Annual Report 2020

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

Deputy Director for Operations
Anna Zolnik

Deputy Director for Finance
Hanna Iwaniukowicz

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Director's note

The year 2020 has changed the world. We have been taken by surprise by the COVID-19 pandemic that has turned our daily lives upside down, the way we work and socialize. It has brought multiple unprecedented challenges that we have had to handle quickly. Personally, I felt like I was taking an accelerated practical course on crisis management of an institution, with outcomes hard to predict. I am sure that this feeling of uncertainty and anxiety about the future has been shared by many. Still, the year 2020 has proven how privileged I am to head an institute with such a great team of co-workers, both scientists and administrative personnel. I would like to thank everybody for their daily work in these unusual circumstances, for taking precautions to ensure the safety of all staff and operations of the IIMCB, for supporting each other and for sharing the feeling of being a community despite the physical distance.

In particular, I would like to thank the "dream team" of my closest collaborators who shared with me the burden of responsibility for the staff and the institution. These are my deputies: Urszula Białek-Wyrzykowska, Hanna Iwaniukowicz, Jacek Jaworski, Anna Zolnik; the head of the HR Unit Katarzyna Fiedorowicz, and my executive assistant Ewa Jack-Górska. Together, throughout the year, we have been elaborating the rules of IIMCB operations and adapting them to the changing epidemiological situation, while communicating regularly with our staff about all relevant developments. "A friend in need is a friend indeed", as the English say. Nobody could have even predicted that we would be responsible for executing a lockdown of the IIMCB in March 2020 and then compelled to keep its activities at a reduced intensity, the opposite of what is normally expected from directors. Still, looking back at the last year, I am proud to say that despite all the difficulties the IIMCB community has succeeded in several endeavors.

It was obvious for us in 2020 that we needed to show our responsibility and solidarity with the society fighting the pandemic, both by our research but also by social activities. Marcin Nowotny has joined the Exscalate4CoV consortium of 18 European partners to search for an effective therapy against SARS-CoV-2. Janusz Bujnicki and Andrzej Dziembowski have engaged in research to elucidate the biology of the virus. This has already resulted in a breakthrough article entitled "Genome-wide mapping of SARS-CoV-2 RNA structures identifies therapeutically-relevant elements", published in *Nucleic Acid Research* by the Bujnicki team in collaboration with Dutch scientists. At the initial outbreak of the pandemic, the Institute lent specialized equipment to set up a certified COVID-19 diagnostic laboratory in a neighboring hospital. Even more importantly, our volunteer scientists worked in this laboratory to perform RT-PCR-based diagnostic tests for COVID-19 infections. We also supported a local nursing home for the elderly.

Despite pandemic-imposed limitations, we have continued our work and studies across the levels of biological organization – from atoms to organisms – to understand the mechanisms of human diseases, in accordance with the IIMCB mission. Many of our projects aim to elucidate the molecular basis for neurodegenerative, neurodevelopmental, metabolic and rare diseases or cancers. In some of these projects, we have collaborated with clinicians to provide the groundwork for novel therapeutic strategies.

In 2020, IIMCB scientists have published 76 articles. Among the most notable reports, in addition to the aforementioned one on SARS-CoV-2 RNA, are structural studies of therapeutic antisense oligonucleotides or investigation of cellular mechanisms of erythrocyte differentiation. Last year, our scientists received several prestigious grants and personal awards. Among community service activities, my predecessor, Prof. Jacek Kuźnicki, has been appointed as the President of the Council of the National Science Center (NCN), the main granting agency for fundamental research in Poland, for the term 2020-2022 (see sections on best paper awards and personal achievements in this Annual Report).

In 2020, we prepared for the arrival of one more research group led by Prof. Gracjan Michlewski, who won the competition to establish a Laboratory of RNA-Protein Interactions as the Dioscuri Centre at the IIMCB. The Dioscuri Programme, initiated by the Max Planck Society and jointly managed with the National Science Centre, is mutually funded by the Polish Ministry of Science and Higher Education and the German Federal Ministry of Education and Research. Its goal is to support the development of lighthouses of scientific excellence in Central and Eastern Europe by promoting outstanding researchers. Prof. Michlewski's research focuses on RNA-protein interactions in innate immune responses to RNA viruses, as well as cellular roles and structural characteristics of novel RNA-binding proteins. We are very happy about Prof. Michlewski joining the IIMCB, as his studies will further strengthen the expertise on RNA biology at the IIMCB, making this research area one of the main pillars of our scientific activities. We wish a lot of success to the new laboratory that started operating in January, 2021.

The year 2020 was, unfortunately, a time of physical distancing. Thus, maintaining communication and the feeling of being a community has become more difficult, but also more crucial than before. We have quickly adopted the possibilities of modern internet technologies to keep in touch and stay abreast of scientific developments. Our External Seminar Series was held online and featured a number of prominent speakers from all over the world. Moreover, we started a second series of Weekly Internal Seminars with IIMCB scientists at all career levels presenting their results. Several yearly events, such as the International Advisory Board Meeting, the PhD Students' Report Session or Lab Leaders' and Directors' Retreat, took place online. The International Young Scientists Conference on Molecular and Cell Biology and the 1st Women in Science Symposium, great bottom-up initiatives of our PhD students and postdocs, respectively, will also take place online in 2021. Likewise, the first year of the IIMCB's membership in the EU-LIFE consortium was marked with remote meetings, both the Strategy and Community Meetings. Despite the online format, our representatives got introduced into the activities of several EU-LIFE working groups, such as those focused on core facilities, IT, recruitment and training, gender equality, or grants and funding strategies. Urszula Białek-Wyrzykowska, as the IIMCB's main representative, coordinates our engagement at the EU-LIFE level and we look forward to intensifying our joint activities in the coming years.

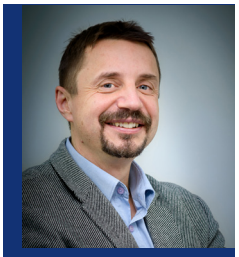
We still do not know how deeply and permanently the pandemic will change our lives. Despite this, we will strive for excellence in research and institutional management independently of the circumstances. The year 2020 has proven that we can do it when we do it together.

Marta Miączyńska
Warsaw, February 2021

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

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 | **Institutional development and partnerships** | **Organizational culture**

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

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

Jo Bury
Flanders Institute for Biotechnology, Belgium

Aaron Ciechanover
Technion - Israel Institute of Technology, Israel

Urszula Hibner
Institute of Genetics and Molecular Medicine, France

Reinhard Jahn
Max Planck Institute for Biophysical Chemistry, Germany

Artur Jarmołowski
Adam Mickiewicz University, Poland

Caroline Kisker
University of Würzburg, Germany

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

EU-LIFE

GENERAL INFORMATION

EU-LIFE is an alliance of research centres whose mission is to support and strengthen European research excellence. EU-LIFE members are leading research institutes in their countries and internationally renowned for producing excellent research, widely transferring knowledge, and nurturing talent. Since its foundation in 2013, EU-LIFE has become a stakeholder in science policy development participating regularly in the European science policy dialogue.

The IIMCB became the first Polish member of the EU-LIFE consortium. The decision was ratified by the EU-LIFE Board of Directors and the IIMCB Director and is in effect as of 1 January 2020. As a member of the EU-LIFE alliance, the IIMCB is working with other 14 other institutes towards excellence in the life sciences, giving high regard to quality and responsible science, as well as turning the spotlight on issues related to European science policies.

ORGANIZATION

The structure of EU-LIFE is based on a Board of Directors, a Strategy Group, several Working Groups & 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 policy making, deciding on the EU-LIFE initiatives, and proposing an action plan. The group is composed of the Board of Directors of the member institutes, the EU-LIFE coordinator, the main representatives, and the chairs of working groups (WG). The IIMCB representatives in the Strategy Group are Marta Miączyńska as the director of the IIMCB and Urszula Białek-Wyrzykowska as the main representative.

The Core Facilities Working Group is a forum for discussion of core facility-specific challenges. Two dominating themes of the current Core Facilities WG activity are comparison of indicators across institutes, and the best practices and expertise in core facility management. In 2021, WG is planning to concentrate on issues related to Core Facilities-specific career development and research data management (in collaboration with IT WG). IIMCB representatives in this WG are Joanna Dodzian and Krzysztof Skowronek.

The Gender Equality Working Group was formed in November 2019 to coordinate the gender equality activities. It has established the following priorities:

- develop anti-harassment/bullying concept
- develop/agree on indicators to monitor gender equality
- implement a second edition of the Career Development Compass (career development training)
- follow-up of the Directors activities

In 2021, Gender Equality WG is planning to organize a Workshop on Bullying and Harassment for institutes' Directors and key HR staff, as well as a training for the entire EU-LIFE community on this topic. IIMCB representative in this WG is Katarzyna Fiedorowicz.

Grants & 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 trainings. In 2021, WG will concentrate works on three distinct areas: sharing best practices, resources & training, and Horizon Europe. IIMCB representatives in this WG are Dorota Libiszowska and Marcin Ogonowski.

The IT Working Group is a community of specialists dedicated to addressing Information Technology challenges. The main domains of 2020 discussion were Research Data Management (RDM) Policies and data storage. In 2021, WG will face the big data analysis issues, network access control research, remote work environment examination and RDM formalization (in collaboration with Core Facilities WG). Paweł Kobylarz represents the IIMCB in this WG.

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
- defining best practices in terms of grants and employment contracts, with a special focus on postdocs

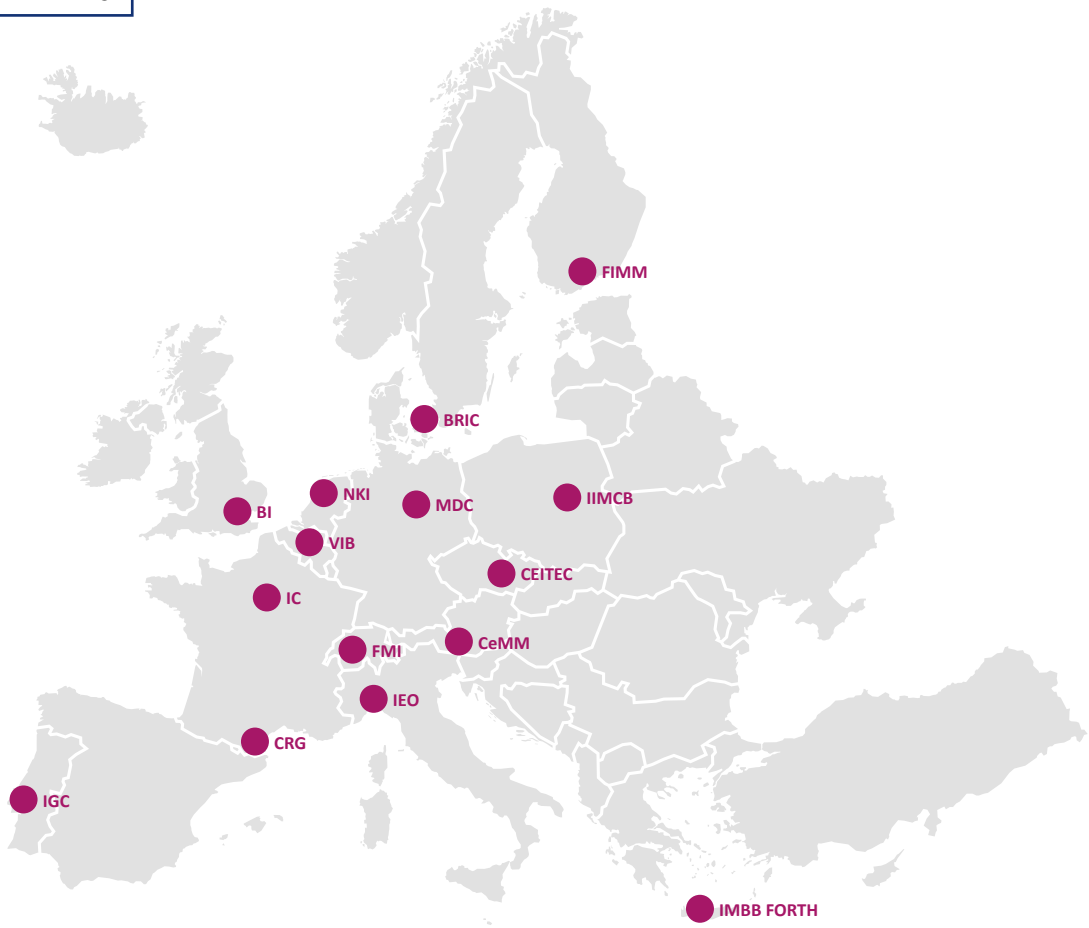
In 2021, Recruitment and Training WG is planning to concentrate on the exchange of best practices in the following areas: recruitment process, administrative support for individual research groups, and career development. Recruitment and Training WG will establish a common platform with Open Access trainings, courses, and seminars, intending to increase the mobility opportunities. IIMCB representatives in this WG are Agnieszka Faliszewska and Elżbieta Purta.

Science Communication Working Group focuses on:

- media and public relations management as well as institutional contacts
- running of the Social Media profiles for scientists and/or for the general public
- public relations and institutional contacts management
- sharing best practices on benchmarking papers

As of the beginning of 2021 Science Communication WG launched a long-term Twitter campaign in which all the EU-LIFE members will be introduced. The campaign contains also the main topics in which EU-LIFE are involved such as: fight against COVID-19, science policy, science for the society, or research excellence. Science Communication WG is also planning to design and implement EU-LIFE internal and external communication strategy. IIMCB representatives in this WG are Daria Goś and Magdalena Krupa.

www.eu-life.eu



Members

BI	**Babraham Institute,** Cambridge, United Kingdom
BRIC	**Biotech Research and Innovation Centre,** Copenhagen, Denmark
CEITEC	**Central European Institute of Technology,** Brno, Czech Republic
CEMM	**Research Center for Molecular Medicine of the Austrian Academy of Sciences,** Vienna, Austria
CRG	**Centre for Genomic Regulation,** Barcelona, Spain
FIMM	**Institute for Molecular Medicine Finland,** Helsinki, Finland
FMI	**Friedrich Miescher Institute for Biomedical Research,** Basel, Switzerland
IC	**Institut Curie,** Paris, France
IEO	**European Institute of Oncology,** Milan, Italy
IGC	**Gulbenkian Science Institute,** Oeiras, Portugal
IIMCB	**International Institute of Molecular and Cell Biology in Warsaw,** Warsaw, Poland
MDC	**Max Delbrück Center for Molecular Medicine in the Helmholtz Association,** Berlin, Germany
NKI	**Netherlands Cancer Institute,** Amsterdam, The Netherlands
VIB	**Flanders Institute for Biotechnology,** Zwijnaarde, Belgium

Associate Partner

IMBB FORTH	**Institute of Molecular Biology & Biotechnology,** Heraklion, Greece

Human Resources Strategy for Researchers

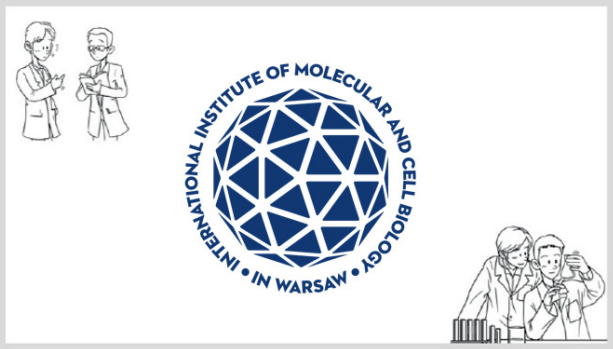
The unexpected COVID-19 pandemic made it necessary to reorganize our work at the IIMCB to ensure the security of our employees and simultaneously maintain scientific and administrative activities of the Institute.

All measures we introduced in 2020 were based on the assumptions of the **HR Strategy** that was implemented in 2019 as part of the MOSaIC project "MOlecular Signaling in Health and Disease - Interdisciplinary Centre of Excellence" and according to provisions of the **Human Resources Strategy for Researchers (HRS4R)**. These measures were intended to ensure the best possible working conditions for research staff at this unusual time. Some activities were also implemented thanks to funding we received from the Polish National Agency for Academic Exchange within the Welcome to Poland Programme.

Despite the circumstances, we were able to achieve most of what had been planned for 2020. All of this was made possible through consultations with numerous groups, including Directors, Lab Leaders, Heads of Administration Units, and representatives of individual scientific groups in online meetings, as well as regular meetings of the HR Logo Working Group. Internationally this was done by the active participation of the IIMCB representatives in the Recruitment and Training Working Group and Gender Equalities Working Group within the EU-LIFE alliance.

The most important activities in 2020

- Preparation of an **exit interview form** and its implementation
- Establishment of a **Buddy Institution** and the preparation of a leaflet on how to introduce new employees
- Preparation of **welcome boxes** for new employees with an information package on the most important regulations of the IIMCB
- Organization of an online **basic statistics course** available to all the IIMCB employees
- **Verification of regulations** that were introduced, including those concerning the periodic evaluation of employees
- Continuous support for researchers in the recruitment process, including the **recruitment of PhD students**
- Presentation of the profiles and scope of activity of ombudspersons at the IIMCB and the preparation of a **movie that promotes effective conflict resolution**
- Modification of **effective document processing** by introducing the possibility of using electronic means of communication
- Regular communication of **COVID-19 internal rules** and **reporting on the pandemic situation** at the Institute
- Keeping members of the Institute apprised of **restrictions being introduced** on a national scale



Horizon 2020 ERA Chairs project at IIMCB

"MOlecular Signaling in Health and Disease – Interdisciplinary Centre of Excellence"

Project coordinator
Jacek Kuźnicki

Implementation period
2018-2023

Funding
2 498 887.50 EUR

Referenced call
H2020 WIDESPREAD-03-2017

Goals

- **Establishment of the ERA Chairs Research Group headed by an outstanding scientist**
- **Structural improvements in science management and HR activities, according to the best international standards**

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

Laboratory of RNA Biology – ERA Chairs Group

The Laboratory of RNA Biology - ERA Chairs Group headed by Professor Andrzej Dziembowski started its activities at the IIMCB on 1st December 2019. The Group studies post-transcriptional regulation of gene expression. They aim to answer the questions of how processive ribonucleases shape the transcriptomes of mammalian cells through RNA degradation and how poly(A) and poly(U) polymerases regulate protein production.

The Laboratory of RNA Biology – ERA Chairs Group has been implementing seven research grants

- TEAM FNP, Andrzej Dziembowski
- TEAM-TECH Core Facility FNP, Andrzej Dziembowski
- GRIEG (EEA and Norway Grants), NCN, Andrzej Dziembowski (Coordinator), University of Warsaw and University of Bergen (Partners)
- MAESTRO NCN, Andrzej Dziembowski
- OPUS NCN, Andrzej Dziembowski
- SONATA NCN, Monika Kusio-Kobiałka
- SONATINA NCN, Tomasz Kuliński

Andrzej Dziembowski hosted two renowned scientists who held open online seminars

- Radislav Sedláček, Institute of Molecular Genetics of the Czech Academy of Sciences
  Czech Centre for Phenogenomics: gateway to a comprehensive description of gene functions
- Torben Heick Jensen, Technical University of Denmark
  Nuclear fates of RNA 3' ends

The ERA Chairs Group's scientific activities resulted in a publication

Bilska A, Kusio-Kobiałka M, Krawczyk PS, Gewartowska O, Tarkowski B, Kobyłecki K, Nowis D, Golab J, **Gruchota J, Borsuk E, Dziembowski A, Mroczek S**. Immunoglobulin expression and the humoral immune response is regulated by the non-canonical poly(A) polymerase TENT5C, *Nature Communications*, 2020; 11(1):2032

Opening Ceremony of the ERA Chairs Research Group, IIMCB, January 9, 2020

Effective science management and improved HR activities

MOSaIC supported open access and ethics practices at the IIMCB

- Support of the IIMCB researchers in preparation of data management plans and ethics for their project proposals
- Securing GMO and GMM work permits for the IIMCB laboratorie
- Open Access publishing process and depositing of publications (CeON) and research data (RePOD) repositories of the Interdisciplinary Centre for Mathematical and Computer Modelling at the University of Warsaw

IIMCB shared its Open Access Policy practices at external events

- National Open Access Workshop, OpenAIRE, June 8, 2020
- UNESCO Regional Consultation on Open Science for Eastern Europe, September 24, 2020

Warsaw PhD School in Natural and BioMedical Sciences

With the MOSaIC's contribution, the IIMCB, together with eight other institutes, established the Warsaw PhD School in Natural and BioMedical Sciences (Warsaw-4-PhD). In the 2020/2021 academic year, the IIMCB has had ten PhD students enrolled at the Warsaw-4-PhD. The IIMCB's dedicated staff has provided efficient management of the school's procedures, recruitment, and promotional activities throughout the year.

Toward translational research by the IIMCB with SPARK

The SPARK Global network promotes translational research all over the world. IIMCB participated in the 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 launched in January 2020. Two of the IIMCB projects that took part in this competition 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 Programme

New developments in the IIMCB HR Strategy

The HR Strategy of the IIMCB, one of the MOSaIC's milestones, has introduced a number of functional procedures and practices. Those introduced in 2020 are listed on page 6.

MOSaIC contributed to specialized training sessions and workshops for the IIMCB staff

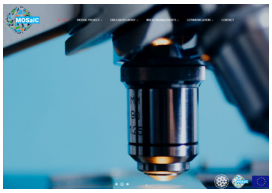
- Advanced course Essential informatics skills and knowledge to begin analysing next generation sequencing data
- Introduction to statistics in R
- Research Data Management Workshop
- Data Management Workshop for Data Stewards
- Open Science & Open Access with particular reference to Horizon 2020
- Web of Science training, How to search and analyze open access journals
- National OpenAIRE Open Access Workshops - DATA
- Basic statistics course

MOSaIC birthday celebration

On November 1, 2020, MOSaIC turned 2 years old!

On this occasion, we distributed MOSaIC bandanas among the IIMCB staff and organized the *MOSaIC Bandana Photo Challenge*. The outcome was a MOSaIC photo collage that decorated IIMCB's 2021 calendar.

MOSaIC website



mosaic.iimcb.gov.pl

In 2020, IIMCB launched MOSaIC website, where you can learn about the project's highlights, objectives, major achievements, and people involved, the ERA Chairs Group and administrative personnel alike.

Organizational structure

Director

- Auresine Strategic Project
- Aging and Longevity Strategic Project
- 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

- Core 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

- Operations Unit
- Public Procurement Unit
- IT Unit

Deputy Director for Finance/Chief Accountant

- Financial and Accounting Unit

RESEARCH GROUPS

Laboratory of Structural Biology

Group Members

Lab Leader
Matthias Bochtler, PhD, Professor

Senior Researcher
Honorata Czapińska, PhD, DSc Habil

Postdoctoral Researchers
Humberto Fernandes, PhD (IBB/IIMCB, until September 2020)
Charles Weige, PhD (until December 2020)
Marek Wojciechowski, PhD

PhD Students
Igor Helbrecht, MSc (IBB)
Magdalena Klimczak, MSc Eng.
Katarzyna Krakowska (formerly Szafran), MSc
Abhishek Pateria, MSc Eng.
Anton Slyvka, MSc
Anna Stroynowska-Czerwińska, MSc Eng.

Other Co-workers
Anna Fedenko, MSc
Norbert Osiński, MSc
Michał Pastor, MSc (IBB)
Dominik Rafalski, MSc Eng.

Technicians
Agnieszka Olszewska (part-time)
Julia Pac, MSc (part-time)

Laboratory Support Specialists
Ewelina Borsuk, PhD (until January 2021)
Aleksandra Jakielaszek, MSc Eng.

Lab Leader:

Matthias Bochtler, PhD, Professor

Curriculum Vitae

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.

Selected Publications

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

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

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

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

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

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

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Mierzejewska K, Siwek W, Czapinska H, Kaus-Drobek M, Radlinska M, **Skowronek K, Bujnicki JM**, Dadlez M, **Bochtler M.** Structural basis of the methylation specificity of R.DpnI. *Nucleic Acids Res*, 2014; 42(13): 8745-54

Description of Current Research

Our laboratory works on DNA modifications and their interplay with chromatin modifications. DNA methylation itself is evolutionarily ancient. This enables us to use well-behaved, prokaryotic models to study biophysical questions. It also allows the discovery of enzymes in bacteria that are useful as tools to study eukaryotic DNA methylation biology. In 2020, the laboratory has worked on the following topics:

SENSOR DOMAINS FOR DNA METHYLATION

Most proteins that sense DNA methylation do so using dedicated domains. SRA domains are the preferred domains for sensing methylation in a single DNA strand (regardless of the complementary strand). SRA domains belong to a larger family that is collectively referred to as the PUA superfamily. Until recently, PUA superfamily domains, with the exception of SRA domains, were believed to be involved in the biology of RNA modifications. In collaboration with Dr. Xu (New England Biolabs), we discovered that PUA domains in prokaryotes are frequently fused to endonuclease domains of the HNH and PD-(D/E)XK types. These domains typically act on DNA and not on RNA. Our findings suggested that naturally occurring fusion proteins could play a role as modification-sensitive restriction endonucleases. In initial biochemical experiments, we confirmed this hypothesis by demonstrating modest *in vitro* and pronounced *in vivo* preference for modified DNA (Lutz et al., *Nucleic Acids Res,* 2019). The PUA superfamily comprises EVE domains, among others. In 2020, we solved structures of the EVE-HNH endonuclease VcaM4I alone and in complex with DNA. The structures demonstrated that the methylated DNA base is flipped and accommodated in a pocket of the EVE domain. The pockets of the EVE and SRA domains are located in equivalent regions. This confirms homology of the fold and modified base recognition, despite pronounced differences in the local environment of the flipped base that are attributable to mutational drift (Pastor et al., *Nucleic Acids Res*, 2021).

REPLICATION OF DNA METHYLATION

As a consequence of semi-conservative DNA replication, each daughter cell inherits one methylated and one non-methylated DNA strand. Traditionally, the re-establishment 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 the 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 for 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 (ripples) in the correlation signal that we are not yet able to reliably correlate with features of the chromosome structure.

REVERSIBILITY OF DNA METHYLATION

In eukaryotes, DNA methylation is reversible even in the absence of DNA replication. The process of DNA methylation reversal relies on the oxidation of methyl groups to hydroxyl, formyl, or carboxyl groups by TET dioxygenases or related enzymes. For adenine methylation, one or two hydroxylation rounds are sufficient to directly restore the non-methylated base, with the concomitant loss of formaldehyde or formate. For cytosine methylation, the oxidized bases are stable but can be enzymatically replaced by the base excision repair pathway, including TDG, optionally in cooperation with NEIL1. In collaboration with Drs. Jurkowski and Hore (Cardiff University and Otaga University), we discovered that dioxygenases have intrinsic sequence preferences and that *in vitro* the "best" target sequences are demethylated > 100-fold faster than the "worst" ones. We confirmed that the preferences are also relevant *in vivo* in embryonic stem cells and the germline. Intriguingly, the sequence preferences of all TET paralogues are similar. This initially puzzling result could be explained by co-crystal structures with most and least optimal substrates. TETs impose a non-canonical DNA structure (with flipped base) that strongly favors some sequence contexts. Additionally, other weaker sequence preferences are more conventionally achieved by interactions between DNA bases and protein residues (manuscript submitted). In related work, we collaborated with Prof. Klimasauskas (Vilnius University) on investigating activity of the TET demethylation pathway toward bulky methylcytosine analogs that are of interest as biochemical probes of the demethylation reaction. The data show that the analogs can be oxidized and also replaced but with efficiencies that decrease with the extent of steric bulk. This indicates that the TET-BER demethylation pathway unlikely eradicates bulky 5-methylcytosine analogs from the genome in mammalian cells (Tomkuvienė et al., *J Mol Biol,* 2020)

METHYLATION IN EPIGENOMICS AND DNA REPAIR

The distinction between dioxygenases that are involved in epigenetics and those that play a role in DNA repair has recently become blurred because of overlapping activities on alkylated nucleobases (Fig. 1). We summarized similarities and differences of TET and ALKBH dioxygenases in a recent invited review (Xu and Bochtler, *Nat Chem Biol*, 2020). Moreover, we wrote an opinionated review that makes the case that 6-methyladenine in mammalian DNA is a form of DNA damage and not an epigenetic signal (Bochtler and Fernandes, *Bioessays*, 2020). We also generated preliminary experimental results on the DNA repair activity of TETs that we are now verifying biochemically and plan to confirm with relevant crystal structures.

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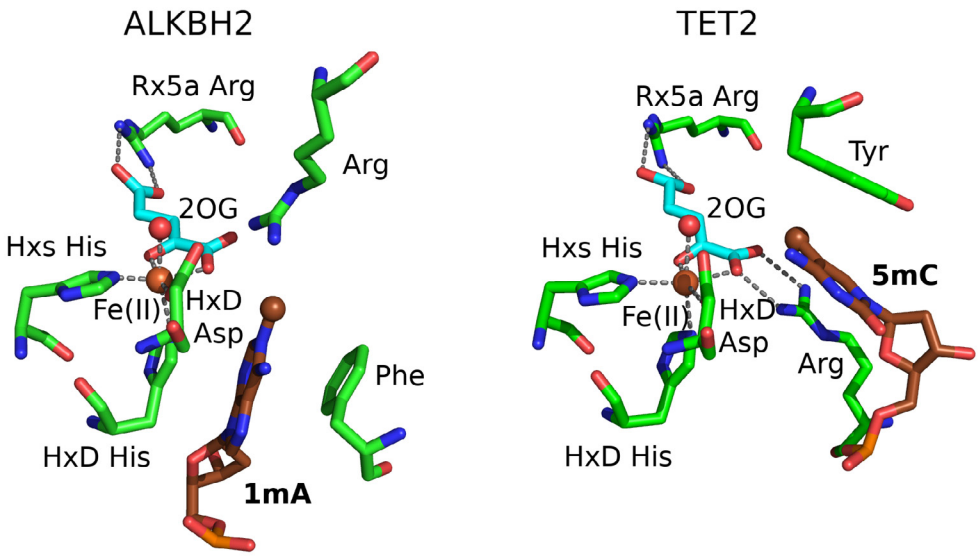

Fig.1 Comparison of active sites of ALKBH2, a DNA repair dioxygenase, and TET2, a demethylase that is involved in epigenetic reprogramming. Both models are idealized from one or more crystal structures by combining ligand information and/or replacing co-substrate analogs with physiologically relevant co-substrates. The glycosidic bond of the substrate 2'-deoxynucleotide is in a favorable *anti*-conformation in the ALKBH complexes but in the unfavorable *syn*-conformation in the TET complexes. The binding of 2'-deoxynucleotides with non-canonical glycosidic bond conformation broadens the substrate repertoire of the dioxygenases.

Laboratory of Bioinformatics and Protein Engineering

Group Members

Lab Leader
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Filip Stefaniak, PhD

Researcher
Michał Boniecki, PhD (until August 2020)

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Nithin Chandran, PhD
Pritha Ghosh, PhD (until September 2020)
Sunandan Mukherjee, PhD
Almudena Ponce Salvatierra, PhD
Tales Rocha de Moura, PhD
Tomasz Wirecki, PhD

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Masoud Amiri Farsani, MSc
Farhang Jaryani, PhD
Seyed Naeim Moafinejad, MSc
Iswarya Pandara Nayaka PJ, MSc
Kanchan Chauhan, MSc (until September 2020)
Ankita Rawat, MSc (until September 2020)

Undergraduate Students
Agata Momot (until September 2020)
Joanna Stachera
Natalia Szulc, MSc (until September 2020)
Jan Wójtowicz (until September 2020)

Other Co-workers
Pietro Boccaletto, MSc
Karolina Bogacka, MSc
Michał Boniecki, PhD
Doni Dermawan, BSc (until September 2020)
Olga Kowalska
Marta Luterek

Technician
Iwona Ptasiewicz (part-time)

Laboratory Support Specialist
Katarzyna Grzelak, MSc

Lab Leader:

Janusz M. Bujnicki, PhD, Professor

Curriculum Vitae

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, University of Warsaw (Chairman, 2019-2020)
2018-Present	Member, Academia Europaea
2017-Present	Member, European Molecular Biology Organization
	Member, 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

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

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

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Magnus M, Antczak M, Zok T, Wiedemann J, Lukasiak P, Cao Y, **Bujnicki JM**, Westhof E, Szachniuk M, Miao Z. RNA-Puzzles toolkit: a computational resource of RNA 3D structure benchmark datasets, structure manipulation, and evaluation tools. ***Nucleic Acids Res***, 2020; 48(2):576-588

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

Miao Z et al. (**Boniecki MJ**, **Bujnicki JM**, **Joshi A**, **Magnus M**, **Piatkowski P**, **Pluta R**.). RNA-Puzzles Round IV: 3D Structure Predictions of Four Ribozymes and Two Aptamers. ***RNA***, 2020; 26(8):982-995

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Ponce-Salvatierra A, **Astha**, **Merdas K**, **Chandran N**, **Ghosh P**, **Mukherjee S**, **Bujnicki JM**. Computational modeling of RNA 3D structure based on experimental data. ***Biosci Rep***, 2019; 39(2):BSR20180430

Stasiewicz J, **Mukherjee S**, **Nithin C**, **Bujnicki JM**. QRNAS: software tool for refinement of nucleic acid structures. ***BMC Struct Biol***, 2019; 19(1):5

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Madan B, **Kasprzak JM**, **Tuszynska I**, **Magnus MM**, **Szczepaniak K**, **Dawson WK**, **Bujnicki JM**. Modeling of protein-RNA complex structures using computational docking methods. ***Methods Mol Biol***, 2016; 1414:353-72

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

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Smietanski M, **Werner M**, **Purta E**, **Kaminska KH**, Stepinski J, Darzynkiewicz E, **Nowotny M**, **Bujnicki JM**. Structural analysis of human 2′-O-ribose methyltransferases involved in mRNA cap structure formation. ***Nature Commun***, 2014; 5:3004

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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 a method for predicting metal ion binding sites in RNA structures (MetalionRNA; http://metalionrna.genesilico.pl), a method for modeling 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/), and a structural classification of known families of structured non-coding RNAs (RNArchitecture; http://iimcb.genesilico.pl/RNArchitecture/).

