (Garcia-Moreno et al., *Mol Cell*, 2019). Over 200 RBPs showed a differential association with RNA upon infection with Sindbis virus. This remodeling of RNA-protein interactions is driven by the host cell to limit virus infection and by the virus to limit the host's innate immune response.

We are currently working toward uncovering the RNA-binding roles of TRIM25 in the innate immune response to RNA virus infections. In our recent preprint, we revealed a new mechanism whereby TRIM25 binds to influenza A mRNA and destabilizes it, contributing to the antiviral response (Choudhury et al., *bioRxiv*, 2021). Concomitantly, we aim to solve the E3 ubiquitin ligase TRIM25/RNA complex structure, which will inform us about novel RNA-binding domains and the role of RNA in stimulating ubiquitination. The outcome of this research will reveal how TRIM25 uses its RNA-binding activity for anti-viral functions. Importantly, TRIM25 belongs to a large (> 80 member) family of tripartite motif-containing proteins that have various functions in cellular processes and disease, including development, apoptosis, autophagy, carcinogenesis, and innate immunity. Thus, our research will open new lines of investigation into other TRIM proteins and their putative RNA-binding roles.

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

microRNAs (miRs) are small noncoding RNAs that negatively regulate the expression of mRNAs. They have defined tissue expression patterns and affect many cellular processes and developmental pathways. Most miRs are transcribed by RNA polymerase II, with a long primary transcript, termed pri-miR, that carries a hairpin structure. The biogenesis of miRs is accomplished by two enzymes, DROSHA and DICER, that catalyze two processing events in the nucleus (from pri-miR to pre-miR) and cytoplasm (from pre-miR to miR duplex), respectively. miR duplexes are incorporated into the RNA-induced silencing complex (RISC) together with Argonaute (AGO) protein, where one strand is selected to become the mature miR. The RISC then recognizes a specific mRNA sequence by complementary base-pairing, resulting in translation inhibition and/or RNA degradation.

Because of the important role of miRNAs in the control of gene expression and organism development, the production of mature miRNAs is tightly regulated at multiple levels, including transcriptional and post-transcriptional steps. We have shown that the biogenesis of miRs can be regulated post-transcriptionally by RBPs that bind to their pri-miRs and pre-miRs (Michlewski et al., *Mol Cell*, 2008; Michlewski and Caceres, *Nat Struct Mol Biol*, 2010; Choudhury et al., *Genes Dev*, 2012; Nowak et al., *Nat Commun*, 2014; Nowak et al., *RNA*, 2017; Kooshapur et al., *Nat Commun*, 2018; Michlewski and Caceres, *RNA*, 2019; Downie Ruiz Velasco et al., *Mol Ther Nucleic Acids*, 2019; Sajini et al., *Nat Commun*, 2019).

We are currently working on regulation of the miR-7 pathway, which is directly involved in Parkinson's disease (PD). Parkinson's disease is an incurable neurodegenerative disease that affects all ages but is most prevalent in the elderly, afflicting over 1% of the population over the age of 60. Approximately 10 million people live with PD, and hundreds of thousands die from the disease each year. One of the main causes of PD is the overproduction and aggregation of α-synuclein (α-Syn) protein in brain cells in affected individuals. A large body of evidence indicates that decreases in α-Syn levels should be beneficial for PD patients. Several clinical trials are now focusing on α-Syn clearance by silencing RNAs or vaccines. Notably, miR-7 has been shown to target α-Syn production, and approaches for miR-7 replacement therapies have been proposed.

We have shown that HuR (ELAVL1) protein is a naturally occurring inhibitor of miR-7 production that binds to its pri-miR-7 (Choudhury et al., *Genes Dev*, 2012). Furthermore, evidence suggests that HuR is upregulated in PD, and binding to the α-Syn mRNA 3'-untranslated region stabilizes the transcript, thereby allowing an increase in α-Syn production. This suggests that disrupting the RNA/HuR complex will have a positive effect on miR-7 and negative effect on α-Syn, suggesting a novel approach for PD therapy. Indeed, we recently reported that the natural compound quercetin inhibited RNA/HuR and downregulated α-Syn levels in human cultured cells (Zhu et al., *Nucleic Acids Res*, 2021).

In recent years, we have been developing a novel drug discovery platform, based on RNA-protein interactions in eukaryotic cell extracts with confocal nanoscanning (RP-CONA), to screen for specific compounds that interfere with the pri-miR-7/HuR complex, thus reactivating miR-7 biogenesis, suppressing α-Syn production, and alleviating PD symptoms (Zhu et al., *Nucleic Acids Res*, 2021). Our strategy is based on an ultra-sensitive RNA-protein interaction assay in cell extracts from human cultured cells (Choudhury and Michlewski et al., *Methods*, 2019) and detecting pri-miR-7/HuR complex disruption by a confocal nanoscanner (Fig. 2). The use of human cultured cell extracts for an ultrasensitive on-bead assay offers great advantages over traditional systems for drug screening that use purified proteins from prokaryotic cells. First, all human proteins, such as HuR, are extensively modified in human cells, which directly influences their structure, activity, and function. Second, RNA-protein complexes, such as pri-miR-7/HuR, are analyzed in the context of other competing pri-miR-7/protein complexes, better reflecting the cellular environment. Our new methodology will allow the discovery of compounds that disrupt the pri-miR-7/HuR complex as close to the human cellular context as possible. Finally, implementation of the RP-CONA screening platform will allow many researchers to explore the possibilities of targeting RNA-protein interactions in research and medical-based projects.

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DIOSCURI
CENTRE FOR RNA-PROTEIN INTERACTIONS IN HUMAN HEALTH AND DISEASE

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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.

PREPRINTS

Slusarczyk P, Mandal PK, Zurawska G, Niklewicz M, Chouhan KK, Macias M, Szybinska A, Cybulska 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. ***bioRxiv***, doi: 10.1101/2022.01.16.476518

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DESCRIPTION OF CURRENT RESEARCH

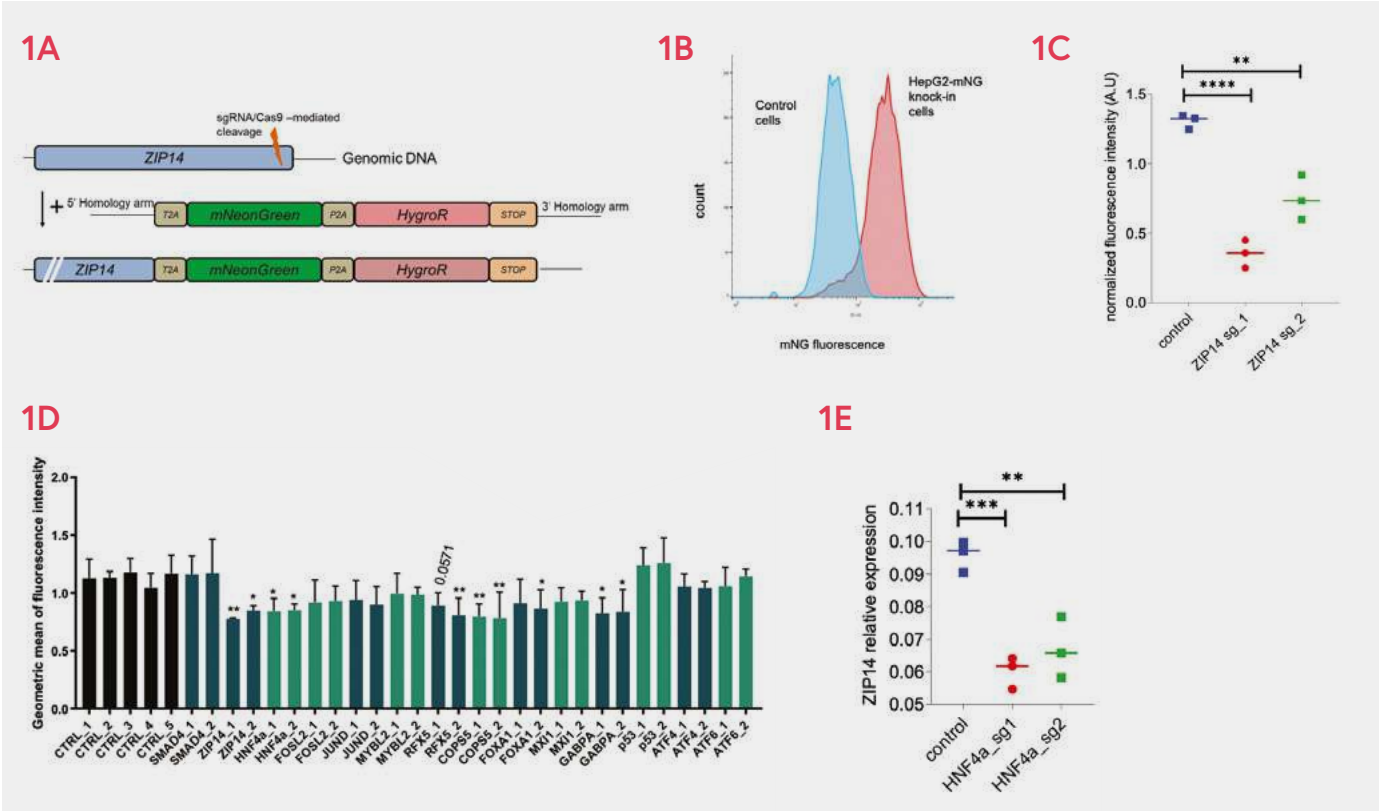
Sufficient iron supplies are critical for vital cellular functions, such as energy production and RNA/DNA processing and repair. In the human body, the vast majority of iron is utilized for hemoglobin synthesis during the daily production of ~200 billion erythrocytes. However, excess free iron can cause oxidative damage and lead to organ failure. The maintenance of iron balance is thus essential for the proper functioning of cells and organisms. Expanding knowledge of the genetic control of iron homeostasis is important for human health. The role of iron redistribution within tissue microenvironments has emerged as a signaling means of controlling (patho)physiological functions of specialized cell types. Our laboratory seeks to define cell type-specific iron regulatory processes both from the perspective of the control of iron levels and by looking at other iron-dependent cellular functions. We expect that the broadening of such knowledge will improve our understanding of human diseases that are directly or indirectly linked to iron dyshomeostasis.

Hepcidin is a master regulator of body iron homeostasis. When iron levels in the body increase, hepcidin production is enhanced to prevent further iron absorption from the diet. To gain insights into the genetic control of iron homeostasis, we previously designed and conducted large-scale RNAi screens for novel hepcidin regulators, generating new knowledge about 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). Nevertheless, despite growing knowledge of the molecular control of iron homeostasis, the genetic basis for variations in body iron parameters is still not fully understood. Identifying elusive factors that modify such processes as iron sensing, iron flux, and iron accumulation has high medical relevance. 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 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 or liver iron levels translate into alterations of Bmp6 mRNA levels in LSECs and how different cell types in the liver contribute to Bmp6 regulation.

Body iron levels increase above homeostatic levels when 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 potentially toxic and currently considered the main contributor to iron-overload disorders. Liver hepatocytes are the primary cell type that acquires NTBI in iron overload pathologies. Therefore, we speculated that communication between LSECs and hepatocytes may contribute to the control of Bmp6 transcription. We established a system in which primary mouse LSECs (immortalized cell line/primary cells) are co-cultured with hepatocytes, treated with NTBI iron, and then separated by fluorescence-activated cell sorting (FACS). Compared with LSECs that were cultured alone, LSECs that were maintained in culture together with hepatocytes induced *Bmp6* mRNA expression in a more pronounced manner in response to iron supplementation. These data indicate that a factor that is secreted by iron-loaded hepatocytes serves as an additional signal to enhance Bmp6 expression. We validated these observations in a mouse model of aging, indicated by iron deposition specifically in liver hepatocytes but not in LSECs. We found that LSECs in aged mice compared with young controls did not exhibit oxidative stress, a condition that was previously linked to iron-triggered Bmp6 induction via the transcription factor NRF2. Consistently, LSECs from aged mice did not exhibit a clear transcriptional signature that is characteristic of activated NFR2-mediated signaling.

Progressive iron accumulation in hepatocytes is a hallmark of hereditary hemochromatosis and some severe anemias (e.g., thalassemias) and accompanies several other common liver diseases. It can lead to impairments in liver function and a higher risk of aggressive hepatocellular carcinoma.

Fig. 1 Generation, validation, and utilization of fluorescent ZIP14 reporter HepG2 cells. (A) Shown is the ZIP14 locus with CRISPR-mediated knock-in of the fluorescent mNeonGreen (mNG) gene together with the hygromycin resistance gene. (B, C) The fluorescence signal intensity from engineered cells (B) decreased upon CRIPSR-mediated knock-out of ZIP14 (C). (D) CRISPR-mediated knock-out in engineered fluorescent cells aided the identification of new ZIP14 regulators, including HNF4a. (E) The control of ZIP14 expression by HNF4a was confirmed on mRNA levels, using CRISPR-mediated HNFa knockout..



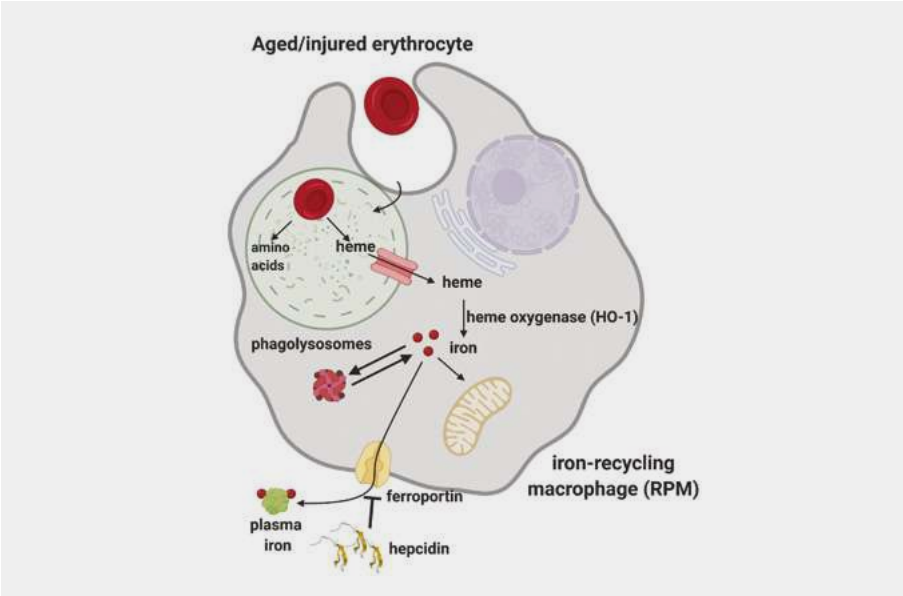


Fig. 2 Depicted is the process of iron-recycling from aged/injured erythrocytes by red pulp macrophages (RPMs) in the spleen. Erythrocytes are first phagocytosed. Upon the formation of phagolysosomes, they are digested, heme is transported to the cytoplasm, and catabolized to iron. Iron is either deposited in iron storing protein ferritin or exported from RPMs via ferroportin to feed the pool of plasma iron. Using mice, we investigate how functions of these specialized cells may be affected by different physiological and environmental conditions, including aging. Figure adapted from Slusarczyk, Mleczko-Sanecka Genes 2021.

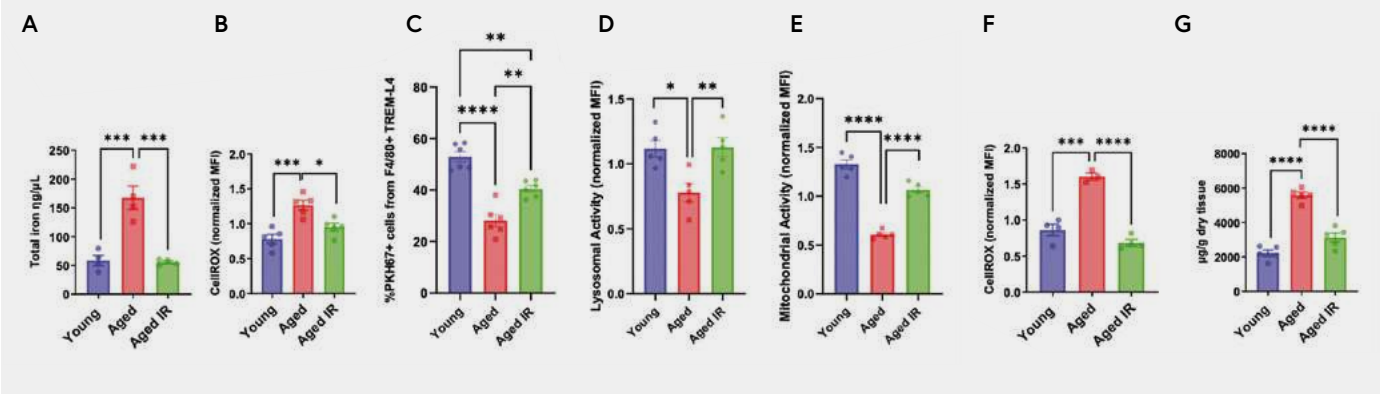
Interestingly, the severity of iron loading, particularly in hemochromatosis, differs substantially between patients. The genetic basis of this variation is still not fully understood. One of our ongoing projects seeks to understand the molecular processes that contribute to NTBI uptake in hepatocytes. 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. Interestingly, the ablation of ZIP14 in zebrafish and mice and ZIP14 mutations in humans were recently reported to affect mainly manganese (Mn) levels. Thus, comprehensive characterization of the ZIP14 regulatory network also has medical relevance to our understanding of Mn homeostasis.

To decipher the regulatory mechanisms of ZIP14, we first employed CRISPR-based gene-editing technology to generate reporter cells that are engineered to monitor endogenous levels of ZIP14 expression using a fluorescence-based readout (Fig. 1A, B). We validated this new-generation reporter system by showing that the CRISPR-based depletion of ZIP14 itself efficiently reduced the fluorescent signal (Fig. 1C). Next, we analyzed the promoter region of the ZIP14 gene using available chromatic accessibility and ChIPseq data. This approach allowed us to identify a region that is further upstream of the transcription start site where liver-enriched transcription factors bind. Together with hints from transcriptomics databases, this guided us to *a priori* select several potential genes that may be involved in the control of ZIP14 mRNA expression levels. These genes were further verified as ZIP14 regulators with a small focused screen using our engineered fluorescent cell line. We identified four genes (*HNF4α*, *COPS5*, *RFX5*, and *GABPA*; Fig. 1D) as potential ZIP14 regulators. We focused our attention on *HNF4α* and validated its involvement in ZIP14 transcriptional control using mRNA levels as a read-out. We next uncovered a signaling mechanism that acts upstream of *HNF4α* as a new signaling mode for ZIP14 regulation that we are currently characterizing in detail using primary hepatocytes and mice.

More than 90% of daily iron needs are met by internal iron recycling from aged erythrocytes. This task is accomplished by macrophages, predominantly red pulp macrophages (RPMs) in the spleen, cells that proficiently recognize and engulf aged erythrocytes, in the erythrophagocytosis process (Fig. 2). Red pulp macrophages release iron via the iron transporter ferroportin to replenish the pool of iron that is necessary for sustaining erythropoiesis. Hallmarks of aging include iron accumulation in tissues, serum iron restriction, and so-called anemia in the elderly. Red pulp macrophages process continuous iron flux. Thus, we hypothesized that their cellular functions might be susceptible to age-dependent decline and hence may have an impact on iron and erythrocyte parameters during aging. We found that 10-to 11-month-old female mice exhibited mild anemia, serum iron deficiency, and iron overload in the spleen and liver compared with young control animals. These were accompanied by iron deposition and oxidative stress in RPMs, lower mitochondrial and lysosomal activity, and a decrease in the rate of erythrophagocytosis. Next, we found that feeding mice a diet with lower iron content alleviated iron loading and oxidative stress in RPMs, restored their lysosomal and mitochondrial functions, and improved their ability to clear erythrocytes (Fig. 3A-E). Consequently, this diet normalized splenic erythrocyte parameters and prevented splenic iron deposition (Fig. 3F, G). Mechanistically, we found that the rate of erythrophagocytosis in aging was suppressed by a combination of excessive labile iron, low activity of the heme catabolizing enzyme HO-1, and endoplasmic reticulum stress in RPMs. Altogether, we identified the dysfunction of phagocytic RPM function as an early iron-dependent hallmark of physiological aging. We further demonstrated that dietary iron reduction improved iron turnover efficacy.

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Fig. 3 Dysfunction of red pulp macrophages (RPMs) is an early iron-dependent hallmark of physiological aging. Shown are total iron levels (B), reactive oxygen species (B), lysosomal (C) and mitochondrial (D) activities and the capacity for erythrophagocytosis (E) in RPMs in young mice compared to aged mice on a standard and iron-reduced (IR) diet. Depicted is the level of reactive oxygen species in erythrocytes isolated from spleens of young mice as compared to aged mice on a standard and IR diet (F) along with the splenic iron content (G).





LABORATORY OF PROTEIN STRUCTURE

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Technician
Iwona Ptasiewicz (part-time)

Laboratory Support Specialist
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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

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
2016 TEAM, Foundation for Polish Science
2015 Jan Karol Parnas Award for the best Polish biochemical publication (with the group of Prof. Janusz M. Bujnicki)
2013 Academia Europea Burgen Scholar
2013 Knight's Cross of the Order of Polonia Restituta
2012 Polish Prime Minister's Award for scientific achievement
2012 Ideas for Poland Award, Foundation for Polish Science
2012 Jan Karol Parnas Award for the best Polish biochemical publication
2012 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's 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.

SELECTED PUBLICATIONS

IIMCB Best Papers Award

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

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DESCRIPTION OF CURRENT RESEARCH

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

Binding of antisense DNA therapeutics by proteins

Antisense oligonucleotide (ASO) technology is an emerging therapeutic approach for inhibiting gene expression through the RNase H1-dependent degradation of cellular mRNAs. The number of approved ASO drugs for the treatment of both rare and common diseases is growing. Nusinersen (Spinraza®) was the first drug that was approved for the treatment of previously untreatable spinal muscular atrophy. Antisense oligonucleotides are complementary to the target mRNA and frequently chemically modified to enhance their therapeutic properties. The most widely used modifications include a phosphorothioate (PS) backbone and modifications of sugar moieties at 2' sites. When administered, therapeutic oligonucleotides interact with numerous plasma and cellular proteins to affect many aspects of their performance, including distribution, tissue delivery, cellular uptake, intracellular trafficking, potency, and toxicity. Molecular mechanisms of the toxicity of chemically modified ASOs are not fully understood.

We reported the first structural study of a complex between an ASO (full PS 2'-OMe DNA gapmer) and a cellular protein using dimeric PC4 protein as a model (Hyjek-Składanowska et al., *J Am Chem Soc*, 2020; Fig. 1). The results provide insights into the molecular forces that govern the greater affinity of ASOs for cellular proteins and provide a potential model of how this nonspecific protein binding can cause cellular toxicity. The structure revealed a possible mechanism of ASO-induced toxic protein aggregation. The ASO is bound in a hairpin-like conformation, and its exposed gapmer part promotes the formation of a dimer of PC4 dimers through base pairing. We also showed that the protein interacts with the PS-nucleic acid through a network of electrostatic and hydrophobic interactions. Importantly, the backbone of the PS ASO is able to form new and more extensive hydrophobic interactions than a natural phosphodiester backbone, thus providing insights into the origins of the enhanced affinity of PS for proteins.

These results are an important step forward in our understanding of ASO-protein interactions, which may contribute to the design of safer and more effective RNA-targeted therapeutics. Studies of the PC4-ASO complex have been performed in cooperation with Ionis Pharmaceuticals (Carlsbad, California, USA).

Structure and mechanism of foamyviral reverse transcriptase

Reverse transcription is a process of DNA synthesis from an RNA template and is used by retroviruses (e.g., human immunodeficiency virus [HIV-1], which causes acquired immunodeficiency syndrome). During reverse transcription, the viral genome that is encoded by RNA is converted to double-stranded DNA, which is then integrated into the host genome. The enzymes that catalyze the reverse transcription process are called reverse transcriptases (RTs). They contain two domains: polymerase and RNase H. Polymerase is responsible for the synthesis of DNA on a DNA or RNA template. RNase H degrades the viral RNA strand of RNA/DNA intermediates.

The activity of RTs is essential for the replication of retroviruses. These viruses are further divided into two subfamilies: Orthoretrovirinae (OV), which includes HIV-1, and Spumaretrovirinae (Spumavirus), which includes simian foamy virus (SFV). Spumaviruses, also known as foamy viruses (FVs), have a unique feature among retroviruses—they do not cause disease, which makes them useful for gene therapy. Moreover, they are the oldest group of retroviruses and have coexisted with their host for over 100 million years, which may have contributed to their non-pathogenicity. One of the distinguishing features of FVs and OV is the different way of replication, mainly related to expression of the *pol* gene. Orthoretrovirinae synthesize Pol protein as a Gag-Pol precursor, which is then cleaved by a retroviral protease into three proteins: protease (PR), RT, and integrase (IN). Foamy viruses, in contrast, synthesize a separate mRNA from which Pol protein is expressed as a precursor of PR-RT-IN, which is later cleaved into two proteins: PR-RT and IN. Thus, FV RT is the only protein of its type that contains an additional PR domain that is located at the N-terminus of the protein.

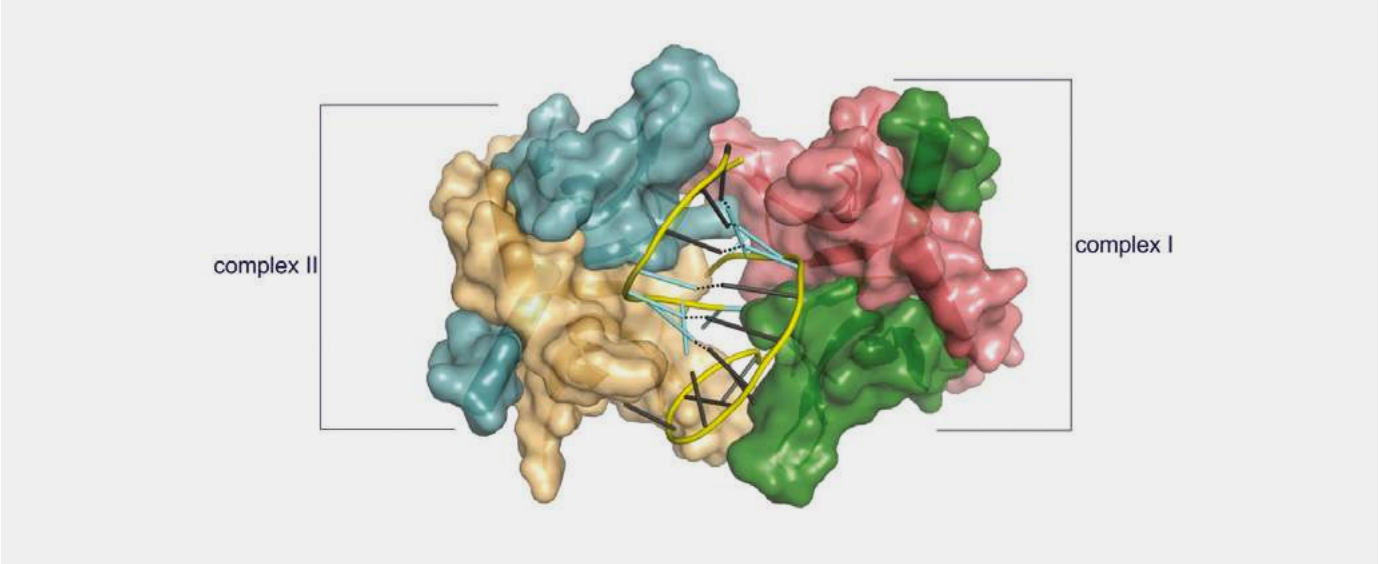


Fig. 1 Structure of PC4 in complex with the ASO PS 2'-OMe DNA gapmer (PTEN ASO), showing the content of the asymmetric unit of the complex crystal. PC4 protomers are color-coded and shown in surface representation. DNA is shown in a cartoon representation. The PS backbone is shown in yellow. 2'-OMe PS nucleotides are shown in aquamarine. DNA gapmer nucleotides are shown in dark gray. Base pairing between nucleotides is shown as black dotted lines.

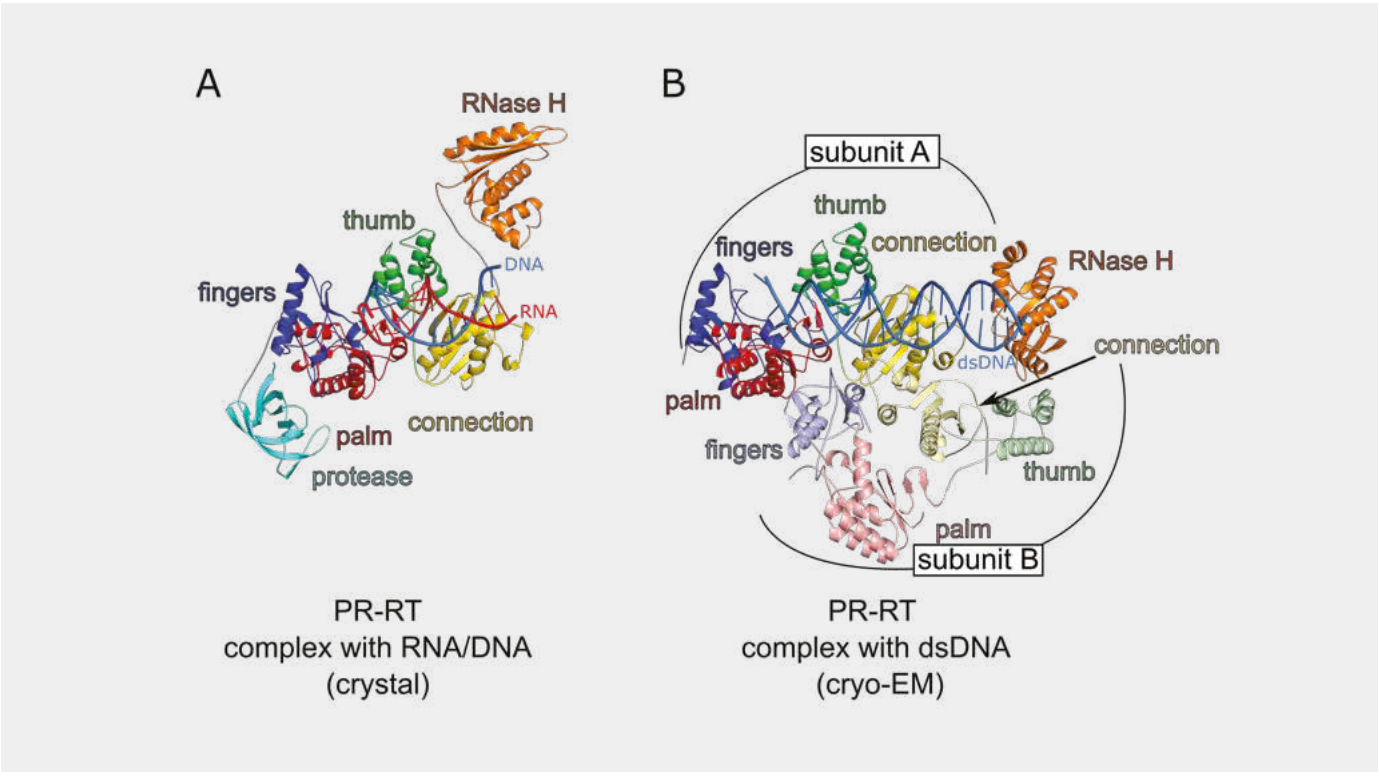


Fig. 2 Structures of nucleic acid complexes of MFV PR-RT. (A) Crystal structure of MFV PR-RT in complex with an RNA/DNA hybrid substrate. (B) Cryo-electron microscopy structure of MFV PR-RT ΔRH in complex with dsDNA. Lighter shades of colors are used for domains/subdomains of subunit B.

For FVs, no structural information for full-length foamy viral PR-RT interacting with nucleic substrates has been reported to date. We solved crystal and cryo-electron microscopy structures of complexes of marmoset foamy virus (MFV) PR-RT with RNA/DNA and dsDNA substrates (Fig. 2). Unexpectedly, the structures and biochemical data revealed that foamy viral PR-RTs have the ability to change their oligomeric state, depending on the type of nucleic acid that is bound. Foamy virus RTs adopt a monomeric configuration in the presence of an RNA/DNA hybrid and an asymmetric dimer arrangement that we observed in the presence of dsDNA. We further corroborated the monomer-dimer switch using biophysical methods. Finally, our advanced biochemical experiments determined the mode of interaction between the mobile RNase H domain of foamy viral PR-RT with its RNA/DNA substrate. Our data showed a novel mode of action of an RT that changes its oligomeric state at different stages of reverse transcription. This work was performed and published in collaboration with Prof. Birgitta Wöhrl (Bayreuth University, Germany; Nowacka et al., *J Virol*, 2021).

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Aleksandra Szybińska, MSc (until August 2021)

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DEGREES

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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

2020 GRIEG, EEA and Norway Grants, and NCN
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

Das A, Thapa P, Santiago U, **Shanmugam N, Banasiak K**, Dabrowska K, Nolte H, **Szulc NA**, Gathungu RM, Cysewski D, Krüger M, Dadlez M, Nowotny M, Camacho CJ, Hoppe T, **Pokrzywa W**. (2021). Heterotypic Assembly Mechanism Regulates CHIP E3 Ligase Activity. ***bioRxiv***. *doi.org/10.1101/2021.08.20.457118*

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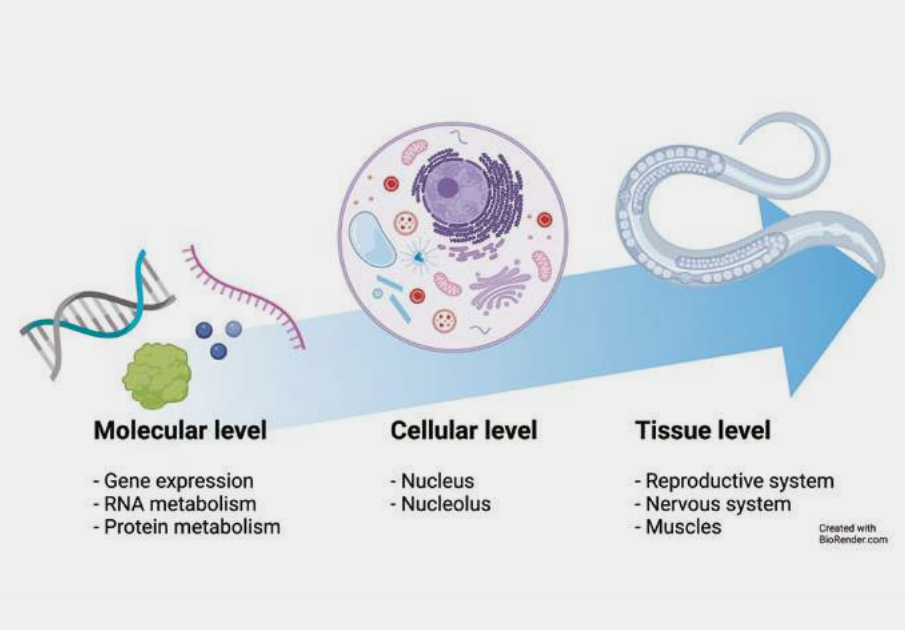
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^ no IIMCB affiliation
* co-first authorship

DESCRIPTION OF CURRENT RESEARCH

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



WE FOCUS MAINLY ON THE FOLLOWING PROJECTS:

E3 ligase complexes in the integration of proteostasis and aging

From their synthesis to destruction, the fate of eukaryotic proteins is supervised by the ubiquitin-proteasome system (UPS). The cooperation of E3 ligases, essential components of the UPS that recognize damaged or misfolded proteins, can lead to the formation of alternative ubiquitylation structures that aid in directing substrate specificity. We investigate how specific E3 ligase pairs determine substrate recruitment and ubiquitin chain formation to coordinate proteolytic networks. Understanding the function of E3 ligases and identifying signals that coordinate interactions among them will provide information on how proteolytic networks are tuned to maintain cellular proteostasis in health and disease.

The regulation of methionine metabolism by the ubiquitin-proteasome system

Methylation is a modification that various cellular molecules, from nucleic acids to proteins to lipids, undergo. It is central to the regulation of many biological processes, including gene expression, signaling, protein synthesis, and lipid metabolism. The long-term goal of this project is to understand how the UPS modulates cellular methylation potential. Congenital methylation disorders comprise a group of rarely described and probably largely unrecognized disorders that involve transmethylation processes. We also model these human diseases in *C. elegans* to understand the molecular basis of the dysfunction of methylation pathway enzymes and their impact on physiology.

Stress-induced myosin folding and assembly mechanisms

Little is known about the regulation of muscle-specific response programs that coordinate protein quality control upon mechanical stress and in human disease. We established a *C. elegans*-centered array of experimental approaches for the in-depth investigation of myosin-directed stress induction mechanisms. The long-term objective of this project is to understand how protein folding and degradation networks are coordinated with dynamics of myosin assembly, muscle integrity, and repair in the context of mechanical stress.

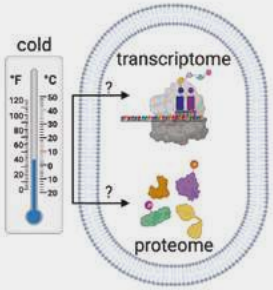
Cellular adaptation to cold temperatures

To counteract cold temperatures, organisms have developed various types of 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 type of cellular messenger RNAs and proteins because these kinds of molecules are critical for the live-or-die decisions of cells. In some disease states, such as stroke, cooling can facilitate a patient's recovery. Thus, understanding how cells adapt to cold has the potential to influence treatments of human disorders.

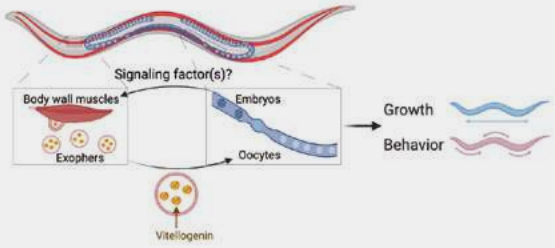
Mechanisms of muscular exopheresis

We discovered that large extracellular vesicles, termed exophers, that are expressed in neurons and cardiomyocytes and carry damaged subcellular components, are released by muscles to support embryonic growth in *C. elegans*. Our results demonstrate that exopher formation (i.e., exopheresis) represents a transgenerational metabolic/resource management system that supports embryos *in utero*. We are currently investigating mechanisms of exopher formation and the regulation of exopheresis at the molecular level.

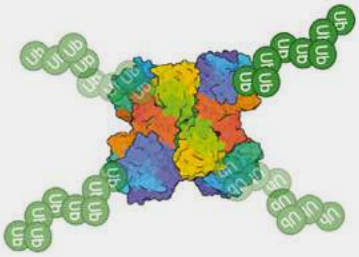
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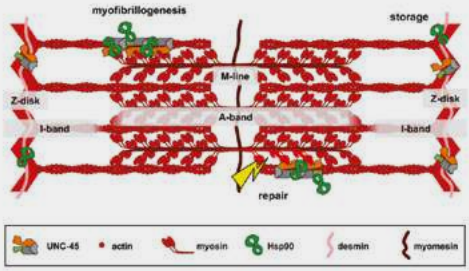
Cellular adaptation to cold



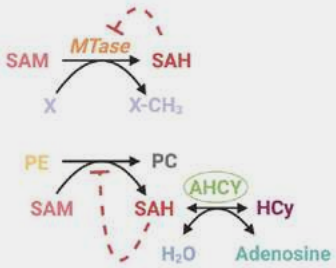
Mechanisms of muscular exopheresis



E3 ligase complexes in the integration of protein homeostasis and aging



Stress-induced myosin folding and assembly mechanisms



Regulation of methionine metabolism by the UPS

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LABORATORY OF ZEBRAFISH DEVELOPMENTAL GENOMICS

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2004 BSc (Honors) in Biology, Department of Biological Sciences, National University of Singapore

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2013-2014 Research Associate, Genome Institute of Singapore (with 2013 research visit to laboratory of Prof. PeterAlestrom, 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

SELECTED PUBLICATIONS

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Abu Nahia K, **Migdał M**, Quinn TA, Poon KL, **Łapiński M**, **Sulej A**, Liu J, Mondal SS, **Pawlak M**, Bugajski Ł, Piwocka K, Brand T, Kohl P, **Korzh V**, **Winata C**. Genomic and physiological analyses of the zebrafish atrioventricular canal reveal molecular building blocks of the secondary pacemaker region. ***Cell Mol Life Sci***, 2021; 78(19-20):6669-87

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Pawlak M, **Niescierowicz K**, **Winata CL**. Decoding the Heart through Next Generation Sequencing Approaches. ***Genes (Basel)***, 2018; 9(6):289

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Winata CL, Kondrychyn I, Korzh V. Changing Faces of Transcriptional Regulation Reflected by Zic3. ***Curr Genomics***, 2015; 16(2):117-27

Kraus P, ^**Winata CL**, Lufkin T. BAC transgenic zebrafish for transcriptional promoter and enhancer studies. ***Meth Mol Biol***, 2015; 1227:245-58

* equal contribution
^ no IIMCB affiliation

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 seeks to understand the mechanism of gene regulation during embryonic development *in vivo* using zebrafish (*Danio rerio*) as a model organism.

ELUCIDATING THE GENOME-WIDE REGULATORY LANDSCAPE OF HEART DEVELOPMENT

We aim 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. We pose our questions within the context of heart development because of the importance of this organ for life. 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.

To gain a comprehensive view of the gene regulatory network that underlies heart development, we investigate two distinct cell types of the heart: cardiomyocytes (CMs) and cardiac pacemaker cells. These two cell types originate from the same progenitor population but are set apart early in the course of heart development through the implementation of distinct genetic programs, resulting in their different properties. Parallel studies in these two cell types provide an additional interesting dimension of differential gene regulation in the context of cell type specification.

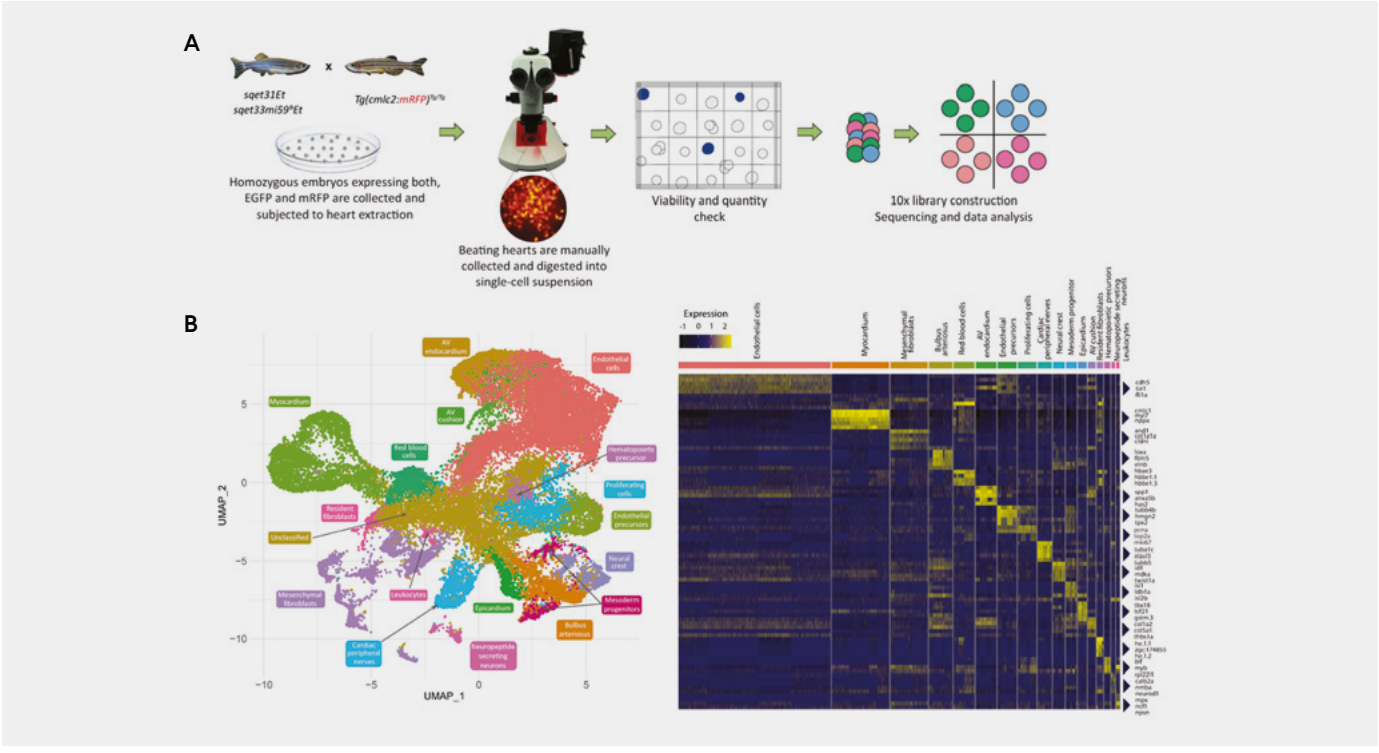
I. Discovery and functional analyses of enhancers in heart development and disease

Large-scale genomics analyses have enabled the discovery of a large number of functional non-coding DNA elements, including thousands of putative enhancers. The field of heart biology has also benefited from these discoveries, with the promise that the knowledge of enhancers that regulate the expression of genes implicated in heart development and function would expand our ability to diagnose and develop more precise treatments for heart disorders. To achieve this, an enormous task is 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-seq) and an assay for chromatin accessibility (ATAC-seq) at several key stages of heart development. Our analyses revealed genetic regulatory hubs that drive crucial events of heart development, which contained key cardiac TFs and are associated with open chromatin regions that are enriched with DNA sequence motifs that belong to the family of corresponding TFs (Pawlak et al., *Genome Res*, 2019). Loss of function of the cardiac TFs Gata5, Tbx5a, and Hand2 affected cardiac regulatory networks and caused global changes in the chromatin accessibility profile. Among regions with differential chromatin accessibility in the mutants were highly conserved non-coding elements that represent putative enhancers that drive heart development. To elucidate the role of these enhancers in heart development and disease, we combine both experimental and computational approaches for discovery and biological validation in the zebrafish model system (Minhas et al., *Dev Dynamics*, 2019). To characterize putative enhancers at higher resolution and ultimately assemble the molecular interaction network between factors that are involved in heart development and disease, we applied various computational and mathematical modeling strategies, including the *in silico* TF footprinting analysis of ATAC-seq data and mathematical modeling of the cardiac transcriptional regulatory network based on genomic and epigenomic data. Modeling-based approaches will enable us to better define and characterize principles of molecular interactions and apply these principles to predicting outcomes of genetic perturbations. Ultimately, we seek to characterize the contribution of the dynamic transcriptional regulatory landscape to heart development and identify novel elements (both genic and nongenic) that are associated with congenital heart disease.

II. Genomic dissection of pacemaker development

The cardiac conduction system is responsible for generating and propagating electrical impulses that are required for the contraction of heart muscle tissues. The cardiac conduction system consists of pacemaker cells, specialized heart muscle cells that ensure rhythmic contractions of the heart. Pacemaker cells possess distinctive morphological and electrophysiological properties that are specialized

Fig. 1 Single cell transcriptome analysis of the developing zebrafish heart. (A) experimental design for single cell transcriptome analysis on the 10x Genomics platform. (B) Clustering of single cells based on their transcriptome profile delineated various cardiac cell types and subtypes, as well as uniquely expressed marker genes for each population.



for their function. They express TFs, such as Tbx2, Tbx3, Tbx18, and Isl1, that prevent their differentiation into CMs. 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, despite the knowledge of key genetic factors that are required for pacemaker cell specification, important questions remain unanswered with regard to the molecular mechanisms that regulate their development and how this translates into proper functioning of the pacemaker. Moreover, inherited forms of arrhythmia are often associated with more common forms of congenital heart malformations that affect other tissue types of the heart, including CMs, implying the interconnectivity of gene regulatory networks that govern their development and function.