Our experimental research focuses on elucidating sequence structure-function relationships in bio-macromolecules (currently mainly RNA and RNA-protein complexes, also with small chemical molecules) using biophysics, biochemistry, molecular biology, and cell biology. 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 X-ray crystallography and low-resolution methods, such as small-angle X-ray scattering (SAXS), structure probing by chemical modification, circular dichroism, mutagenesis, etc. Most recently, our group began using cryo-electron microscopy, and we have collected our first datasets and begun data processing using established dedicated software and tools that were developed in-house.

RECENT HIGHLIGHTS

The SARS-CoV-2 coronavirus genome RNA structure was studied in detail by researchers from the International Institute of Molecular and Cell Biology in Warsaw, the University of Groningen, and Leiden University. The viral RNA structures could be targets for the development of drugs against the virus.

Throughout history, mankind has always suffered from infectious diseases that are caused by bacteria and viruses. Over the last 18 years, many deaths have occurred worldwide because of severe acute respiratory syndromes that are caused by coronaviruses, including SARS and MERS. Together with the ongoing COVID-19 pandemic that has already taken more than one million lives, this demonstrates the urgent need for new ways of combating coronavirus infections. COVID-19 is caused by SARS-CoV-2, a betacoronavirus with a linear single-stranded, positive-sense RNA genome. For other RNA viruses, the characterization of genomic RNA structures of SARS-CoV-2 is expected to play a crucial role in revealing how coronavirus replicates in human cells. To date, only a handful of functionally relevant coronavirus structural RNA elements have been studied.

The collaborative study involved RNA structure probing to obtain single-base resolution secondary structure maps of the full SARS-CoV-2 coronavirus genome both *in vitro* and in living infected cells. Importantly, the structure of the entire coronavirus RNA, one of the longest viral RNAs of approximately 30,000 nucleotides, was determined for the first time. The team identified at least 87 regions in the SARS-CoV-2 RNA sequence that appear to form well-defined compact structures, of which at least 10% are under strong evolutionary selection among coronaviruses, hinting at their functional relevance. An article that reports results of these analyses was published in *Nucleic Acids Research*, and reviewers who evaluated the manuscript nominated it as a “Breakthrough paper".

Publication:

Manfredonia I, **Nithin C, Ponce-Salvatierra A, Ghosh P, Wirecki TK**, Marinus T, Ogando NS, Snijder EJ, van Hemert MJ, **Bujnicki JM**, Incarnato D. Genome-wide mapping of SARS-CoV-2 RNA structures identifies therapeutically-relevant elements. ***Nucleic Acids Res.*** 2020; 48(22):12436-12452

DNAmoreDB, a database of DNAzymes

Deoxyribozymes, DNA enzymes or simply DNAzymes, are single-stranded oligodeoxyribonucleotide molecules that, like proteins and ribozymes, have the ability to perform catalysis. Although DNAzymes have not yet been found in living organisms, they have been isolated in the laboratory through *in vitro* selection. Selected DNAzyme sequences have the ability to catalyze a broad range of chemical reactions, utilizing DNA, RNA, peptides, or small organic compounds as substrates.

We developed DNAmoreDB, a comprehensive database resource for DNAzymes that collects and organizes the following types of information: sequences, conditions of the selection procedure, catalyzed reactions, kinetic parameters, substrates, cofactors, structural information (where available), and literature references. Currently, DNAmoreDB contains information about DNAzymes that catalyze 20 different reactions. We included a form for the submission of new data, a REST-based API system that allows users to retrieve database contents in a machine-readable format, and keyword and BLASTN search functions. The database is publicly available at https://www.genesilico.pl/DNAmoreDB.

Publication:

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

RNAProbe: a web server for the normalization and analysis of RNA structure probing data

Knowledge of structural characteristics of RNA molecules allows a better understanding of the mechanisms of their action. RNA chemical probing allows studies of the susceptibility of nucleotides to chemical modification. The obtained information can then be used to guide secondary structure prediction. These experimental results can be analyzed using various computational tools, which often requires additional and often tedious steps (e.g., further normalization of reactivities and visualization of the results), for which there are no fully automated methods.

We developed RNAProbe, a web server that facilitates the normalization, analysis, and visualization of low-pass SHAPE, DMS, and CMCT probing results with modification sites that are detected by capillary electrophoresis. RNAProbe automatically analyzes chemical probing output data and turns otherwise tedious manual work into a one-minute assignment. RNAProbe performs normalization based on a well-established protocol, utilizes recognized secondary structure prediction methods, and generates high-quality images with structure representations and reactivity heatmaps. It summarizes the results in the form of a spreadsheet, which can be used for comparative analyses between experiments. Results of predictions with normalized reactivities are also collected in text files, providing interoperability with bioinformatics workflows. RNAProbe is available at https://rnaprobe.genesilico.pl/.

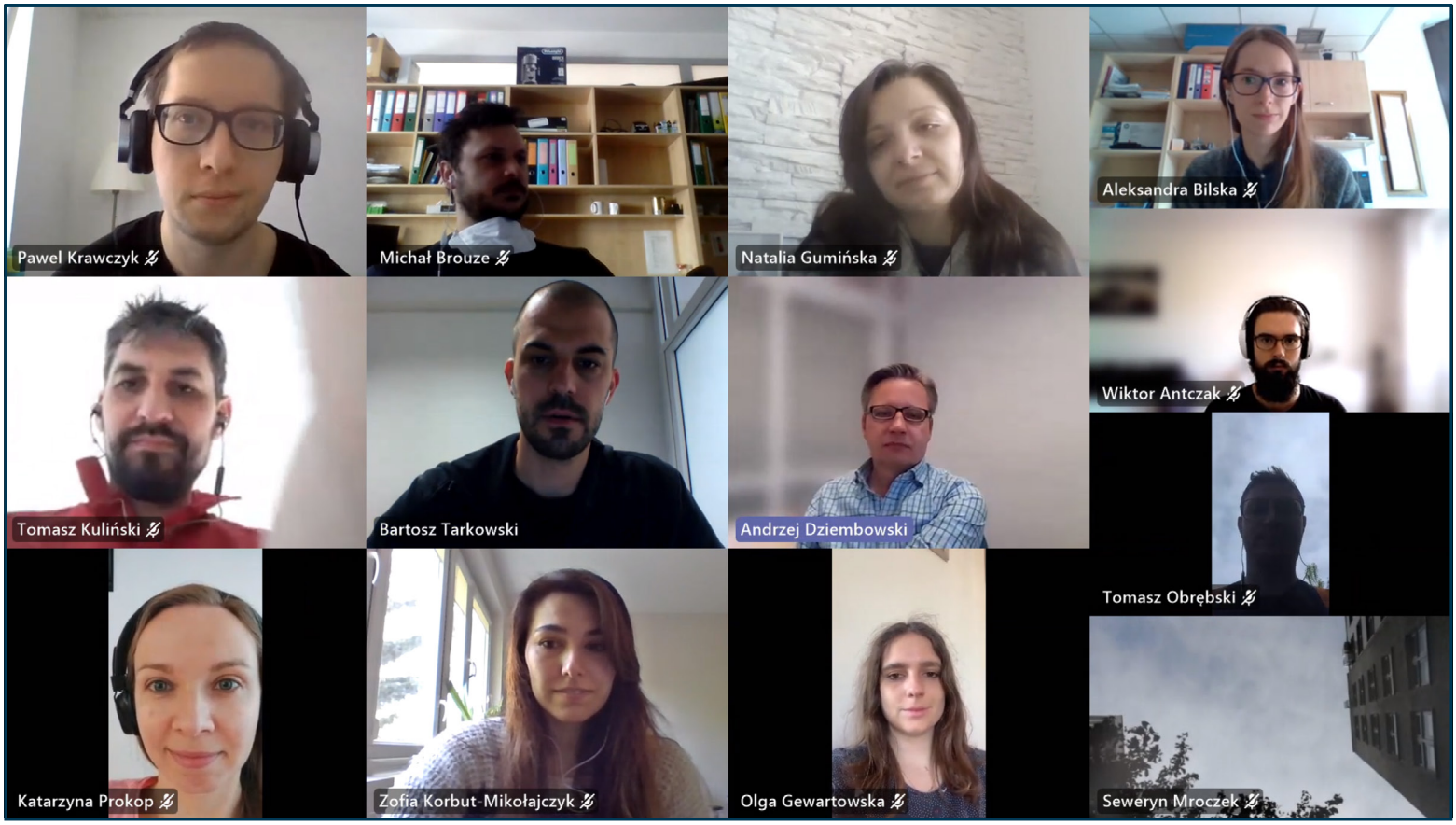
Publication:

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

www.iimcb.gov.pl/bujnicki-lab

www.genesilico.pl

Laboratory of RNA Biology – ERA Chairs Group



Group Members

Lab Leader
Andrzej Dziembowski, PhD, Professor

Senior Researchers
Ewa Borsuk, Professor (part-time)
Seweryn Mroczek, PhD (part-time)

Postdoctoral Researchers
Olga Gewartowska, PhD
Natalia Gumińska, PhD
Anna Hojka-Osińska, PhD
Paweł Krawczyk, PhD
Tomasz Kuliński, MD PhD
Monika Kusio-Kobiałka, PhD
Vladyslava Liudkovska, PhD
Katarzyna Matylla-Kulińska, PhD
Bartosz Tarkowski, PhD

Research Specialists
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Jakub Gruchota, MSc
Karolina Piechna, MSc
Katarzyna Prokop, MSc
Marcin Szpila, MSc

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Michał Brouze, MSc
Zuzanna Mackiewicz, MSc
Michał Mazur, MSc
Tomasz Obrębski, MSc
Karolina Wróbel, MSc

Undergraduate Students
Wiktor Antczak
Magdalena Orzyłowska
Julia Sygocka

Other Co-workers
Hanna Grzesik
Kamil Jachacy

Technician
Alina Zielińska, BSc (part-time)

Laboratory Support Specialist
Zofia Korbut-Mikołajczyk, MSc

Lab Leader:

Andrzej Dziembowski, PhD, Professor

Curriculum Vitae

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 (currently 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

2020 GRIEG (EEA and Norway Grants), National Science Centre
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

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

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

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

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

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

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

Mroczek S, Chlebowska J, Kuliński TM, Gewartowska O, Gruchota J, Cysewski D, Liudkovska V, Borsuk E, Nowis D, **^Dziembowski A**. The non-canonical poly(A) polymerase FAM46C acts as an onco-suppressor in multiple myeloma. ***Nat Commun***, 2017; 8(1):619 Kalisiak K, Kuliński TM, Tomecki R, Cysewski D, Pietras Z, Chlebowski A, Kowalska K, **^Dziembowski A**. A short splicing isoform of HBS1L links the cytoplasmic exosome and SKI complexes in humans. ***Nucleic Acids Res***, 2017; 45(4):2068-80

Łabno A, Warkocki Z, Kuliński T, Krawczyk PS, Bijata K, Tomecki R, **^Dziembowski A**. Perlman syndrome nuclease DIS3L2 controls cytoplasmic noncoding RNAs and provides surveillance pathway for maturing snRNAs. ***Nucleic Acids Res***, 2016; 44(21):10437-53

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

Szczepińska T, Kalisiak K, Tomecki R, Labno A, Borowski LS, Kulinski TM, Adamska D, Kosinska J, **^Dziembowski A**. DIS3 shapes the RNA polymerase II transcriptome in humans by degrading a variety of unwanted transcripts. ***Genome Res***, 2015; 25(11):1622-33

Wrobel L, Topf U, Bragoszewski P, Wiese S, Sztolsztener ME, Oeljeklaus S, Varabyova A, Lirski M, Chroscicki P, Mroczek S, Januszewicz E, **^Dziembowski A**, Koblowska M, Warscheid B, Chacinska A. Mistargeted mitochondrial proteins activate a proteostatic response in the cytosol. ***Nature***, 2015; 524(7566):485-8

Lubas M, Andersen PR, Schein A, **^Dziembowski A**, Kudla G, Jensen TH. The human nuclear exosome targeting complex is loaded onto newly synthesized RNA to direct early ribonucleolysis. ***Cell Rep***, 2015; 10(2):178-92

Mathys H, Basquin J, Ozgur S, Czarnocki-Cieciura M, Bonneau F, Aartse A, **^Dziembowski A**, Nowotny M, Conti E, Filipowicz W. Structural and biochemical insights to the role of the CCR4-NOT complex and DDX6 ATPase in microRNA repression. ***Mol Cell***, 2014; 54(5):751-65

Tomecki R, Drazkowska K, Kucinski I, Stodus K, Szczesny RJ, Gruchota J, Owczarek EP, Kalisiak K, **^Dziembowski A**. Multiple myeloma-associated hDIS3 mutations cause perturbations in cellular RNA metabolism and suggest hDIS3 PIN domain as a potential drug target. ***Nucleic Acids Res***, 2014; 42(2):1270-90

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

Gene expression in eukaryotes is regulated at multiple levels, from chromatin structure, transcription, pre-mRNA processing, and mRNA export from the nucleus to mRNA stability and translation. The primary research interest of the laboratory is the regulation of gene expression at the posttranscriptional level. In the past, we were interested in mechanistic aspects of RNA metabolism. We are currently studying RNA biology at the organismal level using transgenic mouse lines as a main research model.

Our research focuses on two areas:

- Analysis of the function of processive ribonucleases that shape transcriptomes of eukaryotic cells through RNA degradation.
- Analysis of cytoplasmic poly(A) and poly(U) polymerases that add nontemplated nucleotides to 3' ends of RNA molecules to affect their stability and biological functions.

ANALYSIS OF THE FUNCTION OF PROCESSIVE RIBONUCLEASES

Processive exoribonucleases play a major role in eukaryotic RNA turnover and processing. Some act in the 3'-to-5' direction, such as the exosome complex or monomeric ribonuclease DIS3L2. Alternatively, RNA molecules can be degraded or processed from the 5' end by enzymes that belong to the XRN family of RNases. Importantly, the dysfunction of exoribonucleases is often associated with human diseases. The nuclear catalytic subunit of the exosome DIS3 is one of the most frequently mutated genes in multiple myeloma, a cancer of plasma cells. Mutations of DIS3L2 are associated with Perlman syndrome, a rare genetic overgrowth disease. We previously analyzed the mechanism of action and substrates of exoribonucleases using cellular model systems. In the past, we identified catalytic subunits of a primary eukaryotic ribonuclease, the exosome both in yeast and humans (Dziembowski et al., *Nat Struct Mol Biol*, 2007; Tomecki et al., *EMBO J*, 2010). We also showed that the complex, in addition to exonuclease activity, is also an endonuclease (Lebreton, Tomecki et al., *Nature*, 2008).

We participated in biochemically and structurally characterizing the exosome, which together with the work of others elucidated its mechanism of action (Drazkowska et al., *Nucleic Acids Res*, 2013; Hernandez et al., *EMBO Rep*, 2006; Lorentzen et al., *Mol Cell*, 2008; Lorentzen et al., *EMBO Rep*, 2007; Malet et al., *EMBO Rep*, 2010). The exosome needs cofactors for its full activity. We described such complexes in human cells (Kalisiak et al., *Nucleic Acids Res*, 2017; Lubas et al., *Cell Rep*, 2015; Lubas et al., *Mol Cell*, 2011). We also determined the nuclear exosome substrates that proved that this complex plays a primary role in shaping the human transcriptome by degrading various pervasive transcription products (Szczepinska et al., *Genome Res*, 2015). Finally, cancer genome projects revealed that the catalytic subunit of the exosome DIS3 is frequently mutated in multiple myeloma. We identified vulnerabilities that are associated with such mutations to propose a novel drug target (Tomecki et al., *Nucleic Acids Res*, 2014). In the future, we will investigate functional interactions between RNA-degrading enzymes and other cellular pathways that are involved in the expression of genetic information. In parallel, we will analyze the role of selected exoribonucleases using transgenic mouse models. Finally, we are interested in the role of mutations of DIS3 in the pathogenesis of multiple myeloma.

ANALYSIS OF CYTOPLASMIC NONCANONICAL POLY(A) AND POLY(U) POLYMERASES

Most mRNA molecules are polyadenylated during classic 3'-end formation by canonical poly(A) polymerases. The poly(A) tail greatly enhances

protein synthesis through its interactions with poly(A) binding proteins, which protect the mRNA 3' end from exoribonucleolytic decay and directly interact with translation-initiation factors to promote translation. It is now known that poly(A) tail dynamics are more complex than previously suspected. Deadenylated mRNAs in the cytoplasm can be degraded, uridylated, or stored in a dormant state to be later re-adenylated to activate protein synthesis. The enzymes that are responsible for modifications of the poly(A) tail are non-canonical poly(A) and poly(U) polymerases. Analyses of the human cytoplasmic poly(U) polymerases TUT4 and TUT7 led us to an unexpected discovery, in which uridylation was found to be a potent restrictor of retrotransposition of the LINE-1 element, the only active autonomous transposon in humans (Warkocki et al., *Cell*, 2018) (Fig. 1). We are currently focusing on cytoplasmic polyadenylation rather than uridylation. Cytoplasmic polyadenylation was mostly studied in the context of gametogenesis and in neuronal synapses, where transcriptional activity is limited. Surprisingly, mouse lines that were devoid of the cytoplasmic poly(A) polymerase GLD2 (TENT2) exhibited no apparent phenotypes. We recently described a novel family of cytoplasmic poly(A) polymerases, TENT5 (FAM46), which comprise four members in vertebrates: TENT5A-D. TENT5C acts as a tumor suppressor in multiple myeloma (Mroczek et al., *Nature Commun*, 2017), whereas mutations of TENT5A lead to a rare genetic disease, osteogenesis imperfecta. We generated knockout and knock-in (GFP/FLAG-tagged) mouse models for all TENT5 family members using CRISPR/Cas9 technology. Although knockouts of these genes are not lethal, we detected a plethora of different phenotypes that affect several organs and biological processes. In the future, we will dissect the functions and mechanisms of cytoplasmic polyadenylation by TENT5 in gametogenesis, innate immunity, hormonal regulation, and neuronal physiology. We will use animal models and advanced transcriptomic and proteomic approaches. An important element of the proposed studies is the refinement of methodology for genome-wide analyses of the global state of polyadenylation.

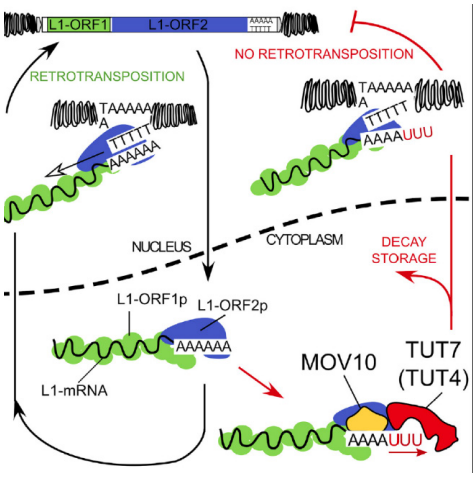

Fig.1 Model of restriction of LINE-1 retrotransposition by uridylation (based on Warkocki et al., *Cell,* 2018).

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

www.iimcb.gov.pl/dziembowski-lab

Mouse Genome Engineering Facility

Mouse Genome Engineering Facility

EXPERIENCE

Our facility is supported by the Foundation for Polish Science TEAM-TECH Core Facility grant. It is based on cooperation between Prof. Andrzej Dziembowski (International Institute of Molecular and Cell Biology in Warsaw) and Prof. Ewa Borsuk (Department of Embryology, Faculty of Biology, University of Warsaw), thus combining knowledge about RNA biology and expertise in manipulating early embryos. We have generated dozens of different mouse lines with a ~97% success rate.

SERVICES

The Mouse Genome Engineering Facility provides customized transgenic mouse models that are generated using CRISPR/Cas9 methodology. The facility is exceptionally efficient in generating knock-in mouse lines with large inserts. We offer many types of genetic modifications: knockout, indels, floxed exons, insertions of N- and C-terminal tags in the locus (FLAG, EGFP, HA, etc.), and insertions of transgenes into ROSA26 and any other locus. Importantly, the facility provides the guarantee of charging clients only if the model is successfully generated. We can generate mouse lines on any desired genetic background. The price for generating of new mouse line starts at ~5,000 Euro. The average timeline to obtain F1 generation mice (heterozygotes ready to be provided to clients) is ~6 months.

DESIGN OF STRATEGY → CONSTRUCTION OF gRNA AND DNA REPAIR TEMPLATE → ZYGOTE INJECTION → FOUNDER SCREENING → BREADING TO OBTAIN F1

Laboratory of Molecular and Cellular Neurobiology

Group Members

Lab Leader
Jacek Jaworski, PhD, Professor

Senior Researchers
Magdalena Błażejczyk, PhD
Ewa Liszewska, PhD
Matylda Macias, PhD (part-time, until July 2020)
Małgorzata Urbańska, PhD
Justyna Zmorzyńska, PhD

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Michalina Wężyk, PhD (until March 2020)

Postdoctoral Researchers
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Andrii Kopach, PhD

Research Assistant
Marcelina Firkowska, PhD (until September 2020)

Research Specialists
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Katarzyna Machnicka, MSc
Aleksandra Zdanowicz, MSc

PhD Students
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Magdalena Mlostek, MSc
Karolina Bogusz (formerly Ryczek), MSc
Aleksandra Tempes, MSc
Oliver Tkaczyk, MSc
Jan Węsławski, MSc
Juan Zeng, MSc

Technician
Alina Zielińska, BSc (part-time)

Laboratory Support Specialist
Angelika Jocek, MSc

Lab Leader:

Jacek Jaworski, PhD, Professor

Curriculum Vitae

DEGREES

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

PROFESSIONAL EXPERIENCE

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

RESEARCH TRAINING

2016	Research visit (3 weeks) with Prof. William Harris, Cambridge University, Cambridge, UK
2011	Research visit (2 weeks) with Dr. Carlo Sala, CNR Institute of Neuroscience and Instituto Neurologico Carlo Besta, Milan, Italy
2006	Research visit (1 month) with Dr. C.C. Hoogenraad, Erasmus Medical Center, Rotterdam, Holland
2002-2005	Postdoctoral Associate with Prof. Morgan Sheng, Picower Center for Learning and Memory, Massachusetts Institute of Technology and Howard Hughes Medical Institute, Cambridge, MA, USA
2000	Research training (1 month) with Dr. J. Guzowski, ARL Division of Neural Systems, Memory, and Aging, University of Arizona, Tucson, USA
1997-2001	Research training (7 months) with Prof. J. Mallet, Laboratoire de Genetique Moleculaire de la Neurotransmission et des Processus Neurodegeneratifs (LGN), UMR 9923, Centre National de la Recherche Scientifique, Paris, France
1996-2002	PhD student (until 2001) and Postdoctoral Associate (until May 2002) with Prof. L. Kaczmarek, Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1995-1996	Master's degree, Prof. P. Węgleński, Department of Genetics, University of Warsaw, Poland

FELLOWSHIPS AND AWARDS

2020	Prime Minister'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

2019	Member, Scientific Advisory Board 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 Advisory Board of the Nencki Institute of Experimental Biology, Polish Academy of Sciences
2011	Member, Neurobiology Committee, Polish Academy of Sciences (terms 2011-2014; 2015-2018; 2019-2020)

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.

Selected Publications

IIMCB Best Papers Award

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

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

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

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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**. GSK3β 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

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^Jaworski J, Mioduszewska B, Sanchez-Capelo A, Figiel I, Habas A, Gozdz A, Proszynki T, Hetman H, Mallet J, Kaczmarek L. Inducible cAMP early repressor, 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 reach 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 in 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.

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 2020, despite the COVID-19 pandemic, we tried to continue our work toward all our research goals. One of the most interesting results of 2020 was obtained in Objective 2, in cooperation with numerous research teams from Europe in a project that was led by Dr. Kathrin Thedieck (Innsbruck University) and Dr. Christiane Opitz (DFKZ, Heidelberg). We jointly demonstrated that G3BP1 protein, a stress granule element, in the absence of cellular stress, is an anchor of the TSC1-TSC2 complex (the loss of which is responsible for TSC; see above) in the lysosome membrane (Prentzell et al., *Cell*, 2021). The research of our team, especially Dr. Justyna Zmorzyńska, Ms. Magdalena Kędra, and Dr. Matylda Macias, has shown that G3BP1 interacts with TSC1-TSC2 in the brain, and the absence of G3bp1 in zebrafish leads to analogous mTOR hyperactivity and "neurological" phenotypes (Fig. 1) as described earlier in the absence of TSC (Kedra et al., *Proc Natl Acad Sci USA*, 2020; e.g., epilepsy). Additionally, using live lightsheet microscopy, we showed that the loss of G3bp1 affects newly born neuron migration (Fig. 2).

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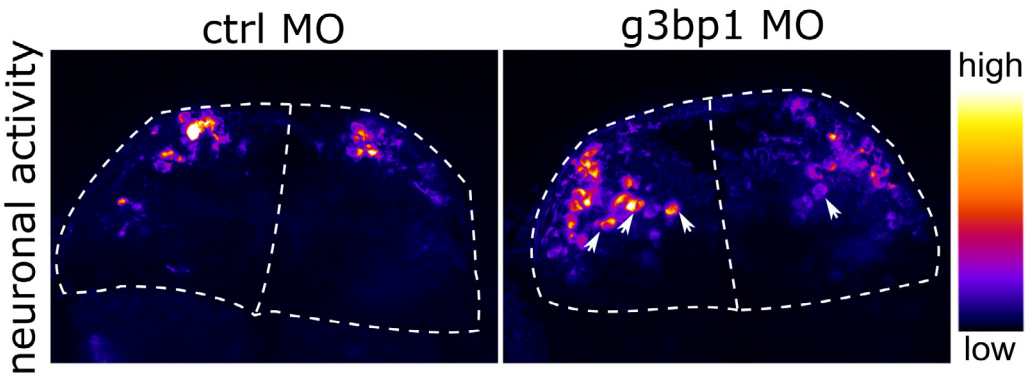


Fig.1 Morpholino- driven knockdown of g3bp1, a newly discovered TSC complex anchor results in hyperactivity of neurons of pallium of zebrafish (Prentzell et al., 2021). Representative example of functional brain imaging using calcium sensors obtained with SPIM (phot. Magdalena Kędra & Justyna Zmorzyńska).

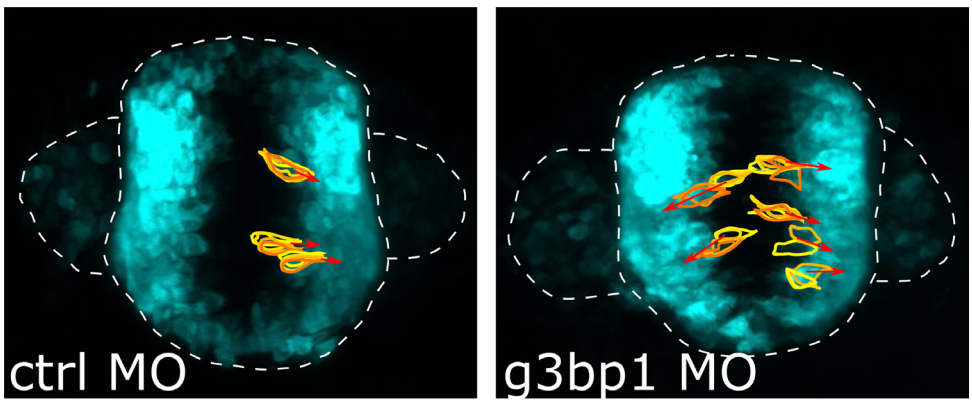
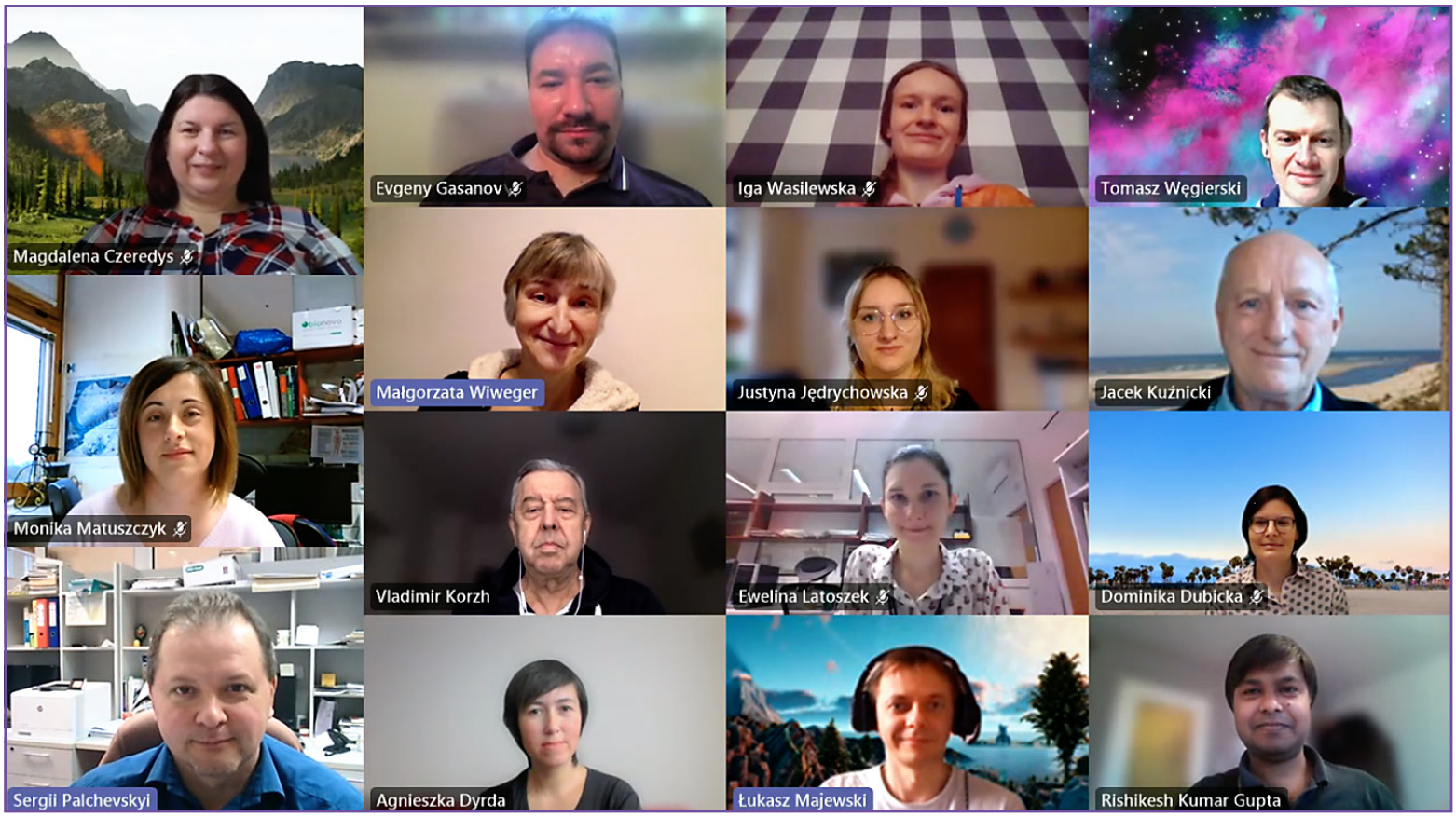


Fig.2 Morpholino-driven knockdown of g3bp1, results in hyperactivity of neurons leads to abberant migration and mislocalization of neurons to the white matter of pallium of zebrafish (Prentzell et al., 2021). Representative example of live brain imaging using calcium sensors obtained with SPIM (phot. Magdalena Kędra & Justyna Zmorzyńska). Color outlines show trajectories of neurons.

Laboratory of Neurodegeneration



Group Members

Lab Leader
Jacek Kuźnicki, PhD, Professor

Senior Researchers
Magdalena Czeredys, PhD
Vladimir Korzh, PhD
Łukasz Majewski, PhD
Tomasz Węgierski, PhD (part-time)
Małgorzata Korzeniowska (formerly Wiweger), PhD

Postdoctoral Researchers
Agnieszka Dyrda, PhD
Evgeny Gasanov, PhD
Oksana Palchevska, PhD (until August 2020)

Research Specialist
Sergii Palchevskyi, PhD (part-time)

PhD Students
Rishikesh Kumar Gupta, MSc Tech.
Justyna Jędrychowska, MSc
Ewelina Latoszek, MSc Eng.
Filip Maciąg, MSc Eng. (until September 2020; PhD defense in December 2020)
Iga Wasilewska, MSc

Trainees
Katarzyna Bagińska (August 2020)
Dominik Bielecki (July-August 2020)
Alicja Jasińska (July 2020)
Monika Kwiatkowska, MSc Eng. (since December 2020) - volunteer from ICHB PAN
Ewelina Latoszek, MSc Eng. (January-September 2020)
Karolina Piwko (September 2020)

Technician
Monika Matuszczyk (part-time)

Laboratory Support Specialist
Dominika Dubicka-Boroch, MSc

Lab Leader:

Jacek Kuźnicki, PhD, Professor

Curriculum Vitae

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 (PAS)
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
2017-2018	Deputy Chair, Council of Provosts, Division II: Biological and Agricultural Sciences, PAS
2016-Present	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
2004-Present	Honorary Chair and co-founder, BioEducation Foundation
2004-Present	Head of Program Board, Centre for Innovative Bioscience Education
1993-2014	Member, Scientific Council, Nencki Institute of Experimental Biology, PAS
1996-1998 & 2000-2002	Vice-President, Biotechnology Committee, PAS
1989-1991	General Secretary, Polish Biochemical Society

HONORS, PRIZES AND AWARDS

2013	Award from theDivision II: Biological and Agricultural Sciences, PAS for series of works on β-catenin
2013	Crystal Brussels Sprout Award
2011	Konorski Award from the Polish Neuroscience Society and Committee on Neurobiology, PAS
2008	Officer's Cross of the Order of Polonia Restituta
2003	Prime Minister's Award for Scientific Achievements
2001	Award from the Division II: Biological and Agricultural Sciences, PAS 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.

Selected Publications

IIMCB Best Papers Award

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

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de Assis GG, Murawska-Cialowicz E, Cieszczyk P, **Gasanov EV**. Respiratory Syndrome Coronavirus Infections: Possible Mechanisms of Neurological Implications-A Systematic Review. ***Front Neurol***, 2020; 11:864, review

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 voltagegated potassium channels in developmental perspective. ***Dev Dyn***, 2019; 248(12):1180-94

Khalil R, Lalai RA, **Wiweger MI**, Avramut CM, Koster AJ, Spaink HP, Bruijn JA, Hogendoorn PCW, Baelde HJ. Glomerular permeability is not affected by heparan sulfate glycosaminoglycan deficiency in zebrafish embryos. ***Am J Physiol Renal Physiol***, 2019; 317(5):F1211-F1216

de Assis GG, **Gasanov EV**. BDNF and Cortisol integrative system - Plasticity vs. degeneration: Implications of the Val66Met polymorphism. ***Front Neuroendocrinol***, 2019; 55:100784, review

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

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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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Czeredys M, Vigont VA, Boeva VA, Mikoshiba K, Kaznacheyeva EV, **Kuznicki J**. Huntingtin-Associated Protein 1A Regulates Store-Operated Calcium Entry in Medium Spiny Neurons From Transgenic YAC128 Mice, a Model of Huntington's Disease. ***Front Cell Neurosci***, 2018; 12:381

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

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

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

Soman S, Keatinge M, Moein M, Da Costa M, Mortiboys H, Skupin A, Sugunan S, **Bazala M**, **Kuznicki J**, Bandmann O. Inhibition of the mitochondrial calcium uniporter rescues dopaminergic neurons in pink1-/- zebrafish. ***Eur J Neurosci***, 2017; 45(4):528-35

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Gruszczynska-Biegala J, **Sladowska M**, **Kuznicki J**. AMPA Receptors Are Involved in Store-Operated Calcium Entry and Interact with STIM Proteins in Rat Primary Cortical Neurons. ***Front Cell Neurosci***, 2016; 10:251

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

Nagalski A, Puelles L, Dabrowski M, **Wegierski T**, **Kuznicki J**, **Wisniewska MB**. Molecular anatomy of the thalamic complex and the underlying transcription factors. ***Brain Struct Funct***, 2016; 221(5):2493-510

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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, rats, and mice as model organisms. The projects focus on proteins that are involved in store-operated Ca^{2+} entry (SOCE) and Ca^{2+} homeostasis in mitochondria, the involvement of potassium 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) is involved in a thapsigargin-induced SOCE-like process, whereas STIM2 is mostly active after the ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (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 α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Gruszczynska-Biegala et al., *Front Cell Neurosci*, 2016). STIM proteins also associate with *N*-methyl-D-aspartate (NMDA) receptors *in vitro*. The results suggest cross-talk between STIM proteins and NMDA receptors and their effect on Ca^{2+} influx through NMDA receptors (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 the level of SOCE components (Wasilewska et al., *Genes*, 2019). We generated *stim2a*, *stim2b*, and *stim2a/stim2b* knockout zebrafish lines and analyzed them using *in vivo* calcium imaging in brain neurons and behavioral tests. Deficiency in Stim2a or Stim2b resulted in a significant increase in locomotor activity 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). Moreover, an RNASeq analysis revealed a new candidate that is sensitive to Stim2a and Stim2b, annexin 3 protein.

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).

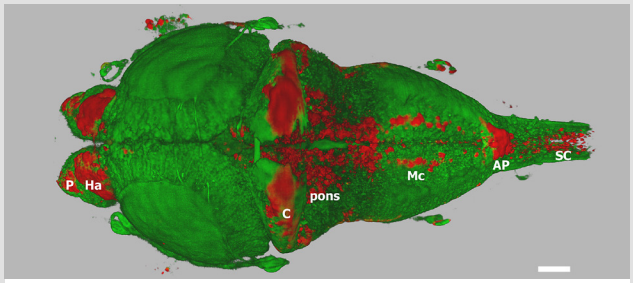

Fig.1 CAMPARI2 calcium fluorescent integrator (Moeyaert et al., 2018) was used to identify brain regions of high neuronal activity in freely swimming 3 dpf zebrafish embryos. Green indicates unconverted CAMPARI2 probe and red indicates converted CAMPARI2 probe in the presence of free calcium ions. The brain regions with the highest free calcium levels were the habenula (Ha) involved in the stress (fear) response, and cerebellum (C) involved in the spatial control. Less intense calcium signal was present in the pallium (P; olfaction), pons (escape response), myelencephalon (Mc), area postrema (AP), and spinal cord (SC). Scale bar denotes 50 µm.

Using quantitative polymerase chain reaction, we compared microRNA (miRNA) profiles in blood plasma from Alzheimer's disease patients with mild cognitive impairment (whose diagnoses were confirmed by cerebrospinal fluid biomarkers), Alzheimer's disease patients, and non-demented, age-matched controls. We adhered to standardized blood and cerebrospinal fluid assays that are recommended by the JPND BIOMARKAPD consortium. Six miRNAs (three not yet reported in the context of Alzheimer's disease and three reported in Alzheimer's disease blood) were selected as the most promising biomarker candidates that can differentiate early Alzheimer's disease from controls with the highest fold changes (Nagaraj et al., *Oncotarget*, 2017; patent pending: PCT/EP 2017/059800).

Our studies of Huntington's disease have focused on the role of CacyBP/SIP protein in β-catenin regulation in medium spiny neurons from YAC128 mice (i.e., a model of Huntington's disease) and in *cacybp* knockout zebrafish. Mutants that stabilize the dimerization domain of CacyBP/SIP had no effect on the Siah-1-dependent β-catenin degradation pathway but increased β-catenin levels in *cacybp* knockout zebrafish embryos. Moreover, we found that CacyBP/SIP plays a role in mutant huntingtin regulation. 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 in the context of medium spiny neuron neurodegeneration using different models of Huntington's disease.

A loss-of-function mutation of *PINK1* causes early-onset Parkinson's disease in humans. In collaboration with Oliver Bandmann (University of Sheffield), we used a *pink1* mutant (*pink*$^{-/-}$) zebrafish line to study alterations of Ca^{2+} homeostasis (Flinn et al., *Ann Neurol*, 2013; Soman et al., *Eur J Neurosci*, 2016). We generated *mcu* knockout zebrafish, which are viable and fertile. 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 the pathology of the *pink1* mutant. We expressed a mitochondrial Ca^{2+} probe (CEPIA2mt) under a pan-neuronal promoter (*elavl3*) to visualize Ca^{2+} levels in the mitochondrial matrix of zebrafish. Lightsheet fluorescence microscopy enabled us to visualize chemically inducible Ca^{2+} flux in zebrafish neurons *in vivo*.

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.,* manuscript submitted), mucopolysaccharidosis type III A (*SGSH*), and calcineurin variant associated with epilepsy (*PPP3CA*; Rydzanicz *et al*., *Eur J Hum Genet*, 2018). In future work, we will focus on Ca^{2+} homeostasis and its impact on the progression of neurodegeneration. For this purpose, we also created several reporter lines under the *elavl3* promoter (e.g., with calcium sensors [G-CEPIAer, GEM-CEPIAer, and CAMPARI2] or NFAT). We use these lines to monitor [Ca^{2+}] in neurons in wild type and mutant zebrafish.

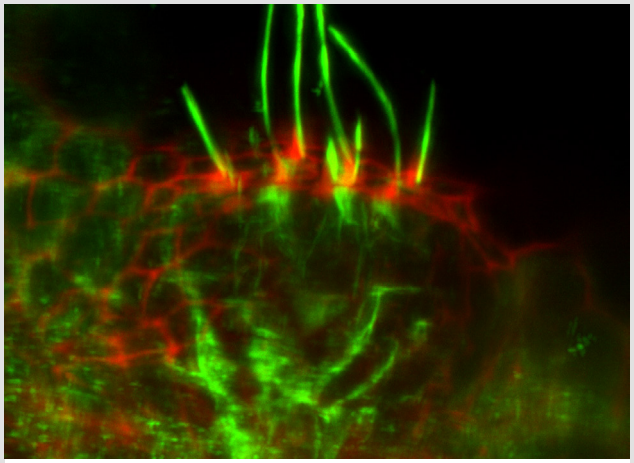

Fig.2 Image represents the inner ear's sensory patch (lateral crista) of the 3 dpf wild type zebrafish larva stained with anti-acetylated tubulin antibody (green) and phalloidin (red). Imaging by the lightsheet fluorescence microscope, 40 x objective.

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 [BVS], 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 BVS cause epilepsy in humans (Jedrychowska and Korzh, *Dev Dynam*, 2019). Formation of the BVS occurs during the early neural development of vertebrates (Korzh, *Cell Mol Life Sci*, 2018). Deficiencies in the BVS have been linked to several neurodegenerative diseases. Formation of the BVS depends on the ependyma (i.e., cells that line the BVS cavity), circumventricular organs, including the choroid plexus (Garcia-Lecea et al., *Front Neuroanat*, 2017; Korzh and Kondrychyn, *Semin Cell Dev Biol*, 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 BVS and ears. To further characterize the role of KCNB1 in development, we demonstrated that it regulates inflation of the ear and the formation of otic stones (i.e., otoliths; Jedrychowska et al., *Dev Biol*, 2020).

www.iimcb.gov.pl/kuznicki-lab

Laboratory of Cell Biology

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Ewelina Szymańska, PhD
Daria Zdżalik-Bielecka, PhD

Researcher
Jarosław Cendrowski, PhD

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Kamil Jastrzębski, PhD (until November 2020)
Krzysztof Kolmus, PhD (until November 2020)
Lidia Wolińska-Nizioł, PhD

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Agata Poświata, MSc
Karolina Wojciechowska, MSc
Marta Wróbel (formerly Kaczmarek), MSc

Undergraduate Students
Purevsuren Erdenbat, BSc (until September 2020)
Karolina Romaniuk, Eng. (until September 2020)

Trainees
Kamila Kozik, MSc Eng. (until November 2020)
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Monika Matuszczyk (part-time)

Laboratory Support Specialist
Renata Wyszyńska, MSc

Lab Leader:

Marta Miączyńska, PhD, Professor

Curriculum Vitae

DEGREES

2013	Professor of Biological Sciences, nomination by the President of the Republic of Poland
2008	DSc Habil in Cell Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1997	PhD in Genetics, University of Vienna, Austria
1992	MSc in Molecular Biology, Jagiellonian University, Cracow, Poland
1991	BSc in Biological Sciences, University of Wolverhampton, UK

PROFESSIONAL EMPLOYMENT

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

RESEARCH TRAINING

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

HONORS, PRIZES AND AWARDS

2021	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
1900-1991	Studentship, European Community Tempus Scheme

DOCTORATES DEFENDED UNDER LAB LEADER'S SUPERVISION

M. Olchowik, A. Urbańska, A. Hupałowska, Ł. Sadowski, A. Mamińska, A. Toruń, K. Jastrzębski, M. Maksymowicz.

Selected Publications

IIMCB Best Papers Award

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:jcs.250951

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^ no IIMCB affiliation

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). Moreover, some endocytic proteins can affect cell proliferation or act as tumor suppressors, or their expression changes in human cancers (Pyrzyńska et al., *Mol Oncol*, 2009).

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 (e.g., lymphotoxin β receptor [LTβR]) can cause their accumulation on endosomal membranes and the ligand-independent activation of nuclear factor-κB (NF-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.

As a follow-up of this work, we further investigated the mechanisms of intracellular trafficking and inflammatory signaling by LTβR. We showed that various types of endolysosomal dysfunction lead to the accumulation of ligand-free LTβR on endosomes, but the exact topology of the receptor within these compartments determines whether NF-κB signaling is induced or prevented (Banach-Orłowska et al., *J Cell Sci*, 2018). We found that plasma membrane cholesterol content is important for proper LTβR internalization to prevent overstimulation of the NF-κB pathway and the overproduction of cytokines (Banach-Orłowska et al., *Cell Commun Signal*, 2019). Most recently, we reported that clathrin- and dynamin-dependent endocytosis limits canonical NF-κB signaling that is triggered by LTβR (Maksymowicz et al., *Cell Commun Signal*, 2020).

In our molecular oncology projects, we discovered synthetic lethality between two paralogous ATPases of the 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. We found that the *VPS4A* gene was a synthetic lethal partner of *VPS4B*. 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, our study revealed that 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. Most recently, we 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.

Finally, we revealed the cell type-specific regulation of membrane transport pathways during erythropoiesis, which could be exploited to modulate the rate of red blood cell production (Cendrowski et al., *eLife*, 2020; Cendrowski et al., *Autophagy*, 2020). Specifically, we identified cellular functions of a relatively poorly studied kinase, BMP2K, and its involvement in erythroid differentiation. 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. BMP2K-L promotes COPII assembly, autophagic degradation, and erythroid maturation, whereas BMP2K-S is an inhibitor of these processes. The maturation of erythroblasts is associated with a greater proportion between the abundance of BMP2K-L and BMP2K-S (i.e., high L/S ratio). Our findings uncover an unusual mechanism of two splicing variants of a kinase that play opposing roles in intracellular processes. We further propose that the BMP2K-L/S regulatory system fine tunes erythroid maturation (Fig. 1).

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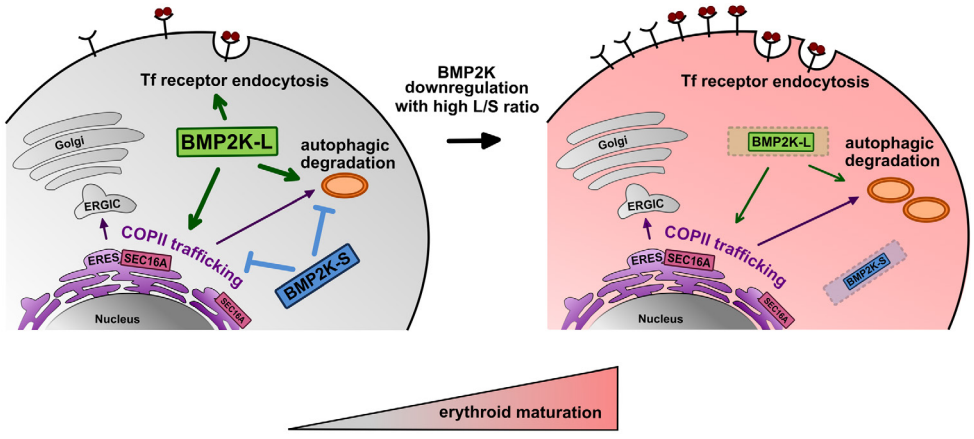
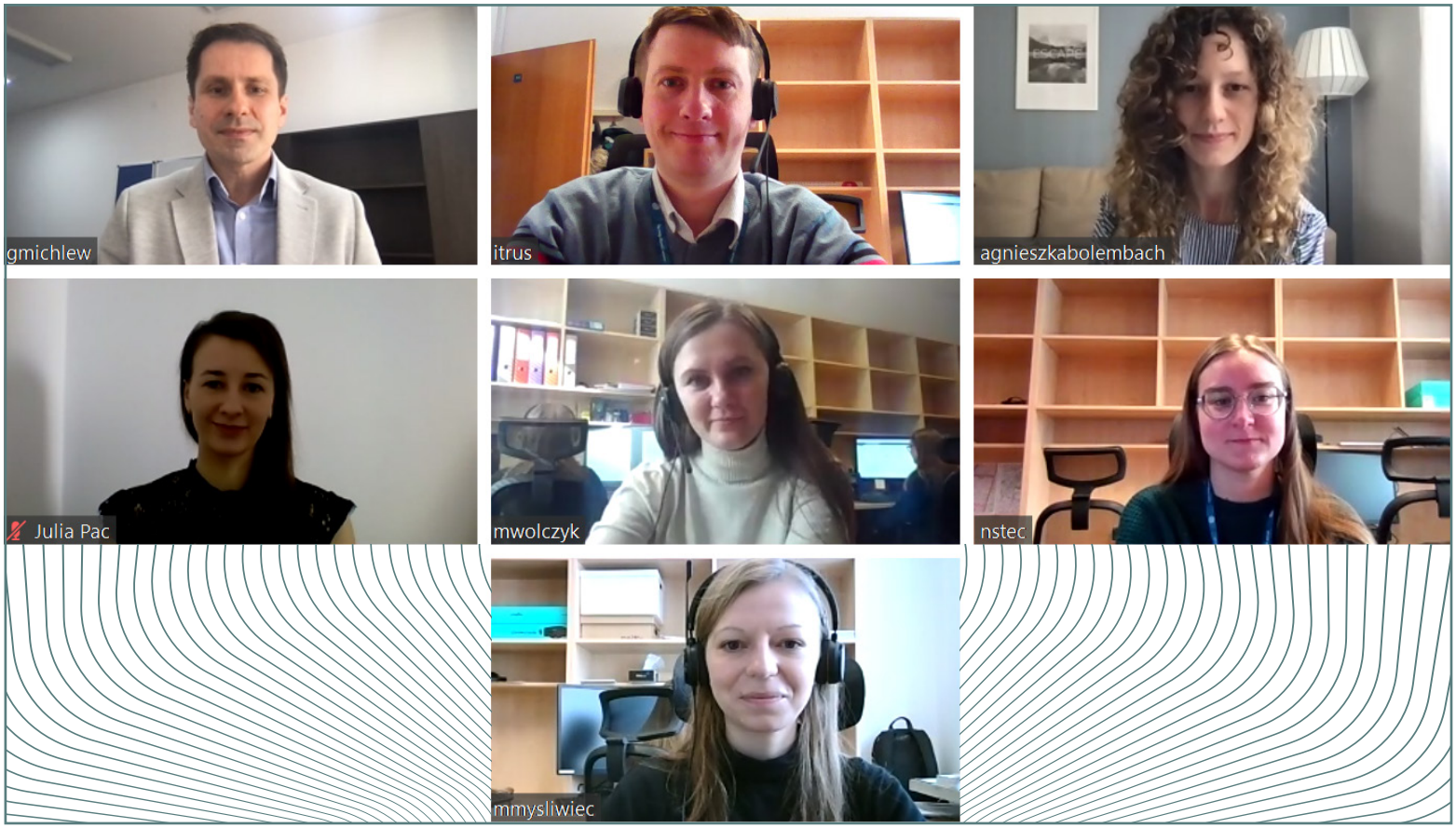


Fig.1 Model for involvement of the BMP2K-L/S system in the regulation of intracellular events that are important for erythroid maturation. BMP2K-L (the activator) and BMP2K-S (the inhibitor) are both highly abundant in immature erythroblasts (left). Hence, the stimulating functions of BMP2K-L in these cells with regard to COPII trafficking and autophagy are inhibited by BMP2K-S. Upon the downregulation of both BMP2K variants with a preserved high L/S ratio, the inhibitory effects of BMP2K-S decline, and the remaining BMP2K-L molecules stimulate COPII production at SEC16A-positive endoplasmic reticulum exit sites (ERES) and promote autophagy. These events facilitate erythroid maturation (right) that is associated with the higher biosynthesis of transferrin (Tf) receptors. ERGIC, endoplasmic reticulum-Golgi intermediate compartment. Authors: Marta Wróbel and Jarosław Cendrowski

Laboratory of RNA-Protein Interactions – Dioscuri Centre



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

Dioscuri Centre of Scientific Excellence. The Programme initiated by the Max Planck Society (MPG), managed jointly with the National Science Centre in Poland and mutually funded by the Polish Ministry of Education and Science (MEiN) and the German Federal Ministry of Education and Research (BMBF)

MAX PLANCK GESELLSCHAFT · NATIONAL SCIENCE CENTRE POLAND · Ministry of Education and Science · Federal Ministry of Education and Research

Lab Leader:

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Curriculum Vitae

DEGREES

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2001	MSc in Biotechnology, Adam Mickiewicz University, Poznań, Poland

PROFESSIONAL EXPERIENCE

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2020	Reader, Infection Medicine, University of Edinburgh, United Kingdom
2018-2020	Associate Professor, Zhejiang University-University of Edinburgh Institute, Haining, China
2018-2020	Senior Lecturer, Infection Medicine, University of Edinburgh, Edinburgh, United Kingdom
2011-2017	Medical Career Award Fellow, Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, United Kingdom
2005-2010	Postdoctoral Fellow, Human Genetics Unit, Medical Research Council, Edinburgh, United Kingdom

HONORS, PRIZES, AND AWARDS

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

DOCTORATES DEFENDED UNDER LAB LEADER'S SUPERVISION

J.S. Nowak, B. Özkan, G. Heikel, A. Downie Ruiz Velasco.

Selected Publications

Choudhury NR, Heikel G, **^Michlewski G**. TRIM25 and its emerging RNA-binding roles in antiviral defense. ***Wiley Interdiscip Rev RNA***, 2020; 11(4):e1588

Zhu S, Rooney S, **^Michlewski G**. RNA-Targeted Therapies and High-Throughput Screening Methods. ***Int J Mol Sci***, 2020; 21(8):2996

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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 the majority of 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 RNA-binding proteins in HeLa cells (Castello et al., *Cell*, 2012). Strikingly, approximately 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 RNA-binding proteins in various cells and tissues. Despite the extensive cataloging of novel RNA-binding proteins, we have a limited understanding of what dictates their molecular function and RNA-related roles in human health and disease.

We are currently focusing on two main topics:

1. Functional and structural characteristics of novel RBPs and RNA-protein interactions in the innate immune response to RNA viruses.
2. Regulation of microRNAs through RBPs for the treatment of Parkinson's disease.

HOST-VIRUS RNA-PROTEIN INTERACTIONS

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 [CC]), 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 [SINV], 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, TRIM25's role in the RIG-I pathway has been recently challenged by numerous reports. We discovered that TRIM25 is

an RNA-binding protein 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 have not yet been explored.

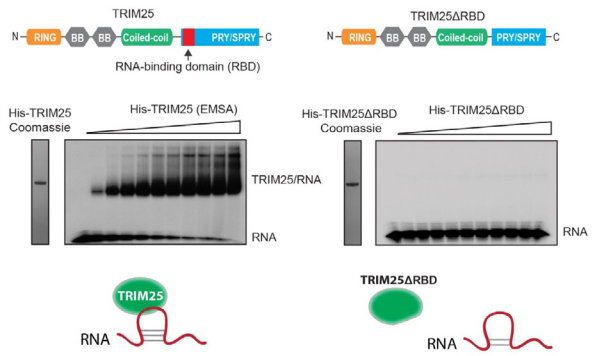

Fig.1 The E3 ubiquitin ligase TRIM25, a key factor in the innate immune response to RNA viruses is an RNA-binding protein. TRIM25 binds RNA through its PRY/SPRY domain (Choudhury et al., *BMC Biol*, 2017).

Within the same theme, we recently co-authored a study that identified global changes in RBP composition and affinity in response to SINV infection (Garcia-Moreno et al., *Mol Cell*, 2019). Over 200 RBPs showed differential association with RNA upon infection with SINV. 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 roles of novel RNA-protein interactions in the innate immune response to RNA virus infections. Concomitantly, we aim to solve the E3 ubiquitin ligase TRIM25/RNA complex structure, which will inform us about novel RNA-binding domain(s) 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. They 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 MICRORNA BIOGENESIS

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, which 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 an 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 a protein called α-synuclein (α-Syn) 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 with 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.

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., *bioRxiv*, 2021). Our strategy is based on using 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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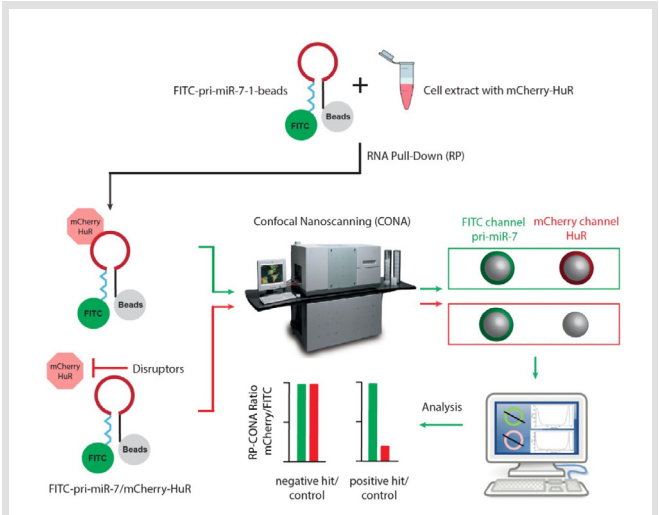
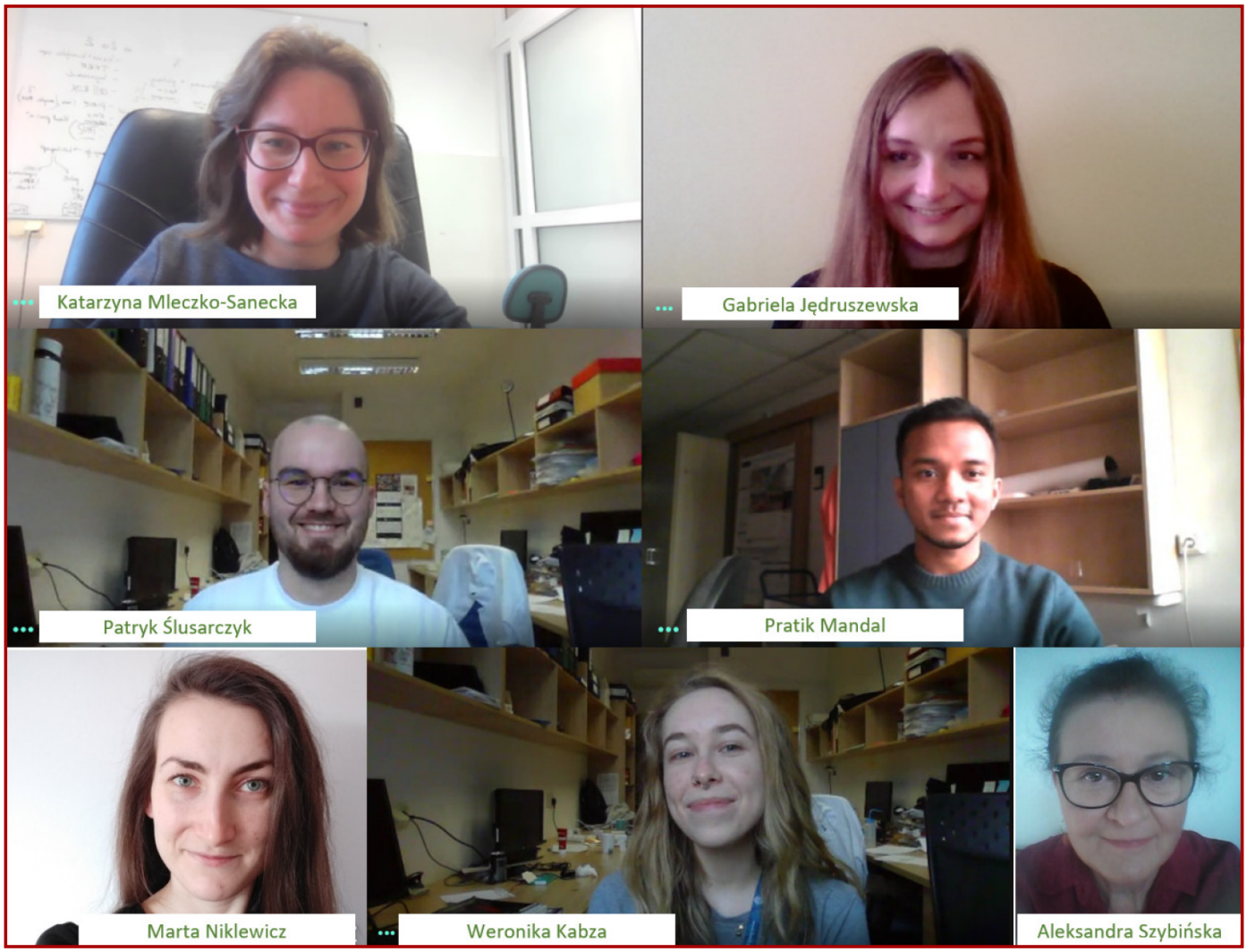


Fig.2 The working scheme of RP-CONA, a novel drug discovery platform for RNA-protein interactions (Zhu et al., *bioRxiv*, 2021).

Laboratory of Iron Homeostasis



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Pratik Kumar Mandal, MSc
Patryk Ślusarczyk, MSc

Undergraduate Student
Weronika Kabza

Technician
Marta Niklewicz, MSc (part-time)

Laboratory Support Specialist
Aleksandra Szybińska, MSc (part-time)

Lab Leader:

Katarzyna Mleczko-Sanecka, PhD

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PROFESSIONAL EXPERIENCE

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

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

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

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

HONORS, PRIZES AND AWARDS

2021 SONATA BIS, National Science Centre

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

2019 OPUS, National Science Centre

2016 POLONEZ, National Science Centre

2014 Independent research grant, University of Heidelberg

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

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

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

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

Selected Publications

Mleczko-Sanecka K, Silvestri L. Cell-type-specific insights into iron regulatory processes. ***Am J Hematol***. 2021; 96(1):110-127

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^ no IIMCB affiliation

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 emerges 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, yielding 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. It also remains unclear how different cell types in the liver contribute to Bmp6 regulation.