The zebrafish heart exhibits remarkable similarities to the human heart in terms of basal heart rate, electrophysiological properties, and action potential shape and duration. Thus, it is an ideal model organism to study the heart pacemaker and model human clinical conditions that affect pacemaker function. To study how the pacemaker develops and functions, we use the zebrafish as a model organism because of its similarity to humans with regard to heart physiology and genetics. We utilized the zebrafish transgenic lines *sqet33mi59BEt* and *sqet31Et* that express green fluorescent protein in the sinoatrial ring (SAR) and atrioventricular canal (AVC), cardiac regions that are known to possess pacemaker activity. These transgenic lines enabled us to isolate cells of these pacemaker regions by FACS and profile its transcriptome by RNA-seq. Results of this study are now presented in two publications, in which we profiled the transcriptome and electrophysiology of the SAR (Minhas et al., *BMC Genomics*, 2021) and AVC (Abu Nahia et al., *Cell Mol Life Sci*, 2021).

The bulk transcriptome profiling of isolated pacemaker cells revealed the expression of genes that define the sinoatrial and atrioventricular nodes, including *isl1*, *tbx2a*, *tbx2b*, *tbx3a*, and *hcn4*. Compared with the rest of the heart, the zebrafish SAR overexpresses *hcn4* and genes that encode calcium- and potassium-gated channels that are implicated in sinoatrial node function in mammals. Moreover, genes that encode components of the BMP and Wnt signaling pathways, as well as members of the Tbx family, that have previously been implicated in pacemaker development, were also overexpressed in the SAR. On the other hand, the zebrafish AVC transcriptome exhibits hallmarks of the mammalian atrioventricular node, including the expression of genes that are implicated in its development and genes that encode connexins that form low-conductance gap junctions. Transcriptome analysis uncovered protein-coding and -noncoding transcripts enriched in AVC, which have not been previously associated with this structure, as well as the dynamic expression of epithelial-to-mesenchymal transition markers and components of the transforming growth factor b, Notch, and Wnt signaling pathways, likely reflecting ongoing AVC and valve development. Abolishing the main pacemaker in the SAR resulted in spontaneous activation in the AVC region, suggesting that it possesses inherent automaticity, although it is insufficient to replace the SAR. Interestingly, we found that the SAR and AVC 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 SAR and AVC 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 SAR- and AVC-overexpressed genes have human homologs that are implicated in various forms of congenital heart diseases, 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 (Fig. 1). Cells of the pacemaker and their subtypes represent populations of rare cell types that are challenging to isolate and study. Therefore, in addition to providing key information that is necessary for meaningfully interpreting our transcriptomics data, 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 POST-TRANSCRIPTIONAL REGULATION OF MATERNAL MRNA EXPRESSION

During embryogenesis, a silent transcriptional period exists from the moment of fertilization to the time of zygotic genome activation, known as the mid-blastula transition (MBT) in zebrafish and frogs. During this period of transcriptional silence, development is regulated by maternally deposited mRNAs that undergo various forms of posttranscriptional modifications to regulate their expression.

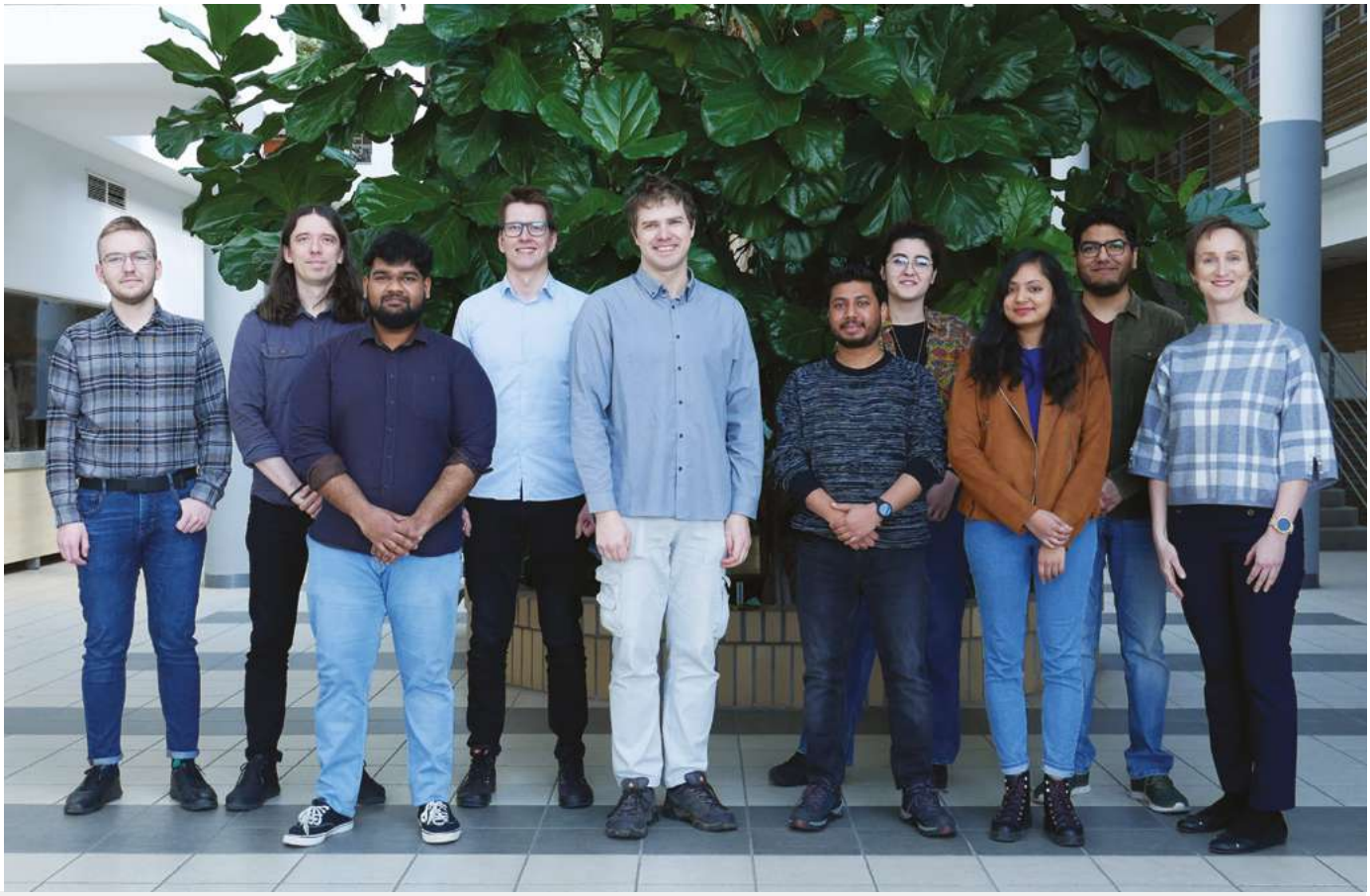
I. Translational control by cytoplasmic polyadenylation

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. Through profiling of the polysome-associated transcriptome, we discovered that cytoplasmically polyadenylated maternal mRNAs are increasingly associated with polysomes, which demonstrates the coupling of translation to cytoplasmic polyadenylation. Furthermore, we found 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. Thus, we established cytoplasmic polyadenylation as a prominent mode of the temporal activation of maternal mRNAs that is necessary for the MBT (Winata et al., *Development*, 2018). Current work in the laboratory focuses on studying the mechanistic basis of cytoplasmic polyadenylation through functional analyses of cytoplasmic polyadenylation element binding proteins (CPEBs) that are known to regulate cytoplasmic polyadenylation and translation. The transcripts of at least three different CPEBs (*cpeb1b*, *cpeb4a*, and *cpeb4b*) are present as maternal mRNAs and associated with polysomes between fertilization and the MBT. Functional studies of these CPEBs are currently underway, and we are developing methods and tools to analyze RNA cytoplasmic polyadenylation, including 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 post-transcriptional 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. A-to-I editing also determines the caste-specific behavior of certain ant species and adaptation to cold temperatures in octopuses, and it is essential for development of the brain and nervous system in *Drosophila* and mice. Extensive A-to-I editing is prevalent in the squid nervous system at significantly higher levels in the giant axon system compared with the cell body, indicating region-specific editing within a neuronal cell. A-to-I RNA editing has also been increasingly recognized as a powerful tool for genome editing apart from CRISPR. However, despite extensive research that has been performed across different organisms, no consensus has been reached on the biological function of this phenomenon. The post-transcriptional mode of RNA modifications is prevalent during the earliest stages of embryogenesis in the absence of zygotic transcription while the progression of critical developmental processes needs to be maintained. The embryo achieves this by means of various modifications to maternal mRNAs that alter their stability and translation rates. RNA editing would thus be a possible candidate as a mode of gene expression regulation. Surprisingly, RNA editing has been seldom considered in the context of embryonic development. 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'-UTR. 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 demonstrated its key role in establishing the antero-posterior and dorso-ventral axes and patterning. Genome-wide editing discovery revealed that Adar knockdown or overexpression affected gene expression and global editing patterns at 12 hpf. Our findings established that RNA editing by Adar is necessary for the earliest steps of embryonic patterning along the zebrafish antero-posterior and dorso-ventral axes. A manuscript reporting these findings is currently under revision and available as a preprint in BioRxiv (https://www.biorxiv.org/content/10.1101/2021.08.26.457081v1).

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LABORATORY OF BIOMOLECULAR INTERACTIONS AND TRANSPORT AMU/IIMCB IN POZNAŃ

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LAB LEADER

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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, 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

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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 Infrastructurefor 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

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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* equal contribution
# corresponding author
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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 the implications of such processes for the 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, both endogenous and exogenous, referred to as the metabolome. 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 channels and tunnels that form inside protein structures (Fig. 1). 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 co-existence of individual chemical species within a single compartment or a whole cell, avoiding overly disruptive interference. Both tunnels and channels 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 the 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 of 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. Together, 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:

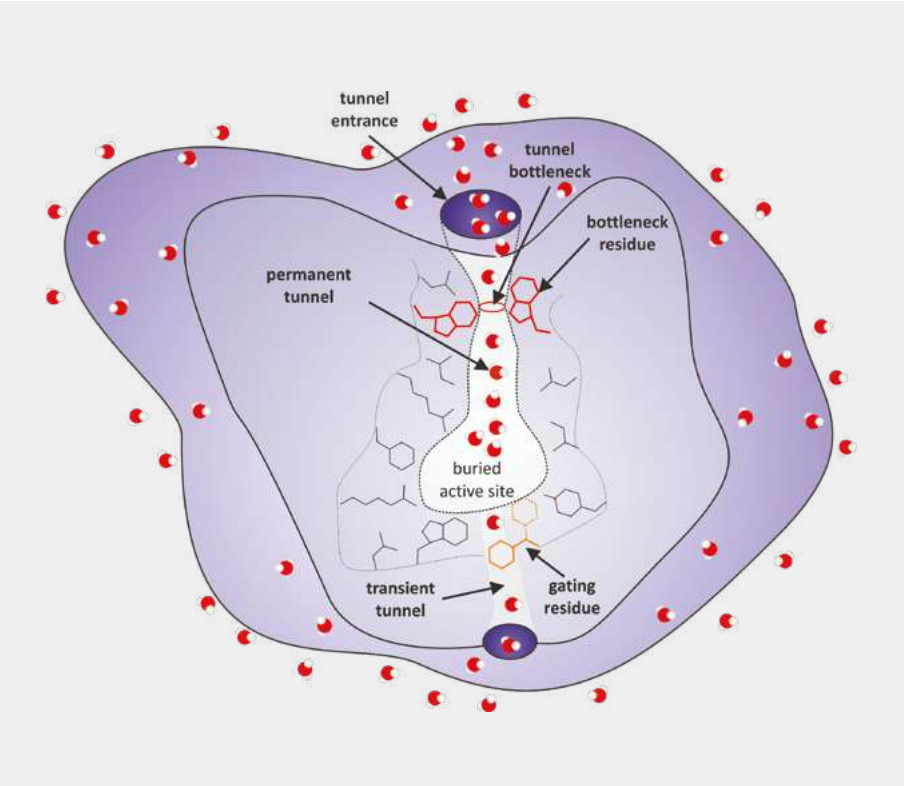
Efficient analysis of ligand transport processes

The primary goal of this project is to enable large-scale studies of the properties and dynamics of functionally relevant transport tunnels. We are currently evaluating and optimizing various approximate dynamics methods to provide ensembles of protein structures with tunnel properties and dynamics that correspond to those that are obtained from rigorous simulations. Another research direction consists of developing various accelerated or adaptive sampling strategies that are tailored to investigations of tunnels and their utilization by small molecules. We developed *TransportTools* (https://github.com/labbit-eu/transport_tools), a software library for high-throughput analyses of internal voids in biomolecules and ligand transport through them (Fig. 2). This library fills an essential technological gap that is connected to high-throughput approaches to molecular simulations that currently tend to result in sets of hundreds and thousands of simulations that are employed for investigating single molecular events. With the application of these methods, we initiated systematic studies of biologically relevant tunnels in a diverse set of proteins to determine specific properties that govern the transport component of protein function, possibly enabling the development of machine learning models to efficiently identify and predict functional tunnels.

Understanding biologically relevant mechanisms in proteins

We apply our developed methods to broaden our detailed understanding of functional aspects of various proteins. We have been analyzing the molecular mechanism that is responsible for the quorum quenching activity of acyl-homoserine lactone acylase from *Pseudomonas aeruginosa* and several other members of the N-terminal serine

Fig. 1 Schema of transport tunnels connecting buried active sites with the bulk solvent. Water molecules are indicated with red-white spheres.



protease superfamily that we found can disrupt the bacterial quorum-sensing process and hence represents a potential antimicrobial agent. We have been investigating the dynamics of their catalytic machinery, the gating of their binding pockets, the formation of reactive complexes, and acylation reaction steps to dissect individual roles of these dynamic factors in the mechanism of quorum quenching. Additionally, we are investigating molecular principles of the selective transport of different phenylpropanoids across cell membranes that utilize ABCG transporter proteins (Fig. 3), which are responsible for the distribution of secondary metabolites, hormone transport, cell detoxification, and the dedicated response to biotic stress in various plants.

Roles of water permeability in enzymatic catalysis

Water molecules are the most abundant and 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. To provide insights into this interesting interplay among different roles of water molecules, we are investigating the nature of minimal tunnels that can be exploited by water molecules to traverse the protein matrix to reach their buried active sites. The early results indicate that such tunnels in several hydrolytic enzymes are responsible for the transport of almost 20% of water molecules, and their utilization can be markedly enhanced by various gain-of-function mutations.

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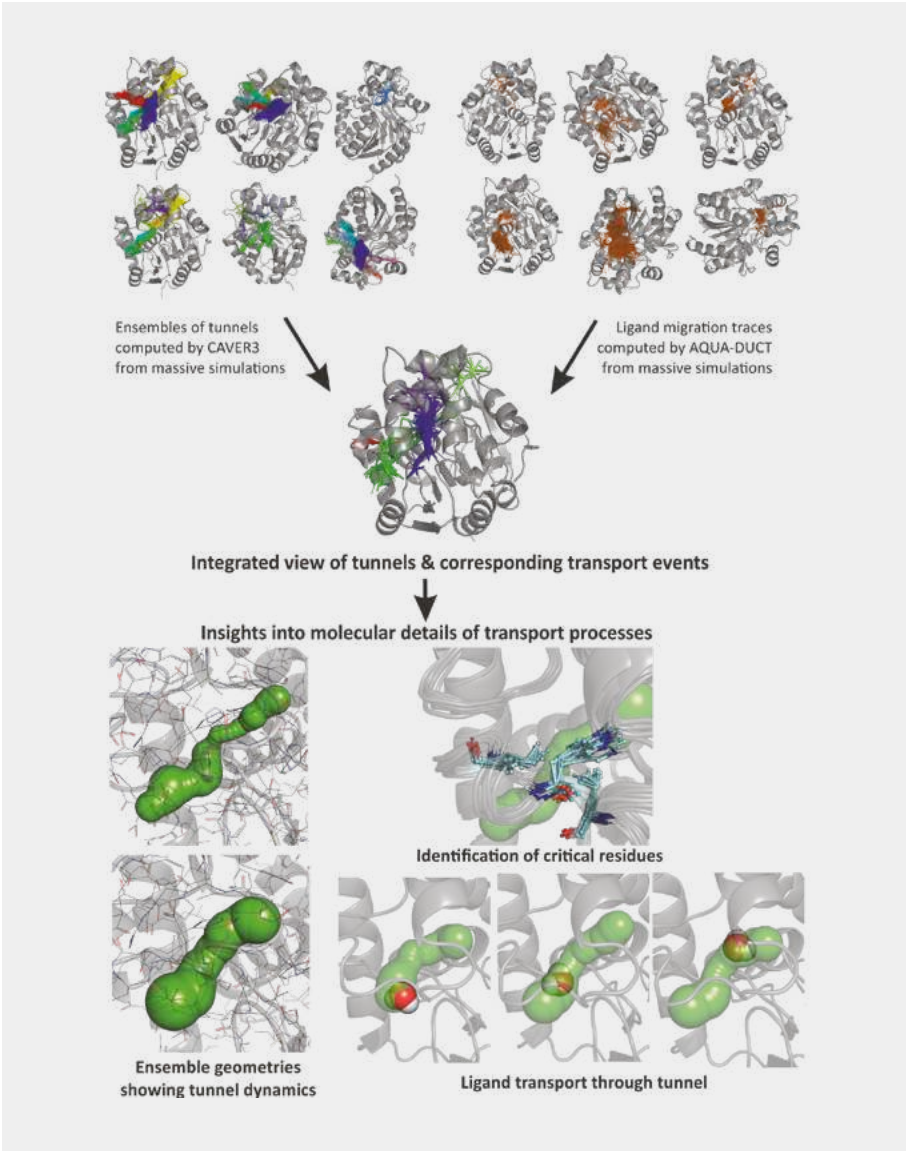
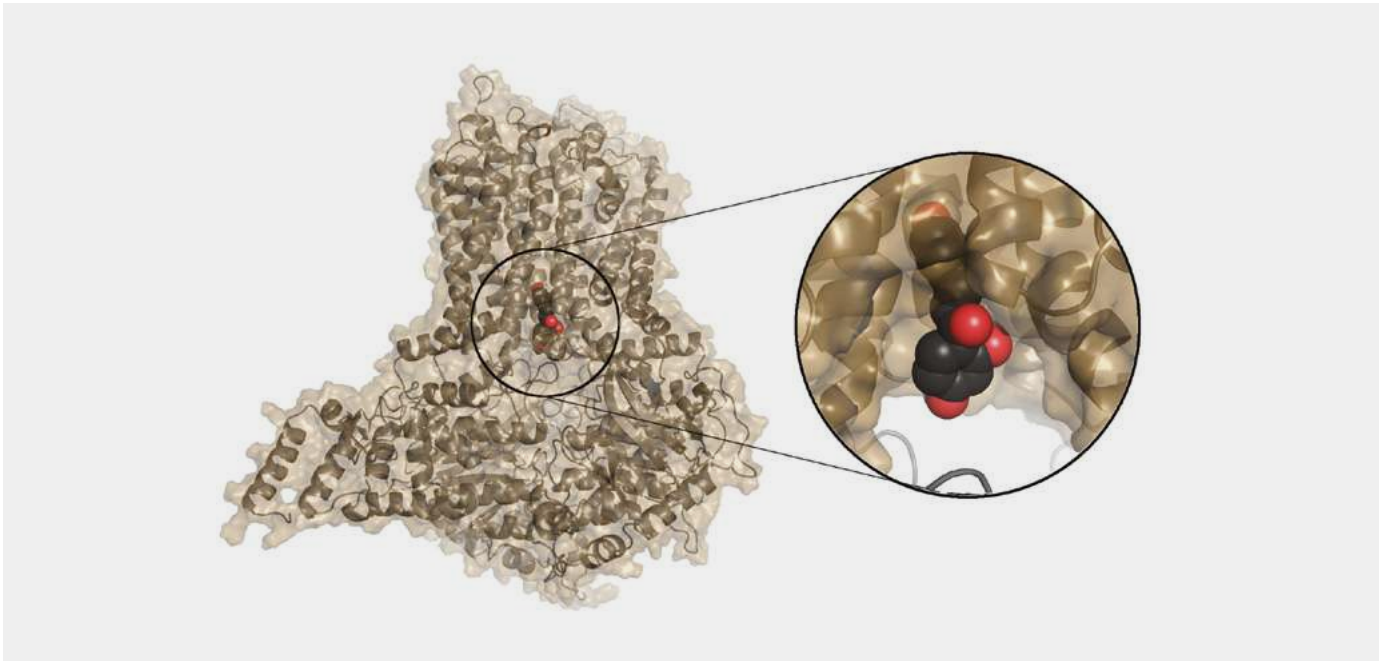


Fig. 2 Schematics of standard analysis workflow using the in-house developed TransportTools library.

Fig. 3 The selectivity filter at the entry to the tunnel of ABCG transporter. The structure of ABCG transporter is depicted as light gray green cartoon and surface, whereas the phenylpropanoid, isoliquiritigenin, is shown in black and red spheres. In the zoomed region, the L-shaped molecule of isoliquiritigenin is bound at the entry to the tunnel unable to effectively proceed further unlike other smaller or more linear phenylpropanoids.



STRATEGIC PROJECTS



STUDY ON AGING AND LONGEVITY

GROUP MEMBERS

Project Coordinator

Małgorzata Mossakowska, PhD, DSc Habil

Project Assistant

Aleksandra Szybalska, PhD (until January 2021)

RESEARCH FOCUS

Research on aging and longevity was launched in 1999 at IIMCB 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), that was commissioned by the Committee for Scientific Research.

The PolSenior project, 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 the national and local levels. In 2021, several papers reported further analyses of PolSenior data, including the causes of anemia (Styszyński et al., *Cells*, 2021), the prevalence of diabetes (Puzianowska-Kuźnicka et al., *Endokrynol Pol*, 2021), hyperuricemia (Winder et al., *Int J Environ Res Public Health*, 2021), and serum uric acid as a predictor of mortality (Winder et al., *J Clin Med*, 2021).

The PolSenior2 project was conducted in 2016-2020, coordinated by the Medical University of Gdańsk. Its methodology was based on the previous PolSenior study (referred to as PolSenior1). The final project report was published in 2021 in Polish as a monograph "Badanie poszczególnych obszarów stanu zdrowia osób starszych, w tym jakości życia związanej ze zdrowiem" (Analysis of health of older adults, including health-related quality of life – PolSenior2 survey). Małgorzata Mossakowska was one of the research editors of the monograph and co-author of 17 chapters. Aleksandra Szybalska was the first author of three chapters, the co-author of additional three, and a language editor and proofreader for the entire book.

Altogether, the PolSenior1 and PolSenior2 projects that characterize older Poles will enable observations of epidemiological trends over the last decade. Researchers who have been involved in implementing PolSenior1 participate in analyzing the data, preparing recommendations for health and social policymakers, as well as presenting and publishing the results of PolSenior2. The first paper that describes socioeconomic risk factors of poor nutritional status has already been published (Krzyminska-Siemaszko et al., *Nutrients*, 2021).

The PolSenior Study Group continued its activities as a member of the NCD Risk Factor Collaboration (NCD-RisC), a worldwide group of scientists from health fields that collaborates closely with the World Health Organization (WHO) to deliver up-to-date, accurate data for 200 countries on non-communicable diseases' (NCDs) risk factors. The results of the pooled data analysis were collected, reanalyzed, and checked by members of the Country and Regional Data Groups. In 2021, the PolSenior project data were included in the pooled analysis of worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019 (NCD-RisC, *Lancet*, 2021) and changes in the population distribution of Body Mass Index and prevalence of obesity and underweight (NCD-RisC, *eLife*, 2021).