Body iron levels increase above the homeostatic level 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 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 (Fig. 1A). These data indicate that a factor that is secreted by iron-loaded hepatocytes rather than iron deposition in LSECs itself serves as a signal to enhance Bmp6 expression. Our initial work with two immortalized LSEC cell lines (termed LSEC-/-p19^{ARF}and TSECs) suggested that one candidate molecule that may perform this function was extracellular ferritin. This protein may shuffle between hepatocytic cells (Hepa1-6) and LSECs (TSECs; Fig. 1B). In our hands, ferritin stimulated Bmp6 in immortalized LSEC models (Fig. 1D). In primary murine cell cultures, we found that ferritin can be efficiently taken up by LSECs (Fig. 1C) and can mildly stimulate Bmp6 expression (Fig. 1E). In our ongoing and future work, we aim to utilize primary liver cell cultures and mice to determine the physiological function of ferritin-dependent intercellular communication between LSECs and hepatocytes.

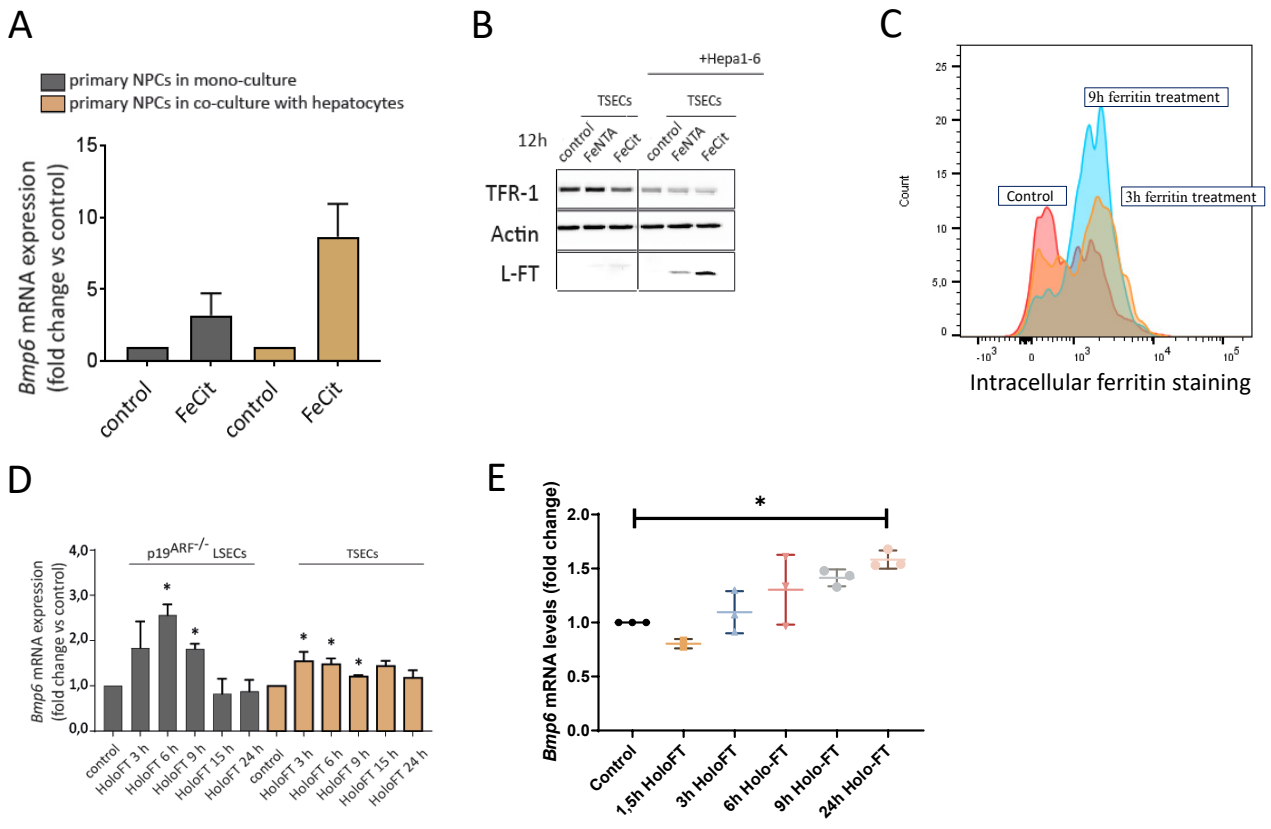

Fig.1 (A) Liver sinusoidal endothelial cells that were sorted from primary liver cell co-cultures responded in a more pronounced manner to iron supplementation than LSECs that were cultured without hepatocytes. (B) Upon iron treatments, ferritin levels are more elevated in immortalized LSECs (TSECs) after co-culture with hepatocytic cells (Hepa1-6). (C) Primary murine LSECs efficiently take up extracellular ferritin. (D, E) Supporting the hypothesis that ferritin is secreted from hepatocytes to stimulate LSEC Bmp6 expression, Bmp6 mRNA levels in LSEC models (immortalized cell lines in D and primary cultures in E) were responsive to ferritin (Holo-FT) treatment.

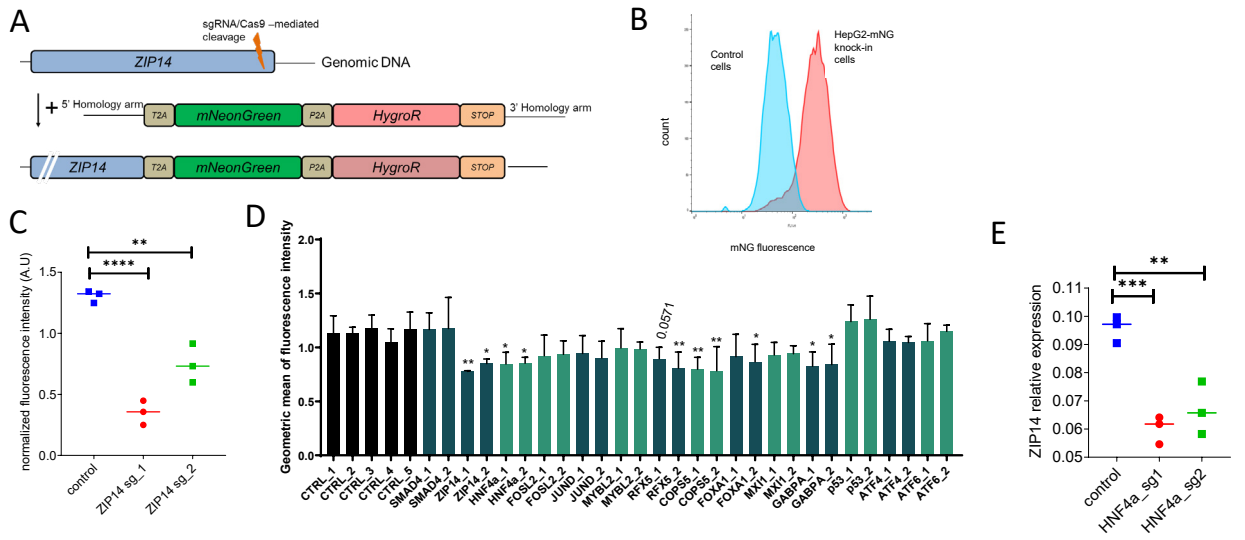

Fig.2 Generation, validation, and utilization of fluorescent ZIP14 reporter HepG2 cells. (A) Shown is the ZIP14 locus with CRISPR-mediated knock-in of the fluorescent mNeonGreen (mNG) gene together with the hygromycin resistance gene. (B, C) The fluorescence signal intensity from engineered cells (B) 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 HNF4α. (E) The control of ZIP14 expression by HNF4α was confirmed on mRNA levels, using CRISPR-mediated HNFα knockout.

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. 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. We aim to identify signaling mechanisms that control or alter the hepatic expression of ZIP14 (which is encoded by *SLC39A14*), a key metal transporter that is responsible for NTBI uptake in the liver. The identification of druggable ZIP14 regulators may contribute to new pharmacological interventions to limit liver iron loading and shed light on underdiagnosed iron-related side effects of some pharmaceutical compounds. Insights into regulatory mechanisms of ZIP14 may also help identify genes that modify the severity of iron overload and serve as diagnostic markers to identify patients who are at risk of developing overt clinical symptoms. Interestingly, the ablation of ZIP14 in zebrafish and mice and ZIP14 mutations in humans were recently reported to lead to hepatic manganese (Mn) deficiency and Mn accumulation in other organs, notably in the brain where Mn deposition causes neurotoxicity. Thus, comprehensive characterization of the ZIP14 regulatory network also has medical relevance to our understanding of Mn homeostasis.

To decipher the ZIP14 regulome, we aim to apply CRISPR-based genetic screens, followed by functional characterization of the most interesting hits in cellular assays and mice. We have already employed CRISPR-based gene-editing technology to generate reporter cells that are engineered to monitor endogenous levels of ZIP14 using a fluorescence-based readout (Fig. 2A, B). We validated this new-generation reporter system by showing that the CRISPR-based depletion of ZIP14 itself efficiently reduced the fluorescent signal (Fig. 2C). To expand our knowledge of the transcriptional control of ZIP14 and possibly identify additional genes that can serve as positive controls for our future screens, 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 using a small focused screen of our engineered fluorescent cell line. We identified four genes (*HNF4α, COPS5*, *RFX5*, and *GABPA*; Fig. 2D) as potential ZIP14 regulators. We focused our attention on *HNF4*α 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 *HNF4*α 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 red blood cells (RBCs). This task is accomplished by macrophages, predominantly red pulp macrophages (RPMs) in the spleen, cells that proficiently recognize and engulf aged RBCs, in the erythrophagocytosis process (Fig. 3). Using mice, we seek to understand how the functions of these specialized cells are affected by different physiological conditions in the body.

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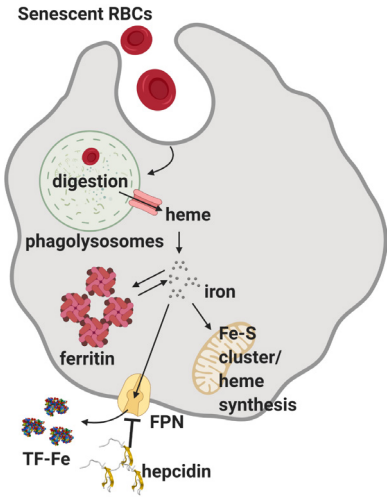
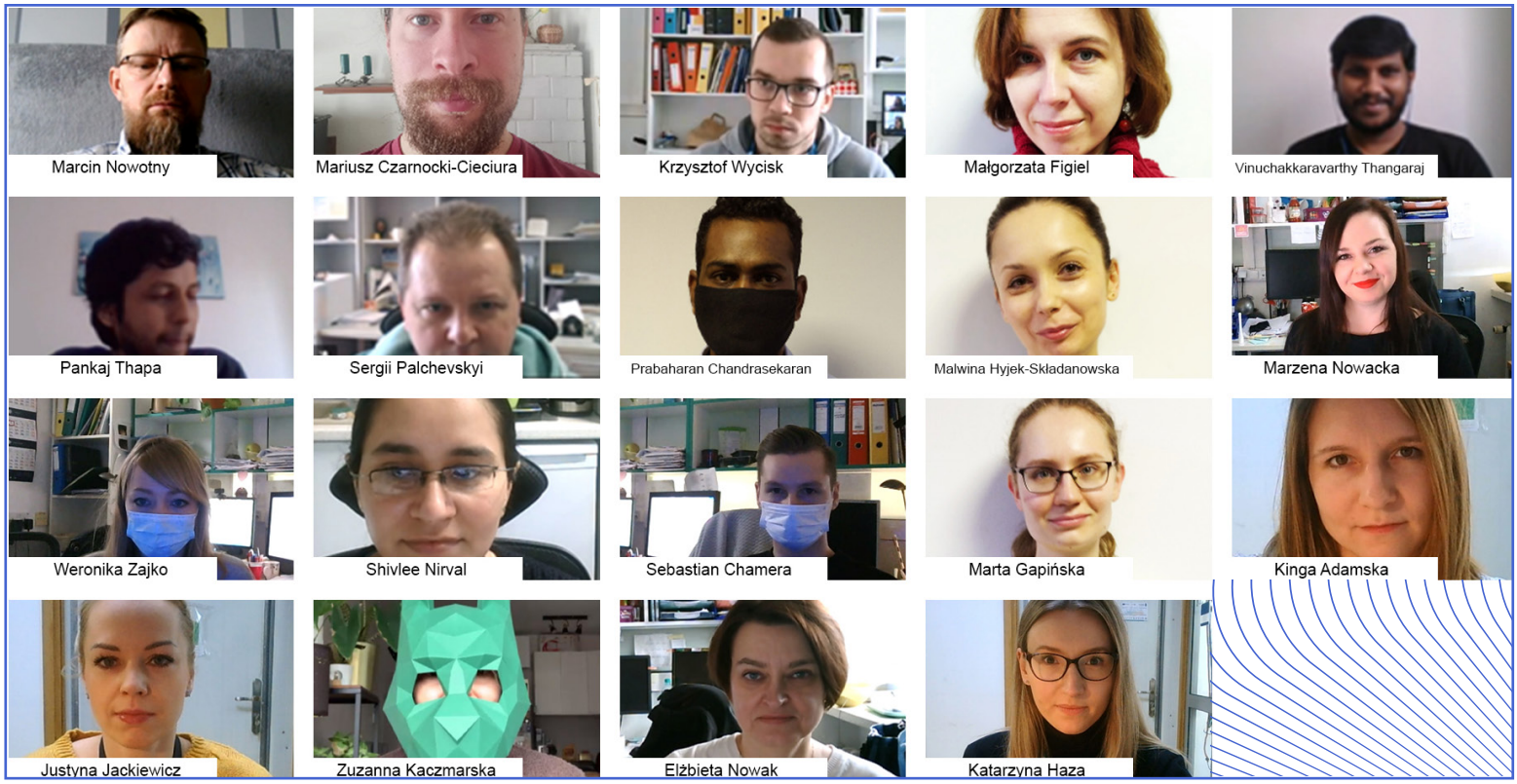


Fig.3 Depicted is the process of iron-recycling from senescent red blood cells (RBCs) by red pulp macrophages in the spleen. RBCs are first phagocytosed. Upon the formation of phagolysosomes, RBCs are digested, heme is transported to the cytoplasm, and catabolized to iron. Iron is either deposited in iron storing protein ferritin or exported from macrophages via ferroportin (FPN). Iron efflux via FPN feeds the pool of serum transferrin-bound iron (TF-Fe) and is tightly regulated by hepcidin. Using mice, we investigate how functions of these specialized cells may be affected by different physiological and environmental conditions.

Laboratory of Protein Structure



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Zuzanna Kaczmarska, PhD
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Sergii Palchevskyi, PhD (part-time)
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Marzena Nowacka, MSc

Technician
Iwona Ptasiewicz (part-time)

Laboratory Support Specialist
Kinga Adamska, MSc

Lab Leader:

Marcin Nowotny, PhD, Professor

Curriculum Vitae

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, 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

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.

Selected Publications

IIMCB Best Papers Award

Jaciuk M*, Swuec P*, **Gaur V***, Kasprzak JM, Renault L , Dobrychłop M, Nirwal S, Bujnicki JM§, Costa A§, **Nowotny M§**. A Combined Structural and Biochemical Approach Reveals Translocation and Stalling of UvrB on the DNA Lesion as a Mechanism of Damage Verification in Bacterial Nucleotide Excision Repair. ***DNA Repair (Amst.)***, 2020; 85:102746

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^**Nowotny M**, Gaidamakov SA, Ghirlando R, Cerritelli SM, Crouch RJ, Yang W. Structure of human RNase H1 complexed with an RNA/DNA hybrid: insight into HIV reverse transcription. ***Mol Cell,*** 2007; 28(2):264-76

^**Nowotny M**, Gaidamakov SA, Crouch RJ, Yang W. Crystal structures of RNase H bound to an RNA/DNA hybrid: substrate specificity and metal-dependent catalysis. ***Cell,*** 2005; 121(7):1005-16

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§ corresponding authors
^ no IIMCB affiliation

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 RNase H1-dependent degradation of cellular mRNAs. The number of approved ASO drugs for the treatment of both rare and common diseases is growing. Spinraza (Nusinersen) was the first drug that was approved for the treatment of previously untreatable spinal muscular atrophy (SMA). 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, which affects 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).

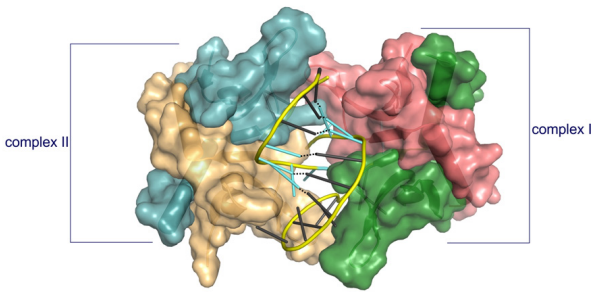

Fig.1 Structure of PC4 in complex with the ASO PS 2'-OMe DNA gapmer (PTEN ASO), showing the content of the asymmetric unit of the complex crystal. PC4 protomers are color-coded and shown in surface representation. DNA is shown in a cartoon representation. The PS backbone is shown in yellow. 2'-OMe PS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

MECHANISM OF DNA DAMAGE VERIFICATION STEP IN BACTERIAL NUCLEOTIDE EXCISION REPAIR

Nucleotide excision repair (NER) is a major pathway of DNA repair. Its main feature is its ability to recognize a wide spectrum of DNA lesions of various sizes and structures. In bacterial NER, the dimeric adenosine triphosphatase UvrA plays the role of a DNA damage sensor. Damage verification is performed by UvrB helicase, and UvrC double nuclease excises the damaged DNA fragment. We previously reported the first crystal structure of UvrA in complex with modified DNA (Jaciuk et al., *Nat Struct Mol Biol*, 2011). It showed that UvrA does not interact with the damage site directly but senses the deformed conformation of the DNA that is induced by the presence of the lesion, including bending, stretching, and unwinding. Building on this work in collaboration with Janusz Bujnicki, we prepared a computational structural model of the UvrA—UvrB—DNA complex that is involved in the DNA damage verification step (Fig. 2A). The model was corroborated experimentally by electron microscopy in collaboration with Alessandro Costa from The Crick Institute (Jaciuk, Swuec, Gaur, et al., *DNA Repair*, 2019). The model showed that UvrB uses a β-hairpin element to clamp one DNA strand, and each of the two UvrB molecules in the complex clamps a different DNA strand. Moreover, UvrB translocates in the 3' direction toward the DNA lesion where the UvrB molecule that clamps the damaged strand stalls and recruits UvrC nuclease (Fig. 2B). This mechanism explains how the initial imprecise localization of the damage by UvrA is converted to precise and strand-specific localization to promote accurate incisions by UvrC.

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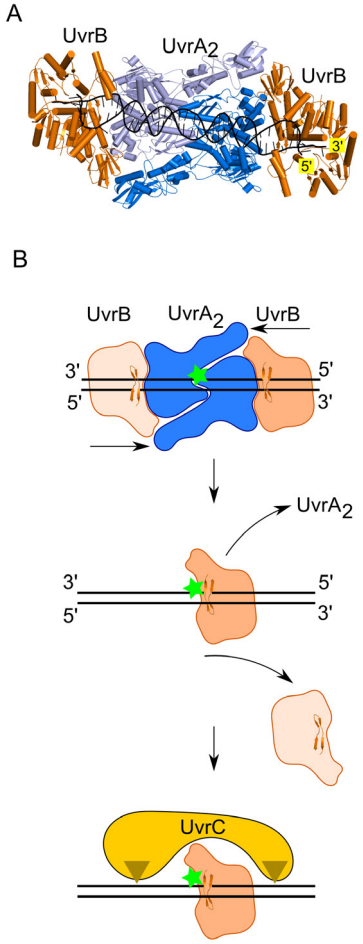
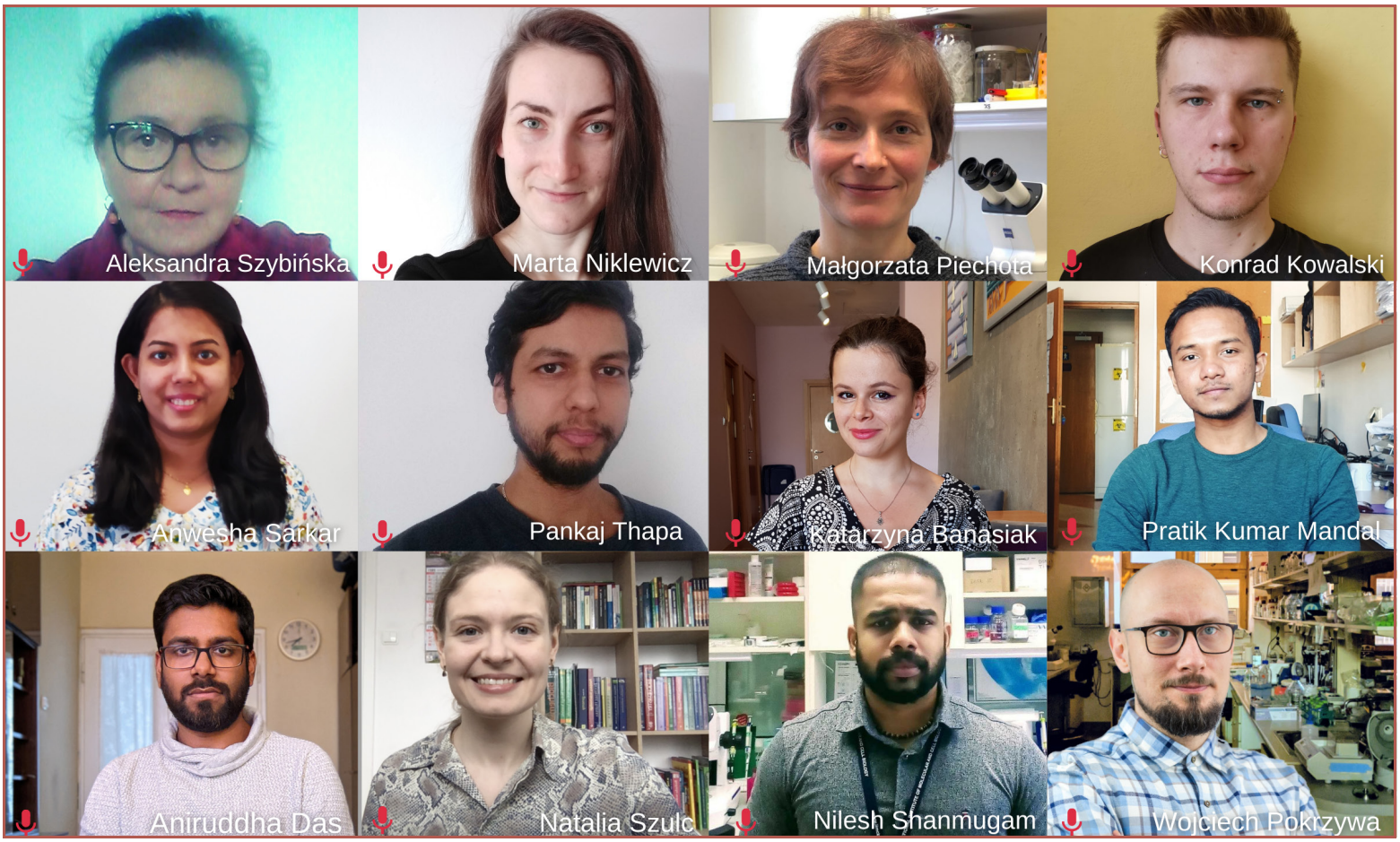


Fig.2 Model of damage verification in bacterial NER. (A) Model of $UvrA_2$—$UvrB_2$—DNA complex. UvrA dimer shown in two shades of blue. UvrB shown in orange. DNA shown in black. (B) Proposed mechanism. The UvrA dimer bound at the site of DNA modification recruits two UvrB molecules. Each UvrB molecule clamps a different DNA strand under the β-hairpin element (upper panel). Both UvrB molecules then translocate toward the lesion with 5' to 3' polarity on the strand under the hairpin. The UvrB molecule that clamps the modified strand will stall at the lesion (green star indicates the site of DNA modification) and the other UvrB molecule (light orange) will dissociate (middle panel). The stalled UvrB recruits UvrC double nuclease (shown in yellow), which makes two incisions indicated with triangles.

Laboratory of Protein Metabolism



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Curriculum Vitae

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PROFESSIONAL EMPLOYMENT

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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), National Science Centre
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2017 OPUS, National Science Centre
2005 PhD Fellowship, FNRS-Fund for Scientific Research, Belgium

Selected Publications

Turek M, **Banasiak K, Piechota M, Shanmugam N, Macias M**, Śliwińska MA, **Niklewicz M, Kowalski K,** Nowak N, Chacińska A, **Pokrzywa W.** Muscle-derived exophers promote reproductive fitness. ***EMBO Rep.*** in press, preprint doi:10.1101/2020.06.17.157230

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Description of Current Research

The proteome is defined as the entire set of proteins that are expressed in a given cell type or organism, which can vary with time and physiological status. Quality control networks support integrity of the cellular proteome. The human protein homeostasis network (proteostasis) involves >1000 accessory factors and regulatory components that govern protein synthesis, folding, and degradation. Defective folding can result in a greater abundance of toxic protein aggregates, which can endanger integrity of the entire proteome. With age, the ability of post-mitotic cells to maintain a stable proteome is gradually compromised, particularly by the downregulation of molecular chaperones and lower efficiency of protein degradation. To maintain the cellular proteome, molecular chaperones and ubiquitin-dependent degradation pathways coordinate protein refolding and remove terminally damaged proteins. Irreversibly affected proteins are recognized by chaperone-assisted E3 ubiquitin ligases that target them for degradation by the ubiquitin-proteasome system (UPS) or autophagy (Fig. 1). Our studies concentrate on achieving a basic understanding of the spatiotemporal regulation of protein quality control activity and its substrate processing. Our research uses a combination of biochemical, microscopic, and molecular genetic techniques and tissue-specific approaches in *Caenorhabditis elegans*.

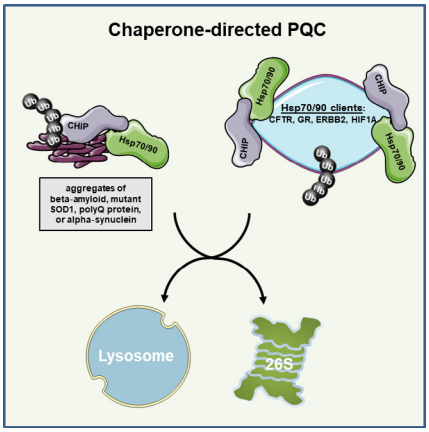

Fig.1 The illustration represents an important component of the protein homeostasis system, the quality control ubiquitin ligase CHIP. The primary function of CHIP is polyubiquitylation of chaperone-bound polypeptides facilitating the switch from chaperone-mediated folding/maturation to proteasomal degradation. In cooperation with chaperones, CHIP ameliorates proteotoxicity in various proteinopathies by marking aggregates of beta-amyloid, mutant SOD1, or alpha-synuclein for degradation. In addition to misfolded proteins, CHIP promotes degradation of a broad array of substrates when bound to chaperones, such as glucocorticoid receptor or hypoxia-inducible factor 1. Figure from Kevei, Pokrzywa, Hoppe. *FEBS Lett*, 2017.

WE FOCUS MAINLY ON THE FOLLOWING PROJECTS:

Identification of signals that coordinate the function of distinct E3 ligases

The fate of eukaryotic proteins, from their synthesis to destruction, is supervised by the UPS. The UPS is the primary pathway that is responsible for the selective proteolysis of intracellular proteins, guided by the covalent attachment of ubiquitin to target proteins by E1 (activating), E2 (conjugating), and E3 (ligating) enzymes in a ubiquitylation process. Despite many structurally unrelated substrates, ubiquitin conjugation is remarkably selective. E3 ubiquitin ligases represent the largest group of proteins within the UPS, linked to their crucial role in substrate selection. A detailed analysis of several classes of E3 ligases identified specific proteins and molecular pathways that they regulate.

Furthermore, the heterotypic oligomerization of E3 ligases might control the specificity and processivity of ubiquitylation. For example, Cullin-RING (CRL) ligase was shown to associate with a mechanistically distinct thioester-forming RBR-type E3, ARIH1, and rely on ARIH1 to directly add ubiquitin chains on CRL substrates. Thus, a combination of E3 ligases could support the formation of alternative ubiquitylation structures in different physiological processes. Our long-term objective is to understand the mechanistic and developmental aspects of protein degradation pathways that are defined by a specific pair of E3 enzymes.

Regulation of methionine metabolism by the ubiquitin proteasome system

S-adenosyl-L-methionine (SAM)-dependent methylation is central to regulating many biological processes, including gene expression, signaling, protein synthesis, and lipid metabolism. SAM is synthesized from L-methionine and adenosine triphosphate in a reaction that is catalyzed by methionine adenosyltransferase (AHCY). Despite the fundamental roles of the SAM cycle in a broad range of biological processes, the mechanisms of its regulation are still enigmatic. We found that AHCY is strictly regulated by UPS network components. We seek to understand the ways in which proteolytic systems modulate methionine and lipid metabolism and methylation potential of the cell using *C. elegans* as a model organism.

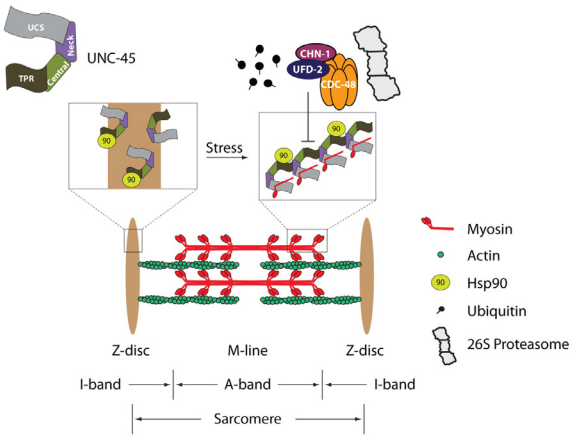

Fig.2 Model for the UNC-45 polymerization in response to stress. The sarcomeric unit is defined by the distance between two Z-discs including the A-band, I-band, and M-line. UNC-45 composes tandem modules that allow the simultaneous binding of Hsp70/Hsp90 and myosin, enabling the folding and assembly of myosin in regular spacing. In the fully developed muscle, monomeric UNC-45 might be stored at the Z-disk, which anchors the thin actin filaments of the I-band. Under stress conditions, UNC-45 is relocated to damaged myosin filaments of the A-band and might assemble into short chaperone chains to maintain the sarcomeric structure especially during muscle regeneration and aging. The conserved CDC-48/UFD-2/CHN-1 ubiquitylation complex might influence the process of UNC-45 chain formation. The ubiquitylation of UNC-45 either reduces the pool of the monomeric form available for chain formation or inhibits UNC-45 polymerization directly by modifying the binding interface. Figure adapted from Pokrzywa and Hoppe, *Worm*, 2013.

Stress-induced myosin folding and assembly mechanisms

The assembly and maintenance of myofilaments require a tightly balanced proteostasis network. One key player in myosin organization and muscle thick-filament formation in health and disease is the Hsp90 co-chaperone UNC-45. The activity and assembly of various myosin subtypes are coordinated by conserved UCS (UNC-45/CRO1/She4p) domain proteins. One founding member of the UCS family is *C. elegans* UNC-45, a protein that is essential for the organization of striated muscle filaments. Moreover, UNC-45 homologs exist in vertebrates, indicating the conserved requirement for myosin-specific co-chaperones. Indeed, abnormal UNC-45 function is associated with severe muscle defects that result in skeletal and cardiac myopathies.

The integrity of sarcomeric structures is permanently challenged upon muscle growth and mechanical stress. In response to eccentric exercise or damage to myofibers, UNC-45 and the chaperone Hsp90 shuttle between impaired myofibers to support their repair (Fig. 2). However, little is known about the coordination of protein homeostasis pathways upon mechanical stress. Therefore, this project's long-term objective is to understand the ways in which the balance between protein folding and degradation networks is coordinated with myosin assembly and muscle integrity. We combine genetic and biochemical approaches to study the conserved function of UNC-45 in myosin assembly and examine the ways in which this function is modulated during mechanical stress. Specifically, we plan to use targeted screening strategies to uncover mechanosensory proteins, chaperones, and UPS and autophagy components that are required for muscle function. The conserved regulation of proteostasis networks is studied in *C. elegans*, C2C12 mouse myoblasts, and human skeletal muscles. Finally, we are investigating the remodeling of UNC-45 folding machinery under conditions of mechanical stress.

Cellular adaptation to cold

Environmental stressors can seriously jeopardize animals' ability to survive and reproduce. One potentially dangerous environmental stressor is acute cold. To counteract cold, affected organisms engage various types of responses, ranging from cold avoidance to adaptation. The latter strategy is used by hibernating animals, which can survive subzero temperatures for many days in extreme cases. We utilize a simple animal model, the nematode *C. elegans*, as a rapid tool to understand cellular adaptations to cold. We focus on mechanisms that alter the abundance and types of cellular messenger RNAs and proteins because these kinds of molecules are critical for deciding cellular fate (Fig. 3A, B). In some disease states, such as stroke, cooling can facilitate a patient's recovery. Moreover, hibernation is of interest to ageing research because animals tend to live longer at lower temperatures. Thus, understanding the ways in which cells adapt to cold has the potential to influence treatments of human disorders.

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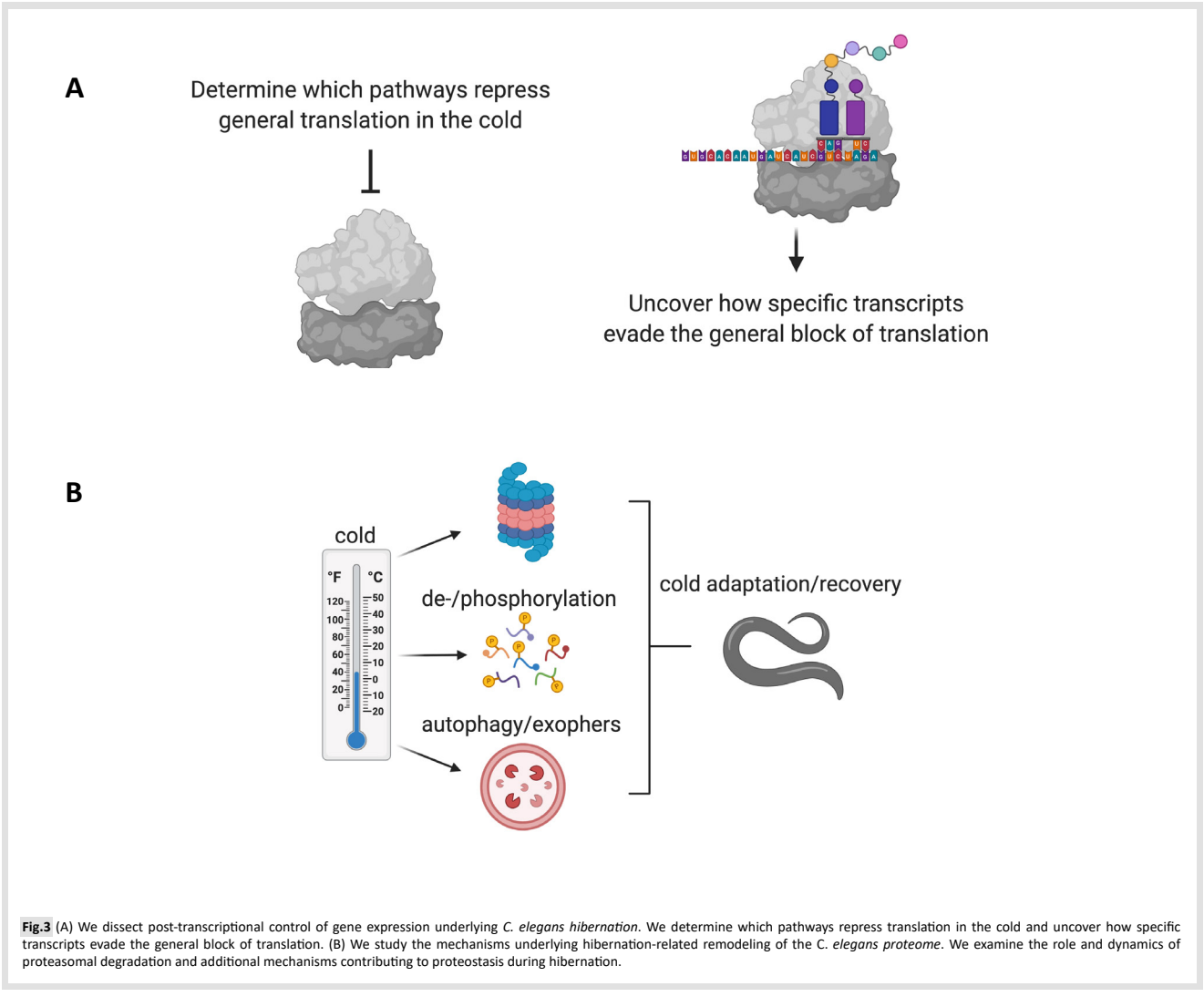

Fig.3 (A) We dissect post-transcriptional control of gene expression underlying *C. elegans hibernation*. We determine which pathways repress translation in the cold and uncover how specific transcripts evade the general block of translation. (B) We study the mechanisms underlying hibernation-related remodeling of the C. *elegans proteome*. We examine the role and dynamics of proteasomal degradation and additional mechanisms contributing to proteostasis during hibernation.

Laboratory of Zebrafish Developmental Genomics

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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

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Selected Publications

IIMCB Best Papers Award

Winata CL, Łapiński **M**, Ismail H, Mathavan S, Sampath P. Exploring Translational Control of Maternal mRNAs in Zebrafish. ***Methods Mol Biol***, 2021; 2218:367-80

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

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Yin A, **^Winata CL**, Korzh S, Korzh V, Gong Z. Expression of components of Wnt and Hedgehog pathways in different tissue layers during lung development in *Xenopus laevis*. ***Gene Expr Patterns***, 2010; 10(7-8):338-44

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* equal contribution ^ no IIMCB affiliation

Description of Current Research

Intricate embryonic patterning is achieved through highly precise regulatory mechanisms that ensure controlled expression of genes 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 seek to understand the mechanism by which transcription factors (TFs) and the epigenetic landscape interact to regulate the development of complex embryonic patterns and structures and how the disruption of this regulation results in congenital malformations in human diseases. We pose our questions within the context of heart development because of the importance of this organ for life. Key genetic factors that regulate the development and function of the heart have been established. However, still unknown are precisely how these factors are regulated and how they interact with each other and with epigenetic factors to orchestrate different phases of heart development.

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. Transcriptional regulatory landscape in developing cardiomyocytes.

Heart muscle cells or CMs are specified early during embryogenesis from a pool of mesodermal progenitors. To elucidate the dynamics of the transcriptional regulatory landscape during heart development, we employed a combination of transcriptome profiling (RNA-seq) and an assay for chromatin accessibility (ATAC-seq) at several key stages of heart development. Our study 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 identify these regulatory elements at higher resolution, we are establishing the Cap Analysis of Gene Expression (CAGE)-seq method (Kodzius et al., *Nat Methods*, 2006). In addition to the identification of potential noncoding determinants of congenital heart defects, the results of this project will also provide valuable insights into the principles of dynamic gene regulation in a developing organism.

At the core of the machinery that regulates each step of heart morphogenesis are cardiac TFs, including Nkx2.5, Gata5, Tbx5, and Hand2. These TFs play a role in establishing the CM identity of mesodermal progenitor cells, regulating the formation and looping of the heart tube and specification of atrial and ventricular CMs. To assemble the molecular interaction network between factors that are involved in heart development and disease, we applied various computational and mathematical modeling strategies, including *in silico* TF footprinting analysis of ATAC-seq data and Boolean 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. This provides a system with which we can test a large number of possible scenarios and ultimately build a biologically relevant genetic regulatory network based on our genomics experiments. Such an approach will allow us to pinpoint crucial links or correlations in the system for targeted validation, thus saving time and resources. 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 heart defects.

II. Genomics 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 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 the 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. In collaboration with Vladimir Korzh (IIMCB), we utilized the transgenic lines ET33mi59B, ET33mi28, and ET31 that express green fluorescent protein (GFP) in subpopulations of pacemaker cells to characterize morphology of the zebrafish pacemaker and isolate pacemaker cells for further genomic analyses to elucidate gene regulatory networks in pacemaker development (Figs. 1 and 2; Abu Nahia et al., in preparation). 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*. 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. 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 the meaningful interpretation of 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 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 fit as a possible candidate for 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 aims to elucidate the biological function of A-to-I RNA editing in early embryonic development.

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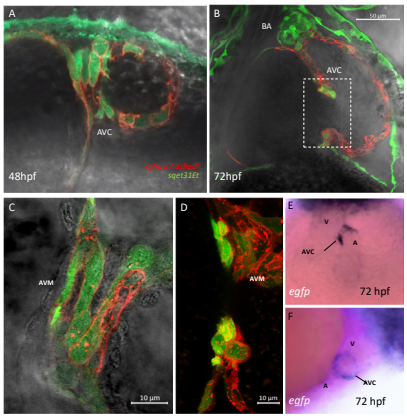

Fig.1 (A, B) Confocal microscope image of the atrioventricular canal (AVC) pacemaker region that expresses GFP in the transgenic line sqet31Et at 48 and 72 hours post-fertilization (hpf). Red fluorescent protein signals indicate cardiomyocytes. (C, D) Magnification of the region marked in panel B at different focal planes, showing the surface (C) and lumen (D) of the AVC. (E, F) Whole-mount *in situ* hybridization of *egfp* in *sqet31Et* transgenic embryos at 72 hpf showing the enrichment of enhanced GFP expression in the AVC relative to the rest of the heart. E - ventral view. D - lateral view. AVM, atrioventricular myocardium.

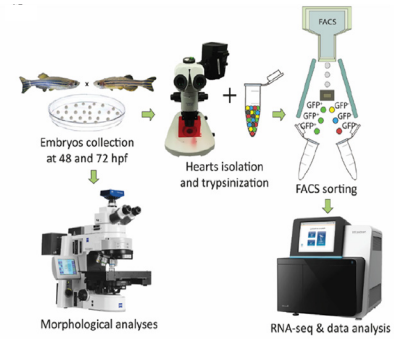

Fig.2 Scheme of experimental design for transcriptome profiling of GFP-positive cells isolated from *sqet31Et*.

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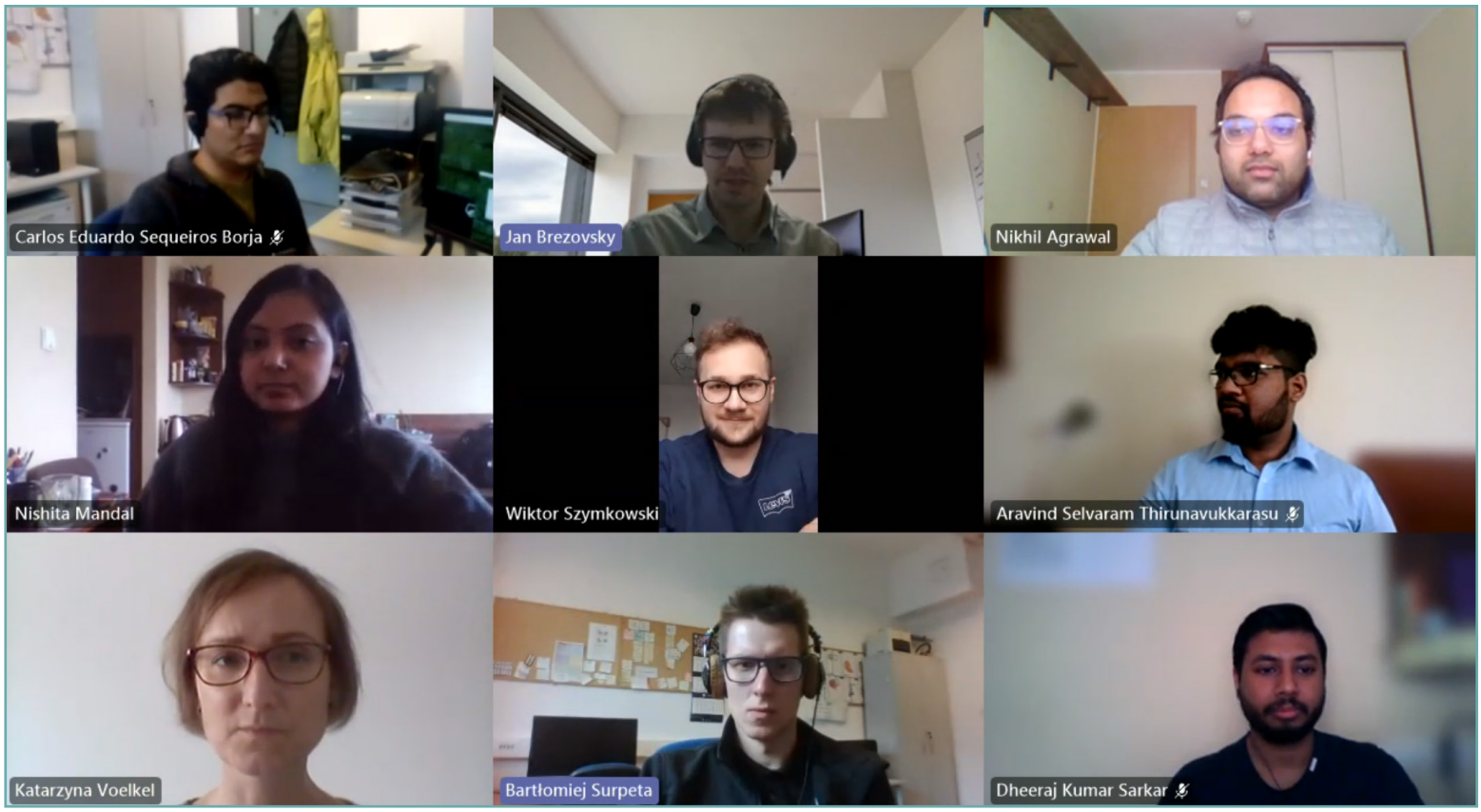

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Aravind Selvaram Thirunavukkarasu, MSc

Undergraduate Student
Wiktor Szymkowski, BSc (AMU)

Research Specialist & Laboratory Support Specialist
Katarzyna Voelkel, MSc

Lab Leader:

Jan Brezovsky, PhD

Curriculum Vitae

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

HONORS, PRIZES AND AWARDS

2020 Member of the FWO Review College

2018 SONATA BIS, National Science Centre

2017 OPUS, National Science Centre

2016 GACR grant, Czech Science Foundation

2015-2016 Elected member of the national node committee of European Life-Science Infrastructure for Biological Information, Czech Republic (ELIXIR-CZ)

2011 Dean's prize for outstanding PhD research, Masaryk University, Brno, Czech Republic

2007 Research grant from Masaryk University, Brno, Czech Republic

Selected Publications

Sequeiros-Borja CE, Surpeta B, Brezovsky J#. Recent advances in user-friendly computational tools to engineer protein function. ***Brief Bioinform***, 2020; doi.org/10.1093/bib/bbaa150. Online ahead of print

Surpeta B, Sequeiros-Borja CE, Brezovsky J#. Dynamics, a Powerful Component of Current and Future in Silico Approaches for Protein Design and Engineering. ***Int J Mol Sci***, 2020; 1(8):2713

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Sykora J*, **^Brezovsky J***, Koudelakova T*, Lahoda M, Fortova A, Chernovets T, Chaloupkova R, Stepankova V, Prokop Z, Smatanova IK, Hof M, Damborsky J. Dynamics and hydration explain failed functional transformation in dehalogenase design. ***Nat Chem Biol,*** 2014; 10(6):428-30

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

* equal contribution
# corresponding author
^ no IIMCB affiliation

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. 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 dynamical 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 notably improved or compromised catalysis. Together, these critical limitations result in very few types of selectivity filters being probed by mutagenesis and prohibit 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 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 direction consists of the development of various accelerated or adaptive sampling strategies that are tailored to investigations of tunnels and their utilization by small molecules. With the application of these methods, we will begin identifying a wide range of biologically relevant tunnels 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 the developed methods to expand 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* that is capable of disrupting the bacterial quorum-sensing process and hence represents a potential antimicrobial agent. Here, we are focusing on plasticity of the active site and binding tunnel, the formation of reactive complexes, and acylation reactions to pinpoint which of these dynamic factors are essential for its function (Fig. 1). Additionally, we are investigating molecular principles of the selective transport of different phenylpropanoids across cell membranes that utilize ABCG transporter proteins, which are responsible for the distribution of secondary metabolites, hormone transport, cell detoxification, and the dedicated response to biotic stresses 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. Additionally, water molecules are 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 (Fig. 2) that can be exploited by water molecules to traverse the protein matrix to reach their buried active sites and the ways in which these tunnels and their utilization change as enzymes progress through different stages of reactions.

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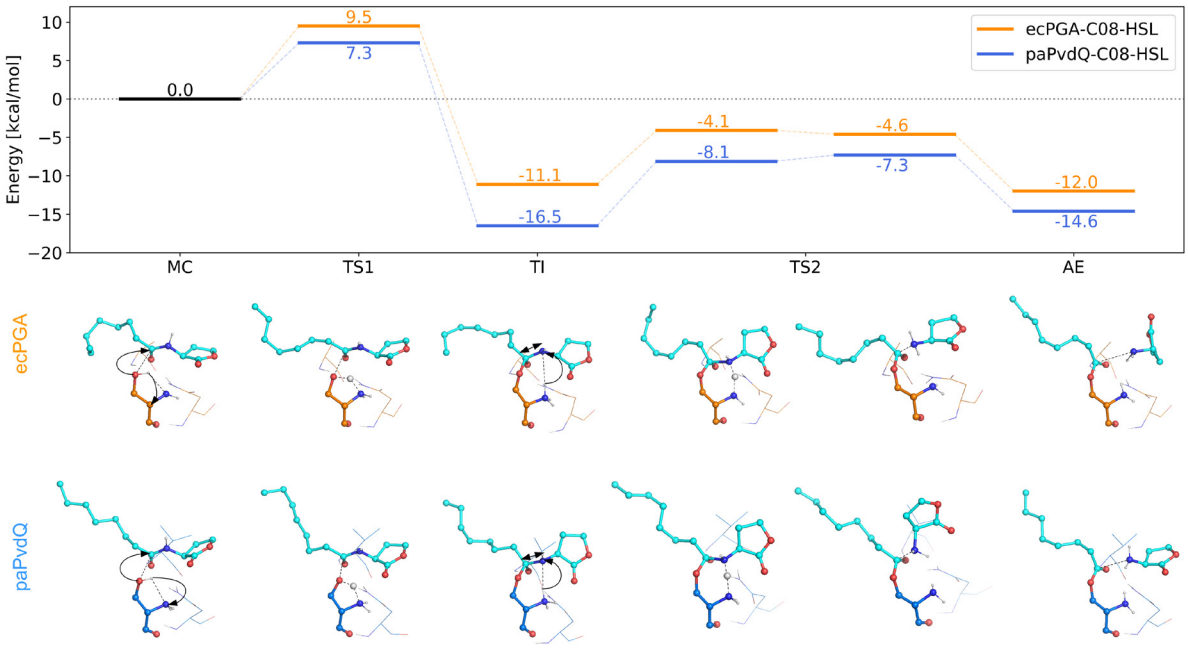

Fig.1 The reaction mechanism of acyl-homoserine lactone acylation catalyzed by ecPGA (orange) and paPvdQ (blue) quorum quenching enzymes. Five geometrically and energetically distinguishable states MC, TS1, TI, TS2 and AE can be recognized during this reaction stage.

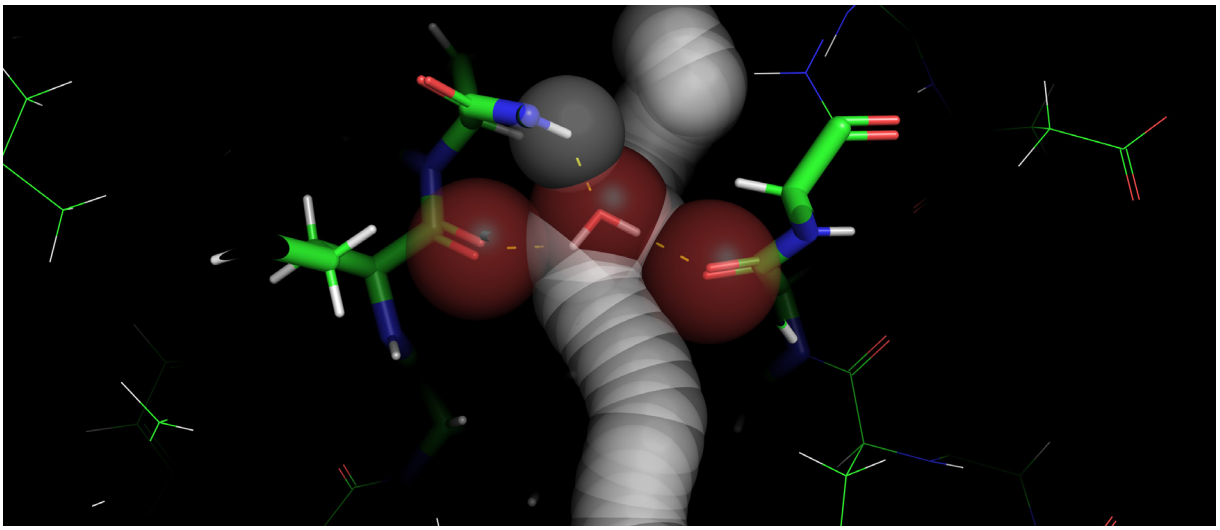

Fig.2 A rare event of water molecule squeezing through a very narrow molecular tunnel towards the active site of epoxide hydrolase enzyme. The water molecule is shown both in sticks as well as transparent spheres, while the body of the tunnel is represented with white spheres. The water molecule is stabilized by three hydrogen bonds (yellow dashed lines) that offset the steric repulsion from the overlapping atoms from surrounding residues (red and white transparent spheres).

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 the IIMCB by a pilot study of Polish centenarians (PolStu99). Data that were gathered in the PolStu99 project formed the basis for further research that was commissioned by the Committee for Scientific Research - "Genetic and Environmental Factors of Longevity of Polish Centenarians" (PolStu2001).

The PolSenior project, conducted in 2007-2012 and coordinated by the 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 was created, as well as a database that includes all information from questionnaires and biochemical and genetic analyses. 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.

POLSENIOR 2

The PolSenior2 project was conducted in 2016-2020 and coordinated by the Medical University of Gdańsk. Its methodology was based on the previous PolSenior study. The aims, methods, scope, and study flow of the PolSenior2 project were described in Wierucki et al., *Arch Med Sci*, 2020.

Together, the two projects characterizing older Poles will enable observations of epidemiological trends over the last 10 years. Researchers who have been involved in implementing the PolSenior project participate in analyzing the data, preparing reports that contain recommendations for health and social policymakers, and presenting and publishing the results of the PolSenior2 project. In 2020, the IIMCB team was involved in elaborating many social and medical topics for preparation of the project's final report that will be presented to the Ministry of Health (the project financing institution) and published in mid-2021.

The PolSenior Study Group continued its activities as a member of the NCD Risk Factor Collaboration (NCD-RisC), a network of health scientists around the world that provides rigorous and timely data on risk factors for non-communicable diseases (NCDs) for 200 countries and territories. The group works closely with the World Health Organisation (WHO). The results of the pooled data analysis were collected, reanalyzed, and checked by members of the Country and Regional Data Groups. In 2020, the PolSenior project data were included in the pooled analysis of repositioning the global epicenter of non-optimal cholesterol (NCD-RisC group, *Nature*, 2020), national trends in total, HDL, and non-HDL cholesterol in Asian and Western countries (NCD-RisC group, *Int J Epidemiol,* 2020), and height and body mass index trajectories (NCD-RisC group, *Lancet,* 2020).

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Auresine

Group Members

Project Coordinator
Izabela Sabała, PhD, DSc Habil

Senior Researcher
Elżbieta Jagielska, PhD

Postdoctoral Researcher
Piotr Małecki, PhD (until November 2020)

PhD Students
Paweł Mitkowski, MSc
Alicja Wysocka, MSc

Undergraduate Students
Paulina Brodacka (until September 2020)
Łukasz Łężniak (until September 2020)
Gabriela Ryk (October-December 2020)

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 2020

We were finalizing research tasks of the TEAM-TECH project "INFECTLESS New generation of antibacterial wound dressing", which focuses on the development of new-generation wound dressings that are functionalized with bacteriolytic enzymes.

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 and has now entered an international Patent Cooperation Treaty procedure at the European Patent Office (patent pending, PCT/PL2020/050075).

We also performed 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 Trinity College Dublin (Ireland), the Applied Molecular Biosciences Unit of the Department of Life Sciences FCT-NOVA (Portugal), the Fraunhofer Institute for Silicate Research (Germany), the Tuebingen University (Germany), and the Institute for Structural Biology (Grenoble, France).

Our results were presented at international meetings and trades: Joint Polish-German Crystallographic Meeting 2020, 4th AMR Conference: Novel Antimicrobials and AMR Diagnostics, Bio International Convention.

Our team was awarded two international grants within the POLNOR 2019 program that is coordinated by National Centre for Research and Development (NCBR). These 3-year projects will be conducted by international consortia that comprise academic and industrial partners from Poland and Norway. These projects will be carried out in the Laboratory of Protein Engineering headed by Dr. Izabela Sabała at the Mossakowski Medical Research Institute Polish Academy of Sciences where our team will move in 2021.

Our Master student, Paulina Brodacka, and Bachelor student, Gabriela Ryk, have successfully completed experimental work and defended their theses at the Warsaw University of Technology. A thesis of Paulina Brodacka was focused on "Development of new bacteriolytic enzymes for medical applications", while Gabriel Ryk was working on "Modification of enzybiotics for intracellular bacteria eradication improvement".

Publication:

Gonzalez-Delgado LS, Walters-Morgan H, Salamaga B, Robertson AJ, Hounslow AM, **Jagielska E**, **Sabała I**, Williamson MP, Lovering AL, Mesnage S. Two-site recognition of Staphylococcus aureus peptidoglycan by lysostaphin SH3b. ***Nat Chem Biol***. 2020; 16(1):24-30

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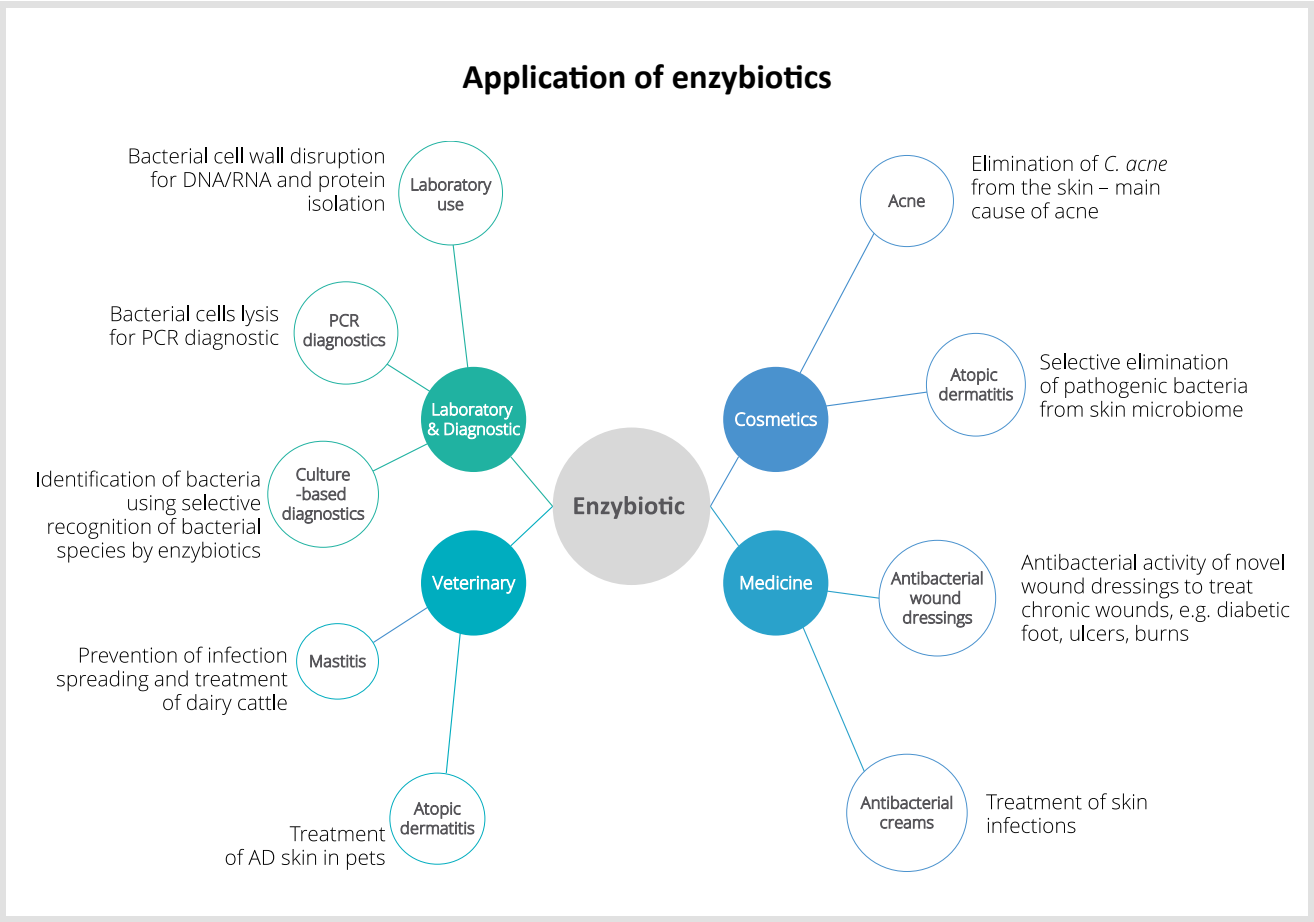
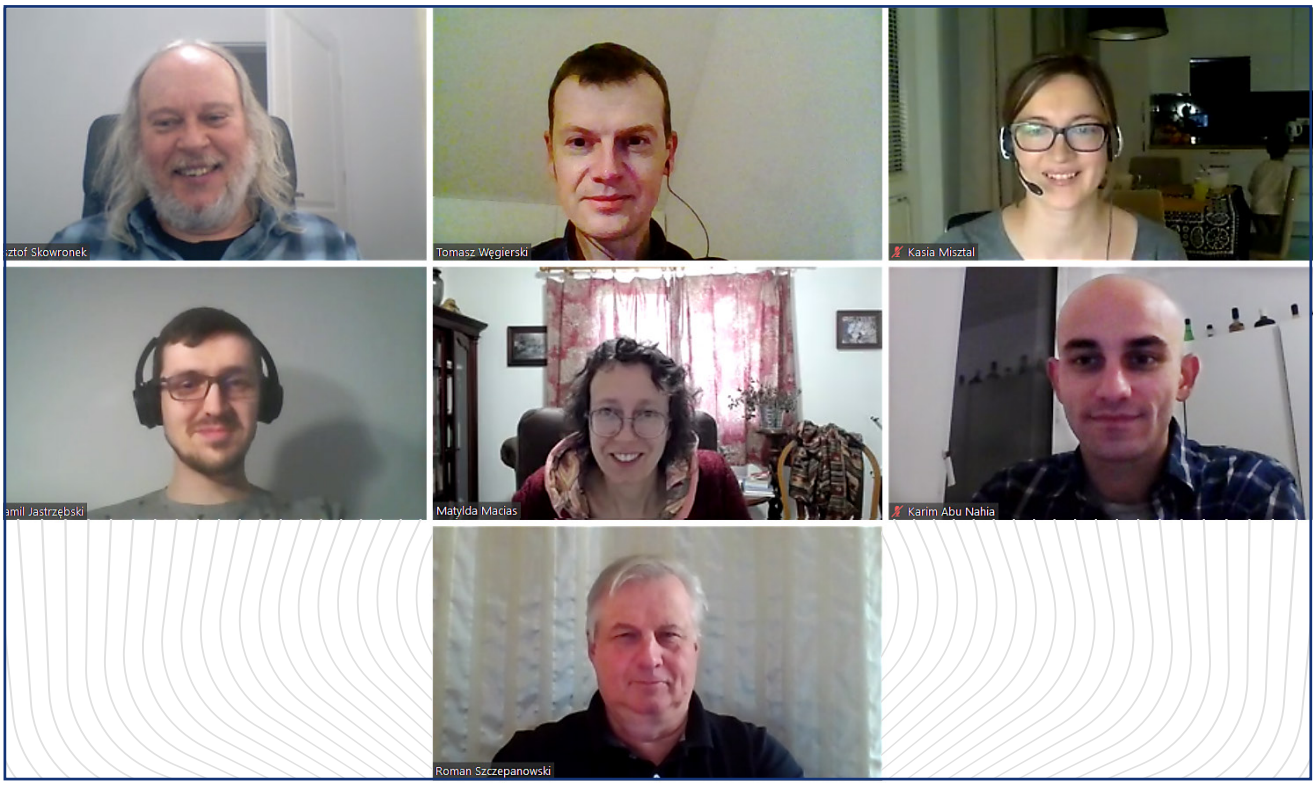



CORE
FACILITIES

Core Facility



Group Members

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Deputy Head
Roman Szczepanowski, PhD

Staff Scientists
Karim Abu Nahia, MSc (contract work since December 2020)
Kamil Jastrzębski, PhD (since December 2020)
Matylda Macias, PhD (part-time)
Katarzyna Misztal, PhD
Tomasz Węgierski, PhD (part-time)

The IIMCB Core Facility was established as a shared research resource that provides access to a broad range of cutting-edge research technology platforms that are extensively used in such diverse areas as structural biology, bioinformatics, and molecular and cell biology. The Core Facility is managed by experienced scientists who devote their time and effort to maintain and operate the most sophisticated equipment. More than 50 pieces of equipment are grouped into several units according to leading technologies and applications.