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AURESINE

GROUP MEMBERS

Project Coordinator

Izabela Sabała, PhD, DSc Habil

Senior Researcher

Elżbieta Jagielska, PhD

PhD Students

Paweł Mitkowski, MSc
Alicja Wysocka, MSc

Undergraduate Student

Gabriela Anna Ryk (January-February 2021)

Project Assistant

Weronika Augustyniak, MSc

RESEARCH FOCUS

Our team investigates the activity of bacteriolytic enzymes and studies their structure, specificity, and stability to make potentially useful antimicrobials for industry, medicine, and veterinary medicine, among others. The enzymes selectively and efficiently eliminate pathogenic bacteria from various environments and can be applied in diagnostic tests, as a food bioprotectant, to decontaminate various surfaces in industry and hospitals, and as a component of hygiene products for animals and humans. Results of basic research on the regulation of enzymatic activity provide a scientific basis for structure-designed enzyme engineering, which further improves antimicrobials and adjusts to new applications and product demands. Our applied research demonstrates the great potential of these enzymes in eliminating pathogenic bacteria, including antibiotic-resistant strains.

MAIN ACHIEVEMENTS IN 2021

A patent application that protects unique features of a new staphylolytic enzyme that is active under physiological conditions was submitted to the Polish Patent Office (patent pending, P.431445) in 2019. In 2021, it entered the international Patent Cooperation Treaty procedure at the European Patent Office (patent pending, PCT/PL2020/050075).

We finalized research tasks of the TEAM-TECH project, "INFECTLESS: New generation of antibacterial wound dressing", which focused on developing new-generation wound dressings that are functionalized with bacteriolytic enzymes.

We are conducting activities that are supported by a grant from the International Academic Partnerships program, funded by the Polish National Agency for Academic Exchange (NAWA). The MolSpec project, "Molecular basis of enzyme specificity and applications", provides the opportunity to intensify our collaborations with the Institute for Structural Biology (France), Trinity College Dublin (Ireland), the Applied Molecular Biosciences Unit of the Department of Life Sciences FCT-NOVA (Portugal), the Fraunhofer Institute for Silicate Research and Tuebingen University (Germany).

In January 2021, we began two new projects within the POLNOR 2019 program that is coordinated by the National Centre for Research and Development (NCBR). These 3-year projects are conducted by international consortia that comprise academic and industrial partners from Poland and Norway. They are performed in the Laboratory of Protein Engineering, headed by Izabela Sabała, at the Mossakowski Medical Research Institute, Polish Academy of Sciences, where our team moved in spring 2021.

Our undergraduate student, Gabriela Anna Ryk, from the Warsaw University of Technology worked on the project "Modification of enzybiotics for intracellular bacteria eradication improvement". As a result of her research, she was awarded the professional title of Engineer after defending her thesis, "Development of new bacteriolytic enzymes for medical applications".

Our results were presented at international and national meetings, including the EMBO Young Scientists' Forum, EMBL Symposium: "New Approaches and Concepts in Microbiology", 5th Institute of Biochemistry and Biophysics Symposium for Young Investigators, IBB PhD seminar series, and IIMCB PhD students' report session.

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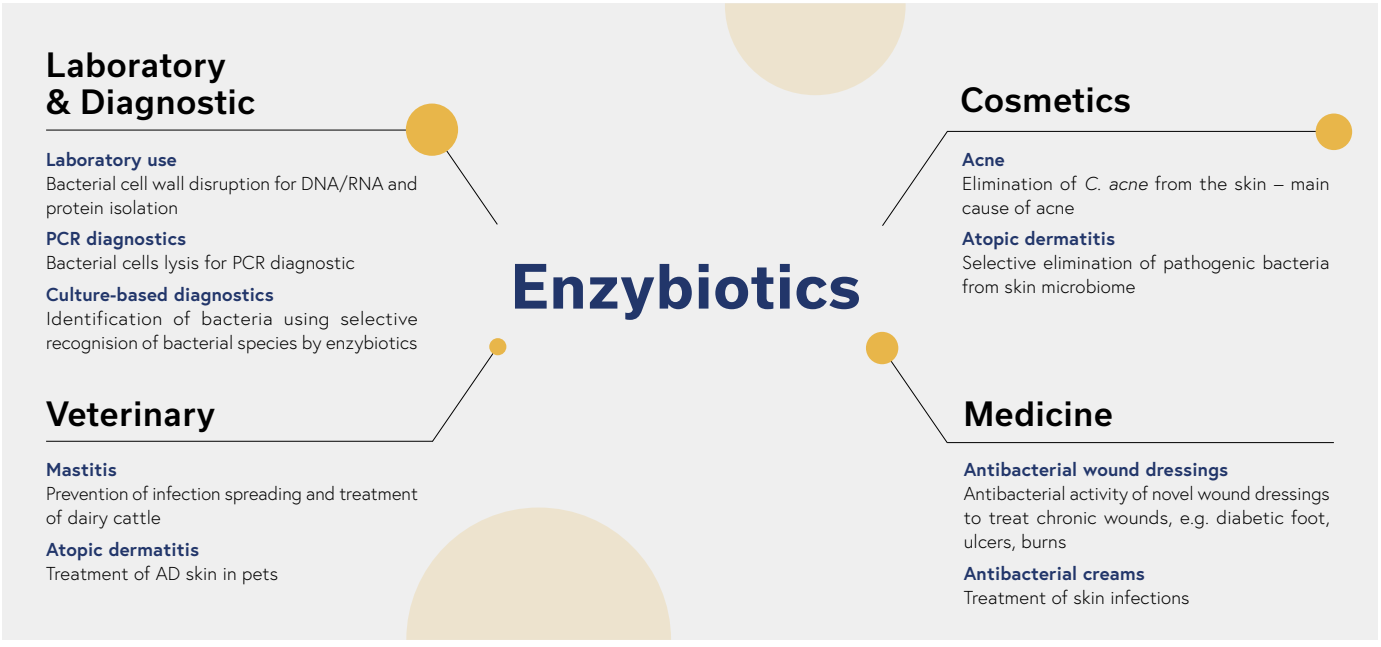
PUBLICATIONS IN 2021

Urbanek O, **Wysocka A**, Nakielski P, Pierini F, **Jagielska E**, **Sabała I**. *Staphylococcus aureus* specific electrospun wound dressings: influence of immobilization technique on antibacterial efficiency of novel enzybiotic. ***Pharmaceutics***, 2021; 13(5):711

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Application of enzybiotics



CORE FACILITIES



BIOPHYSICS AND STRUCTURAL BIOLOGY FACILITY

GROUP MEMBERS

Head

Krzysztof Skowronek, PhD, DSc Habil

Deputy Head

Roman Szczepanowski, PhD

The IIMCB Biophysics and Structural Biology Facility (BSBF) was created in 2021 by splitting the existing Core Facility into two more specialized ones. It provides access to a broad range of cutting-edge research technology platforms that are extensively used in structural biology, biochemistry, and molecular biology. The BSBF is managed by experienced scientists who devote their time and effort to maintain and operate the most sophisticated equipment that can be grouped into several sections.

The Structural Biology section is one of the most advanced in Poland. Proteins that are purified by research laboratories undergo crystallization trials using two crystallization robots and ~1,000 crystallization conditions (buffers). The crystallization process is performed in a crystallization hotel at 4°C or 18°C, and progress is tracked by a charge-coupled device (CCD) camera. The crystals are analyzed using an X-ray generator (Proteum Bruker) that is equipped with a CCD detector (Platinum 135) and cryostream cooler (Oxford Cryosystems series 700). This facility allows the collection of a complete set of diffraction data within a few hours. The Structural Biology section also shares an FEI Tecnai T12 transmission electron microscope with the Microscopy and Cytometry Facility for cryo-electron microscopy (cryo-EM) analyses of protein complexes. The system is combined with a TemCam F-Series camera and mostly used for structural biology and analyses of protein complexes both conventionally and with cryo-EM. One of the greatest advantages of cryo-EM relative to conventional structural biology techniques is its ability to analyze large, complex, and flexible structures that often cannot be crystallized. The T12 transmission electron microscope can be used to investigate polymers, thin films, fibers, ceramics, powders, and single molecules. The transmission electron microscope is supplemented with a Quorum Q150T ES, which is necessary for sample preparation (e.g., the hydrophilization [wetting] of films and grids) for transmission electron microscopy (TEM). The Q150T ES also allows the deposition of layers of carbon on grids. As part of our cryo-TEM workflow, we have a Vitrobot FEI that offers fully automated cryo-fixation (vitrification) under constant physical and mechanical conditions. This ensures high-quality cryo-fixation results and high-throughput sample preparation prior to cryo-TEM observations.

The Molecular Bioanalytics 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 (VP-differential scanning calorimetry and VP-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 Bioanalytics section is also equipped with a wide selection of multiwell plate luminometers and spectrometers, including spectrophotometers, spectrofluorometers, a circular dichroism spectropolarimeter, and a Fourier transform infrared spectrometer. We also offer access to an ultra-performance liquid chromatography system equipped with ultraviolet/visible and fluorescence detectors and an assortment of reverse-phase and SEC columns for precise qualitative and quantitative analyses of proteins, nucleic acids, and small molecules.

The Mass Spectrometry section has two mass spectrometers: matrix-assisted laser desorption ionization tandem time-of-flight (MALDI-TOF-TOF; ultrafleXtreme, Bruker) and liquid chromatography-electrospray ionization (LC-ESI)-Ion Trap (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 macromolecules 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, 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. We also operate one MinION instrument (third-generation sequencing) in the Oxford Nanopore MinION access program.

The General Use Equipment section gathers all other equipment that is administered by BSBF and available for 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 BSBF 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, BSBF collaborated with biotech companies, including Adamed, Captor Therapeutics, Celon Pharma, OncoArendi Therapeutics, and Polfa. BSBF 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).

BSBF 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.

Members of BSBF 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.



GENOME ENGINEERING UNIT

GROUP MEMBERS

Head

Olga Gewartowska, PhD

Senior Specialist

Katarzyna Prokop, MSc

Specialists

Katarzyna Sałas, MSc
Marcin Szpila, MSc

The Genome Engineering Unit (GEU) is the newest addition to the portfolio of IIMCB Core Facilities. It has three main branches of activities: generating transgenic mouse models using CRISPR/Cas9 technology, genotyping, and plasmid cloning. These methodologies have been developed for years in the Laboratory of RNA Biology – ERA Chairs Group and now are widely available to scientists from IIMCB and external clients.

Transgenic mouse generation and assisted reproduction technologies

Development of the transgenic mouse service was possible thanks to cooperation between Prof. Andrzej Dziembowski (IIMCB) and Prof. Ewa Borsuk (University of Warsaw Faculty of Biology, Department of Embryology) and support from the Foundation for Polish Science through the TEAM-TECH Core Facility grant (2018-2021).

The GEU seeks to provide high-quality, cutting-edge services for new mouse line generation and other embryological services (*in vitro* fertilization, embryo transfer, rederivation, embryo cryopreservation, vasectomy). Around 20 novel mouse lines are generated annually using CRISPR/Cas9 technology under licenses to patents from ERS Genomics. 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, degron systems), CRE-expressing mice, conditional (LoxP) knockouts, and mice that express large transgenes from the ROSA26 locus (up to 11 kb). 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 if the model is successfully generated. To date, we have generated several dozen different mouse lines with a ~95% success rate. Typical project duration is 6-8 months.

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 principles 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.

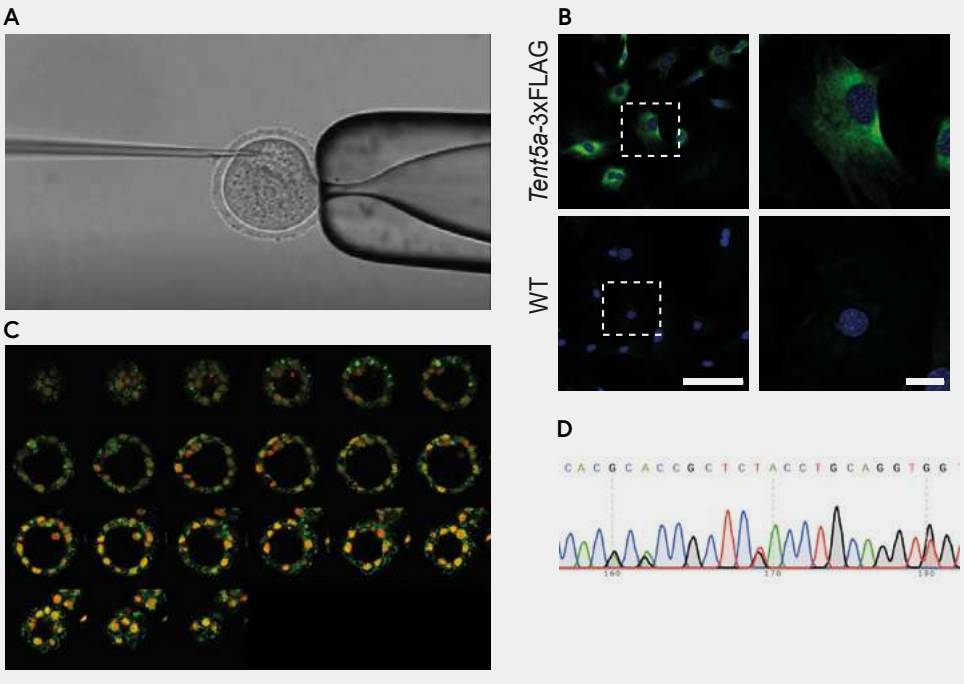
Genotyping

The GEU routinely provides transgenic mouse genotyping services using standard polymerase chain reaction, restriction fragment length analysis, sequencing, and high resolution melting (HRM) analysis.

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 250 plasmids per year with a predicted increase to 400 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 at once, with a total upper size limit of 20 kb. In addition to standard plasmids, we are also able to 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.

Fig. 1 Examples of results obtained using mouse models generated by the Genome Engineering Unit.
A. Procedure of oocyte microinjection (M. Brouze)
B. Immunohistochemical staining for FLAG in osteoblasts isolated from Tent5a-3xFLAG and WT controls with FLAG in green and Hoechst in blue (Gewartowska et al. 2021)
C. Confocal imaging of Dis3L-GFP blastocyst with Dis3L-GFP in green and Hoechst in red (M. Brouze, unpublished)
D. Exemplary chromatogram of heterozygotic knock-in mouse harboring several point mutations





MICROSCOPY AND CYTOMETRY FACILITY

GROUP MEMBERS

Head

Tomasz Węgierski, PhD

Senior Specialists

Kamil Jastrzębski, PhD (until December 2021)
Matylda Macias, PhD

Specialists

Katarzyna Misztal, PhD
Aleksandra Szybińska, MSc (since September 2021)

The IIMCB Microscopy and Cytometry Facility (MCF) split from the IIMCB Core Facility in March 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 a Becton Dickinson FACSAria II cell sorter. The sorter is equipped with three lasers (violet, blue, and red) and nine fluorescence detectors. Up to four populations can be sorted simultaneously. With four nozzle sizes, a very broad range of samples can be processed. Cell sorting 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 services are also possible on the most sophisticated microscopy platforms.



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 pre-trimmed 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 the latter 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).

LIST OF PUBLICATIONS

Poźniak M, Porębska N, **Jastrzębski K**, Krzyścik MA, Kucińska M, Zarzycka W, Barbach A, Zakrzewska M, Otlewski J, Miączyńska M, Opaliński Ł. Modular self-assembly system for development of oligomeric, highly internalizing and potent cytotoxic conjugates targeting fibroblast growth factor receptors. ***J Biomed Sci***, 2021;28(1):69

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

Poźniak M, Porębska N, Krzyścik MA, Sokołowska-Wędzina A, **Jastrzębski K**, Sochacka M, Szymczyk J, Zakrzewska M, Otlewski J, Opaliński Ł. The cytotoxic conjugate of highly internalizing tetravalent antibody for targeting FGFR1-overproducing cancer cells. ***Mol Med***, 2021;27(1):46

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Pozniak M, Sokolowska-Wedzina A, **Jastrzebski K**, Szymczyk J, Porebska N, Krzyscik MA, Zakrzewska M, Miaczynska M, Otlewski J, Opalinski L. FGFR1 clustering with engineered tetravalent antibody improves the efficiency and modifies the mechanism of receptor internalization. ***Mol Oncol***, 2020;14(9):1998-2021



ZEBRAFISH CORE FACILITY

The Zebrafish Core Facility (ZCF) has been in existence since 2012. It is a licensed breeding and research facility (District Veterinary Inspectorate in Warsaw registry no. PL14656251; Ministry of Science and Higher Education record no. 064 and 0051). The ZCF was established to introduce a new vertebrate model, *Danio rerio*, to research that is conducted at IIMCB. As the first in Poland, the ZCF joined the prestigious **European Zebrafish Society** (EZS) and is registered in the **Zebrafish Information Network database** (ZFIN). Since 2019, the ZCF has been included in IIMCB's Research Infrastructure of Molecules and Cells (IN-MOL-CELL) within the **Polish Roadmap for Research Infrastructures**. In 2020, the ZCF became a member of the Core Facility Working Group of the **EU-LIFE alliance**, actively participating in various initiatives, 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 originated in South Asia. Several key features, including high genetic similarity to humans, relatively short generation time, and transparent embryos that are readily accessible to genetic manipulations, have led to the rapid growth of zebrafish as an excellent model for biomedical research. Moreover, the wide sharing of techniques and collections of mutant/transgenic animals, together with low maintenance costs make zebrafish an attractive world-wide alternative to mammalian *in vivo* models that can be used to adhere to the Reduce, Refine, Replace (3R) principles of animal research. **We boast the largest zebrafish collection in Poland, which consists of wildtype and genetically modified lines**, including transgenics and mutants.

Among the ZCF collection are zebrafish expressing modified genes that are involved in transcription regulation, heart development, neuropathology, and neurodegenerative disorders. Thanks to our ongoing activities, IIMCB researchers can use zebrafish in innovative projects on genetics, developmental biology, and molecular mechanisms of human diseases. The **ZCF offers services for 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. Additionally, because of our international reputation and scientific collaborations, we export zebrafish lines to European and American scientific institutes.

Maintaining such a large number of zebrafish would not be possible without a suitable infrastructure. Our fish are currently housed in **1,210 tanks** (eight independent, automated aquatic systems). The ZCF is equipped with **incubators, microscopes, and microinjection systems** for zebrafish embryos. Additionally, Zebrafish Core Facility users have at their disposal a laboratory that is dedicated to **behavioral testing**. The room is equipped with automated systems for observing and tracking larval and adult zebrafish. The ZCF also performs **sperm freezing** and ***in vitro* fertilization** to guarantee the preservation of zebrafish 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, which allows us to **continuously monitor health status of the fish colony and maintain the highest standards of animal welfare**.

Scientists who use zebrafish for research purposes are obligated by law (Act of 15 January 2015 on the Protection of Animals Used for Scientific or Educational Purposes) to be certified to work with this animal model. **All research and breeding activities at the ZCF are performed in compliance with fundamental ethical principles** (Act of 15 January 2015 and European/International guidelines on animal welfare, including Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes and instructions of the Federation of European Laboratory Animal Science Associations [FELASA]).

The ZCF team consists of 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.

www.iimcb.gov.pl/zebrafish-core-facility

zcf-team@iimcb.gov.pl



SCIENTIFIC REPRESENTATION

SCIENTIFIC REPRESENTATION

SENIOR RESEARCHERS COUNCIL

The Senior Researchers Council is an association of researchers and senior researchers from all laboratories. In 2021, the group was represented by Daria Zdżalik-Bielecka and Jarosław Cendrowski.

The key responsibilities of our members, in addition to designing and performing scientific projects, are: to ensure that the expertise of the IIMCB is maintained, educate younger members of the laboratories, and assist lab leaders in organizational matters. Those of us who hold a DSc Habil degree engage in various scientific activities of the Institute, such as recruitment and serving as supervisors of PhD students. Our goal is to obtain scientific results and gain experience to be better prepared to start our own research groups in other institutions or to pursue non-academic careers.

GRANTS

In 2021 researchers and senior researchers received funding or lead the following projects:

- Filip Stefaniak, OPUS NCN "The new methodology for better understanding of ligand-RNA interactions"
- Vladimir Korzh, OPUS NCN "Experimental analysis of molecular determinants involved in epilepsy"
- Vladimir Korzh, OPUS NCN "Building a genomic atlas of human inner ear malformations: focus on novel genes and functional non-coding regions" [in collaboration with Dr. M. Oldak, Institute of Physiology and Pathology of Hearing (IFPS)]
- Ewelina Szymańska, OPUS NCN "Identification of novel vulnerabilities of VPS4B-deficient cancers cells"
- Justyna Zmorzyńska, OPUS NCN "Rac1 contribution to brain connectivity impairments and neuropsychiatric disorders in Tuberous Sclerosis Complex"

PUBLICATIONS

Last year our group co-authored 38 publications, and in many of them, researchers or senior researchers were either first or corresponding authors. Importantly, publications that won Best Papers Award 2021 were prepared with the prominent contribution of researchers and senior researchers.

FELLOWSHIPS

Elżbieta Purta received Scholarship of the Director of the Polish National Agency for Academic Exchange in the selection procedure of the Bekker Programme (edition 2020) to implement the project "Structural studies of lncRNAs *in vivo*" during a scientific visit at the University of Groningen.

EXTERNAL LECTURES

- Ewelina Szymańska, *Investigating cancer-associated VPS4B loss – in search of therapeutic targets for personalized oncology* (Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, April 2021)
- Jarosław Cendrowski, *Membrane trafficking during erythroid differentiation in normal erythropoiesis or upon stress* (5th International ReMedy Minisyposium, Warsaw, April 2021)
- Filip Stefaniak, three lectures: *Before a new pastille is made* (Warsaw Festival of Science, September 2021)

MENTORING

Auxiliary supervisors of PhD students:

- Jarosław Cendrowski for Marta Wróbel
- Honorata Czapińska for Anna Fedenko
- Magdalena Czeredys for Ewelina Latoszek
- Elżbieta Jagielska for Paweł Mitkowski and Alicja Wysocka
- Kamil Jatrzębski for Karolina Wojciechowska
- Vladimir Korzh for Justyna Jędrychowska and Ruchi Prakash Jain
- Seweryn Mroczek for Aleksandra Bilska
- Elżbieta Nowak for Marzena Nowacka
- Filip Stefaniak for Pietro Boccaletto
- Ewelina Szymańska for Malwina Grębowicz-Maciukiewicz
- Małgorzata Urbańska for Magdalena Mlostek and Juan Zeng
- Daria Zdżalik-Bielecka for Marta Jakubik and Agata Poświata
- Justyna Zmorzyńska for Olga Doszyń and Magdalena Kędra

COMMUNITY SERVICE

Editors: Vladimir Korzh is an editor for *Scientific Reports* and the *International Journal of Molecular Sciences*.

REVIEWERS

- Vladimir Korzh: *Scientific Reports, Zebrafish, Molecular Biology and Evolution*
- Filip Stefaniak: *Nucleic Acid Research and Bioinformatics*
- Małgorzata Korzeniowska: *MethodsX, Molecular Toxicology, Frontiers in Cell and Developmental Biology, Water*
- Ewelina Szymańska: *BMC Biology, Acta Neurobiologiae Experimentalis*
- Justyna Zmorzyńska: *Neurochemical Research*

EXPERTS

Vladimir Korzh evaluated grant applications for the French National Research Agency (ANR), and Elżbieta Nowak for the National Science Centre (NCN).

Małgorzata Korzeniowska is among the experts at the Ministry of Education and Science who participate in inspections of users performing animal experiments, which are carried out by district veterinarians.

Notably, our group initiated efforts to enable the employment of experienced scientists, who are more than 7 years after defending PhD thesis, in grants funded by the National Science Centre (NCN). Thanks to our efforts, the NCN introduced a new position of Senior Researcher to OPUS and MAESTRO grants. Currently, we are trying to convince the NCN Council to change the rules for the employment of Senior Researchers in grants funded by the NCN. To this end, we prepared an open letter addressed to the NCN Council and we are currently collecting signatures supporting our initiative.