The Structural Biology Unit 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 collection of a complete set of diffraction data within a few hours. The Structural Biology Unit also shares an FEI Tecnai T12 transmission electron microscope (TEM) with the Bioimaging Unit 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, which often cannot be crystallized. The T12 TEM can be used to investigate polymers, thin films, fibers, ceramics, powders, and single molecules. The TEM is supplemented with a Quorum Q150T ES, which is necessary for sample preparation (e.g., the hydrophilization [wetting] of films and grids) for TEM. The Q150T ES also allows 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 Unit 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-DSC and VP-ITC), surface plasmon resonance (SPR), 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 Unit is also equipped with a wide selection of spectrometers, including spectrophotometers, spectrofluorometers, a circular dichroism spectropolarimeter, and a Fourier transform infrared spectrometer. The list of instruments has recently been broadened by a new Biacore S200 SPR system, which replaced the Biacore 3000. 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 Unit 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 analysis (i.e., protein identification and protein integrity assays) for internal users, which are vital for crystallography projects, the Mass Spectrometry Unit provides non-standard analyses of macromolecules other than proteins, particularly analyses of RNA samples and nucleosides.

The Bioimaging and High-Throughput Screening Unit offers fluorescence-based imaging systems that are suited for cell biology applications. Our microscopes either work in wide-field mode or use one of several optical sectioning techniques: confocal, two-photon, lightsheet, and total internal reflection fluorescence (TIRF). The newest acquisition is Opera Phenix, a high-content screening system from Perkin-Elmer for the large-scale imaging of cells in wide-field or confocal mode (e.g., in RNAi-based microscopy screens). Our equipment 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 (SPIM) for the imaging of fluorescently labeled zebrafish larvae, an Olympus CellR/ScanR imaging station for intracellular calcium measurements and the semi-high-throughput quantitative analysis of fluorescence signals, 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 and Harmony. The Bioimaging and High-Throughput Screening Unit has two cytometry systems: BD FACSAria II for cell sorting and BD FACSCalibur for the quantitative analysis of suspension cells. The FEI Tecnai T12 transmission electron microscope is shared by the Bioimaging Unit (for the conventional imaging of cells and tissue samples) and Structural Biology Unit (for the Cryo-EM of protein complexes). For the conventional TEM of cells and tissue samples, the Core Facility offers a Leica EM tissue processor. This is a tool that was designed for electron microscopy and light microscopy resin processing under constant temperature while avoiding exposure to toxic substances. After saturation with resin, tissue and cell samples are cut on our Ultramicrotome Leica EM UC7, which enables the easy preparation of semi- and ultrathin sections and perfect, smooth surfaces of biological and industrial samples for TEM, scanning electron microscopy, atomic force microscopy, and light microscopy examination.

The Genomics Unit 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 Unit 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 Core Facility provides flexible assistance with methodological principles, experimental design, initial training, 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, Core Facility collaborated with biotech companies, including Adamed, Celon Pharma, Polfa, OncoArendi Therapeutics, and Captor Therapeutics. The Core Facility is also available to scientists from other institutions. We have conducted research in collaboration with scientists from the University of Gdańsk, the University of Warsaw, the Medical University of Lublin, the Nencki Institute of Experimental Biology PAS, the Institute of Biochemistry and Biophysics PAS, the Mossakowski Medical Research Institute PAS, the University of Wrocław, the Adam Mickiewicz University and the University of Veterinary Medicine (Vienna, Austria).

core-facility.iimcb.gov.pl

ARBRE MOBIEU
BETWEEN ATOM AND CELL

The Molecular Bioanalytics Unit of the Core Facility is one of the founding members of the Association of Resources for Biophysical Research in Europe (ARBRE) and the Core Technologies for Life Sciences (CTLS) networks. We are participating in the organization of the next **Core Technologies for Life Sciences Association Congress, Lisbon, 2021 https://www.ctls2021.pt/**.

Core Technologies for Life Sciences

Zebrafish Core Facility

The Zebrafish Core Facility (ZCF) has been existing 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 facility was established to introduce a new vertebrate model to research that is conducted at the 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 is included in the IIMCB's Research Infrastructure of Molecules and Cells (IN-MOL-CELL) placed in the **Polish Roadmap for Research Infrastructures.** Moreover, the ZCF has recently become a member of the Core Facility Working Group of the **EU-LIFE alliance**. The ZCF actively participates in discussions on specific core facility challenges and shares its best practices and expertise in core facility management.

Zebrafish are small (3-5 cm) tropical freshwater fish. Thanks to their high genetic similarity to humans, very short reproduction cycle, and the generation of transparent embryos, zebrafish are an excellent model for biomedical research. Moreover, access to experimental manipulations, an extensive collection of mutant/transgenic animals, and low maintenance costs make zebrafish an attractive alternative to mammalian *in vivo* models and can be used to adhere to the Reduce, Refine, Replace (3R) principles of animal research. **Our zebrafish collection consists of wild type and genetically modified lines**. Numerous zebrafish mutants were generated using methods that are based on engineered endonucleases, such as transcription activator-like effector nucleases (TALENs) and the bacterial type II clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. Among the ZCF collection are animals that express modified genes involved in the mTOR signaling pathways, mitochondrial processes, heart development, and neurodegenerative disorders. The ZCF and research groups use zebrafish in innovative projects on genetics, developmental biology, and molecular mechanisms of human diseases. The **ZCF offers services for the IIMCB researchers and external users**, including research groups from the Centre of New Technologies at the University of Warsaw, the Medical University of Warsaw, the Warsaw University of Life Sciences, the Nencki Institute of Experimental Biology, the Medical University of Lublin, the University of Warmia and Mazury in Olsztyn, the University of Wrocław, and the Institute of Industrial Organic Chemistry in Pszczyna. 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 fish would not be possible without a suitable infrastructure. Our fish are currently housed in **1 210 tanks** (eight independent, automated aquatic systems). Moreover, the ZCF is equipped with **incubators, microscopes, and microinjection systems** for zebrafish embryos. Zebrafish Core Facility users have at their disposal a laboratory that is dedicated to **behavioral testing**. The room is equipped with automated systems for observations and the tracking of 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 conducted 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 **constantly monitor the 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 of the 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 the instructions of the Federation of European Laboratory Animal Science Associations [FELASA]).

The ZCF team consists of eight members, including the head of the facility, six 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.

ZEBRAFISH STOCK COLLECTION OVER THE YEARS

2013

300 tanks **(6 racks)** | **2** individual aquatic systems | **6 000** fish | **30** lines

2020

1 210 tanks **(23 racks)** | **8** individual aquatic systems | **~13 000** fish | **>150** lines

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

zcf-team@iimcb.gov.pl

WILD TYPE LINES	MUTANT LINES			TRANSGENIC LINES
Name	Name	Affected genomic region	Genomic feature	Name(construct)
AB	albino	slc45a2	unknown	Tg(nccr.cne12030-Mmu.Fos:EGFP)waw101Tg*
TL	casper	(roy x nacre)	unknown	Tg(nccr.cne12032-Mmu.Fos:EGFP)waw102Tg*
ABxTL	dackel	ext2	point mutation	Tg(10xHis-Eco.BirA)waw103Tg*
TU	fmr1	fmr1	point mutation	Tg(vas:EGFP)
	gba1	gba1	small deletion	Tg(ptf1a:GFP)
	hand2	hand2	insertion	Tg(nkx2.5:EGFP)
	mtor(ztor)	mtor(ztor)	transgenic insertion	Tg(myl7:EGFP)
	nacre	mitfa	unknown	Tg(mnx1:TagRFP-T)
	npc2	npc2	point insertion	Tg(kdr-l:mCherry-CAAX)
	npc2	npc2	point deletion	Tg(hand2:GFP)
	pink1	pink1	point mutation	Tg(-14.8gata4:GFP)
	ppp3ca	ppp3ca	small deletion	Tg(gata1:dsRed;globin:GFP)
	ppp3ca	ppp3ca	small insertion	Tg(gata1:dsRed)
	rptor1del	rptor	deletion	Tg(fli:EGFP)
	sgsh	sgsh	small deletion	Tg(fabp10a:dsRed)
	sgsh	sgsh	small insertion	Tg(CMV:GFP-map1lc3b)
	sgsh	sgsh	in-frame small deletion	Tg(cmlc2:mRFP)
	tsc1a2del	tsc1a	deletion	Tg(cmlc2:GFP)
	tsc1b7del	tsc1b	deletion	Tg(brn3c:mGFP)
	tsc2	tsc2	point mutation	Tg(ath5:gap43GFP)
	waw201*	tet1	insertion	
	waw202*	tet2, cxxc4	complex	
	waw203*	tet3	small deletion	
	waw301	stim2b	insertion	

Table 1. Selection of zebrafish lines from the ZCF stock (note that MTAs limit some lines' usage) *- Lines distributed to EZRC (European Zebrafish Resource Center)

SCIENTIFIC REPRESENTATION

Scientific Representation

PhD Students Council

The PhD Students Council consists of PhD students from all laboratories at the IIMCB. In 2020, the Council representatives were Justyna Jędrychowska and Maciej Migdał. Since November 2020, Abhishek Pateria and Jan Węsławski are our new representatives. The role of the PhD Students Council is to bring forward initiatives, provide networking and learning opportunities, find solutions to problems, and facilitate communication with the IIMCB authorities.

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

- Postgraduate School of Molecular Medicine (Medical University of Warsaw - SMM),
- 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)
- Warsaw PhD School in Natural and BioMedical Sciences (Warsaw-4-PhD).

IIMCB representatives in these schools are: Justyna Jędrychowska (SMM), Katarzyna Banasiak and Anna Stroynowska-Czerwińska (IBB), Jan Węsławski (Nencki Institute) and Zuzanna Mackiewicz (Warsaw-4-PhD).

The academic year 2020-2021 has been peculiar for all PhD students due to the COVID-19 pandemic. But despite the hardships, our PhD students continued to actively and extensively participate in various events, conferences, and symposiums throughout the year. In 2020, our PhD students were co-authors of 21 publications.

Report Session 2020

The yearly PhD students' report session was organized online through the ZOOM platform on 10-11.09.2020 and 5-6.10.2020. In total, 46 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): Maciej Łapiński, Carlos Eduardo Sequeiros Borja, Oliver Tkaczyk and Jan Węsławski.

Awards and scholarships

Anton Slyvka was a laureate of the prestigious START fellowship granted by the Foundation for Polish Science to young, talented and successful researchers working in Poland.

Participation in conferences

- Karolina Bogusz and Jan Węsławski participated in the *EMBO Conference Microtubules: From Atoms to Complex Systems*, 3-6.06.2020. Karolina Bogusz co-authored a virtual poster presented during the meeting
- Aniruddha Das and Pankaj Thapa participated and presented posters during the *European Worm Meeting 2020*, 22-23.06.2020
- Aniruddha Das delivered an oral presentation during the *4th IBB Symposium for Young Investigators*, 4.09.2020 and participated in the *Ubiquitin & Friends Symposium*, 14-15.05.2020
- Justyna Jędrychowska delivered oral presentations during the *SMM report session*, 28-30.09.2020 and the *Neuronus IBRO Neuroscience Forum*, 8-11.12.2020
- Filip Maciąg participated and presented a poster during the *ENS Forum 2020*, 11-15.07.2020. To enable his participation, he was awarded a travel grant by the Polish Neuroscience Society
- Nishita Mandal, Dheeraj Kumar Sarkar and Aravind Thirunavukarasu participated in the *Horizons in Molecular Biology Symposium*, 14-17.09.2020. During the meeting, Dheeraj Kumar Sarkar presented a poster
- Dheeraj Kumar Sarkar and Aravind Selvaram Thirunavukkarasu participated in the conference *BioLOGIES: Bioinspiration, Inventions and Breakthrough technologies*, organized at the Faculty of Biology of Adam Mickiewicz University, 22-24.09.2020
- Anna Stroynowska-Czerwińska participated and presented a poster during the *14th EMBL Conference Transcription and Chromatin*, 27-29.08.2020. She received the EMBL Corporate Partnership Programme Registration Fee Waiver
- Natalia Szulc presented a poster during the *Autumn Workshop PTBI 2020* of the Polish Bioinformatics Society, 26-27.11.2020
- Karolina Wojciechowska participated and delivered an oral presentation during the *4th IBB Symposium for Young Investigators*, 4.09.2020. She was awarded the title of best oral presentation. She participated in a workshop *Python from Scratch*. In addition, she delivered the mandatory Doctoral Seminar at the IBB on 22.10.2020
- Juan Zeng participated and presented a poster during the *Neuronus IBRO Neuroscience Forum*, 8-11.12.2020

Many PhD students actively participated in organizing the International Young Scientists Conference on Molecular and Cell Biology 2021, as members of the Organizing Committee: Gabriela Jędruszewska, Justyna Jędrychowska, Rishikesh Kumar Gupta, Małgorzata Maksymowicz, Pratik Kumar Mandal, Maciej Migdał, Karim Abu Nahia, Agata Poświata, Anton Slyvka, Eugeniusz Tralle, Jan Węsławski, and Marta Wróbel.

Postdoctoral Council

The Postdoctoral Council gathers postdoctoral researchers from all laboratories at the IIMCB. In 2020, the Council representatives were Małgorzata Figiel and Almudena Maria Ponce Salvatierra. The new representatives, appointed in September 2020, are Anna Hojka-Osińska and Andrii Kopach.

Our mission is to provide resources for career and personal development of postdoctoral researchers by organizing courses, training and networking activities. Another goal of the Council is to facilitate communication between postdocs, support newcomers as well as strengthen scientific interactions and collaboration between postdocs and other researchers in Poland and abroad. In 2020, the postdocs co-authored 25 papers and obtained 6 grants as Principal Investigators.

Our biggest achievement in 2020 was launching a series of scientific meetings called **Spotlight Talks**, an initiative jointly organized by the IIMCB postdoc representatives Anna Hojka-Osińska & Andrii Kopach and the IIMCB alumni Anna Bajur (King's College London). This initiative brings together postdocs and senior researchers from different countries into an interdisciplinary network.It is important to increase understanding of science being done in other fields & disciplines and also to learn about cutting-edge tools. There is also a need to bring together people with a wide range of expertise ready to solve complex scientific challenges in the future. The Spotlight Talks are open to everyone and are regularly advertised via Facebook/LinkedIn/Twitter.

Spotlight Talks in 2020

- Andrii Kopach, IIMCB: *From Phase Separation Towards the Mental Health*, 28.10.2020
- Anna Hojka-Osińska, IIMCB: *Landscape of Functional Interactions of Human Processive Ribonucleases Revealed by High-Throughput siRNA Screenings*, 18.11.2020
- Rohit Suratekar, IIMCB: *Dissecting the Cardiac Regulatory Network in Zebrafish using Mathematical Modelling*, 25.11.2020
- Anna Bajur, King's College London: *A Single-Molecule Approach to Study B Cell Antigen Recognition and Affinity Discrimination*, 23.12.2020

Individual achievements of postdocs in 2020

- Malwina Hyjek-Składanowska was invited by Roche Innovation Center Copenhagen in Denmark to give an online lecture on 26.06.2020 based on her paper Hyjek-Składanowska et al., *J Am Chem Soc,* 2020
- Mariusz Czarnocki-Cieciura and Filip Stefaniak participated in the 24th Science Festival in Warsaw by giving popular science lectures (18-27.09.2020)

The 1st Women in Science Symposium (WiSS) was an initiative of the postdoc community at the IIMCB. It aimed to increase the awareness of young scientists of their own potentials and opportunities, with special emphasis on women's presence in the scientific world. The organization of WiSS was an effort of great team work supervised by Małgorzata Figiel and Almudena Maria Ponce Salvatierra.

Senior Researchers Council

The Senior Researchers Council is an association of researchers and senior researchers from all laboratories at the IIMCB. In 2020, the group was represented by Magdalena Czeredys and Daria Zdżalik-Bielecka.

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 & Publications

In 2020 researchers and senior researchers received funding for the following projects:

- Elżbieta Jagielska, NCBR POLNOR: "Sustainable and safe food production by novel control strategies of bacteria in the food chain"
- Izabela Sabała, NCBR POLNOR: "OneHealth approach to sustainable prevention and treatment of infectious diseases"
- Ewelina Szymańska, NCN OPUS19: "Identification of novel vulnerabilities of VPS4B-deficient cancers cells"
- Justyna Zmorzyńska, NCN OPUS19: "Rac1 contribution to brain connectivity impairments and neuropsychiatric disorders in Tuberous Sclerosis Complex"

Last year our group co-authored 21 publications, and in most of them, researchers or senior researchers were either first or corresponding authors. Importantly, publications that won Best Papers Award 2020 were prepared with the prominent contribution of researchers and senior researchers.

Awards

Award of the Division II: Biological and Agricultural Sciences, Polish Academy of Sciences in 2020 for the scientific team of the IIMCB composed of: J. Jaworski, M. Kędra, B. Tarkowski, M. Urbańska, and J. Zmorzyńska for the series of publications on *New molecular mechanisms of mTORopathy and epilepsy*.

External Lectures

Małgorzata Wiweger, *How can zebrafish help patients with rare diseases?*, Rare Diseases Day: Patients in Art, Science and Medicine (Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, 28.02.2020).

Małgorzata Wiweger was a lecturer at courses by the Polish Society for Science on Laboratory Animals (PolLASA) for people who use animals for research and education: *Fish as laboratory and experimental animals*; February, July & October 2020.

Filip Stefaniak was among four scientists who gave three lectures *Before a new pastille is made*, Warsaw Festival of Science, 18-27.09.2020.

Mentoring

Auxiliary supervisors of PhD students that defended their thesis in 2020:

- Magdalena Banach-Orłowska (for Małgorzata Maksymowicz)
- Łukasz Majewski (for Filip Maciąg)
- Filip Stefaniak (for Pietro Boccaletto)

Supervisors of MSc students:

- Filip Stefaniak (for Natalia Szulc)
- Elżbieta Jagielska (for Paulina Brodacka)

Community service

Editors: Vladimir Korzh is an editor for *Scientific Reports* and the *International Journal of Molecular Sciences*. Izabela Sabała is a member of the Editorial Board of *Scientific Reports*.

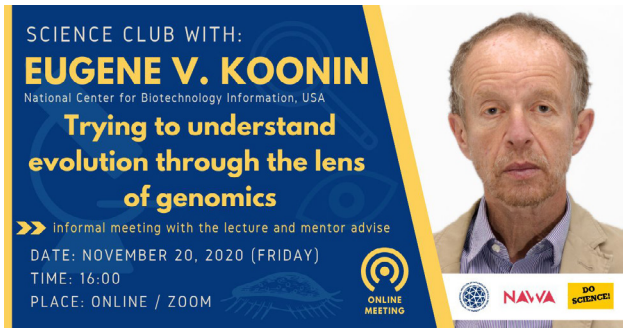
Reviewers: Vladimir Korzh: *Briefings in Functional Genomics*, *Computer Methods and Programs in Biomedicine*, *Development*, *Frontiers in Genetics*, *Journal of Fish Biology*, *Micropublication*, *Mechanisms of Development*, and *Scientific Reports*. Elżbieta Jagielska: *Scientific Reports,* Łukasz Majewski: *International Journal of Medical Sciences* and *Journal of Physiology,* Filip Stefaniak: *Nucleic Acid Research* and *Journal of Physical Chemistry*, Ewelina Szymańska: *British Journal of Cancer*, and Justyna Zmorzyńska: *Journal of Pediatric Genetics*.

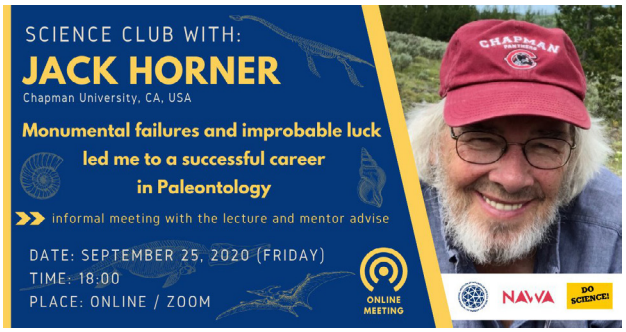
Experts: Izabela Sabała evaluated grant applications in the Marie Skłodowska-Curie Actions Research Fellowship Programme (EC), and Elżbieta Nowak for the National Science Centre (NCN).

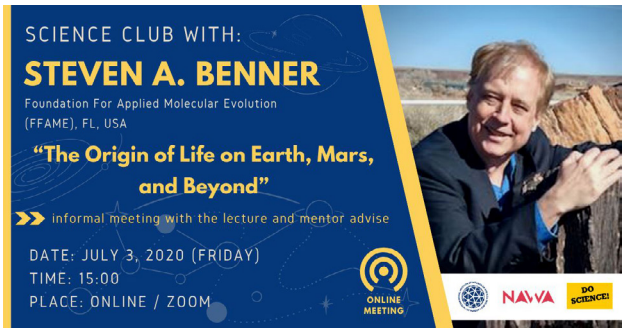
Małgorzata Korzeniowska is among the experts at the Ministry of Science and Higher Education who participate in inspections of users performing animal experiments, which are carried out by district veterinarians.

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 NCN. To this end, we prepared an open letter addressed to the NCN Council and collected signatures supporting our initiative. Thanks to these efforts, the NCN introduced a new position of Senior Researcher to OPUS and MAESTRO grants.

Science Club







POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme

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:

- The Nencki Institute of Experimental Biology of the Polish Academy of Sciences - leader
- The International Institute of Molecular and Cell Biology in Warsaw
- The Institute of Organic Chemistry of the Polish Academy of Sciences
- The Institute of Physical Chemistry of the Polish Academy of Sciences
- The Institute of Physics of the Polish Academy of Sciences
- The Center for Theoretical Physics of the Polish Academy of Sciences
- The Institute of High Pressure Physics of the Polish Academy of Sciences
- The Maria Sklodowska-Curie National Institute of Oncology - State Research Institute
- The Institute of Psychiatry and Neurology

Education at the School is delivered through four types of classes

- Specialization lectures related to a given discipline
- Specialization tutorials/training - practical classes providing education within the scope of skills, methods or research tools and procedures related to a given discipline
- Specialization seminars - research seminars related to a given discipline
- Electives - classes that develop the researcher's skills regardless of the discipline selected.

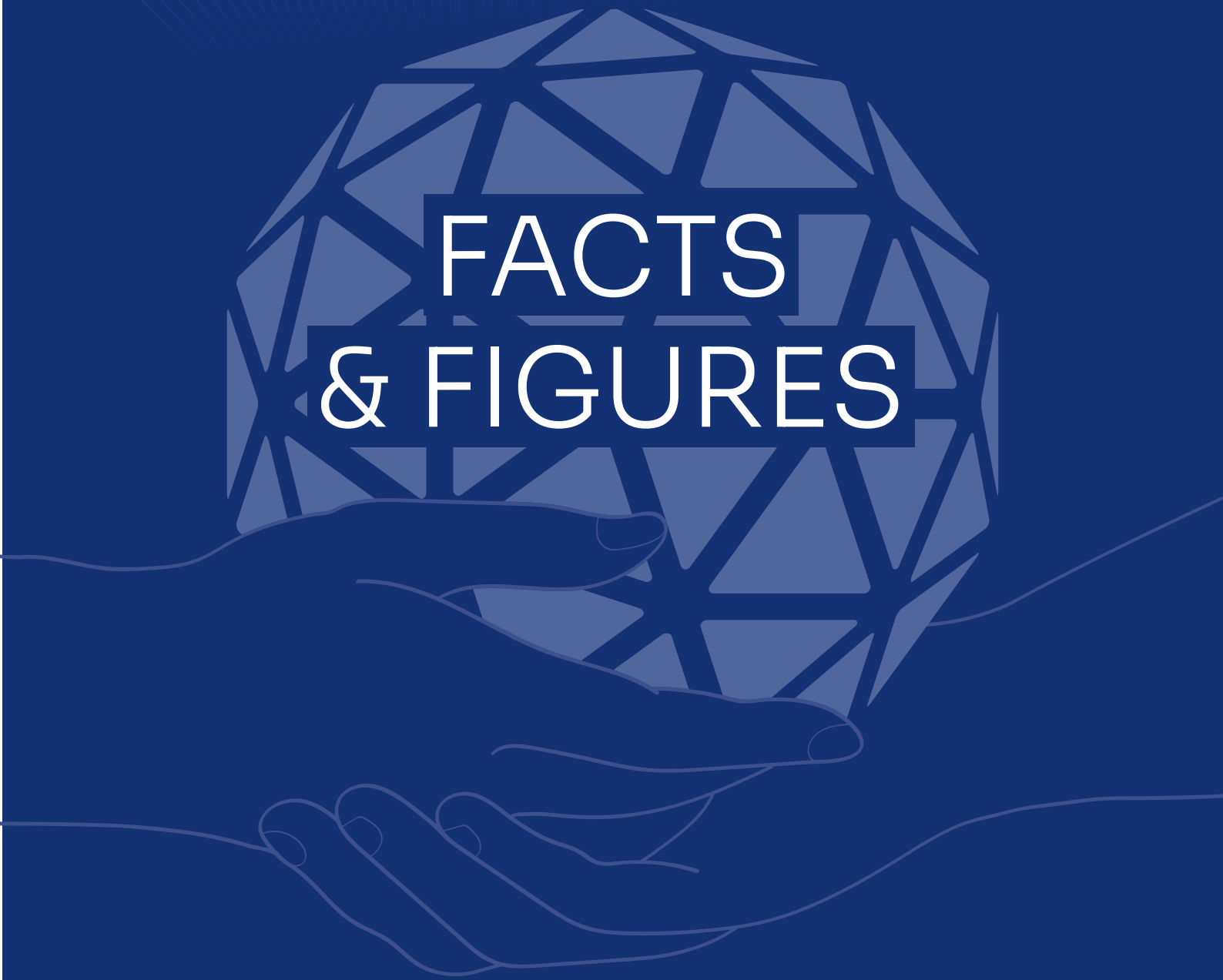
Admission to Warsaw-4-PhD is preceded by an open international competition in which the leading criterion is the candidate's excellence and predisposition to conduct groundbreaking research. Doctoral students, under the guidance of their supervisors, implement individual research plans and develop their 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, tight-knit international community, where their research and social needs are looked after. We encourage PhD students to participate in international happenings, ranging from research secondments and conferences to workshops and training, by financing their trips. PhD students who choose to apply for competitive funding are helped in this process by our administrative staff every step of the way. Additionally, students have access to a basic private medical package, as well as a social benefits fund provided by the IIMCB.

We know that students' voices matter. With this in mind, the PhD Students Council, which gathers all doctoral students was appointed. The Council selects two representatives to provide this voice for them among the IIMCB directors. The Council also has annual meetings where open discussions with the International Advisory Board take place.

www.warsaw4phd.eu/en/

FACTS & FIGURES



HR Data

267 STAFF

165	Female	48	Foreigners
102	Male	16	Nations

12	Lab Leaders	59	PhD Students
2	Project Coordinators	14	Undergraduate Students
20	Senior Researchers	7	Trainees
4	Researchers	11	Core Facilities Staff
42	Postdoctoral Researchers	6	Technicians
5	Research Assistants	12	Laboratory Support Specialists
18	Research Specialists	42	Administration
2	Project Assistants	11	Others

Personal Achievements

Marta Miączyńska appointed **new Member of the EMBO Council**

Jacek Kuźnicki appointed **new President of the Council of the National Science Centre**

Jacek Jaworski awarded the **Prime Minister's Prize** for achievements in the category: scientific activity

Michał Rażew awarded the **Prime Minister's Prize** in the category: distinguished doctoral dissertation

Janusz M. Bujnicki received the **Award of the Minister of Science and Higher Education** in the 2nd degree for significant achievements in the field of organizational activity

Jacek Kuźnicki has become an expert advisor to the **European Partnerships in Horizon Europe**

Andrzej Dziembowski has become an expert advisor to the **Health Cluster in Horizon Europe**

Katarzyna Mleczko-Sanecka won a **Scholarship For Outstanding Young Scientists** of the Minister of Science and Higher Education

Anton Slyvka received a **scholarship within the START program** of the Foundation for Polish Science

Jacek Jaworski received the **Konorski Award** (PTBUN/ Neurobiology Committee of the Polish Academy of Sciences) for the best publication in the field of neurobiology: *Amot and Yap1 regulate neuronal dendritic tree complexity and locomotor coordination in mice.* Rojek KO, Krzemień J, Doleżyczek H, Boguszewski PM, Kaczmarek L, Konopka W, Rylski M, **Jaworski J**, Holmgren L, Prószyński TJ. *PLoS Biol*. 2019; 17(5):e3000253

Jacek Jaworski, **Magdalena Kędra**, **Bartosz Tarkowski**, **Małgorzata Urbańska** and **Justyna Zmorzyńska** received the **award of Division II: Biological and Agricultural Sciences, Polish Academy of Sciences** for a series of scientific works on *New molecular mechanisms of mTORopathy and epilepsy*

Leszek Pryszcz received the **Bassalik Award** (Molecular Cell Biology Committee of the Polish Academy of Sciences) for the best publication in the field of microbiology done in a Polish laboratory for the paper: *Transcriptome analyses of cells carrying the Type II Csp231I restriction-modification system reveal cross-talk between two unrelated transcription factors: C protein and the Rac prophage repressor*. Negri A, Jąkalski M, Szczuka A, **Pryszcz LP**, Mruk I. *Nucleic Acids Res*. 2019; 47(18):9542-9556

Scientific Promotions

Marcin Nowotny, Professor of Biological Sciences

Elżbieta Nowak, DSc Habil in Biological Sciences

Wojciech Pokrzywa, DSc Habil in Biological Sciences

Filip Maciąg, PhD in Biological Sciences, *The role of STIM and ORAI proteins in selected aspects of neuronal physiology*, thesis advisor: J. Kuźnicki

Małgorzata Maksymowicz*, PhD in Biological Sciences, *Characterization of endocytic transport of lymphotoxin β receptor (LTβR) and its impact on the activation of the NF-κB pathway*, thesis advisor: M. Miączyńska

Katarzyna Poleszak, PhD in Biological Sciences, *Characteristics of the MLH1-MBD4 complex, formed by proteins involved in DNA repair,* thesis advisor: J.M. Bujnicki

Sreedevi Sugunan, PhD in Biological Sciences, *Localized translation of nuclear encoded mitochondrial proteins in zebrafish*, thesis advisors: C.L. Winata & A. Chacińska

Aleksandra Szybalska*, PhD in Health Sciences, *Sociodemographic factors and selected health status indicators and the utilization of medical rehabilitation and health resort treatment services by people aged 65 years and over based on the PolSenior study,* thesis advisor: M. Mossakowska

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**promotion with honors*

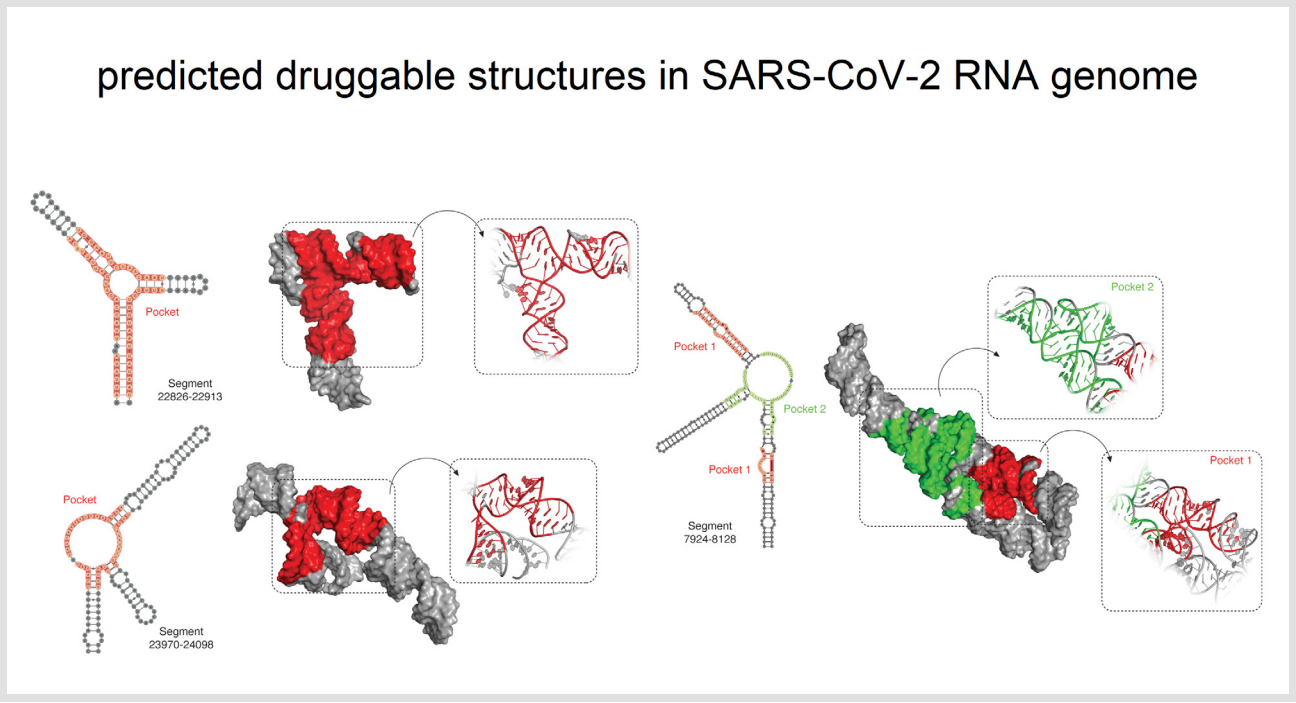
Best Papers Award 2020

As in the previous years, the best papers with the IIMCB affiliation in 2020 have been awarded. The best papers 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. Of course they 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. The financial prizes are divided among the authors with the IIMCB affiliation. The best papers at the IIMCB in 2020 are listed below.

1st Place

Manfredonia I*, **Nithin C***, **Ponce-Salvatierra A***, **Ghosh P***, **Wirecki TK***, Marinus T, Ogando NS, Snijder EJ, van Hemert MJ§, **Bujnicki JM§**, Incarnato D§. Genome-wide mapping of SARS-CoV-2 RNA structures identifies therapeutically-relevant elements. ***Nucleic Acids Research***, 2020; 48(22):12436-12452. doi: 10.1093/nar/gkaa1053, * equal contributors, § corresponding authors

The IIMCB researchers from the Laboratory of Bioinformatics and Protein Engineering and Dutch scientists described the first comprehensive experimental structural analysis of the entire SARS-CoV-2 coronavirus genome, which is also the very first such a study for any coronavirus genome. Briefly, in a collaborative project carried out jointly by three laboratories (the IIMCB laboratory, and two laboratories from the Netherlands), RNA structure probing was performed to obtain single-base resolution secondary structure maps of the full SARS-CoV-2 coronavirus genome both in vitro and in living infected cells. Probing data recapitulated the previously described coronavirus RNA elements (5' UTR and s2m), and revealed new structures, of which some are conserved among coronaviruses. Based on experimental structural information, the researchers modeled the 3D structures of these segments and identified putative druggable pockets. They also identified intrinsically flexible (mostly single-stranded) segments, showing high sequence conservation, suitable for the development of antisense oligonucleotide therapeutics. This work lays the foundation for the development of RNA-targeted therapeutic strategies to fight SARS-related infections.



2nd Place

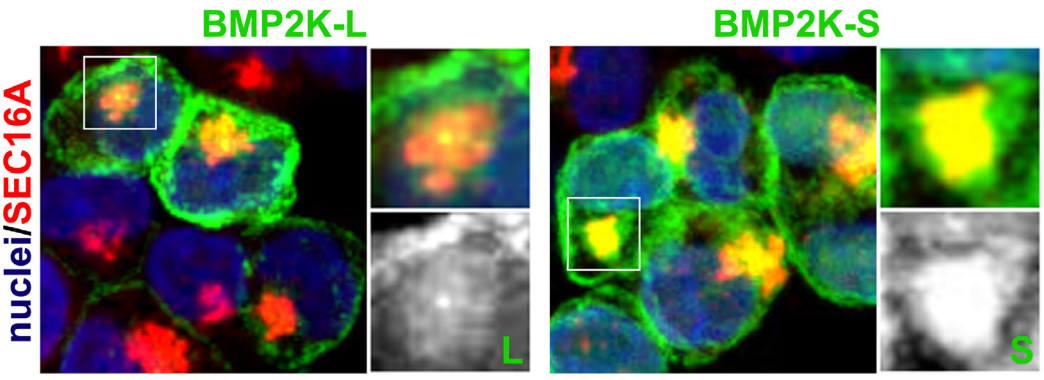
Hyjek-Składanowska M, Vickers TA, **Napiórkowska A**, Anderson BA, Tanowitz M, Crooke ST, Liang XH, Seth PP, **Nowotny M**. Origins of the Increased Affinity of Phosphorothioate-Modified Therapeutic Nucleic Acids for Proteins. ***Journal of the American Chemical Society***, 2020; 142(16):7456-7468. doi: 10.1021/jacs.9b13524

Scientists from the Laboratory of Protein Structure published structural insights in the increased affinity of phosphorothioate-modified therapeutic nucleic acids for proteins. The work was performed in cooperation with Ionis Pharmaceuticals – a leading company in RNA-targeted therapeutics. Antisense oligonucleotides (ASOs) comprise an emerging class of advanced therapeutics such as Spinraza (Nusinersen) - the first clinically approved drug for the previously untreatable spinal muscular atrophy (SMA). ASOs interact with a number of plasma and cellular proteins, which affects their distribution, tissue delivery, cellular uptake, intracellular trafficking and consequently potency and toxicity. This work describes the first structural study of a complex between an ASO and a protein, identifying the molecular forces that govern the enhanced affinity of therapeutic oligonucleotides for proteins and presenting a model for how protein-ASO interactions can cause cellular toxicity. The presented results are an important step forward in the understanding of the interactions of phosphorothioate ASOs with proteins. This work provides information that can be instrumental in the design of improved nucleic acid-based drugs.

3rd Place

Cendrowski J, **Kaczmarek M**, **Mazur M**, **Kuzmicz-Kowalska K**, **Jastrzebski K**, Brewinska-Olchowik M, Kominek A, Piwocka K, **Miaczynska M**. Splicing variation of BMP2K balances abundance of COPII assemblies and autophagic degradation in erythroid cells. ***Elife***, 2020; 9:e58504. doi: 10.7554/eLife.58504

Researchers from the Laboratory of Cell Biology of IIMCB and from the Nencki Institute reported the identification of a novel molecular regulatory system that controls maturation of red blood precursor cells. During erythropoiesis, red blood precursor cells undergo profound rearrangements of membrane transport pathways, that requires existence of erythroid-specific mechanisms and regulators of membrane trafficking, so far poorly characterized. The authors discovered a cell-type specific regulation of membrane transport pathways during erythropoiesis, which could be potentially exploited to modulate the rate of red blood cell production. They uncovered an unusual mechanism of two splicing variants of a kinase playing opposing roles in intracellular processes, thereby constituting a regulatory system that fine-tunes erythroid maturation. Specifically, the study demonstrates that variants of BMP2K have different interactomes and play opposing roles in regulation of erythroid differentiation. Although BMP2K kinase has been suspected to act primarily in endocytosis, this publication shows that BMP2K splicing variants differentially regulate other processes important for erythropoiesis, including SEC16A-dependent trafficking of COPII vesicles, involved in protein secretion and autophagic degradation. The results of this research lay grounds for studying whether pharmacological modulation of BMP2K function could be beneficial for patients suffering from anemia or leukemia.



Publications in 2020

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

No	Authors	Title	Journal	5-Year IF	Journal Category	Quartile in Category
1	**Hyjek-Składanowska M**, Vickers T, **Napiórkowska A**, Anderson B, Tanowitz M, Crooke ST, Liang XH, Seth PP, **Nowotny M**.	Origins of the increased affinity of phosphorothioate-modified therapeutic nucleic acids for proteins.	J Am Chem Soc. 2020; 142(16):7456-68 doi: 10.1021/jacs.9b13524	14.549	CHEMISTRY, MULTIDISCIPLINARY	Q1
2	Xu G-L, **Bochtler M.**	Reversal of nucleobase methylation by dioxygenases.	Nat Chem Biol. 2020; 16:1160-9 doi: 10.1038/s41589-020-00675-5	13.824	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
3	Bilska A, Kusio-Kobiałka M, **Krawczyk PS**, **Gewartowska O, Tarkowski B**, Kobyłecki K, Nowis D, Golab J, **Gruchota J, Borsuk E, Dziembowski A, Mroczek S.**	Immunoglobulin Expression and the Humoral Immune Response Is Regulated by the Non-Canonical poly(A) Polymerase TENT5C.	Nat Commun. 2020; 11(1):2032 doi: 10.1038/s41467-020-15835-3	13.611	MULTIDISCIPLINARY SCIENCES	Q1
4	**Cendrowski J, Miaczynska M.**	Splicing variants of an endocytic regulator, BMP2K, differentially control autophagic degradation in erythroid cells.	Autophagy. 2020; 16(12):2303-4 doi: 10.1080/15548627.2020.1833501	11.966	CELL BIOLOGY	Q1
5	**Kisiala M, Kowalska M, Pastor M**, Korza HJ, **Czapinska H, Bochtler M.**	Restriction endonucleases that cleave RNA/DNAheteroduplexes bind dsDNA in A-like conformation.	Nucleic Acids Res. 2020; 48(12):6954-69 doi: 10.1093/nar/gkaa403	11.797	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
6	**Magnus M**, Antczak M, Zok T, Wiedemann J, Lukasiak P, Cao Y, **Bujnicki JM**, Westhof E, Szachniuk M, Miao Z.	RNA-Puzzles toolkit: a computational resource of RNA 3D structure benchmark datasets, structure manipulation, and evaluation tools.	Nucleic Acids Res. 2020; 48(2):576-88 doi: 10.1093/nar/gkz1108	11.797	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
7	**Malik D**, Kobyłecki K, **Krawczyk P**, Poznański J, **Jakielaszek A**, **Napiórkowska A**, Dziembowski A, Tomecki R, **Nowotny M.**	Structure and mechanism of CutA, RNA nucleotidyl transferase with an unusual preference for cytosine.	Nucleic Acids Res. 2020;48(16):9387-405 doi: 10.1093/nar/gkaa647	11.797	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
8	Manfredonia I, **Nithin C**, **Ponce-Salvatierra A, Ghosh P, Wirecki TK**, Marinus T, Ogando NS, Snijder EJ, van Hemert MJ, **Bujnicki JM,** Incarnato D.	Genome-wide mapping of SARS-CoV-2 RNA structures identifies therapeutically-relevant elements.	Nucleic Acids Res. 2020; 48(22):12436-52 doi: 10.1093/nar/gkaa1053	11.797	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
9	**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 doi: 10.1093/nar/gkaa396.	11.797	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
10	**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 U S A. 2020; 117(4):2170-9 doi: 10.1073/pnas.1910834117	10.620	MULTIDISCIPLINARY SCIENCES	Q1
11	**Szymańska E, Nowak P, Kolmus K**, Cybulska M, Goryca K, Derezińska-Wołek E, Szumera-Ciećkiewicz A, Brewińska-Olchowik M, Grochowska A, Piwocka K, Prochorec-Sobieszek M, Mikula M, **Miączyńska M.**	Synthetic lethality between VPS4A and VPS4B triggers an inflammatory response in colorectal cancer.	EMBO Mol Med. 2020; 12:e10812 doi: 10.15252/emmm.201910812	10.366	MEDICINE, RESEARCH & EXPERIMENTAL	Q1
12	**Cendrowski J, Kaczmarek M, Mazur M, Kuzmicz-Kowalska K, Jastrzebski K,** Brewinska-Olchowik M, Kominek A, Piwocka K, **Miaczynska M.**	Splicing variation of BMP2K balances abundance of COPII assemblies and autophagic degradation in erythroid cells.	eLife. 2020; 9:e58504 doi: 10.7554/eLife.58504	8.176	BIOLOGY	Q1
13	**Sequeiros-Borja CE, Surpeta B, Brezovsky J.**	Recent advances in user-friendly computational tools to engineer protein function.	Brief Bioinform. 2020; bbaa150 doi: 10.1093/bib/bbaa150	7.468	BIOCHEMICAL RESEARCH METHODS	Q1
14	**Korzh V**, Kondrychyn I.	Origin and development of circumventricular organs in living vertebrate.	Semin Cell Dev Biol. 2020; 102:13-20 doi: 10.1016/j.semcdb.2019.10.010	6.629	CELL BIOLOGY	Q1
15	**Fricke T,** Smalakyte D, **Lapinski M, Pateria A, Weige C**, Pastor M, **Kolano A, Winata C,** Siksnys V, Tamulaitis G, **Bochtler M.**	Targeted RNA Knockdown by a Type III CRISPR-Cas Complex in Zebrafish.	CRISPR J. 2020; 3(4):299-313 doi: 10.1089/crispr.2020.0032	5.343	GENETICS & HEREDITY	Q1

16	**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 doi: 10.3390/cells9010160	5.276	CELL BIOLOGY	Q2
17	**Gupta RK, Kuznicki J.**	Biological and Medical Importance of Cellular Heterogeneity Deciphered by Single-Cell RNA Sequencing.	Cells. 2020; 9(8):1751 doi: 10.3390/cells9081751	5.276	CELL BIOLOGY	Q2
18	**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 doi: 10.3390/cells9051285	5.276	CELL BIOLOGY	Q2
19	**Czeredys M.**	Dysregulation of Neuronal Calcium Signaling via Store-Operated Channels in Huntington's Disease.	Front Cell Dev Biol. 2020; 8:611735 doi: 10.3389/fcell.2020.611735	5.186	DEVELOPMENTAL BIOLOGY	Q1
20	**Thapa P, Shanmugam N, Pokrzywa W.**	Ubiquitin Signaling Regulates RNA Biogenesis, Processing, and Metabolism.	Bioessays. 2020; 42(1):e1900171 doi: 10.1002/bies.201900171	4.827	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
21	**Maksymowicz M, Miączyńska M, Banach-Orłowska M.**	Clathrin- and dynamin-dependent endocytosis limits canonical NF-κB signaling triggered by lymphotoxin β receptor.	Cell Commun Signal. 2020; 18(1):176 doi: 10.1186/s12964-020-00664-0	4.812	CELL BIOLOGY	Q2
22	**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):E6198 doi: 10.3390/ijms21176198	4.653	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
23	**Majewski L, Maciąg F,** Boguszewski PM, **Kuznicki 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 doi.org/10.3390/ijms21030842	4.653	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
24	**Surpeta B, Sequeiros-Borja CE, Brezovsky J.**	Dynamics, a Powerful Component of Current and Future in Silico Approaches for Protein Design and Engineering.	Int J Mol Sci. 2020; 21(8):2713 doi: 10.3390/ijms21082713	4.653	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
25	**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 doi: 10.1111/jnc.15010	4.350	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q2
26	Dey K, **Bazala MA, Kuznicki J.**	Targeting mitochondrial calcium pathways as a potential treatment against Parkinson's disease.	Cell Calcium. 2020; 89:102216 doi: 10.1016/j.ceca.2020.102216	4.154	CELL BIOLOGY	Q2
27	**Miaczynska M,** Munson M.	Membrane Trafficking: Vesicle Formation, Cargo Sorting and Fusion.	Mol Biol Cell. 2020; 31(6):399-400 doi: 10.1091/mbc.E19-12-0680	3.979	CELL BIOLOGY	Q2
28	**Jaciuk M,** Swuec P, **Gaur V, Kasprzak JM,** Renault L, Dobrychłop M, **Nirwal S, Bujnicki JM,** Costa A, **Nowotny M.**	A combined structural and biochemical approach reveals translocation and stalling of UvrB on the DNA lesion as a mechanism of damage verification in bacterial nucleotide excision repair.	DNA Repair (Amst). 2020; 85:102746 doi: 10.1016/j.dnarep.2019.102746	3.790	GENETICS & HEREDITY	Q2
29	de Assis GG, Murawska-Cialowicz E, Cieszczyk P, **Gasanov EV.**	Respiratory Syndrome Coronavirus Infections: Possible Mechanisms of Neurological Implications - A Systematic Review.	Front Neurol. 2020; 11:864 doi: 10.3389/fneur.2020.00864	3.164	CLINICAL NEUROLOGY	Q2
30	**Goś D.**	Be Healthy as a Fish educational program - Presenting how zebrafish can improve our understanding of human diseases.	Dev Biol. 2020; 457(2):169-171 doi: 10.1016/j.ydbio.2019.01.012	3.090	DEVELOPMENTAL BIOLOGY	Q2
31	**Jedrychowska J, Gasanov EV, Korzh V.**	Kcnb1 plays a role in development of the inner ear.	Dev Biol, 2020; 471:65-75 doi: 10.1016/j.ydbio.2020.12.007	3.090	DEVELOPMENTAL BIOLOGY	Q2
32	**Winata CL, Dodzian J, Bialek-Wyrzykowska U.**	The zebrafish as a model for developmental and biomedical research in Poland and beyond.	Dev Biol. 2020; 457(2):167-168 doi: 10.1016/j.ydbio.2019.11.003	3.090	DEVELOPMENTAL BIOLOGY	Q2
33	Stawowczyk E, Kawalec P, Kowalska-Duplaga K, **Mossakowska M.**	Productivity Loss Among Parents of Children With Inflammatory Bowel Diseases in Relation to Disease Activity and Patient's Quality of Life.	J Pediatr Gastroenterol Nutr. 2020; 71(3):340-345 doi:10.1097/MPG.0000000000002801	2.934	GASTROENTEROLOGY & HEPATOLOGY	Q3
34	**Hyjek-Składanowska M,** Stasińska AR, **Napiórkowska-Gromadzka A, Bartłomiejczak A,** Seth PP, Chmielewski MK, **Nowotny M.**	Disulfide bridge cross-linking between protein and the RNA backbone as a tool to study RNase H1.	Bioorg Med Chem. 2020; 28(23):115741 doi: 10.1016/j.bmc.2020.115741	2.916	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q3
35	**Bochtler M.**	Arrhenius-law-governed homo- and heteroduplex dissociation.	Phys Rev E. 2020; 101(3-1):032405 doi: 10.1103/PhysRevE.101.032405	2.287	PHYSICS, MATHEMATICAL	Q1
36	**Skowronek KJ, Bochtler M.**	In Vitro Directed Evolution of a Restriction Endonuclease with More Stringent Specificity.	J Vis Exp. 2020; (157) doi: 10.3791/60807	1.539	MULTIDISCIPLINARY SCIENCES	Q3
37	**Wirecki TK, Nithin C, Mukherjee S, Bujnicki JM, Boniecki MJ.**	Modeling of Three-Dimensional RNA Structures Using SimRNA.	Methods Mol Biol. 2020; 2165:103-25 doi: 10.1007/978-1-0716-0708-4_6			

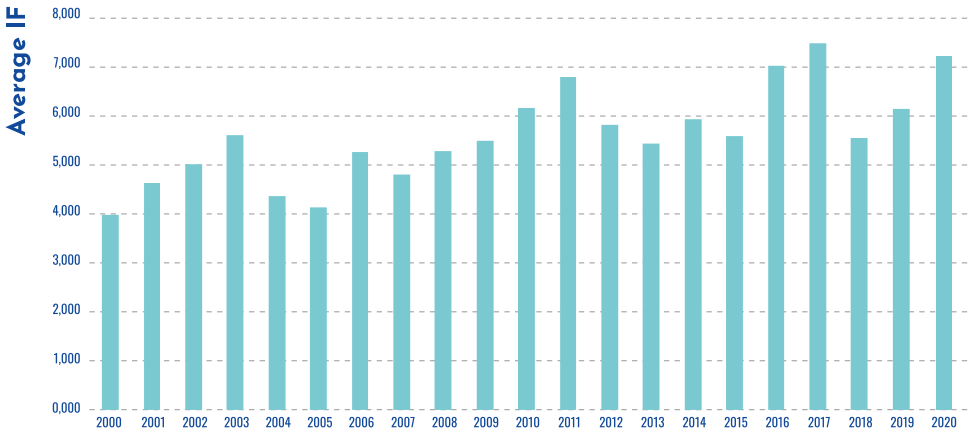
List of papers without IIMCB-affiliated main authors (first and/or corresponding)

No	Authors	Title	Journal	5-Year IF	Journal Category	Quartile in Category
1	NCD Risk Factor Collaboration (**Mossakowska M** and **Ślusarczyk P**)	Height and body-mass index trajectories of school-aged children and adolescents from 1985 to 2019 in 200 countries and territories: a pooled analysis of 2181 population-based studies with 65 million participants.	Lancet. 2020; 396(10261):1511-24 doi: 10.1016/S0140-6736(20)31859-6	59.345	MEDICINE, GENERAL & INTERNAL	Q1
2	NCD Risk Factor Collaboration (**Mossakowska M** and **Ślusarczyk P**)	Repositioning of the global epicentre of non-optimal cholesterol.	Nature. 2020; 582(7810):73-7 doi: 10.1038/s41586-020-2338-1	46.488	MULTIDISCIPLINARY SCIENCES	Q1
3	Gonzalez-Delgado LS, Walters-Morgan H, Salamaga B, Robertson AJ, Hounslow AM, **Jagielska E, Sabała I,** Williamson MP, Lovering AL, Mesnage S.	Two-site recognition of Staphylococcus aureus peptidoglycan by lysostaphin SH3b.	Nat Chem Biol. 2020; 16(1):24-30 doi: 10.1038/s41589-019-0393-4	13.824	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
4	Chorostecki U, Molina M, **Pryszcz LP,** Gabaldón T.	MetaPhOrs 2.0: Integrative, Phylogeny-Based Inference of Orthology and Paralogy Across the Tree of Life.	Nucleic Acids Res, 2020; 48(W1):W553-W557 doi: 10.1093/nar/gkaa282	11.797	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
5	Cieśla M, Turowski TW, **Nowotny M,** Tollervey D, Boguta M.	The expression of Rpb10, a small subunit common to RNA polymerases, is modulated by the R3H domain-containing Rbs1 protein and the Upf1 helicase.	Nucleic Acids Res. 2020; 48(21): 12252-68 doi: 10.1093/nar/gkaa1069	11.797	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
6	Szewczyk M, **Malik D**, Borowski LS, Czarnomska SD, Kotrys AV, Klosowska-Kosicka K, **Nowotny M**, Szczesny RJ.	Human REXO2 Controls Short Mitochondrial RNAs Generated by mtRNA Processing and Decay Machinery to Prevent Accumulation of Double-Stranded RNA.	Nucleic Acids Res. 2020; 48(10):5572-90 doi: 10.1093/nar/gkaa302	11.797	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
7	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 doi: 10.1038/s41436-020-0823-4	10.435	GENETICS & HEREDITY	Q1
8	Donkervoort S, Kutzner CE, Hu Y, Lornage X, Rendu J, Stojkovic T, Baets J, Neuhaus SB, Tanboon J, Maroofian R, Bolduc V, Mroczek M, Conijn S, Kuntz NL, To¨pf A, Monges S, Lubieniecki F, McCarty RM, Chao KR, Governali S, Bo¨hm J, Boonyapisit K, Malfatti E, Sangruchi T, Horkayne-Szakaly I, Hedberg-Oldfors C, Efthymiou S, Noguchi S, Djeddi S, Iida A, di Rosa G, Fiorillo C, Salpietro V, Darin N, Faure´ J, Houlden H, Oldfors A, Nishino I, de Ridder W, Straub V, **Pokrzywa W**, Laporte J, Foley R, Romero NB, Ottenheijm C, Hoppe T, Bönnemann CG.	Pathogenic Variants in the Myosin Chaperone UNC-45B Cause Progressive Myopathy with Eccentric Cores.	Am J Hum Genet. 2020; 107(6):1078-95 doi: 10.1016/j.ajhg.2020.11.002	10.344	GENETICS & HEREDITY	Q1
9	NCD Risk Factor Collaboration (**Mossakowska M** and **Ślusarczyk P**).	National Trends in Total Cholesterol Obscure Heterogeneous Changes in HDL and non-HDL Cholesterol and total-to-HDL Cholesterol Ratio: A Pooled Analysis of 458 Population-Based Studies in Asian and Western Countries.	Int J Epidemiol. 2020; 49(1):173-192 doi: 10.1093/ije/dyz099	9.305	PUBLIC, ENVIRONMENTAL & OCCUPATIONAL HEALTH	Q1
10	Kuzniewska B, Cysewski D, Wasilewski M, **Sakowska P**, Milek J, **Kulinski TM**, Winiarski M, **Kozielewicz P,** Knapska E, Dadlez M, **Chacinska A, Dziembowski A**, Dziembowska M.	Mitochondrial protein biogenesis in the synapse is supported by local translation.	EMBO Rep. 2020; 21(8):e48882 doi: 10.15252/embr.201948882	9.214	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
11	Veerapathiran S, Teh C, Zhu S, Kartigayen I, **Korzh V,** Matsudaira PT, Wohland T.	Wnt3 distribution in the zebrafish brain is determined by expression, diffusion and multiple molecular interactions.	eLife. 2020; 9:e59489 doi: 10.7554/eLife.59489	8.176	BIOLOGY	Q1
12	Koralewski R, Dymek B, Mazur M, Sklepkiewicz P, Olejniczak S, Czestkowski W, Matyszewski K, Andryianau G, Niedziejko P, Kowalski M, Gruza M, Borek B, Jedrzejczak K, Bartoszewicz A, Pluta E, Rymaszewska A, Kania M, Rejczak T, Piasecka S, Mlacki M, Mazurkiewicz M, Piotrowicz M, Salamon M, Zagozdzon A, **Napiorkowska-Gromadzka A, Bartlomiejczak A**, Mozga W, Dobrzański P, Dzwonek K, Golab J, Nowotny M, Olczak J, Golebiowski A.	Discovery of OATD-01, a First-in-Class Chitinase Inhibitor as Potential New Therapeutics for Idiopathic Pulmonary Fibrosis.	J Med Chem. 2020; 63(24):15527-40 doi: 10.1021/acs.jmedchem.0c01179	6.521	CHEMISTRY, MEDICINAL	Q1

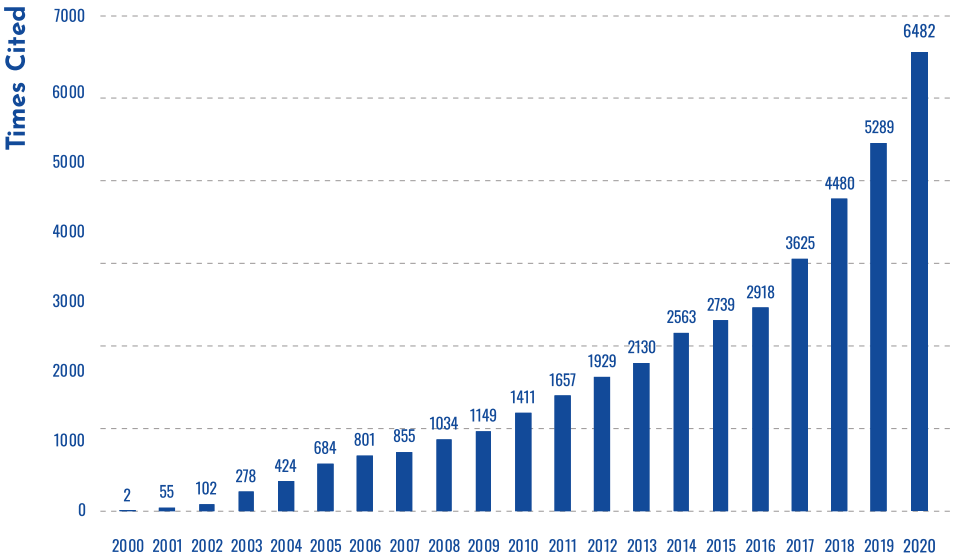
13	Albert M-C, Brinkmann K, **Pokrzywa W**, Günther SD, Krönke M, Hoppe T, Kashkar H.	CHIP ubiquitylates NOXA and induces its lysosomal degradation in response to DNA damage.	Cell Death Dis. 2020; 11(9):740 doi: 10.1038/s41419-020-02923-x	6.486	CELL BIOLOGY	Q1
14	Sojka DR, Gogler-Pigłowska A, Klarzyńska K, **Klimczak M, Zylicz A**, Głowala-Kosińska M, Krawczyk Z, Scieglinska D.	HSPA2 Chaperone Contributes to the Maintenance of Epithelial Phenotype of Human Bronchial Epithelial Cells but Has Non-Essential Role in Supporting Malignant Features of Non-Small Cell Lung Carcinoma, MCF7, and HeLa Cancer Cells.	Cancers (Basel). 2020; 12(10):E2749 doi: 10.3390/cancers12102749	6.433	ONCOLOGY	Q1
15	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 doi: 10.1002/1878-0261.12740	6.287	ONCOLOGY	Q1
16	Adusumilli L, Facchinello N, Teh C, Busolin G, Le MT, Yang H, Beffagna G, Campanaro S, Tam WL, Argenton F, Lim B, **Korzh V**, Tiso N.	miR-7 Controls the Dopaminergic/ Oligodendroglial Fate through Wnt/ β-catenin Signaling Regulation.	Cells. 2020; 9(3):711 doi: 10.3390/cells9030711	5.276	CELL BIOLOGY	Q2
17	Chen L, **Olszewski MB**, Kruithof-de MJ, Snaar-Jagalska BE.	Zebrafish Microenvironment Elevates EMT and CSC-Like Phenotype of Engrafted Prostate Cancer Cells.	Cells. 2020; 9(4):797 doi: 10.3390/cells9040797	5.276	CELL BIOLOGY	Q2
18	Lutz T, **Czapinska H**, Fomenkov A, Potapov V, Heiter DF, Cao B, Dedon P, **Bochtler M,** Xu S.	Protein Domain Guided Screen for Sequence Specific and Phosphorothioate-Dependent Restriction Endonucleases.	Front Microbiol. 2020; 11:1960 doi: 10.3389/fmicb.2020.01960	4.927	MICROBIOLOGY	Q1
19	Lambert AR, Hallinan JP, Werther R, **Głów D**, Stoddard BL.	Optimization of Protein Thermostability and Exploitation of Recognition Behavior to Engineer Altered Protein-DNA Recognition.	Structure. 2020; 28(7):760-775.e8 doi: 10.1016/j.str.2020.04.009	4.827	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
20	Kuciński J, **Chamera S**, **Kmera A**, Rowley MJ, Fujii S, Khurana P, **Nowotny M, Wierzbicki AT.**	Evolutionary History and Activity of RNase H1-Like Proteins in Arabidopsis thaliana.	Plant Cell Physiol. 2020; 61(6):1107-19 doi: 10.1093/pcp/pcaa040	4.799	CELL BIOLOGY	Q2
21	Tomkuvienė M, Ikasalaitė D, **Slyvka A**, Rukšėnaitė A, Ravichandran M, Jurkowski TP, **Bochtler M**, Klimašauskas S.	Enzymatic Hydroxylation and Excision of Extended 5-Methylcytosine Analogues.	J Mol Biol. 2020; 432(23):6157-67 doi: 10.1016/j.jmb.2020.10.011	4.783	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
22	Wilk P, Kuśka K, Wątor E, **Małecki PH**, Woś K, Tokarz P, Dubin G, Grudnik P.	Structural Characterization of Glycerol Kinase fromthe Thermophilic Fungus Chaetomium thermophilum.	Int J Mol Sci. 2020 Dec 16;21(24):9570. doi: 10.3390/ijms21249570	4.653	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
23	McFarlane O, Kozakiewicz M, Kędziora-Kornatowska K, Gębka D, **Szybalska A,** Szwed M, Klich-Rączka A.	Blood Lipids and Cognitive Performance of Aging Polish Adults: A Case-Control Study Based on the PolSenior Project.	Front Aging Neurosci. 2020; 12:590546 doi: 10.3389/fnagi.2020.590546	4.638	GERIATRICS & GERONTOLOGY	Q1
24	Miao Z, Adamiak RW, Antczak M, **Boniecki MJ, Bujnicki JM**, Chen SJ, Cheng CY, Cheng Y, Chou FC, Das R, Dokholyan NV, Ding F, Geniesse C, Jiang Y, **Joshi A**, Krokhotin A, **Magnus M**, Mailhot O, Major F, Mann TH, **Piatkowski P, Pluta R**, Popenda M, Sarzynska J, Sun L, Szachniuk M, Tian S, Wang J, Wang J, Watkins AM, Wiedemann J, Xiao Y, Xu X, Yesselman JD, Zhang D, Zhang Y, Zhang Z, Zhao C, Zhao P, Zhou Y, Zok T, Zyla A, Ren A, Batey RT, Golden BL, Huang L, Lilley DM, Liu Y, Patel DJ, Westhof E.	RNA-Puzzles Round IV: 3D Structure Predictions of Four Ribozymes and Two Aptamers.	RNA. 2020; 26(8):982-95 doi: 10.1261/rna.075341.120	4.549	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q2
25	Szala K, Ogonowska H, Lugowska B, Zmijewska B, **Wyszynska R**, Dmochowska-Boguta M, Orczyk W, Nadolska-Orczyk A.	Different sets of TaCKX genes affect yieldrelated traits in wheat plants grown in acontrolled environment and in field conditions.	BMC Plant Biol. 2020 Oct 29;20(1):496. doi: 10.1186/s12870-020-02713-9	4.494	PLANT SCIENCES	Q1
26	Grabowska W, Achtabowska N, Klejman A, **Skowronek K**, Calka M, Bielak-Zmijewska A.	IQGAP1-dysfunction leads to induction of senescence in human vascular smooth muscle cells.	Mech Ageing Dev. 2020; 190:111295 doi: 10.1016/j.mad.2020.111295	4.315	GERIATRICS & GERONTOLOGY	Q1
27	Kaus-Drobek M, Mücke N, **Szczepanowski RH,** Wedig T, **Czarnocki-Cieciura M**, Polakowska M, Herrmann H, Wysłouch-Cieszyńska A, Dadlez M.	Vimentin S-glutathionylation at Cys328 Inhibits Filament Elongation and Induces Severing of Mature Filaments in Vitro.	FEBS J. 2020; 287(24):5304-22 doi: 10.1111/febs.15321	4.267	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q2
28	Fatima A, Irmak D, Noormohammadi A, Rinschen MM, **Das A**, Leidecker O, Schindler C, Sánchez-Gaya V, Wagle P, **Pokrzywa W**, Hoppe T, Rada-Iglesias A, Vilchez D.	The ubiquitin-conjugating enzyme UBE2K determines neurogenic potential through histone H3 in human embryonic stem cells.	Commun Biol. 2020; 3(1):262 doi: 10.1038/s42003-020-0984-3	4.165	BIOLOGY	Q1
29	Macnar JM, **Szulc N**, Kryś JD, Badaczewska-Dawid AE, Gront D.	BioShell 3.0: Library for Processing Structural Biology Data.	Biomolecules. 2020; 10(3):461 doi: 10.3390/biom10030461	4.082	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q2
30	Cozzuto L, Liu H, **Pryszcz LP**, Pulido TH, Delgado-Tejedor A, Ponomarenko J, Novoa EM.	MasterOfPores: A Workflow for the Analysis of Oxford Nanopore Direct RNA Sequencing Datasets.	Front Genet. 2020; 11:211 doi: 10.3389/fgene.2020.00211	4,007	GENETICS & HEREDITY	Q1
31	Andryianau G, Kowalski M, Piotrowicz MC, Rajkiewicz AA, Dymek B, Sklepkiewicz PL, Pluta E, **Stefaniak F,** Czestkowski W, Olejniczak S, Mazur M, Niedziejko P, Koralewski R, Matyszewski K, Gruza M, Zagozdzon A, Salamon M, Rymaszewska A, Welzer M, Dzwonek K, Golab J, Olczak J, Bartoszewicz A, Golebiowski A.	Benzoxazepine-Derived Selective, Orally Bioavailable Inhibitor of Human Acidic Mammalian Chitinase.	ACS Med Chem Lett. 2020; 11(6):1228-35 doi: 10.1021/acsmedchemlett.0c00092	3.881	CHEMISTRY, MEDICINAL	Q2
32	Hamann L, Szwed M, **Mossakowska M**, Chudek J, Puzianowska-Kuznicka M.	First Evidence for STING SNP R293Q Being Protective Regarding Obesity-Associated Cardiovascular Disease in Age-Advanced Subjects - A Cohort Study.	Immun Ageing. 2020; 17:7 doi: 10.1186/s12979-020-00176-y	3.308	GERIATRICS & GERONTOLOGY	Q2