POSTDOCTORAL COUNCIL

The Postdoctoral Council gathers postdoctoral researchers from all IIMCB laboratories. In 2021, the Council representatives were Andrii Kopach and Natalia Gumińska. A new representative, Angana Ray, replaced Andrii Kopach and was appointed in October 2021.

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 of different groups at IIMCB and strengthen scientific interactions and collaborations with researchers across Poland and abroad.

SPOTLIGHT TALKS



The idea of Spotlight Talks is to present scientific projects in an understandable manner for people from outside the field. The speakers are mostly postdocs and senior researchers involved in life science research. Talks are usually 15-minutes-long, followed by discussions. This initiative is open to the public and regularly advertised via Facebook/LinkedIn/Twitter. In 2021 18 meetings were organized (see page 96).

RNA CLUB REACTIVATION

(resumed in December 2021)

The RNA Club was revived by two of our postdocs (Vladyslava Liudkovska and Tomasz Kuliński, Laboratory of RNA Biology – ERA Chairs Group) in collaboration with a PhD student (Zuzanna Mackiewicz, Laboratory of RNA Biology – ERA Chairs Group) and Lab Leaders (Janusz Bujnicki, Andrzej Dziembowski, and Gracjan Michlewski). The first talk of this series, *Regulation of mRNA translation and decay*, was given by Prof. David Bartel from Whitehead Institute/MIT/Howard Hughes Medical Institute, Cambridge, USA, December 2021.

POSTDOC TEAMS CREATED ON MICROSOFT TEAMS

This is a common forum where all postdocs at IIMCB can communicate with each other. Postdoc Teams has four channels: (i) Career opportunities (discuss and share our information on various career options that postdocs can avail), (ii) Post Spotlights (share information about Spotlight Talks, especially if anyone would like to invite a particular speaker), (iii) Software (share information about different software that is accessible to different groups so that postdocs of different groups can share resources), and (iv) General (discuss issues and integrate with each other).

THE FIRST OPEN-TO-ALL POSTDOC MEETING IN NOVEMBER 2021

We organized an online meeting with all postdocs at IIMCB so that we can learn about their needs and develop a plan to address them. Such topics as mentoring, resource sharing, and career opportunities were discussed. We also shared the outcome of the meeting with lab leaders (Janusz Bujnicki, Andrzej Dziembowski, Jacek Jaworski, and Gracjan Michlewski).

INPUT TO MENTORSHIP PROGRAM

The Directors and lab leaders introduced us to the idea of a mentorship program. We initiated communication with lab leaders about establishing such a program. This involved initial brainstorming among postdocs (discussed during the open-to-all postdoc meeting) and communicating our concerns and expectations to lab leaders.

OTHER ACTIVITIES

In addition to the aforementioned major activities, we strived to improve communication between postdocs. We now have an official e-mail (postdocrepresentatives@iimcb.gov.pl) for the Postdoctoral Council. From this e-mail, we send out periodic e-mails to welcome new postdocs, make everyone aware of current developments, and share new information. We also encourage postdocs to reply about their grievances, concerns, or suggestions so that postdoc representatives can raise these issues with relevant authorities.

PUBLICATIONS

Last year postdoctoral researchers at the IIMCB co-authored 15 publications, including first-authorship of publications in renowned journals (*Cell Reports, Journal of Molecular Biology*). There was one preprint deposited in *bioRxiv*.

EDITORIALS

Agata Sulej was a co-editor of a Special Issue of the *Briefings in Functional Genomics*, entitled Zebrafish Swims into Post-Genomic Era, 2021; 20(6).

INVITED TALKS

Olga Gewartowska gave a lecture *Mouse models of human disease - opportunities associated with the CRISPR-Cas9 method* during the meeting of Warsaw Section of the Polish Genetics Association, November 2021.

POSTERS

Paweł Krawczyk presented a poster *Global view on the metabolism of RNA poly(A) tails in yeast Saccharomyces cerevisiae* at the RiboClub Annual Meeting.

Ivan Trus presented two posters at the International Nidovirus Symposium (NIDO 2021): Xie J, et al. *Comparison of primary virus isolation in PAMs and four different continuous cell lines for type 1 and type 2* PRRSV; Xie J, et al. *Spatial and temporal dynamics of PRRSV-1 spreading in Belgium and the rest of Europe between 1992 and 2018.*

ABSTRACTS

Ivan Trus prepared an abstract for the Annual Meeting of the American Society for Virology (ASV 2021): Udenze D, et al. *Silent in utero Zika virus infection causes systemic immune activation and inflammation in fetuses.*

MENTORING

Auxiliary supervisors of PhD students:

- Monika Kusio-Kobiałka for Michał Mazur
- Vladislava Liudkovska for Zuzanna Mackiewicz
- Małgorzata Piechota for Anwesha Sarkar
- Magdalena Wołczyk for Jacek Szymański

PHD STUDENTS COUNCIL

The IIMCB PhD Students Council serves as the voice for an entire PhD students body and actively works with Institute authorities to promote a better learning environment. The Council aims to stay informed about and to play an active role within important developments that may concern PhD students. Their members have an advisory role concerning PhD-related issues at the Institute level. The PhD Students 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 meeting sessions.

In 2021, the Council representatives were Abhishek Pateria and Jan Węsławski, replaced in October by Olga Doszyń and Tomasz Obrębski.

The PhD students of the IIMCB attend 4 different doctoral schools:

- Warsaw PhD School in Natural and BioMedical Sciences (Warsaw-4-PhD)
- School of Molecular Biology (Institute of Biochemistry and Biophysics Polish Academy of Sciences - IBB)
- PhD studies of the Nencki Institute of Experimental Biology Polish Academy of Sciences (Nencki Institute)
- Postgraduate School of Molecular Medicine (Medical University of Warsaw - SMM)

IIMCB representatives in these schools are: Zuzanna Mackiewicz (Warsaw-4-PhD), Katarzyna Banasiak (IBB), Jan Węsławski (Nencki Institute) and Maciej Migdał (SMM).

Despite the continued COVID-19 pandemic, affecting the organization of events as well as our day-to-day work, our PhD students have participated in online conferences and symposiums, and made scientific progress as evidenced by several awards, scholarships, and publications. In 2021, our PhD students were co-authors of 15 publications and 2 preprints.

REPORT SESSION 2021

The yearly PhD students' report session was organized online through the ZOOM platform on the 2nd of September, 24th of September, 7th of October and 8th of October 2021. In total, 41 PhD students attended the meetings and had the opportunity to present their work and report their scientific progress. Four students received the best presentation awards (by popular vote): Natalia Szulc from the Laboratory of Protein Metabolism, Pratik Mandal from the Laboratory of Iron Homeostasis, Karolina Bogusz from the Laboratory of Molecular and Cellular Neurobiology, and Gabriela Żurawska from the Laboratory of Iron Homeostasis.

AWARDS AND SCHOLARSHIPS

- Katarzyna Banasiak and Anna Stroynowska-Czerwińska have received the IBB Director's scholarship for best performing PhD students in the academic year 2020/2021
- Carlos Sequeiros has secured a PRELUDIUM grant from the National Science Centre for the project titled "Towards automatic derivation of geometry-based descriptors as surrogates for complex structural approaches in enzyme-substrate prediction"
- Natalia Szulc has secured a PRELUDIUM grant from the National Science Centre for the project titled "Living on the edge: evolutionary adaptation of substrate-recruiting subunits of the cullin-RING ubiquitin ligase complexes to avoid premature degradation"
- Alicja Wysocka undertook a research visit at the Cell Wall and Antibiotic Resistance Team, Institute of Structural Biology (Grenoble, France), funded by NAWA programme International Academic Partnerships within the project "Molecular basis of enzyme specificity and applications"

PARTICIPATION IN CONFERENCES

- Katarzyna Banasiak participated in the 5th IBB Young Investigators Symposium, June 2021; Extracellular Vesicle Studies: From Benchtop to Therapeutics conference organized by the American Society for Biochemistry and Molecular Biology, July 2021, and the Mechanical Stress Protection: Mechanobiology Meets Proteostasis international conference of the research unit DFG FOR 2743, September 2021
- Karolina Bogusz presented a poster at the International Young Scientists Conference on Molecular and Cell Biology, February 2021, and at the 5th IBB Young Investigators Symposium, June 2021
- Katarzyna Krakowska participated in the 23rd Heart of Europe Bio-Crystallography Meeting (HEC23), September 2021
- Nishita Mandal has given an oral presentation at the 4th Central European Biomedical Congress (CEBC), June 2021
- Michał Mazur has participated in the Eukaryotic mRNA Processing conference, August 2021
- Agata Poświata took part in organising and presenting at the International Young Scientists Conference on Molecular and Cell Biology, February 2021 (online), and participated in the 1st Women in Science Symposium, March 2021
- Carlos Sequeiros presented a poster at LatinXChem, September 2021
- Anna Stroynowska-Czerwińska presented a poster at the EMBL Chromatin & Epigenetics conference, May 2021
- Natalia Szulc gave oral presentations at the 26th Annual Meeting of the RNA Society, May/June 2021; the Autumn Conference PTBI 2021, September 2021; the EMBO Young Scientists' Forum, October 2021, and presented a poster at the 23rd EMBL PhD Symposium, December 2021
- Aravind Selvaram Thirunavukarasu presented a poster at the 45th FEBS congress, July 2021, and an oral presentation at the annual PhD Conference COMPASS, October 2021
- Alicja Wysocka presented a poster at the EMBO Young Scientists' Forum, October 2021, and at the EMBL Symposium: New Approaches and Concepts in Microbiology, July 2021, and gave oral presentations at the 5th IBB Symposium for Young Investigators, June 2021; the IBB PhD seminar series, October 2021, and at the NMR group meeting, December 2021
- Gabriela Żurawska gave two oral presentations at the European Iron Club Zoomposium, September 2021

OTHER ACTIVITIES

- Zuzanna Mackiewicz was one of the organisers of the RNA Club Warsaw
- Maciej Migdał and Eugeniusz Tralle, with the help of other students, organised the International Young Scientists Conference on Molecular and Cell Biology 2021
- Agata Poświata was the co-originator and active participant of "International cooperation and diversity management in research careers", a soft skills course for PhD students
- Alicja Wysocka co-authored an international patent application "Recombinant polypeptide for use as a medicine, antiseptic agent, antibacterial agent, anti-inflammatory agent, compositions comprising it and uses thereof" (PCT/PL2020/050075)

WARSAW PHD SCHOOL IN NATURAL AND BIOMEDICAL SCIENCES

Warsaw PhD School in Natural and BioMedical Sciences (Warsaw-4-PhD) started its operations in the 2019/2020 academic year. The School is an organized form of PhD student education in four disciplines: biology, chemistry, physic and medical sciences, preparing students to obtain a PhD degree. The School is formed by nine institutions:

- Nencki Institute of Experimental Biology of the Polish Academy of Sciences (Nencki Institute) – leader
- International Institute of Molecular and Cell Biology in Warsaw (IIMCB)
- Institute of Organic Chemistry of the Polish Academy of Sciences (IOC PAS)
- Institute of Physical Chemistry of the Polish Academy of Sciences (IPC PAS)
- Institute of Physics of the Polish Academy of Sciences (IP PAS)
- Center for Theoretical Physics of the Polish Academy of Sciences (CTP PAS)
- Institute of High Pressure Physics of the Polish Academy of Sciences (IHPP PAS "Unipress")
- Maria Sklodowska-Curie National Institute of Oncology – State Research Institute (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. Enrolment occurs three times a year and candidates commence their education in either the winter or the summer semester. Doctoral students, under the guidance of their supervisors, implement individual research plans and develop their research skills. The education in the Warsaw-4-PhD ends with the submission of a dissertation. The next step is to obtain a doctoral degree in a separate procedure conducted outside the School.

The IIMCB offers their PhD students the chance to work in a vibrant, inclusive but 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 lab leaders and doctoral students in their journey to PhD. We encourage PhD students to participate in international activities, ranging from research secondments 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 every step of the way. Additionally, IIMCB provides PhD students access to a private medical package, as well as social benefits equal to the employees.

We know that students' voices matter. With this in mind, appreciation of our PhD students' opinion is shown through the regular meetings of the PhD Students Council representatives with the IIMCB directors and International Advisory Board members. Consequently, the Institute endeavors to address the concerns of doctoral students and support their initiatives.

Being the largest institute-based doctoral school in Poland we are 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 STER programme. Project will be implemented for three years starting January 2022. Main project tasks and responsible institutes:

- 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, advanced lecture series with international experts, spotlight talks – IP PAS

www.warsaw4phd.eu/en/

Disciplines and scientific institutions

Biology

Chemistry

Physics

Medicine

FACTS & FIGURES

HR DATA

270 Staff

163 Female

107 Male

73 Foreigners

18 Nations

12	Lab Leaders
1	Project Coordinator
15	Senior Researchers
1	Researcher
35	Postdoctoral Researchers
6	Research Assistants
18	Research Specialists
55	PhD Students

4	Undergraduate Students
19	Trainees
15	Core Facilities Staff
2	BioCEN Staff
6	Technicians
11	Laboratory Support Specialists
41	Administration
29	Others

PERSONAL ACHIEVEMENTS

- **Marta Miączyńska**: the **Prime Minister's Award** for outstanding scientific achievements in 2020
- **Marcin Nowotny, Marta Gapińska, Vineet Gaur**, **Karolina Górecka**, **Shivlee Nirwal**, **Aleksandra Szlachcic**, **Weronika Zajko**, Jiri Šponer, Miroslav Krepl, Jarosław Poznański: the **Team Award of the Minister of Education and Science** for significant achievements in scientific activities – groundbreaking discoveries describing the mechanism of cleavage of Holliday structures
- **Marta Miączyńska**: **new Member of the EMBO Council**
- **Gracjan Michlewski**: **AIMS Award** for the research focused on identification of small molecule binders of ELAVL1
- **Andrzej Dziembowski**: **Leader of the "Horizon for Excellence in messenger RNA applications in immunoOncology" (acronym: HERO) Project**, Virtual Research Institute (WIB), Polish Science Fund
- **Aleksandra Bilska: START scholarship** Foundation for Polish Science program

SCIENTIFIC PROMOTIONS

- **Gracjan Michlewski**, Professor of Biological Sciences
- **Cecilia Winata**, DSc Habil in Biological Sciences
- **Justyna Jędrychowska***, PhD in Medicine and Health Sciences, *The role of voltage-gated potassium channels in a development of the ear*, thesis advisor: J. Kuźnicki
- **Deepshikha Malik***, PhD in Biological Sciences, *Mechanism of action of exonuclease REXO2 and non-canonical nucleotidyltransferase CutAin mRNA decay*, thesis advisor: M. Nowotny
- **Anton Slyvka***, PhD in Biological Sciences, *Specific recognition of chemically modified cytosines in the DNA*, thesis advisor: M. Bochtler
- **Aleksandra Tempes**, PhD in Biological Sciences, *Impact of mTOR kinase on canonical and non-canonical functions of AP2 adaptor complex*, thesis advisor: J. Jaworski
- **Iga Wasilewska**, PhD in Medicine and Health Sciences, *Involvement of Stim2 protein in the neurodegeneration in Danio rerio*, thesis advisor: J. Kuźnicki

* promotion with honors

BEST PAPERS AWARD 2021

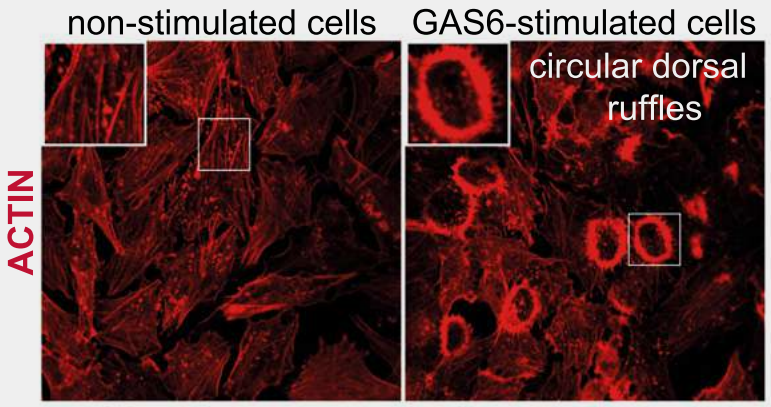
Following the tradition of our Institute, the best papers with IIMCB affiliation in 2021 have been awarded. The best publications are selected by the Institute's Lab Leaders based on contents and significance, but not the bibliometric data. A full list and the pdf files of all the papers submitted for the Award (together with the supporting statements) are sent to all Lab Leaders. They look through all of them and choose those which in their view deserve the Award. Leaders cannot vote for the papers from their own laboratory. The results are discussed during Lab Leaders' meeting and the final list of winning papers is approved.

1st PLACE

authors affiliated with the IIMCB are in bold

Zdżalik-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(28):e2024596118

Study of researchers from the Laboratory of Cell Biology and their colleagues from Institute for Cancer Research, Oslo University Hospital, and Nencki Institute of Experimental Biology Polish Academy of Sciences, reveals intracellular processes that are activated by ligand-stimulated AXL receptor. This receptor tyrosine kinase is overexpressed in late-stage, metastatic or drug-resistant cancers of various origins. Although the first AXL inhibitors are in clinical trials, the cellular mechanisms of AXL action have remained unknown. By identifying a proximity interactome of AXL scientists discovered that AXL primarily activates remodeling of actin. This potentiates membrane ruffling and macropinocytosis that results in increased invasion and nutrient acquisition of cancer cells.



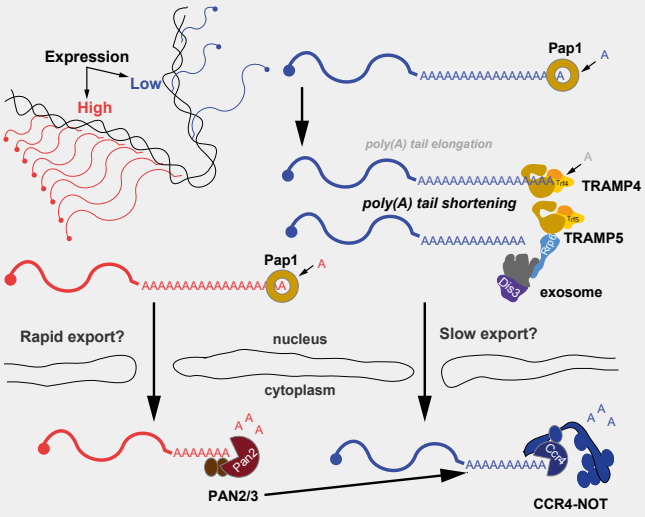
2nd PLACE

authors affiliated with the IIMCB are in bold

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

Researchers from the Laboratory of RNA Biology - ERA Chairs Group and co-authors from Danish and Polish science centers performed a first transcriptome-wide analysis of poly(A) tail lengths in budding yeast, *Saccharomyces cerevisiae*, revealing the factors involved in shaping poly(A) tail length and abundance of all RNA biotypes. Polyadenylation of the RNA 3'-end is a ubiquitous modification playing a pivotal role in the control of the transcript's stability. The transcriptome-wide distribution of poly(A) tails was never comprehensively studied in perturbed states, mainly because of technological limitations. To circumvent this, the authors used nanopore direct RNA sequencing to analyze poly(A) tails in yeast devoid of enzymes relevant for poly(A) tail metabolism. They have shown that nuclear exosome-associated nucleases and cytoplasmic deadenylases can trim the tails in a transcript-specific manner, shaping up to 2/3 of the coding transcriptome. De-novo produced mRNA poly(A) tails were demonstrated to be ~50 adenosines long and rarely exceed 200. Comparable lengths also apply to poly(A)-tails

produced by non-canonical poly(A) polymerases, Trf4/5, at non-coding RNAs such as ribosomal and small nuclear RNA precursors, which were till-now believed to be oligoadenylated. Finally, they revealed that growth conditions, such as temperature and nutrient content, have a significant impact on poly(A) tails. This work confirmed many previously suggested trends regarding poly(A) tails but also challenged the established knowledge in the field.

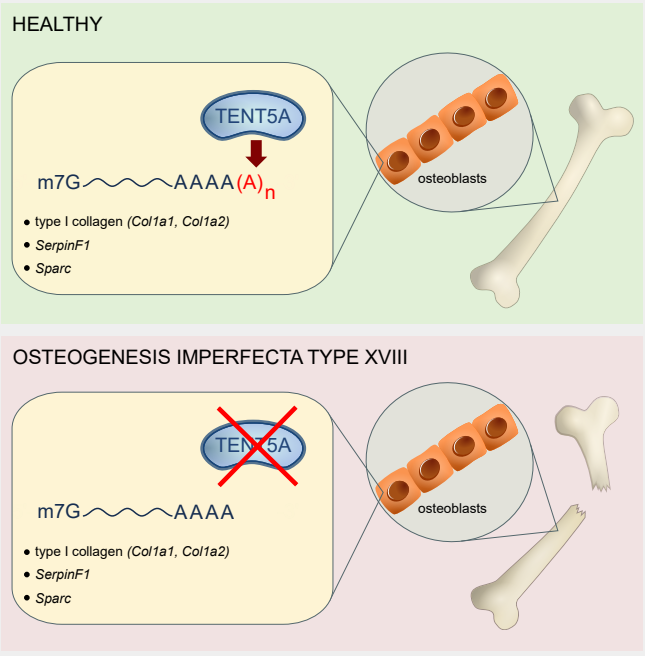


3rd PLACE

authors affiliated with the IIMCB are in bold

Gewartowska O, Aranaz-Novaliches G, **Krawczyk PS, Mroczek S, Kusio-Kobiałka M, Tarkowski B,** Spoutil F, Benada O, Kofroňová 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

Researchers from the Laboratory of RNA Biology - ERA Chairs Group and co-authors from Czech and Polish scientific institutions were investigating the role of cytoplasmic polyadenylation in bone formation. Collagens are the most abundant proteins on our planet. Bone forming cells - osteoblasts secrete fibrillar collagen, a scaffold on which bones mineralize. Using transgenic mice models, complex functional analysis, and direct RNA sequencing, scientists demonstrated that TENT5A poly(A) polymerase regulates the expression of collagen chains, stabilizing mRNA encoding them, which is essential for proper bone formation. In the absence of TENT5A activity, collagen fibres are thinner and improperly structured, resulting in bone susceptibility to fracture. Mutations in TENT5A were previously described in a group of patients with a congenital bone disease (osteogenesis imperfecta). For the first time the link between poly(A) polyadenylation and bone fragility was described. The severe course of the disease is explained by both quantitative and qualitative collagen defects. This work also emphasizes the importance of cytoplasmic poly(A) polyadenylation in posttranscriptional regulation of gene expression.



PUBLICATIONS IN 2021

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	Tudek A*, **Krawczyk PS***, **Mroczek S**, Tomecki R, Turtola M, **Matylla-Kulińska K**, Jensen TH, **Dziembowski A**. * *co-first authorship*	Global view on the metabolism of RNA poly(A) tails in yeast Saccharomyces cerevisiae.	Nat Commun, 2021; 12(1):4951. doi: 10.1038/s41467-021-25251-w	15.805	MULTIDISCIPLINARY SCIENCES	1
2	**Ponce-Salvatierra A**, **Boccaletto P**, **Bujnicki JM**.	DNAmoreDB, a database of DNAzymes.	Nucleic Acids Res, 2021; 49(D1):D76-81. doi: 10.1093/nar/gkaa867	15.542	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
3	**Pastor M**, **Czapinska H**, **Helbrecht I**, **Krakowska K**, Lutz T, Xu S, **Bochtler M**.	Crystal structures of the EVE-HNH endonuclease VcaM4I in the presence and absence of DNA.	Nucleic Acids Res, 2021; 49(3):1708-23. doi: 10.1093/nar/gkaa1218	15.542	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
4	**Zhu S**, Choudhury NR, Rooney S, Pham NT, Koszela J, Kelly D, Spanos C, Rappsilber J, Auer M, **Michlewski G**.	RNA pull-down confocal nanoscanning (RP-CONA) detects quercetin as pri-miR-7/HuR interaction inhibitor that decreases α-synuclein levels.	Nucleic Acids Res, 2021; 49(11):6456-73. doi: 10.1093/nar/gkab484	15.542	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
5	**Zdżalik-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(28):e2024596118. doi: 10.1073/pnas.2024596118	12.291	MULTIDISCIPLINARY SCIENCES	1
6	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. doi: 10.15252/embr.202052071	10.716	BIOCHEMISTRY & MOLECULAR BIOLOGY / CELL BIOLOGY	1
7	**Gewartowska O**, Aranaz-Novaliches G, **Krawczyk PS**, **Mroczek S**, **Kusio-Kobiałka M**, **Tarkowski B**, Spoutil F, Benada O, Kofroňová 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. doi: 10.1016/j.celrep.2021.109015	10.394	CELL BIOLOGY	1
8	**Sequeiros-Borja CE**, **Surpeta B**, **Brezovsky J**.	Recent advances in user-friendly computational tools to engineer protein function.	Brief Bioinform, 2021; 22(3):bbaa150. doi: 10.1093/bib/bbaa150	10.288	BIOCHEMICAL RESEARCH METHODS	1
9	**Abu Nahia K**, **Migdał M**, Quinn TA, Poon KL, **Łapiński M**, **Sulej A**, Liu J, Mondal SS, **Pawlak M**, Bugajski Ł, Piwocka K, Brand T, Kohl P, **Korzh V**, **Winata C**.	Genomic and physiological analyses of the zebrafish atrioventricular canal reveal molecular building blocks of the secondary pacemaker region.	Cell Mol Life Sci, 2021; 78(19-20):6669-87. doi: 10.1007/s00018-021-03939-y	9.074	BIOCHEMISTRY & MOLECULAR BIOLOGY / CELL BIOLOGY	1
10	**Liudkovska V**, **Dziembowski A**.	Functions and mechanisms of RNA tailing by metazoan terminal nucleotidyltransferases.	Wiley Interdiscip Rev RNA, 2021; 12(2):e1622. doi: 10.1002/wrna.1622	8.247	CELL BIOLOGY	1
11	**Mleczko-Sanecka M**, Silvestri L.	Cell-type-specific insights into iron regulatory processes.	Am J Hematol, 2021; 96(1):110-27. doi: 10.1002/ajh.26001	7.954	HEMATOLOGY	1
12	**Latoszek E**, **Czeredys M**.	Molecular Components of Store-Operated Calcium Channels in the Regulation of Neural Stem Cell Physiology, Neurogenesis, and the Pathology of Huntington's Disease.	Front Cell Dev Biol, 2021; 9:657337. doi: 10.3389/fcell.2021.657337	7.219	DEVELOPMENTAL BIOLOGY	1
13	**Herok M**, **Wawrzynow B**, **Maluszek MJ**, **Olszewski MB**, **Zylicz A**, **Zylicz M**.	Chemotherapy of HER2- and MDM2-Enriched Breast Cancer Subtypes Induces Homologous Recombination DNA Repair and Chemoresistance.	Cancers (Basel), 2021; 13(18):4501. doi: 10.3390/cancers13184501	6.999	ONCOLOGY	1
14	**Urbanek O**, **Wysocka A**, **Nakielski P**, **Pierini F**, **Jagielska E**, **Sabała I**.	Staphylococcus aureus Specific Electrospun Wound Dressings: Influence of Immobilization Technique on Antibacterial Efficiency of Novel Enzybiotic.	Pharmaceutics, 2021; 13(5):711. doi: 10.3390/pharmaceutics13050711	6.734	PHARMACOLOGY & PHARMACY	1
15	**Wysocka A**, **Jagielska E**, **Łężniak Ł**, **Sabała I**.	Two New M23 Peptidoglycan Hydrolases With Distinct Net Charge.	Front Microbiol, 2021; 12:719689. doi: 10.3389/fmicb.2021.719689	6.320	MICROBIOLOGY	1
16	**Małecki PH**, **Mitkowski P**, **Jagielska E**, **Trochimiak K**, Mesnage S, **Sabała** I.	Structural Characterization of EnpAD,L-Endopeptidase from Enterococcus faecalis Prophage Provides Insights into Substrate Specificity of M23Peptidases.	Int J Mol Sci, 2021; 22(13):7136. doi: 10.3390/ijms22137136	6.132	BIOCHEMISTRY & MOLECULAR BIOLOGY	1

No	Authors	Title	Journal	5-Year IF	Journal Category	Quartile in Category
17	**Parisi C**, **Vashisht S**, **Winata CL**.	Fish-Ing for Enhancers in the Heart.	Int J Mol Sci, 2021; 22(8):3914. doi: 10.3390/ijms22083914	6.132	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
18	**Kolmus K**, **Erdenebat P**, **Szymańska E**, **Stewig B**, Goryca K, Derezinska-Wolek E, Szumera-Cieckiewicz A, Brewinska-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(1):jcs250951. doi: 10.1242/jcs.250951	6.032	CELL BIOLOGY	2
19	de Assis GG, Hoffman JR, Bojakowski J, Murawska-Ciałowicz E, Cięszczyk P, **Gasanov EV**.	The Val66 and Met66 Alleles-Specific Expression of BDNF in Human Muscle and Their Metabolic Responsivity.	Front Mol Neurosci, 2021; 14:638176. doi: 10.3389/fnmol.2021.638176	5.760	NEUROSCIENCES	1
20	**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. doi: 10.3389/fncel.2021.647860	5.574	NEUROSCIENCES	1
21	**Hojka-Osinska A**, Chlebowski A, Grochowska J, Owczarek EP, Affek K, Kłosowska-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. doi: 10.1016/j.isci.2021.103036	5.458	MULTIDISCIPLINARY SCIENCES	1
22	**Bochtler M**.	Distinction between self and non-self in restriction modification: The mysterious case of type IIL enzymes.	Structure, 2021; 29(6):512-14. doi: 10.1016/j.str.2021.05.007	5.434	BIOCHEMISTRY & MOLECULAR BIOLOGY / CELL BIOLOGY	2
23	**Banasiak K**, **Szulc NA**, **Pokrzywa W**.	The Dose-Dependent Pleiotropic Effects of the UBB+1 Ubiquitin Mutant.	Front Mol Biosci, 2021; 8:650730. doi: 10.3389/fmolb.2021.650730	5.389	BIOCHEMISTRY & MOLECULAR BIOLOGY	2
24	**Stefaniak F**, **Bujnicki JM**.	AnnapuRNA: A scoring function for predicting RNA-small molecule binding poses.	PLoS Comput Biol, 2021; 17(2):e1008309. doi: 10.1371/journal.pcbi.1008309	5.379	BIOCHEMICAL RESEARCH METHODS	1
25	**Nowacka M**, **Nowak E**, **Czarnocki-Cieciura M**, **Jackiewicz J**, **Skowronek K**, **Szczepanowski R**, Wöhrl BM**, Nowotny M**.	Structures of substrate complexes of foamy viral protease-reverse transcriptase.	J Virol, 2021; 95(18):e0084821. doi: 10.1128/JVI.00848-21	5.078	VIROLOGY	1
26	**Bochtler M**, Fernandes H.	DNA adenine methylation in eukaryotes: Enzymatic mark or a form of DNA damage?	BioEssays, 2021; 43(3):e2000243. doi: 10.1002/bies.202000243	5.022	BIOCHEMISTRY & MOLECULAR BIOLOGY	2
27	**Gasanov EV**, **Jędrychowska J**, **Kuźnicki J**, **Korzh V**.	Evolutionary context can clarify gene names: Teleosts as a case study.	BioEssays, 2021; 43(6):e2000258. doi: 10.1002/bies.202000258	5.022	BIOCHEMISTRY & MOLECULAR BIOLOGY	2
28	**Koscielny A**, **Liszewska E**, **Machnicka K**, **Wezyk M**, Kotulska K, **Jaworski J**.	mTOR controls endoplasmic reticulum-Golgi apparatus trafficking of VSVg in specific cell types.	Cell Mol Biol Lett, 2021; 26(1):18. doi: 10.1186/s11658-021-00262-z	4.833	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
29	Yang S, Emelyanov A, You MS, Sin M, **Korzh V**.	Camel regulates development of the brain ventricular system.	Cell Tissue Res, 2021; 383(2):835-52. doi: 10.1007/s00441-020-03270-1	4.588	CELL BIOLOGY	2
30	**Migdał M**, **Tralle E**, **Abu Nahia K**, Bugajski Ł, **Kędzierska KZ**, Garbicz F, Piwocka K, **Winata CL**, **Pawlak M**.	Multi-omics analyses of early liver injury reveals cell-type-specific transcriptional and epigenomic shift.	BMC Genomics, 2021; 22(1):904. doi: 10.1186/s12864-021-08173-1	4.478	GENETICS & HEREDITY	2
31	**Minhas R**, Loeffler-Wirth H, **Siddiqui YH**, **Obrębski T**, **Vashisht S**, **Abu Nahia K**, **Paterek A**, **Brzozowska A**, Bugajski L, Piwocka K, **Korzh V**, Binder H, **Winata CL**.	Transcriptome profile of the sinoatrial ring reveals conserved and novel genetic programs of the zebrafish pacemaker.	BMC Genomics, 2021; 22(1):715. doi: 10.1186/s12864-021-08016-z	4.478	GENETICS & HEREDITY	2
32	**Slusarczyk P**, **Mleczko-Sanecka K**.	The Multiple Facets of Iron Recycling.	Genes (Basel), 2021; 12(9):1364. doi: 10.3390/genes12091364	4.339	GENETICS & HEREDITY	2
33	**Jedrychowska J**, **Gasanov EV**, **Korzh V**.	Kcnb1 plays a role in development of the inner ear.	Dev Biol, 2021; 471:65-75. doi: 10.1016/j.ydbio.2020.12.007	3.587	DEVELOPMENTAL BIOLOGY	2
34	**Czapinska H**, Winiewska-Szajewska M, Szymaniec-Rutkowska A, **Piasecka A**, **Bochtler M**, Poznański J.	Halogen Atoms in the Protein-Ligand System. Structural and Thermodynamic Studies of the Binding of Bromobenzotriazoles by the Catalytic Subunit of Human Protein Kinase CK2.	J Phys Chem B, 2021; 125(10):2491-503. doi: 10.1021/acs.jpcb.0c10264	3.051	CHEMISTRY, PHYSICAL	3
35	**Liszewska E**, Majchrowicz L, Krogulec E, Kotulska K, Kaczmarek L, Kalita K, Dobrzyń A, **Jaworski J**.	Establishment of two hiPSC lines (IIMCBi001-A and IIMCBi002-A) from dermal fibroblasts of healthy donors and characterization of their cell cycle.	Stem Cell Res, 2021; 52:102225. doi: 10.1016/j.scr.2021.102225	2.523	BIOTECHNOLOGY & APPLIED MICROBIOLOGY / CELL BIOLOGY	4
36	**Gasanov EV**, **Jędrychowska J**, **Pastor M**, **Wiweger M**, Methner A**, Korzh V**.	An improved method for precise genome editing in zebrafish using CRISPR-Cas9 technique.	Mol Biol Rep, 2021; 48(2):1951-7. doi: 10.1007/s11033-020-06125-8	2.357	BIOCHEMISTRY & MOLECULAR BIOLOGY	4
37	**Dyrda A**, **Kuznicki J**, **Majewski L**.	Annexin A3: a newly identified player in store-operated calcium entry.	Acta Neurobiol Exp, 2021; 81:307-13. doi:10.21307/ane-2021-004	1.734	NEUROSCIENCES	4
38	Korzh S, **Winata CL**, Gong Z, **Korzh V**.	The development of zebrafish pancreas affected by deficiency of Hedgehog signaling.	Gene Expr Patterns, 2021; 41:119185. doi: 10.1016/j.gep.2021.119185	1.478	DEVELOPMENTAL BIOLOGY	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	NCD Risk Factor Collaboration (incl. **Mossakowska M**.)	Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants.	Lancet, 2021; 398(10304):957-80. doi: 10.1016/S0140-6736(21)01330-1	77.237	MEDICINE, GENERAL & INTERNAL	1
2	ENCODE Project Consortium (incl. **Uszczyńska-Ratajczak B**.)	Expanded encyclopaedias of DNA elements in the human and mouse genomes.	Nature, 2021; 583(7818):699-710. doi: 10.1038/s41586-020-2493-4	54.637	MULTIDISCIPLINARY SCIENCES	1
3	Begik O, Lucas MC, **Pryszcz LP**, Ramirez JM, Medina R, Milenkovic I, Cruciani S, Liu H, Vieira HGS, Sas-Chen A, Mattick JS, Schwartz S, Novoa EM.	Quantitative profiling of pseudouridylation dynamics in native RNAs with nanopore sequencing.	Nat Biotechnol, 2021; 39(10):1278-91. doi: 10.1038/s41587-021-00915-6	50.516	BIOTECHNOLOGY & APPLIED MICROBIOLOGY	1
4	Prentzell MT, Rehbein U, Cadena Sandoval M, De Meulemeester AS, Baumeister R, Brohée 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.e27. doi: 10.1016/j.cell.2020.12.024	46.899	BIOCHEMISTRY & MOLECULAR BIOLOGY / CELL BIOLOGY	1
5	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. doi: 10.1038/s41467-021-21382-2	15.805	MULTIDISCIPLINARY SCIENCES	1
6	Kuepper A, McLoughlin NM, Neubacher S, Yeste-Vázquez A, Collado Camps E, **Nithin C**, **Mukherjee S**, Bethge L, **Bujnicki JM**, Brock R, Heinrichs S, Grossmann TN.	Constrained peptides mimic a viral suppressor of RNA silencing.	Nucleic Acids Res, 2021; 49(22):12622-33. doi: 10.1093/nar/gkab1149	15.542	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
7	Mukhopadhyay S, Das T, Bose M, Jain CK, Chakraborty M, **Mukherjee S**, Shikha K, Das AK, Ganguly A.	Residues at the interface between zinc binding and winged helix domains of human RECQ1 play a significant role in DNA strand annealing activity.	Nucleic Acids Res, 2021; 49(20):11834-54. doi: 10.1093/nar/gkab968	15.542	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
8	Wegrzyn K, Zabrocka E, Bury K, Tomiczek B, Wieczor M, Czub J, Uciechowska U, Moreno-Del Alamo M, Walkow U, Grochowina I, Dutkiewicz R, **Bujnicki JM**, Giraldo R, Konieczny I.	Defining a novel domain that provides an essential contribution to site-specific interaction of Rep protein with DNA.	Nucleic Acids Res, 2021; 49(6):3394-408. doi: 10.1093/nar/gkab113	15.542	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
9	Turtola M, Manav MC, Kumar A, Tudek A, **Mroczek S**, **Krawczyk PS**, **Dziembowski A**, Schmid M, Passmore LA, Casañal A, Jensen TH.	Three-layered control of mRNA poly(A) tail synthesis in Saccharomyces cerevisiae.	Genes Dev, 2021; 35(17-18):1290-303. doi: 10.1101/gad.348634.121	12.029	CELL BIOLOGY / DEVELOPMENTAL BIOLOGY	1
10	Kotulska K, Kwiatkowski DJ, Curatolo P, Weschke B, Riney K, Jansen F, Feucht M, Krsek P, Nabbout R, Jansen AC, Wojdan K, Sijko K, Głowacka-Walas J, Borkowska J, Sadowski K, Domańska-Pakieła D, Moavero R, Hertzberg C, Hulshof H, Scholl T, Benova B, Aronica E, de Ridder J, Lagae L, Jóźwiak S; EPISTOP Investigators (incl. **Jaworski J**, **Błażejczyk M**, **Tempes A**, **Urbańska M**.)	Prevention of Epilepsy in Infants with Tuberous Sclerosis Complex in the EPISTOP Trial.	Ann Neurol, 2021; 89(2):304-14. doi: 10.1002/ana.25956	11.832	NEUROSCIENCES	1
11	Höhfeld J, Benzing T, Bloch W, Fürst DO, Gehlert S, Hesse M, Hoffmann B, Hoppe T, Huesgen PF, Köhn M, Kolanus W, Merkel R, Niessen CM, **Pokrzywa W**, Rinschen MM, Wachten D, Warscheid B.	Maintaining proteostasis under mechanical stress.	EMBO Rep, 2021; 22(8):e52507. doi: 10.15252/embr.202152507	10.715	BIOCHEMISTRY & MOLECULAR BIOLOGY / CELL BIOLOGY	1
12	Ciesiolka A, **Stroynowska-Czerwinska A**, Joachimiak P, Ciolak A, Kozlowska E, Michalak M, Dabrowska M, Olejniczak M, Raczynska KD, Zielinska D, Wozna-Wysocka M, Krzyzosiak WJ, Fiszer A.	Artificial miRNAs targeting CAG repeat expansion in ORFs cause rapid deadenylation and translation inhibition of mutant transcripts.	Cell Mol Life Sci, 2021; 78(4):1577-96. doi: 10.1007/s00018-020-03596-7	9.074	BIOCHEMISTRY & MOLECULAR BIOLOGY / CELL BIOLOGY	1

No	Authors	Title	Journal	5-Year IF	Journal Category	Quartile in Category
13	Koralewska N, Szczepanska A, Ciechanowska K, Wojnicka M, Pokornowska M, Milewski MC, Gudanis D, Baranowski D, **Nithin C**, **Bujnicki JM**, Gdaniec Z, Figlerowicz M, Kurzynska-Kokorniak A.	RNA and DNA G-quadruplexes bind to human dicer and inhibit its activity.	Cell Mol Life Sci, 2021; 78(7):3709-24. doi: 10.1007/s00018-021-03795-w	9.074	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
14	NCD Risk Factor Collaboration (incl. **Mossakowska M**.)	Heterogeneous contributions of change in population distribution of body mass index to change in obesity and underweight.	Elife, 2021; 10:e60060. doi: 10.7554/eLife.60060	9.059	BIOLOGY	1
15	Nowicka U, **Chroscicki P**, Stroobants K, **Sladowska M**, **Turek M**, Uszczynska-Ratajczak B, Kundra R, Goral T, Perni M, Dobson CM, Vendruscolo M, Chacinska A.	Cytosolic aggregation of mitochondrial proteins disrupts cellular homeostasis by stimulating the aggregation of other proteins.	Elife, 2021; 10:e65484. doi: 10.7554/eLife.65484	9.059	BIOLOGY	1
16	Manohar K, **Gupta RK**, Gupta P, Saha D, Gare S, Sarkar R, Misra A, Giri L.	FDA approved L-type channel blocker Nifedipine reduces cell death in hypoxic A549 cells through modulation of mitochondrial calcium and superoxide generation.	Free Radic Biol Med, 2021; 177:189-200. doi: 10.1016/j.freeradbiomed.2021.08.245	7.934	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
17	Ruiz L, **Kaczmarska Z**, Gomes T, Aragon E, Torner C, Freier R, Baginski B, Martin-Malpartida P, de Martin Garrido N, Marquez JA, Cordeiro TN, Pluta R, Macias MJ.	Unveiling the dimer/monomer propensities of Smad MH1-DNA complexes.	Comput Struct Biotechnol J, 2021; 19:632-46. doi: 10.1016/j.csbj.2020.12.044	7.409	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
18	Poźniak M, Porębska N, **Jastrzębski K**, Krzyścik MA, Kucińska M, Zarzycka W, Barbach A, Zakrzewska M, Otlewski J, **Miączyńska M**, Opaliński Ł.	Modular self-assembly system for development of oligomeric, highly internalizing and potent cytotoxic conjugates targeting fibroblast growth factor receptors.	J Biomed Sci, 2021; 28(1):69. doi: 10.1186/s12929-021-00767-x	7.340	CELL BIOLOGY	1
19	De Ridder J, Verhelle B, Vervisch J, Lemmens K, Kotulska K, Moavero R, Curatolo P, Weschke B, Riney K, Feucht M, Krsek P, Nabbout R, Jansen AC, Wojdan K, Domanska-Pakieła D, Kaczorowska-Frontczak M, Hertzberg C, Ferrier CH, Samueli S, Benova B, Aronica E, Kwiatkowski DJ, Jansen FE, Jóźwiak S, Lagae L; EPISTOP consortium (incl. **Błażejczyk M**, **Jaworski J**, **Tempes A**, **Urbańska M**.)	Early epileptiform EEG activity in infants with tuberous sclerosis complex predicts epilepsy and neurodevelopmental outcomes.	Epilepsia, 2021; 62(5):1208-19. doi: 10.1111/epi.16892	7.121	CLINICAL NEUROLOGY	1
20	Tomecki R, Kobylecki K, Drazkowska K, **Hyjek-Skladanowska M**, **Dziembowski A**.	Reproducible and efficient new method of RNA 3'-end labelling by CutA nucleotidyltransferase-mediated CC-tailing.	RNA Biol, 2021; 18(sup2):623-39. doi: 10.1080/15476286.2021.1999104	6.774	BIOCHEMISTRY & MOLECULAR BIOLOGY	2
21	Porebska N, Pozniaka M, Krzyscik MA, Knapika A, Czyrek A, Kucinska M, **Jastrzebski K**, Zakrzewska M, Otlewski J, Opalinski L.	Dissecting biological activities of fibroblast growth factor receptors by the coiled-coil-mediated oligomerization of FGF1.	Int J Biol Macromol, 2021; 180:470-83. doi: 10.1016/j.ijbiomac.2021.03.094	6.737	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
22	Styszyński A, Chudek J, **Mossakowska M**, Lewandowski K, Puzianowska-Kuźnicka M, Klich-Rączka A, Więcek A, Wieczorowska-Tobis K.	Causes of Anemia in Polish Older Population-Results from the PolSenior Study.	Cells, 2021; 10(8):2167. doi: 10.3390/cells10082167	6.663	CELL BIOLOGY	2
23	Mazurek S, Oleksiewicz U, Czerwińska P, Wróblewska J, **Klimczak M**, Wiznerowicz M.	Disruption of RING and PHD domains of TRIM28 evokes differentiation in human iPSCs.	Cells, 2021; 10(8):1933. doi: 10.3390/cells10081933	6.663	CELL BIOLOGY	2
24	Krzymińska-Siemaszko R, Deskur-Śmielecka E, Kaluźniak-Szymanowska A, Kaczmarek B, Kujawska-Danecka H, Klich-Rączka A, **Mossakowska M**, Małgorzewicz S, Dworak LB, Kostka T, Chudek J, Wieczorowska-Tobis K.	Socioeconomic Risk Factors of Poor Nutritional Status in Polish Elderly Population: The Results of PolSenior2 Study.	Nutrients, 2021; 13(12):4388. doi: 10.3390/nu13124388	6.352	NUTRITION & DIETETICS	1
25	Szmeja S, Gubica T, Ostrowski A, Zalewska A, Szeleszczuk Ł, Zawada K, Zielińska-Pisklak M, **Skowronek K**, **Wiweger M**.	Caffeine-Cyclodextrin Complexes as Solids: Synthesis, Biological and Physicochemical Characterization.	Int J Mol Sci, 2021; 22(8):4191. doi: 10.3390/ijms22084191	6.132	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
26	Pankiewicz P, Szybiński M, Kisielewska K, Gołębiowski F, Krzemiński P, Rutkowska-Włodarczyk I, Moszczyński-Pętkowski R, Gurba-Bryśkiewicz L, Delis M, Mulewski K, Smuga D, Dominowski J, Janusz A, Górka M, Abramski K, **Napiórkowska A**, **Nowotny M**, Dubiel K, Kalita K, Wieczorek M, Pieczykolan J, Matłoka M.	Do Small Molecules Activate the TrkB Receptor in the Same Manner as BDNF? Limitations of Published TrkB Low Molecular Agonists and Screening for Novel TrkB Orthosteric Agonists.	Pharmaceuticals, 2021; 14(8):704. doi: 10.3390/ph14080704	5.850	CHEMISTRY, MEDICINAL / PHARMACOLOGY & PHARMACY	1
27	Poźniak M, Porębska N, Krzyścik MA, Sokołowska-Wędzina A, **Jastrzębski K**, Sochacka M, Szymczyk J, Zakrzewska M, Otlewski J, Opaliński Ł.	The cytotoxic conjugate of highly internalizing tetravalent antibody for targeting FGFR1-overproducing cancer cells.	Mol Med, 2021; 27(1):46. doi: 10.1186/s10020-021-00306-2	5.236	BIOCHEMISTRY & MOLECULAR BIOLOGY	1
28	Warminski M, Kowalska J, **Nowak E**, Kubacka D, Tibble R, Kasprzyk R, Sikorski PJ, Gross JD, **Nowotny M**, Jemielity J.	Structural Insights into the Interaction of Clinically Relevant Phosphorothioate mRNA Cap Analogs with Translation Initiation Factor 4E Reveal Stabilization via Electrostatic Thio-Effect.	ACS Chem Biol, 2021; 16(2):334-43. doi: 10.1021/acschembio.0c00864	5.159	BIOCHEMISTRY & MOLECULAR BIOLOGY	2

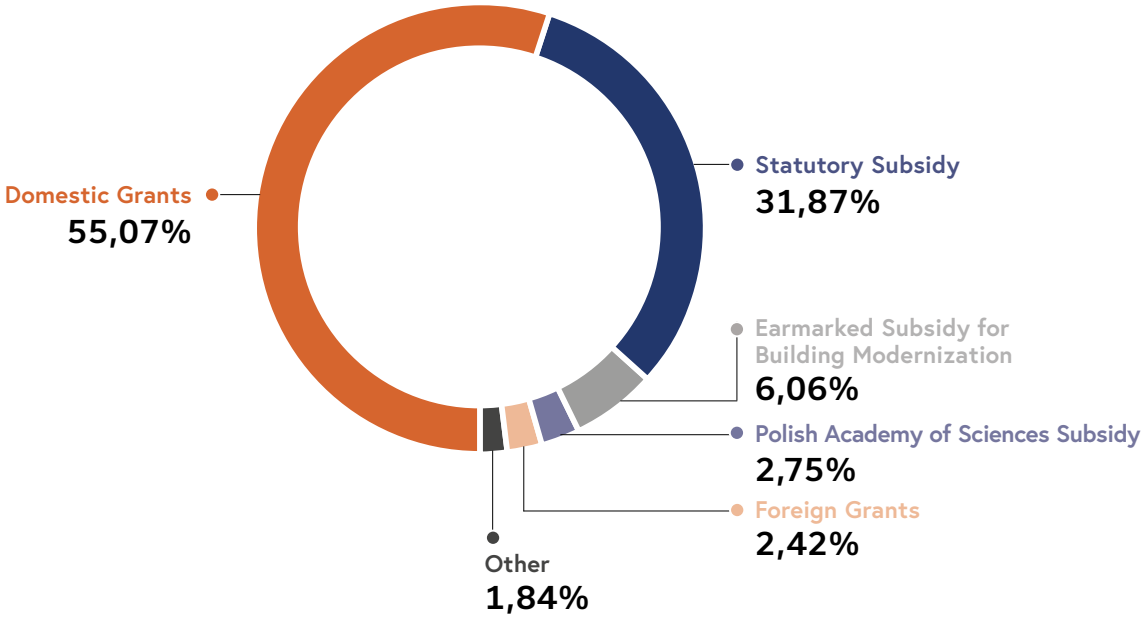
No	Authors	Title	Journal	5-Year IF	Journal Category	Quartile in Category
29	Perzanowska O, Majewski M, Strenkowska M, Głowala P, **Czarnocki-Cieciura M**, Mazur M, Kowalska J, Jemielity J.	Nucleotide-decorated AuNPs as probes for nucleotide-binding proteins.	Sci Rep, 2021; 11(1):15741. doi: 10.1038/s41598-021-94983-y	5.134	MULTIDISCIPLINARY SCIENCES	1
30	Hulshof HM, Slot EMH, Lequin M, Breuillard D, Boddaert N, Jozwiak S, Kotulska K, Riney K, Feucht M, Samueli S, Scholl T, Krsek P, Benova B, Braun KPJ, Jansen FE, Nabbout R; EPISTOP consortium. (incl. **Błażejczyk M**, **Jaworski J**, **Tempes A**, **Urbańska M**.)	Fetal Brain Magnetic Resonance Imaging Findings Predict Neurodevelopment in Children with Tuberous Sclerosis Complex.	J Pediatr, 2021; 233:156-62.e2. doi: 10.1016/j.jpeds.2021.02.060	5.042	PEDIATRICS	1
31	Winder M, Owczarek AJ, **Mossakowska M**, Holecki M, Broczek K, Grodzicki T, Zdrojewski T, Chudek J.	Serum Uric Acid Is a Weak Independent Predictor of Overall Survival in Older Adults.	J Clin Med, 2021; 10(19):4505. doi: 10.3390/jcm10194505	4.567	MEDICINE, GENERAL & INTERNAL	1
32	Jha U, Kondrychyn I, **Korzh V**, Thirumalai V.	High behavioural variability mediated by altered neuronal excitability in auts2 mutant zebrafish.	eNeuro, 2021; 8(5):ENEURO.0493-20.2021. doi: 10.1523/ENEURO.0493-20.2021	4.432	NEUROSCIENCES	2
33	Balatskyi VV, Vaskivskyi VO, Myronova A, Avramets D, **Nahia KA**, Macewicz LL, Ruban TP, Kucherenko DY, Soldatkin OO, Lushnikova IV, Skibo GG, **Winata CL**, Dobrzyn P, Piven OO.	Cardiac-specific β-catenin deletion dysregulates energetic metabolism and mitochondrial function in perinatal cardiomyocytes.	Mitochondrion, 2021; 60:59-69. doi: 10.1016/j.mito.2021.07.005	4.245	GENETICS & HEREDITY	3
34	Winder M, Owczarek AJ, **Mossakowska M**, Broczek K, Grodzicki T, Wierucki Ł, Chudek J.	Prevalence of Hyperuricemia and the Use of Allopurinol in Older Poles-Results from a Population-Based PolSenior Study.	Int J Environ Res Public Health, 2021; 18(2):387. doi: 10.3390/ijerph18020387	3.789	ENVIRONMENTAL SCIENCES	2
35	Czapski GA, Cieślik M, **Białopiotrowicz E**, Lukiw WJ, Strosznajder JB.	Down-regulation of cyclin D2 in amyloid β toxicity, inflammation, and Alzheimer's disease.	PLoS One, 2021; 16(11):e0259740. doi: 10.1371/journal.pone.0259740	3.788	MULTIDISCIPLINARY SCIENCES	2
36	Piotrowicz K, Pac A, Skalska A, **Mossakowska M**, Chudek J, Zdrojewski T, Więcek A, Grodzicki T, Gąsowski J.	Patterns of multimorbidity in 4588 older adults. Implications for non-geriatrician specialist.	Pol Arch Intern Med, 2021; 131(12). doi: 10.20452/pamw.16128	2.790	MEDICINE, GENERAL & INTERNAL	2
37	Babkiewicz E, **Surga K**, Gliwicz M, Maszczyk P	The effect of temperature on the spatial learning rate of zebrafish (Danio rerio).	Ethology, 2021; 127:632-642. doi: 10.1111/eth.13197	1.752	ZOOLOGY	2
38	Puzianowska-Kuźnicka M, Januszkiewicz-Caulier J, Kuryłowicz A, **Mossakowska M**, Zdrojewski T, **Szybalska A**, Skalska A, Chudek J, Franek E.	Prevalence and socioeconomic predictors of diagnosed and undiagnosed diabetes in oldest-old and younger Caucasian seniors: results from the PolSenior study.	Endokrynol Pol, 2021; 72(3):249-55. doi: 10.5603/EP.a2021.0029	1.431	ENDOCRINOLOGY & METABOLISM	4
39	Gentilucci M, **Parisi C**, Coppola MR, Majdoubi F-Z, Madonna A, Guerriero G.	Influence of Mediterranean Sea Temperature Increase on Gaeta Gulf (Tyrrhenian Sea) Biodiversity.	Proc Zool Soc, 2021; 74(1):91-103. doi: 10.1007/s12595-020-00334-6	0.000		0

DIVERSITY OF FUNDING

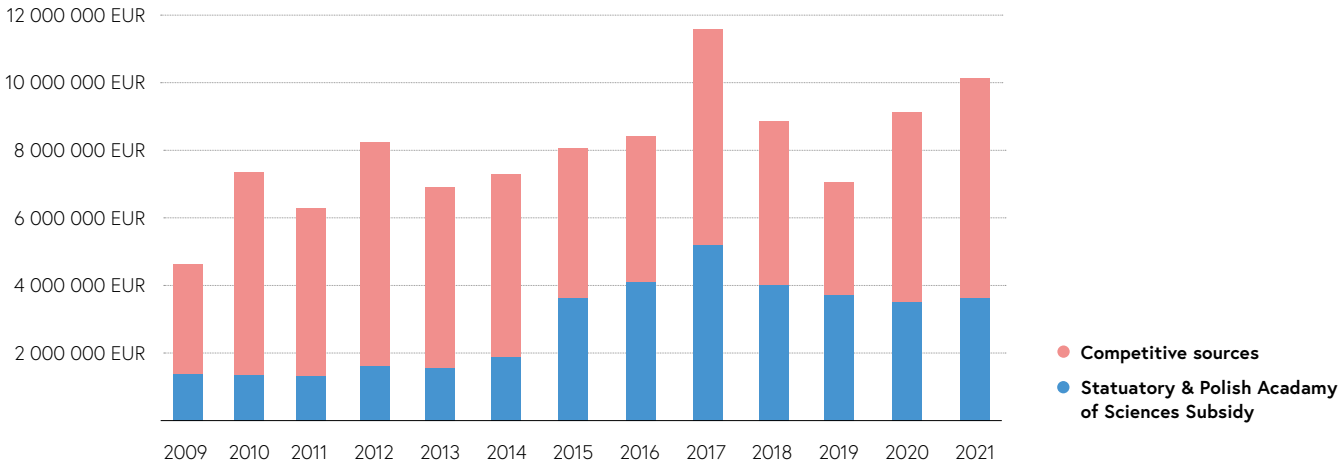
SOURCES OF FUNDING IN 2021

	PLN	EUR*
Statutory Subsidy	14 762 205	3 209 594
Polish Academy of Sciences Subsidy	1 274 000	276 993
Domestic Grants	25 504 548	5 545 190
Foreign Grants	1 120 572	243 634
Earmarked Subsidy for Building Modernization	2 805 202	609 906
Other	850 222	184 855
Total	**46 316 749**	**10 070 172**

* 1 EUR – 4,5994 @ 31st Dec'2021



ANNUAL INCOME 2009-2021



GRANTS RUNNING IN 2021

65 grants with total awarded funding
133 599 945 PLN

NATIONAL SCIENCE CENTRE

NATIONAL SCIENCE CENTRE POLAND

41 projects **75 788 909 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**

COVID FAST TRACK

- "Determination of the 3D structure of key regulatory regions at the 5' and 3' termini of SARS-CoV-2 RNA and identification of small molecule compounds that prevent the formation of these structures" (2020/01/0/NZ1/00232); 999 000 PLN; 2020-2021; **J.M. Bujnicki**

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

- "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**
- "The impact of cytoplasmic polyadenylation on local translation in neurons" (2019/34/H/NZ3/00733); 1 935 625 PLN; 2020-2023; **A. Dziembowski**

DAINA: POLISH-LITHUANIAN FUNDING INITIATIVE

- "CRISPR tools for the study of embryonic development in zebrafish" (2017/27/L/NZ2/03234); 1 634 500 PLN; 2018-2021; **M. Bochtler**; Partner: Vilnius University, Lithuania

OPUS

- "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/NZ2/01010); 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; 2018-2021; **J. Jaworski**
- "Development of new methods for designing RNA molecules that fold into desired spatial structures and their use for development of new functional RNAs and for prediction of noncoding RNAs in transcriptome sequences" (2017/25/B/NZ2/01294): 1 494 250 PLN; 2018-2021; **J.M. Bujnicki**
- "Enabling routine and reliable analysis of transport tunnels in proteins" (2017/25/B/NZ1/01307); 1 375 050 PLN; 2018-2021; **J. Brezovsky**
- "Exploring Baltic Sea cyanobacteria for small-molecule inhibitors of microRNA function" (2017/25/B/NZ9/00202); 27 000 PLN (total grant budget: 1 410 100 PLN); 2018-2021; **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**
- "Role of STIM2 isoforms in regulation of neuronal calcium channels in *Danio rerio*" (2016/23/B/NZ3/03142); 2 085 031 PLN; 2017-2021; **J. Kuźnicki**
- "mTOR kinase impact on cellular functions of selected molecular motors" (2016/21/B/NZ3/03639); 1 336 250 PLN; 2017-2021; **J. Jaworski**
- "Finding novel determinants of the brain ventricular system" (2016/21/B/NZ3/00354); 1 294 885 PLN; 2017-2021; **V. Korzh**
- "The role of E3 ligase complexes in integration of protein homeostasis and aging" (2016/23/B/NZ3/00753); 1 116 875 PLN; 2017-2021; **W. Pokrzywa**
- "A coarse-grained method for RNA 3D structure modeling, with emphasis on noncanonical base pairing" (2016/23/B/ST6/03433); 741 250 PLN; 2017-2021; **M. Boniecki**

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

- "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/NZ2/02837); 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; 2020-2023; **M. Kusio-Kobiałka**
- "Bridging the gap: DNA catalysis explained" (2018/31/D/NZ2/01883); 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; **L. 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

- "The role of mu2-adaptin serine 45 and serine 309 phosphorylation in clathrin mediated endocytosis" (2017/25/N/NZ3/01280); 120 000 PLN; 2018-2021; **A. Tempes**

MINIATURA

- "Study of the function and regulation of the key molecular chaperone UNC-45 in the development of CIM myopathy" (2019/03/X/NZ3/00824); 49 401 PLN; 2019-2021; **M.J. Piechota**

FOUNDATION FOR POLISH SCIENCE

FNP Foundation for Polish Science

10 projects **37 151 887 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; 2018-2022; **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; 2018-2022; **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. **TEAM** "Modeling of dynamic interactions between RNA and small molecules and its practical applications" (POIR.04.04.00-00-3CF0/16-00); 3 499 541 PLN; 2017-2021; **J.M. Bujnicki**
- SG OP 4.4. **TEAM** "Cellular consequences of endosomal dysfunction for proteostasis, metabolism and cancer biology" (POIR.04.04.00-00-20CE/16-00); 3 497 520 PLN; 2017-2021; **M. Miączyńska**
- SG OP 4.4. **TEAM-TECH** "INFECTLESS New generation of antibacterial wound dressing" (POIR.04.04.00-00-3D8D/16-00); 3 463 780 PLN; 2017-2021; **I. Sabała**
- 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**
- SG OP 4.4. **FIRST TEAM** "Genomics dissection of the heart pacemaker in zebrafish" (POIR.04.04.00-00-1AF0/16-00); 2 590 760 PLN; 2017-2021; **C.L. Winata**
- SG OP 4.4 **TEAM-TECH CORE FACILITY** "Mouse Genome Engineering Facility – generation of animal models for biomedical research and preclinical studies" (POIR.04.04.00-00-436A/17-00); 3 499 980 PLN; 2018-2021; **A. Dziembowski**

European Funds Smart Growth | Republic of Poland | FNP Foundation for Polish Science | European Union European Regional Development Fund

EU FRAMEWORK PROGRAMMES & COST

cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

5 projects **11 553 329 PLN**

HORIZON 2020

- **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 (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 (total grant budget: 3 095 829 EUR); 2020-2024; **J.M. Bujnicki**
- **SOCIETAL CHALLENGES** EXSCALATE4CoV "EXaSCale smArt pLatform Against paThogEns for Corona Virus" (101003551); 70 625 EUR (total grant budget: 2 970 875 EUR); 2020-2021; **M. Nowotny**

COST

- EPITRAN "European Epitranscriptomics Network" (CA16120); 2017-2021; **J.M. Bujnicki**, **E. Purta**

NATIONAL CENTRE FOR RESEARCH AND DEVELOPMENT

The 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 (total grant budget: 16 847 247 PLN); 2017-2022; **J. Jaworski** (partner); Coordinator: Medical University of Warsaw

POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE

NAWA POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE

6 projects **4 908 000 PLN**

- **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**
- **Foreign Promotion Programme** "Excellent Institute for excellent Scientists - international promotion of IIMCB" (PPI/PZA/2019/1/00079/U/00001); 99 900 PLN; 2019-2021; **A. Skaruz**
- **International Academic Partnerships** "Molecular basis of enzyme specificity and applications" (PPI/APM/2018/1/00034/U/001); 2 000 000 PLN; 2018-2021; **M. Bochtler**, **I. Sabała**

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

EMBO excellence in life sciences

2 projects **1 109 700 PLN**

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**

EMBO Bridging Fund "UFD-2 modulates the E4 activity of the chaperone-assisted E3 ligase CHIP to regulate organismal proteostasis and lipid metabolism" (3917), 4 000 EUR, 2021, **W. Pokrzywa**

PATENT GRANTED IN 2021

MICRORNA BIOMARKERS IN BLOOD FOR DIAGNOSIS OF ALZHEIMER'S DISEASE

Application number:
P.416956 (25 Apr 2016), PCT/EP2017/059800 (25 Apr 2017), US 16096219 (24 Oct 2018)

Patent number:
EP 3449009 (18 Aug 2021)

Patent inventors:
Urszula Wojda, Katarzyna Laskowska-Kaszub, Tomasz Gabryelewicz, **Jacek Kuźnicki**

The IIMCB is a co-applicant of this patent with the Nencki Institute of Experimental Biology PAS. This invention provides a panel biomarker microRNAs (miRNAs) for diagnosing Alzheimer's disease (AD). It is based on identification of novel miRNAs which showed a consistently changed expression in blood plasma of patients with prodromal AD, as compared to healthy subjects (Nagaraj et al., Oncotarget, 2017). The discovery primarily includes 9 human miRNAs: 483-5p, 486-5p, 30b-5p, 151a-5p, 33a-5p, 18a-5p, 320a, 320b, 320c, with additional 10 miRNAs. The profile of selected biomarker miRNAs is intended for use in diagnosing early stages of the disease (subjective and mild cognitive impairment due to AD). This method is less invasive, less expensive, faster and more accessible in a clinical setting than any other approved method for AD diagnostic. The test involves several steps: collection of 0.6 ml of blood from a patient, obtaining a plasma/serum sample through centrifugation, isolating miRNAs, performing analysis of 9 miRNAs using qRT-PCR. By including additional miRNAs such test can differentiate between early and later AD stages. The method allows patients to be sub-classified for further targeted or personalized therapy and monitoring treatment effectiveness.

SCIENTIFIC EVENTS

OPENING OF THE LABORATORY OF RNA-PROTEIN INTERACTIONS – DIOSCURI CENTRE AT IIMCB

September 30, 2021, hybrid event

A new research group at IIMCB, the Laboratory of RNA-Protein Interactions – Dioscuri Centre, headed by **Gracjan Michlewski**, was officially opened. Gracjan Michlewski (IIMCB) and invited guests (**Juan Valcárcel**, Center for Genomic Regulation, Spain; **Krzysztof Sobczak**, Adam Mickiewicz University, Poland) presented the topic of RNA-protein interactions. A total of 75 people attended the opening in-person. Additionally, IIMCB provided online participation opportunities on Zoom and via social media channels (live stream on Facebook and YouTube). The Dioscuri programme was initiated by the Max Planck Society to support the development of lighthouses of scientific excellence in Central and Eastern Europe and promote outstanding researchers who want to conduct their research within the region. This is a joint programme of the Polish Ministry of Education and Science and the German Federal Ministry of Education and Research and is designed to strengthen scientific exchange between Poland and Germany. Gracjan Michlewski, as every leader of the Dioscuri Centre, has a German partner: **Juri Rappsilber** from the Institute of Biotechnology, Technische Universität Berlin.

1st WOMEN IN SCIENCE SYMPOSIUM (WISS)

March 4-5, 2021, remote meeting

This symposium was initiated by the IIMCB postdoc community to increase the awareness of young scientists of their own potential and opportunities, with special emphasis on women's presence in the scientific world. The invited keynote speakers were **Maya Schuldiner** (Weizmann Institute of Science, Rehovot, Israel) and **Magdalena Żernicka-Goetz** (University of Cambridge, United Kingdom). Over 300 participants from nearly 30 countries attended the meeting. The symposium was financed by the "Excellent science – support for scientific conferences" program of the Polish Ministry of Education and Science.

WiSS was supported by the MOSaIC project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 810425

INTERNATIONAL YOUNG SCIENTISTS CONFERENCE ON MOLECULAR AND CELL BIOLOGY 2021 (IYSC'21)

February 25-26, 2021, remote meeting

This conference was organized by IIMCB PhD students and staff members and dedicated to PhD, master's, and undergraduate students who are interested in molecular and cell biology. The invited keynote speakers were **Anne Bertolotti** (MRC Laboratory of Molecular Biology, United Kingdom), **Patrick Cramer** (Max Planck Institute of Biophysical Chemistry, Germany), **Noam Kaplan** (Technion Israel Institute of Technology, Israel), **Kaska Koltowska** (Uppsala University, Sweden), and **Christophe Lamaze** (Institut Curie, France). The keynote lectures were followed by 21 talks, 20 mentoring meetings, and 104 scientific posters. The event was attended by 218 people and became a great platform for early-stage researchers to present their research, network, and share their knowledge. The conference was financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme.

IYSC'21 was supported by the MOSaIC project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 810425

CEREMONIAL LAUNCH OF GRIEG PROJECTS

January 20, 2021, remote meeting

The ceremonial launch of Andrzej Dziembowski's and Wojciech Pokrzywa's GRIEG projects, co-financed by Norway Grants, was an opportunity to present their projects and disseminate information about the Norwegian Financial Mechanism. Norway Grants are the non-refundable financial aid from Norway to new EU countries. **Andrzej Dziembowski** received funds to conduct research under the project "The impact of cytoplasmic polyadenylation on local translation in neurons" which is implemented jointly with the Centre of New Technologies, University of Warsaw, and Department of Biomedicine, University of Bergen, Norway. **Wojciech Pokrzywa** was awarded for the project "Cellular adaptation to cold" implemented with his partners from the Department of Biosciences, University of Oslo, Norway. Nearly 70 participants attended the meeting.

OPEN IIMCB SEMINARS

Artur Jarmołowski (Adam Mickiewicz University, Poland) *Regulatory mechanisms of miRNA expression: an emerging role of m6A.* 07.01.2021

Oded Rechavi (Tel Aviv University, Israel) *Transgenerational memories in C. elegans nematodes.* 14.01.2021

David Vilchez (University of Cologne, Germany) *Proteostasis of aging and stem cells.* 21.01.2021

Laurence H. Pearl (University of Sussex, United Kingdom) *Phosphorylation-dependent assembly of the DNA damage response.* 04.02.2021

Elizabeta Nemeth (University of California, USA) *From anemia to iron overload and infection: pathobiology of iron.* 11.02.2021

Valerio Orlando (King Abdullah University of Science and Technology, Kingdom of Saudi Arabia) *Retrotransposons activity in the soma: from development to potential new treatments for age-related disorders.* 18.02.2021

Cosimo Commisso (Sanford Burnham Prebys Medical Discovery Institute, USA) *Mechanisms of metabolic stress tolerance in Ras-mutant cancers.* 18.03.2021

Krzysztof Jóźwiak (Medical University of Lublin, Poland) *How structural biology redefines current medicinal chemistry of G protein-coupled receptors.* 25.03.2021

Petr Svoboda (Institute of Molecular Genetics ASCR, Czech Republic) *Something new about small RNAs.* 01.04.2021

Seth Blackshaw (Johns Hopkins University School of Medicine, USA) *Building and rebuilding the hypothalamus one cell at a time.* 08.04.2021

Oscar Llorca (Spanish National Cancer Research Center, Spain) *Cryo-EM of RUVBL1-RUVBL2 chaperone complexes.* 14.04.2021

Herwig Baier (Max Planck Institute of Neurobiology, Germany) *A changing view of vision: from molecules to behavior in zebrafish.* 15.04.2021

Fernando Moreno Herrero (National Centre for Biotechnology/Spanish National Research Council, Spain) *Single-molecule biophysics of protein machines.* 21.04.2021

Tomasz Kamiński (University of Warsaw, Poland) *Single-cell assays in microdroplets.* 22.04.2021

Kinga Kamieniarz-Gdula (Adam Mickiewicz University, Poland) *Knowing when to stop: Pol II transcription termination in vertebrates.* 29.04.2021

Juri Rappsilber (Technical University of Berlin, Germany) *In cell integrative structural biology by crosslinking mass spectrometry and cryo-electron tomography.* 06.05.2021

Mirana Ramialison (Monash University, Australia) *Multi-omics strategies for tackling congenital heart disease.* 13.05.2021

Danny Incarnato (University of Groningen, The Netherlands) *"Static" and dynamic structures in the SARS-CoV-2 genome.* 20.05.2021

Amy Buck (University of Edinburgh, United Kingdom) *RNA in communication: new insights into host-pathogen interactions.* 17.06.2021

Jiemin Weng (East China Normal University, China) *Regulation of DNA methylation homeostasis and inheritance in mammalian cells.* 24.06.2021

Robert Huber (Max Planck Institute of Biochemistry, Germany) *Structural biology and its key role in the life sciences and in medicine.* 17.09.2021

Scott Sternson (HHMI's Janelia Research Campus & University of California, USA) *Molecular insights into neural coding of appetite and other behavioral states.* 07.10.2021

Michał Gdula (Adam Mickiewicz University, Poland) *How to shut down a whole chromosome? - SmcHD1, epigenetics and 4D genome in X inactivation.* 21.10.2021

Alfred Vertegaal (Leiden University Medical Center, The Netherlands) *Signal transduction by small ubiquitin-like modifiers.* 04.11.2021

Helen Bateup (University of California, USA) *The multi-faceted roles of TSC-mTOR signaling in neuronal development, function and disease.* 18.11.2021

Alexander Drakesmith (University of Oxford, United Kingdom) *Control of immune responses by iron.* 25.11.2021

Matthias Hentze (European Molecular Biology Laboratory, Germany) *A new continent of the RNA World.* 02.12.2021

Marcin Zagórski (Jagiellonian University, Poland) *How is patterning precision achieved in developing tissues?* 09.12.2021

INTERNAL SEMINAR SERIES

Malwina Hyjek-Składanowska (Nowotny Lab) *Origins of the increased affinity of phosphorothioate-modified therapeutic nucleic acids for proteins.* 05.02.2021

Aleksandra Tempes (Jaworski Lab) *Regulation of microtubule-based transport: a novel role for mTOR kinase.* 05.02.2021

Vladimir Korzh (Kuźnicki Lab) *Scoliosis: on royals and spines.* 12.02.2021

Michał Pastor (Bochtler Lab) *THYN1- new histone binding protein or just an artifact.* 12.02.2021

Marzena Nowacka (Nowotny Lab) *Biochemical and structural studies of foamyviral reverse transcriptases.* 19.02.2021

Marta Wróbel (Miączyńska Lab) *ESCoRTing lysosomal homeostasis and Ca2+ signaling.* 19.02.2021

Janusz Bujnicki (Bujnicki Lab) *SARS-CoV-2 coronavirus RNA genome structure.* 12.03.2021

Justyna Jędrychowska (Kuźnicki Lab) *The role of voltage-gated potassium channels in the development of the ear in zebrafish.* 19.03.2021

Justyna Zmorzyńska (Jaworski Lab) *G3BP1 enables the function of the TSC1-TSC2 complex.* 19.03.2021 and 23.04.2021

Daria Zdżalik-Bielecka (Miączyńska Lab) *GAS6-AXL signaling triggers actin remodeling that drives membrane ruffling, macropinocytosis and cancer cell invasion.* 26.03.2021

Gabriela Jędruszewska (Mleczko-Sanecka Lab) *LSECs constitute an emergency pathway for hemoglobin clearance during hemolysis.* 26.03.2021

Magdalena Czeredys (Kuźnicki Lab) *Role of CacyBP/SIP in the regulation of mutant huntingtin aggregation in the Huntington's disease.* 09.04.2021

Paweł Mitkowski (Auresine) *Bacteriolytic enzymes biochemistry, structure, engineering and application in wound dressings.* 09.04.2021

Katarzyna Mleczko-Sanecka (Mleczko-Sanecka Lab) *Novel insights into functional alterations of iron-recycling macrophages.* 16.04.2021

Agata Sulej (Winata Lab) *On the rocky path to elucidating the gene regulatory network of the zebrafish pacemaker.* 23.04.2021

Pawel Krawczyk (Dziembowski Lab) *Nanopore direct RNA sequencing as a perfect tool for transcriptome-wide poly(A) tails profiling* 07.05.2021

Mariusz Czarnocki-Cieciura (Nowotny Lab) *Electron microscopy in structural biology – what kind of questions can it address?* 07.05.2021

Jacek Kuźnicki (Kuźnicki Lab) *Zebrafish models of human diseases.* 14.05.2021

Wojciech Pokrzywa (Pokrzywa Lab) *Activation of the quality control E3 Ligase CHIP by UFD-2 binding regulates lipid metabolism and proteostasis.* 21.05.2021

Marcin Nowotny (Nowotny Lab) *Holliday junction resolvases.* 18.06.2021

Maciej Łapiński (Winata Lab) *Elucidating the mechanism of translational control of maternal transcripts through cytoplasmic polyadenylation.* 25.06.2021

Anna Stroynowska-Czerwińska (Bochtler Lab) *How KMT2/MLL proteins target chromatin using CXXC and PHD reader domains?* 25.06.2021

Karolina Bogusz (Jaworski Lab) *Rapamycin-induced p150glued and β2-adaptin interaction requires CLIP-170 protein and autophagy.* 01.10.2021

Sergii Palchewski (Nowotny Lab) *MscS, Kv and TRP channels as positive control for validation of the reconstitution into nanodiscs.* 01.10.2021

Gracjan Michlewski (Michlewski Lab) *TRIM25 inhibits Influenza A infection by destabilising its mRNA and is redundant for the RIG-I pathway.* 08.10.2021

Dominik Rafalski (Bochtler Lab) *Sequence specificity of Tet dioxygenases.* 22.10.2021

Cecilia Winata (Winata Lab) *RNA Editing - a new player in zebrafish development?* 29.10.2021

Marcin Nowotny (Nowotny Lab) *Reverse transcriptases.* 05.11.2021

Rishikesh Kumar Gupta (Kuźnicki Lab) *Stim2 in zebrafish brain.* 19.11.2021

Oliver Tkaczyk (Jaworski Lab) *The role of TBC1D5 phosphorylation in neurodevelopment.* 19.11.2021

Lidia Wolińska-Nizioł (Miączyńska Lab) *Searching for the function of Tollip in zebrafish.* 26.11.2021

Aneta Jończy (Mleczko-Sanecka Lab) *Deciphering Zip14 regulation by glucocorticosteroids.* 26.11.2021

Shikha Vasisht (Winata Lab) *Computational discovery of non-coding SNPs associated with congenital heart disease.* 03.12.2021

Jan Węsławski (Jaworski Lab) *Screen for kinesins critical in mTOR-dependent neuronal growth.* 03.12.2021

Nagendar Badepally (Bujnicki Lab) *3D structure determination of riboswitches.* 17.12.2021

SPOTLIGHT TALKS

James Cleland (Max Planck Institute for Biophysical Chemistry, Germany) *Regeneration: how can some animals regrow lost body parts, when we humans cannot?* 07.01.2021

Adam Klosin (Max Planck Institute of Molecular Cell Biology and Genetics, Germany) *Phase separation provides a mechanism to reduce noise in cells.* 19.01.2021

Elena Taverna (Max Planck Institute for Evolutionary Anthropology, Germany) *Stem Cells Meet Robotics. A robot to study the cell biology of brain development and evolution.* 03.02.2021

Lenka Bielicova (Max Planck Institute of Molecular Cell Biology and Genetics, Germany) *Re-engineering tissue architecture in vivo.* 17.02.2021

Marija Matejcic (Institute for Bioengineering of Catalonia, Spain) *Intestine without the third dimension: investigating intestinal epithelial organisation and function using organoid monolayers.* 03.03.2021

Andrii Kopach (IIMCB, Poland) *Towards an understanding of de novo microtubule nucleation in dendrites using phase separation as an approach.* 17.03.2021

Jarosław Cendrowski (IIMCB, Poland) *Opposing roles of splicing variants as a key to understanding the function of BMP2K kinase in membrane trafficking and erythroid differentiation.* 31.03.2021

Akanksha Jain (ETH Zurich, Switzerland) *4D quantitative light sheet imaging to study tissue morphogenesis: From embryos to organoids.* 14.04.2021

Justyna Zmorzyńska (IIMCB, Poland) *Can we model neuropsychiatric disorders in zebrafish?* 28.04.2021

Stephan Daetwyler (UT Southwestern Medical Center, USA) *Multi-sample light-sheet imaging of development and disease such as cancer.* 12.05.2021

Franziska Decker (ETH Zurich, Switzerland) *A gelation transition enables the self-organization of bipolar metaphase spindles.* 26.05.2021

Pawel Trzaskoma (National Institute of Arthritis and Musculoskeletal and Skin Diseases, USA) *Imaging in genetics – what can microscopy tell us about chromatin organization?* 09.06.2021

Marti Cadena Sandoval (University of Innsbruck, Austria) *The complex signaling of G3BP proteins: from stress granules to lysosomes.* 15.09.2021

Albert Thommen (The Francis Crick Institute, United Kingdom) *Systemic metabolism in normal and abnormal growth: from body size variation to metabolic interactions between tumour and host in liver cancer.* 29.09.2021

Agnieszka Pastuła (Jagiellonian University, Poland) *The role of fibroblasts as Niche Cells in homeostasis and disease.* 13.10.2021

Sílvia Llonch Armengol (Centre for Genomic Regulation, Spain) *A walk through human egg biology.* 10.11.2021

Anna Malik (Nencki Institute, Poland) *The secret lives of VPS10P receptors, or how to unravel the intracellular sorting machinery in glia.* 24.11.2021

Agnieszka Łukaszewicz (Memorial Sloan Kettering Cancer Center, USA) *Germline mutagenesis at recombination hotspots in mice.* 08.12.2021

DO SCIENCE CLUB

Melissa Moore (Moderna Therapeutics, USA) *mRNA as medicine.* 22.01.2021

Anne Martel (Institute Laue-Langevin, France) *Small angle neutron scattering for structural biology.* 02.02.2021

Sergei Grudinin (Nano-D-Inria Grenoble, France) *Structural Bioinformatics in the area of Big Data.* 16.03.2021

Łukasz Kaczmarek (Adam Mickiewicz University in Poznań, Poland) *Water bears, the toughtest animals on Earth. How to survive in extreme environments?* 23.03.2021

RNA CLUB WARSAW

David Bartel (Whitehead Institute/MIT/Howard Hughes Medical Institute, Cambridge, USA) *Regulation of mRNA translation and decay.* 07.12.2021

SCIENCE POPULARIZATION



CENTRE FOR INNOVATIVE BIOSCIENCE EDUCATION

GROUP MEMBERS

Head

Patrycja Dołowy, PhD

Project Manager

Katarzyna Tomaszewska, MSc

Laboratory Manager

Aleksandra Olszańska, BEng

Volunteer Representatives

Mikołaj Cup
Miłosz Majka
Małgorzata Orłowska

The Centre for Innovative Bioscience Education (BioCEN) was established in 2002 by the International Institute of Molecular and Cell Biology in Warsaw, Nencki Institute of Experimental Biology Polish Academy of Sciences, Institute of Biochemistry and Biophysics Polish Academy of Sciences, and Science Festival in Warsaw. BioCEN works to bridge the gap between the scientific community and society by providing educational activities that popularize modern 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.

In 2021, the global pandemic influenced our activities. We brought most of them onto the internet within the framework of the BioCEN online laboratory and the ZOOM platform. In June 2021, we began to conduct outdoor workshops in city parks and school yards. When possible, we organized live laboratory workshops for groups with support from the University of Warsaw Faculty of Biology and Warsaw University of Life Sciences Institute of Biology. BioCEN has also held workshops in various schools in Warsaw and outside the city, including schools in small towns and villages.

BioCEN receives financial support from the International Institute of Molecular and Cell Biology in Warsaw, which has been BioCEN's Strategic Sponsor since 2015. In addition to IIMCB's support, BioCEN is subsidized by the Nencki Institute of Experimental Biology Polish Academy of Sciences, Institute of Biochemistry and Biophysics Polish Academy of Sciences, University of Warsaw Faculty of Biology, and BioEducation Foundation.

ACTIVITIES

A total of 5 615 students participated in laboratory and outdoor workshops in 2021, and our materials reached ~8 000 recipients. Moreover, various BioCEN online activities accumulated over 178 000 views.

Our online courses that are organized for biology teachers are building a connection between them and scientists so they feel they are an important part of 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 the school setting. During the pandemic, we created many additional materials that can be helpful in both the home learning environment and schools.

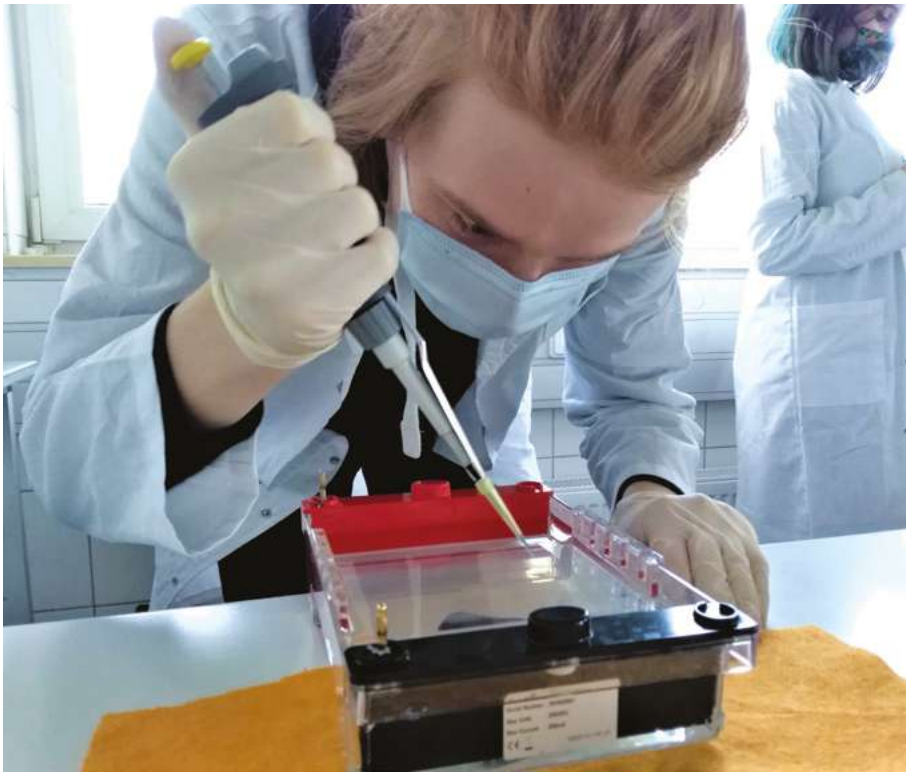
LABORATORY WORKSHOPS

BioCEN workshops cover various areas of biology and life sciences and focus on practical and experimental approaches. Our goal is to cover several scientifically and educationally important topics, such as molecular and cellular 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. This year BioCEN held workshops outside its headquarters at the University of Warsaw, the Warsaw University of Life Sciences, and schools in Mazowieckie, Podkarpackie, Podlaskie, Kujawsko-Pomorskie, Małopolskie, and Lubelskie voivodships.

HYBRID CLASSES IN THE EDUCATIONAL LABORATORY AND ONLINE

New materials and syllabi were created within the framework of the projects, co-funded by the Education Department of the Capital City of Warsaw, including "I Am Experimenting in a Science Laboratory! Biology Laboratory Workshops for Students of Warsaw Elementary Schools and High Schools," "Nematodes, or How Model Organisms Support Scientific Research. Biology Laboratory Workshops for Warsaw High Schools," and "How Model Organisms Support Science Research," funded by the Polish Academy of Sciences. These projects have been conducted in the form of hybrid learning. Educational kits for studying three different topics were sent to students and teachers to be used during interactive classes online via the ZOOM platform.

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?," "How are Euglenae studied?," "The Immune System: The Power Behind the Throne of Health and Illness," "How Medicines Are Developed," "Obesity and Health," "Vaccinations," "The Beginnings of Eukaryotes," "The First Multicellular Organisms," and (2) online science demonstrations with elements of theory for high schools, including "In Vitro Cell Cultures, Synergy, Redox: The Quintessence of Life," "Scientific Experiment: It Isn't Difficult!," "Melatonin: Polluted with Light," "The Microcosm: The World of Bacteria," "The Brain: The Orchestra Between the Ears," and for elementary schools, including "Synergy," "Scientific Experiment – It's Not Difficult!," "The World of Chemical Reactions,"



ONLINE EVENTS

The BioCEN online laboratory is a remote space that was created especially for educational purposes (https://biocen.edu.pl/projekty/laboratorium-biocen-on-line/). Interactive materials were made accessible as free downloads in the form of video demonstrations, including virtual reality, mini-scripts, experience protocols, and podcasts. In 2021, the materials had approximately 8 000 views. The platform is dedicated to youth and adults and includes interactive materials that are simultaneously published on Facebook where they accumulated 178 000 views.

"The Microcosm: The World of Bacteria." In 2021, 132 classes were held.

Since October 2021, BioCEN has organized online meetings in the form of interviews with invited scientists, hosted by Patrycja Dołowy. Teachers and students choose the topic, and BioCEN proposes the scientist. Among the invited guests were two astrobiologists, a climate ecologist, an ancient DNA researcher, a neurologist, an oncologist, and a cancer biologist.

WORKSHOPS FOR MIGRANTS, REFUGEES, AND PEOPLE AT RISK OF SOCIAL EXCLUSION

In collaboration with the Council for the Promotion of the Public Understanding of Science Polish Academy of Sciences, we organized science cafes for adults and science workshops for children (7-12 years old) who are refugees who live in the refugee center in Łuków, Poland. Workshops on microbiology and ecology were held.

Within the project, "Vaccine Means Health," BioCEN, together with the Council for the Promotion of the Public Understanding of Science Polish Academy of Sciences, Association for Legal Intervention, Ukrainian House in Warsaw, and a biology teacher from the Chechen community in Poland, has conducted meetings and workshops on vaccines for adults and children for groups who are at risk of social exclusion, including people with low education, poor people, migrants, and refugees. The scripts were released in four languages: English, Polish, Russian, and Ukrainian.

WORKSHOPS DEDICATED TO HIGH SCHOOL STUDENTS

Together with the Elab Education Laboratory, we introduced GETinMED courses for students who are going to study medicine abroad. In 2021, we conducted six workshops ("Workshops for Erasmus High School Students from European Countries") for two groups in the laboratory of the Warsaw University of Life Sciences Institute of Biology. Students from Belgium, Czech Republic, France, Great Britain, Italy, Slovakia, Slovenia, and Spain were invited to Poland within the Erasmus+ Program in October 2021. BioCEN conducted four workshops for them on cell biology and genetics at the University of Warsaw Faculty of Biology.

Science Cafe for High School Seniors, BioCEN, together with Karrot Cafe, created classes that were dedicated to high school seniors. Mikołaj Cup and Stanisław Szleszkowski from our team are teaching students how to pass the graduation exam for biology.

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 2021, BioCEN, together with the Nencki Institute of Experimental Biology Polish Academy of Sciences, organized an annual symposium in Warsaw for teachers and educators online on the ZOOM platform.

20th Educational Symposium for Biology Teachers online

This annual symposium has become one of our most important events. This year's jubilee symposium was offered online. A total of 138 people participated in the event. During this meeting, biology teachers from all over Poland had the opportunity to receive up-to-date information on frontline discoveries in neuroscience and become more familiar with cutting-edge studies, such as those related to Nobel prizes in Physiology and Medicine, and Chemistry. The symposium program included a workshop for teachers to teach them how to cope with stress that is related to professional life in times of a pandemic and practical training presentations.

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 (https//biocen.edu.pl/en/experimental-kits/). All of the 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. The following experimental kits are available:

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

We also emphasize the notion of "learning while playing." We produce high-quality and genuine BioCEN educational board games for schools:

- Re-action! Lost Experiment
- By the Trails of Evolution

OTHER ACTIVITIES

BIOCAST PODCASTS

BioCAST podcasts are provided by the Centre for Innovative BioScience Education, based on talks on biology and science in general and prepared according to Mikołaj Cup's and Jasiek Malinowski's idea, both from the BioCEN team. The second season has 10 episodes that focus on crucial discoveries in science. BioCAST is posted on BioCEN's website and other websites, including Apple Podcasts, Google Podcasts, Spotify, RadioPublic, Pocket Casts, Mixcloud, Breaker Podcasts, Anchor, and Listen Notes. BioCEN already has regular listeners and continues to gain new ones with each episode. In 2021, the podcasts have been heard by more than 5 600 listeners.

During Summer 2021, within the City Hall of Warsaw "Summer in the City" Program, we conducted 16 outdoor science workshops for 313 children in Warsaw parks.

BioCEN participated in science popularization events in 2021, including the 2nd Night Science Festival online in Kamienica Polska in April, 9th Intercollegiate Biotechnology Symposium "Symbioza" in May, 4th School Science Picnic in Józefosław and 25th Festival of Science in Warsaw (in collaboration with the International Institute of Molecular and Cell Biology in Warsaw) in September, Eksploracje Science Picnic in Rzeszów and School Science Festival in Grajewo in October, and Nature and Art Conference at the Zachęta Gallery in Warsaw in December.



Together with the March for Science Foundation, BioCEN held 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 "Słoneczniki 2021" competition for the most developmental initiative for children. BioCEN was awarded for its science education project, "Be the Health Hero" (an interactive comic), which gave children science and art tools to understand processes that are related to the COVID-19 pandemic.

BioCEN was an Orły Kształcenia (Eagles of Education) Prize 2021 Laureate in Warsaw. Awarded companies are top companies in education, based on internet user ratings. During the competition, 46 023 companies were examined, 4 667 676 ratings were taken into consideration, and 6 001 026 websites were browsed.

BIOCEN ANIMATORS AND CO-WORKERS

Important members of the BioCEN team include animators and coworkers without whom the educational activities would not be possible. In 2021, the following individuals collaborated with BioCEN: animators (Julia Brzykcy, Rafał Jabłuszewski, Jakub Janiec, Marcin Kleibert, Aleksandra Kowalczyk, Mikołaj Kuska, Aleksandra Maciejczuk, Miłosz Majka, Małgorzata Malczewska, Paweł Morga, Małgorzata Orłowska, Malina Pełka, Stanisław Szleszkowski), senior trainers (Mikołaj Cup, Andrzej Gruza, Aleksandra Owczarek), laboratory support (Tamara Aleksandrzak-Piekarczyk, Izabela Kern-Zdanowicz, Anna Osinka), designer (Paulina Czyż), public relations (Jan Malinowski), and Management Board members (Kalina Burnat, Maciej Kotliński).

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RESEARCH SUPPORT UNITS



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Senior Specialists

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Aleksandra Janicka
Aneta Walas
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Marcin Ogonowski

Senior Specialists

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Agata Skaruz
Justyna Szopa
Justyna Mitan-Piłat
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(until December 2021)
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Specialist

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Adam Kucharski



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(part-time) – not in the picture

Senior Specialist

Jakub Skaruz

Specialists

Łukasz Munio
Michał Taperek

Junior Specialist

Kacper Kolasiński



SELF-CONTAINED POSITION FOR VETERINARY AFFAIRS

Chief Specialist

Piotr Korzeniowski

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