33	Brzuzan P, Mazur-Marzec H, Florczyk M, **Stefaniak F**, Fidor A, Konkel R, Woźny M.	Luciferase reporter assay for small-molecule inhibitors of MIR92b-3p function: Screening cyanopeptolins produced by Nostoc from the Baltic Sea.	Toxicol In Vitro. 2020; 68:104951 doi: 10.1016/j.tiv.2020.104951	3.049	TOXICOLOGY	Q2
34	Balatskyi VV, **Palchevska OL**, Bortnichuk L, Gan AM, Myronova A, Macewicz LL, Navrulin VO, Tumanovska LV, Olichwier A, Dobrzyn P, Piven OO.	β-Catenin Regulates Cardiac Energy Metabolism in Sedentary and Trained Mice	Life (Basel). 2020 Dec 17;10(12):357. doi: 10.3390/life10120357	2.991	BIOLOGY	Q2
35	de Assis GG, Hoffman JR, **Gasanov EV.**	BDNF Val66Met Polymorphism, the Allele-Specific Analysis by qRT-PCR - a Novel Protocol.	Int J Med Sci. 2020; 17(18):3058-64 doi: 10.7150/ijms.50643	2.759	MEDICINE, GENERAL & INTERNAL	Q2
36	Abdel-Gawad FK, Khalil WKB, Bassem SM, Kumar V, **Parisi C**, Inglese S, Temraz TA, Nassar HF, Guerriero G.	The Duckweed, Lemna minor Modulates Heavy Metal-Induced Oxidative Stress in the Nile Tilapia, Oreochromis niloticus.	WATER. 2020; 12(11):2983 doi: 10.3390/w12112983	2.709	WATER RESOURCES	Q2
37	Wierucki Ł, Kujawska-Danecka H, **Mossakowska M**, Grodzicki T, Błędowski P, Chudek J, Kostka T, Więcek A, Hajduk A, Bandosz P, Zagożdżon P, Wojtyniak B, Zdrojewski T.	Health status and its socio-economic covariates in the older population in Poland: the assumptions and methods of the nationwide, cross-sectional PolSenior2 survey.	Arch Med Sci. 2020; doi: 10.5114/aoms.2020.100898	2.430	MEDICINE, GENERAL & INTERNAL	Q2
38	Babkiewicz E, **Bazała M**, Urban P, Maszczyk P, Markowska M, Gliwicz M.	The effects of temperature on the proxies of visual detection of Danio rerio larvae: observations from the optic tectum.	Biol Open. 2020; 9(7):bio047779 doi: 10.1242/bio.047779	2.307	BIOLOGY	Q2
39	Tudek A, Czerwińska J, Kosicki K, **Zdżalik-Bielecka D**, Ghahe SS, Bażlekowa-Karaban M, Borsuk EM, Speina E.	DNA damage, repair and the improvement of cancer therapy - A tribute to the life and research of Barbara Tudek.	Mutat Res. 2020; 852:503160 doi: 10.1016/j.mrgentox.2020.503160	2.107	GENETICS & HEREDITY	Q2
40	Kupisz-Urbańska M, Broczek K, Galus K, **Mossakowska M**, Marcinowska-Suchowierska E.	Age-related differences in vitamin D status in Polish centenarians compared with 65-year-olds.	Pol Arch Intern Med. 2020; 130:853-9 doi: 10.20452/pamw.15460	1.788	MEDICINE, GENERAL & INTERNAL	Q2

Average IF of journals with IIMCB's publications 2000–2020



Sum of Times Cited per Year

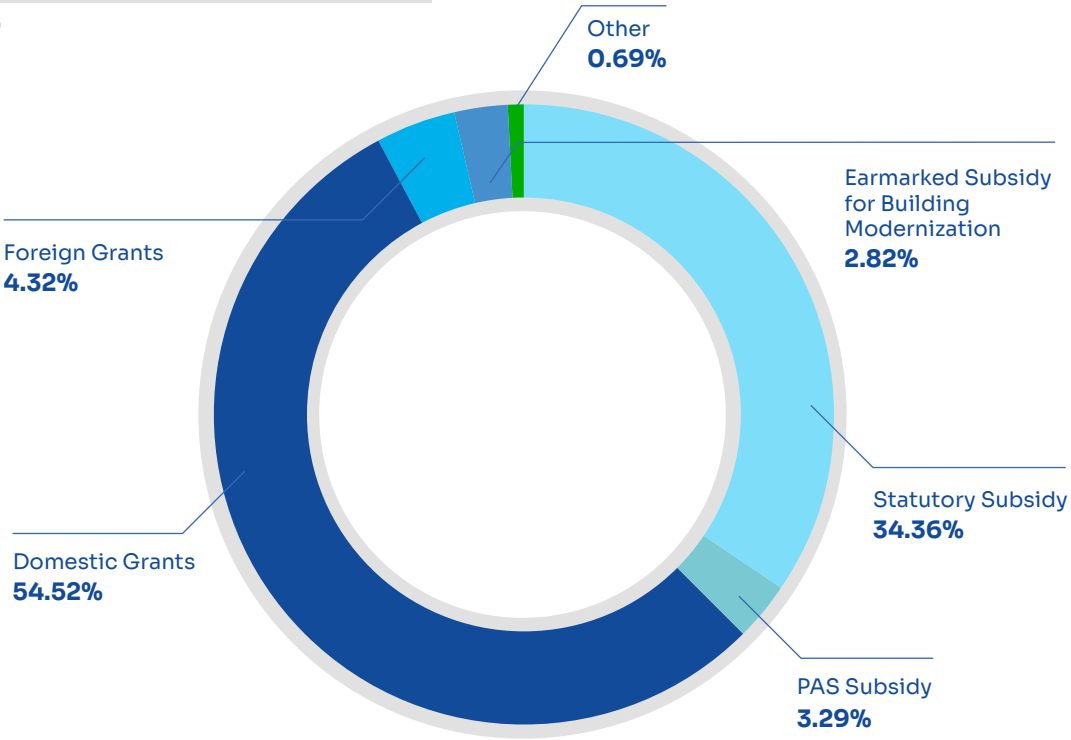


Diversity of Funding

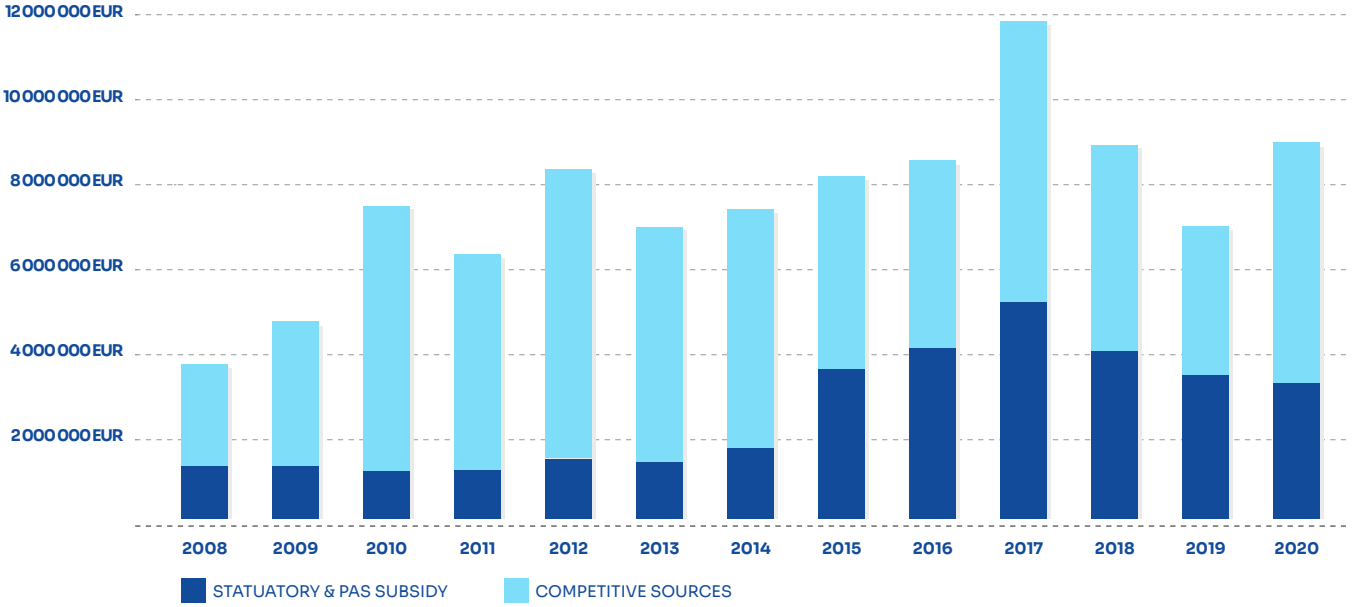
Sources of Funding in 2020

	PLN	EUR*
Statutory Subsidy	14 299 700	3 098 661
PAS Subsidy	1 369 000	296 654
Domestic Grants	22 690 688	4 916 939
Foreign Grants	1 798 306	389 682
Earmarked Subsidy for Building Modernization	1 174 000	254 399
Other	288 897	62 602
Total	**41 620 591**	**9 018 937**

* 1 EUR - 4,6148 @ 31st Dec'2020



Annual Income 2008-2020



Grants running in 2020

57 grants with total awarded funding **108 530 569 PLN**

NATIONAL SCIENCE CENTRE

NATIONAL SCIENCE CENTRE POLAND

36 projects **53 141 133 PLN**

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

- "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**

SYMFONIA

- "Mitochondrial RNA decay and surveillance – comprehensive interdisciplinary studies" (2014/12/W/NZ1/00463); 2 953 248 PLN (total grant budget: 6 879 968 PLN); 2014-2020; **M. Nowotny**; Coordinator: IIMCB

GRIEG (EEA and Norway Grants)

- "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

- "Reconstructing cardiovascular cell lineage evolution, one cell at a time" (2019/35/B/NZ2/02548); 2 631 552 PLN; 2020-2024; **C.L. Winata**
- "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**
- "Linking abnormal Ca^{2+} 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**
- "Shedding new light on genome's dark matter: identification of novel long non-coding RNAs in zebrafish" (2018/31/B/NZ2/01940); 1 896 000 PLN; 2019-2022; **B. Uszczyńska-Ratajczak**
- "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**
- "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
- "Enabling routine and reliable analysis of transport tunnels in proteins" (2017/25/B/NZ1/01307); 1 375 050 PLN; 2018-2021; **J. Brezovsky**
- "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**
- "Biochemical and structural studies of retroviral reverse transcriptases evolution" (2016/21/B/NZ1/02757); 1 145 000 PLN; 2017-2022; **E. Nowak**
- "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**
- "The impact of intracellular distribution and endocytic transport of lymphotoxin beta receptor (LTbetaR) on its signalling" (2016/21/B/NZ3/03637); 996 125 PLN; 2017-2020; **M. Banach-Orłowska**
- "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**
- "Identification of genes controlling brain development through genomic analysis of patients" (2015/19/B/NZ2/01824); 162 960 PLN (total grant budget: 1 539 596 PLN); 2016-2020; **C.L. Winata** (partner); Coordinator: Institute of Mother and Child, Warsaw, Poland

SONATA

- "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**
- "Characterizing the functions and molecular mechanisms of VPS4B action in biology of colorectal cancer (CRC) cells and in CRC pathogenesis" (2016/21/D/NZ3/00637); 791 850 PLN; 2017-2020; **E. Szymańska**
- "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**
- "Uncovering the molecular mechanisms of heart regeneration in zebrafish through profiling of contributing genomic factors" (2016/21/D/NZ2/03843); 556 708 PLN; 2017-2020; **K. Nieścierowicz**
- "Endocytosis of AXL receptor and its role in AXL-mediated signaling" (2015/19/B/NZ3/03270); 762 929 PLN; 2016-2019; **D.P. Zdżalik-Bielecka**

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

- "STIM2 protein oxidative status in mouse brain" (2019/03/X/NZ3/00628); 49 500 PLN; 2019-2020; **O.L. Palchevska**
- "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

10 projects **37 101 887 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** "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); 3 449 541 PLN; 2017-2021; **J.M. Bujnicki**
- 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** "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 **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**
- 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**

EU FRAMEWORK PROGRAMMES & COST

6 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**
- **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**
- **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**

COST

- EPITRAN "European Epitranscriptomics Network" (CA16120); 2017-2021; **J.M. Bujnicki**, **E. Purta**
- MOBIEU "Between Atom and Cell: Integrating Molecular Biophysics Approaches for Biology and Healthcare" (CA15126); 2016-2020; **K. Skowronek**, **R. Szczepanowski**

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); 2017-2021; **J. Jaworski** (partner); Coordinator: Medical University of Warsaw

POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE

3 projects **2 554 100 PLN**

- **Welcome to Poland Programme** "Integrated suport programme for foreigners at IIMCB" (PPI/WTP/2019/1/00054/U/00001); 454 200 PLN; 2019-2021; **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

1 project **1 092 000 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**

SCIENTIFIC LECTURES

Open IIMCB Seminars

Matthew Seaman (Cambridge Institute for Medical Research, UK) *The Retromer complex, what is it? What does it do? and Why is it important?* 02.12.2020

Martin Denzel (Max Planck Institute for Biology of Ageing, Germany) *Mutagenesis screens define new roles of metabolism and protein synthesis in longevity*. 19.11.2020

Ralf Halbach (Roche Diagnostics, Poland) *SARS-CoV-2 & COVID-19. What do we know regarding Testing & Treating this new Virus*. 12.11.2020

Sushma N. Grellscheid (University of Bergen, Norway) *Computational Modelling of Stress-Triggered Cytoplasmic Phase Separation*. 05.11.2020

Torben Heick Jensen (Aarhus University, Denmark) *Nuclear fates of RNA 3'ends*. 22.10.2020

Erik Arner (RIKEN Center for Integrative Medical Sciences, Japan) *Studies of dynamic enhancer usage - from system-wide studies to disease loci*. 15.10.2020

Guillermo Montoya (University of Copenhagen, Denmark) *Dissecting the CRISPR-Cas Type III immune mechanism at atomic detail*. 08.10.2020

Olga Garashchuk (University of Tübingen, Germany) *Counteracting the brain aging*. 24.09.2020

Agata Starosta (Maria Curie-Skłodowska University in Lublin, Poland) *Translation regulation in bacteria: from Bacillus to lake sediments – a story of my lab*. 25.06.2020

Christopher G. Tate (MRC Laboratory of Molecular Biology, UK) *From transporters to receptors: a life in the greasy world of membrane proteins.* 18.06.2020 (organized jointly with Do Science)

Rafał Butowt (Ludwik Rydygier Collegium Medicum in Bydgoszcz Nicolaus Copernicus University in Toruń, Poland) *Why do COVID-19 patients experience olfactory deficits - a potential mechanism and further research perspectives*. 04.06.2020

Alexander Zholos (Taras Shevchenko National University of Kyiv, Ukraine) *Ion channels then and now: from the beginning of life on Earth to the major class of molecular drug targets*. 21.05.2020

Kathrin Thedieck (University of Innsbruck, Austria) *Stress granule proteins meet TSC-mTOR signaling: an unexpected mechanism of lysosomal TSC tethering*. 14.05.2020

Monika Puzianowska-Kuźnicka (Mossakowski Medical Research Institute PAS, Poland) *Aging: why, how and can we do anything about it?* 21.04.2020

Leszek Kaczmarek (Nencki Institute of Experimental Biology, Poland) *Molecular biology to approach the mind*. 05.03.2020

Radislav Sedláček (Czech Centre for Phenogenomics; Institute of Molecular Genetics of the ASCR, Czech Republic) *Czech Centre for Phenogenomics: gateway to comprehensive description of gene functions*. 27.02.2020

Marcin Nowotny (Laboratory of Protein Structure, IIMCB, Poland) *Indirect conformational readout of nucleic acids by proteins*. 28.01.2020

Janusz Bujnicki (Laboratory of Bioinformatics and Protein Engineering, IIMCB, Poland) *Science & Policy In A Complex World*. 21.01.2020

NEW Internal Seminar Series

Nithin Chandran (Bujnicki Lab) *RNA and RNA-protein complexes: A saga of modeling, docking, and benchmarking.* 11.12.2020

Agnieszka Brzozowska (Jaworski Lab) *Effect of AP2 μ2 serine 45 phosphorylation on clathrin-mediated endocytosis*. 11.12.2020

Małgorzata Wiweger (Kuźnicki Lab) *The npc2 zebrafish model for Nieman Pick Type C*. 04.12.2020

Tomasz Kuliński (Dziembowski Lab) *Oncogenic mechanisms of DIS3 mutations in Multiple Myeloma*. 04.12.2020

Humberto Fernandes (Bochtler Lab) *DNA methylation in plants: maintenance and fidelity*. 27.11.2020

Agata Poświata (Miączyńska Lab) *Proximity interactome of AXL provides insights into its intracellular trafficking*. 20.11.2020

Filip Stefaniak (Bujnicki Lab) *Modeling of RNA-Ligand interactions*. 20.11.2020

Bartłomiej Surpeta (Brezovsky Lab) *Understanding molecular determinants of quorum quenching activity in N-terminal serine hydrolases*. 06.11.2020

Evgeny Gasanov (Kuźnicki Lab) *kcng4b potassium channel subunit in the ear mechanosensory system development*. 06.11.2020

Marek Wojciechowski (Bochtler Lab) *Epigenomic landscapes in honeybee caste determination*. 30.10.2020

Vladyslava Liudkovska (Dziembowski Lab) *TENT5 cytoplasmic non-canonical poly(A) polymerases regulate the innate immune response in animals*. 30.10.2020

Deepshikha Malik (Nowotny Lab) *Mechanism of action of Exonuclease and nucleotidyl transferase in mRNA decay*. 23.10.2020

Małgorzata Piechota (Pokrzywa Lab) *Function and regulation of muscular exophers*. 23.10.2020

Rohit Suratekar (Winata Lab) *Dissecting the Cardiac Regulatory Network in Zebrafish using Mathematical Modelling*. 16.10.2020

Jarosław Cendrowski (Miączyńska Lab) *BMP2K kinase controls intracellular membrane trafficking and erythropoiesis in human, mouse and zebrafish*. 16.10.2020

NEW Spotlight Talks

Anna Bajur (King's College London, UK) *A Single-Molecule Approach to Study B Cell Antigen Recognition and Affinity Discrimination*. 23.12.2020

Rohit Suratekar (IIMCB, Poland) *Dissecting the Cardiac Regulatory Network in Zebrafish using Mathematical Modelling*. 25.11.2020

Anna Hojka-Osińska (IIMCB, Poland) *Landscape of Functional Interactions of Human Processive Ribonucleases Revealed by High-Throughput siRNA Screenings*. 18.11.2020

Andrii Kopach (IIMCB, Poland) *From Phase Separation Towards the Mental Health*. 28.10.2020

Science Club

Eugene V. Koonin (National Center for Biotechnology Information, MD, USA) *Trying to understand evolution through the lens of genomics*. 20.11.2020

Lars Juhl Jensen (Technical University of Denmark) *Specialization? Big dirty data*. 30.10.2020

Jack Horner (Chapman University, CA, USA) *Monumental failures and improbable luck led me to a successful career in Paleontology*. 25.09.2020

Steven Benner (Foundation For Applied Molecular Evolution, FL, USA) *The origin of life on Earth, Mars, and beyond*. 03.07.2020

NAWA POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE

The Project is financed by the Polish National Agency for Academic Exchange under the Foreign Promotion Programme

EDUCATION

Centre for Innovative Bioscience Education

Group Members

Head
Patrycja Dołowy, PhD

Project Manager
Katarzyna Tomaszewska, MSc

Laboratory Manager
Aleksandra Olszańska, BEng

The Centre for Innovative Bioscience Education (BioCEN) was established in 2002. 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 the topics of interest. We have professionally equipped laboratories in Warsaw and also deliver our workshops in various places outside of the city, especially in schools in small towns and villages. Our activities are based on sound scientific results. Due to the lockdown in March 2020, BioCEN faced a challenge - how to continue the popularization of science and, at the same time, stay in line with the idea: see, listen, touch, while both the popularizers and the participants had been locked in their homes? This is how the BioCEN on-line Laboratory was created, in the framework of which, demonstration lessons on the zoom platform are being conducted for young people and teachers from all over Poland (and even from all over the world), some of them, with the participation of guests who would be inaccessible under ordinary circumstances. Among them: a NASA employee in California, a Nobel prize winner from Tel Aviv, or scientists working on COVID-19. Interactive video materials are being published, including those made in VR technology, visual and textual ones, as well as BioCAST podcasts. For children aged 6-12, a special educational platform with an interactive educational and science comic strip “Become a Health Hero” was created, and for young people who are over the age of 12, there is a game popularizing science, a type of an escape room one, which comes with science experiments kits.

BioCEN receives financial support from the International Institute of Molecular and Cell Biology in Warsaw (IIMCB), 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, the University of Warsaw Faculty of Biology, and the BioEducation Foundation.

ACTIVITIES

BioCEN workshops cover various areas of life sciences to support the Polish education system and serve to remediate the lack of contact with real experimental sciences in both children and adults. Over the last 18 years, approximately 50 000 students have taken advantage of the workshops that are offered by BioCEN. Over 6 thousand people have participated in real-time on-line classes in 2020, whereas the materials have reached almost 11.4 thousand recipients. At the same time, various BioCEN on-line activities have gathered over 80 thousand views.

During courses that are organized for biology teachers (this year on-line courses), we try to build 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 within their schools. We train and equip them with classroom scenarios and affordable experimental kits, equipment, and reagents that can be used in the school setting. During the pandemic we have created many additional materials which can be helpful both in the home learning environment, as well as later, when used by teachers at schools.

HYBRID CLASSES IN THE EDUCATIONAL LABORATORY AND ON-LINE

In the framework of the projects, which were co-funded by the Education Department of the Capital City of Warsaw: "I Am Experimenting in a Science Laboratory! Biology Laboratory Workshops for the Students of the Warsaw Elementary Schools and High Schools" and "Nematodes, or How Model Organisms Support Scientific Research. Biology Laboratory Workshops for the Warsaw High Schools", new materials and syllabuses have been created. The projects have been conducted in the form of hybrid learning. From September to mid-October 2020, i.e. until the day of school closures, BioCEN conducted laboratory workshops, under the strict sanitary regime. 151 students of the Warsaw elementary schools and high schools, and 12 teachers/guardians of the groups participated in our on-site workshops. At the same time, an on-line program was offered, rich in interactive forms of communication. A prototype of the Rube Goldberg machine was designed, illustrating the course of expression of different genes responsible for the physiological processes, to serve as a teaching aid for the classes "See the DNA", addressed to the youngest participants of the BioCEN courses. The Rube Goldberg machine has been designed in such a way that it can be used both as an interactive aid during laboratory workshops on-site, and during on-line demonstrations as well. For the older participants (high school), an educational kit for studying *Caenorhabditis elegans* nematode has been created. It was designed in two forms - as individually packed small kits which are to be used during interactive classes on-line and are intended for immediate shipment for students, or as a large kit for school use, which will be helpful to biology teachers, after schools reopen.

BIOCEN ON-LINE LABORATORY

Due to the situation caused by the COVID-19 pandemic, BioCEN had to look for new means of fulfilling its mission of popularizing the world of science and the scientific method, through participation in real laboratory experiments. This is why BioCEN on-line Laboratory was established, and in the framework of it, in the internet space that was created especially for this purpose (https://biocen.edu.pl/projekty/laboratorium-biocen-on-line/), interactive educational and popularizing science materials were made accessible as free downloads, free to use, in the form of video demonstrations, including VR, mini-scripts, experience protocols and podcasts. So far, the materials have had approximately 7 500 views. The platform is dedicated to the youth and to adults, it includes interactive materials which are simultaneously published on Facebook, where, altogether, they have had 80 thousand views.

ON-LINE CLASSES VIA ZOOM PLATFORM

In the framework of BioCEN on-line Laboratory, we also offered 1.5 hour classes which took place in real-time, via the zoom platform. Such classes were conducted since April 2009, initially for all those who were interested (signing up was mandatory), in groups ranging from 5 to 25 participants. Starting from September 2020, classes have been offered only for organized groups, on different levels of education. They are conducted in two formulas: (1) in the form of interactive seminars (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 are Medicines Developed, Obesity and Health, Vaccinations, The Beginnings of Eukaryotes, The First Multicellular Organisms) - these classes are available for people 14+, (2) in the form of on-line science demonstrations with elements of the theory, in real-time (for high school - In Vitro Cell Cultures, Synergy, Redox - the Quintessence of Life, Scientific Experiment - It Isn't Difficult!, Melatonine - Polluted with Light, The Microcosm - the World of Bacteria, the Brain - the Orchestra Between the Ears. For elementary schools, the topics included: Synergy, Scientific Experiment - It's Not Difficult! The World of Chemical Reactions, Microcosm - the World of Bacteria). Starting from April 2020, 56 classes have taken place, and 1 318 students have taken part in them, altogether.

BIOCAST PODCAST

BioCAST is a series of podcasts done by the Centre for Innovative BioScience Education, based on talks about biology, and further, about science, prepared according to ideas of Mikołaj Cup and Jan Malinowski, both of whom are from the BioCEN team. Other people from the team are invited to prepare particular episodes, and the experts are guests from the world of science, mostly scientists from partner institutes. The first season consists of 10 episodes, including those that focus on evolution and molecular evolution, and also special episodes. The second season focuses on crucial discoveries in science, which, thanks to the way they are narrated, refer to the most contemporary and burning issues, e.g. the COVID-19 pandemic. BioCAST is available on BioCEN's website, and also on other 8 sites (Apple Podcasts, Google Podcasts, Spotify, RadioPublic, Pocket Casts, Mixcloud, Breaker Podcasts, Anchor Listen Notes). BioCEN has already regular listeners, and continues to gain new ones with each episode. Altogether it has been heard by more than 4 000 listeners. Until September 2020, BioCAST was also released on Radio Spectrum.

THE WORLD IS SMALL - INTERNATIONAL COOPERATION

Together with Division II: Biological and Agricultural Sciences, Polish Academy of Sciences and the Students' Molecular Biophysics Circle, BioCEN organized on-line meetings, entitled "The World is Small in the BioCEN Laboratory". It includes a series of online meetings and lectures delivered by scientists and popularizers of science from different parts of the world, whom we can practically host at our homes, thanks to the on-line form of the classes. Among the invited guests there are those who represent untypical specializations in biology, award-winning researchers, and also some former associates of BioCEN. In 2020, altogether, there were 7 live BioCEN meetings on Facebook, and 5 meetings hosted by the Students' Molecular Biophysics Circle. In total, all the lectures have had 5 582 views.

BE A HEALTH HERO co-operation with GD events

"Be a Health Hero" - an interactive comic strip was created in co-operation with GD Events PR agency. Partners of this project include the Polish Academy of Sciences, Science Festival, Digital Centre, Kosmos Magazine, and the Innowatorium Foundation. It is an educational initiative which helps children understand what is going on at the moment in connection to the COVID-19 epidemiological emergency situation. Every episode is a detective adventure and refers to a different issue - washing hands, studying bacteria and viruses, and how they cause diseases, how to protect yourself against them, what epidemics is, what WHO stands for, what it is responsible for, and many, many other topics. The Centre for Innovative BioScience Education watched over the substantive aspect of the project and supervised devising the experiments, which, accompany particular episodes in the form of video shows, along with home assignments. The access to the platform is free.

FLYING SCIENCE CAFES AND WORKSHOPS FOR REFUGEES on-line

In collaboration with the Council for the Promotion of the Public Understanding of Science (Polish Academy of Sciences) and SPACES Foundation, we organized science cafes for adults and science workshops for children (7-12 years old) who are refugees living in refugee centers in Poland. Workshops concerned DNA and RNA, and ecology.

"Re-action! Lost Experiment" BOARD GAME

"Wejdź w Re-Akcję! Zaginiony eksperyment" ("Re-action! Lost Experiment") is a board game based on the idea of card escape room game, containing an experimental kit, which not only lets the gamers imagine the plot, but really becomes a part of the story, by enabling them to do the experiments with their own hands. The idea was to provide the form of laboratory to our target - teenagers locked in their homes because of pandemic. We wanted to give them the substitute of the laboratory workshops, usually available for them. We decide to create the hybrid of escape room game and experimental kit because this form makes users engage in the story and experiments which are an integral part of the game. The game consists of a deck of cards with riddles and quizzes, which allows players to participate in exciting laboratory investigation and experimental kit of reagents and tools to plan and conduct about 10 biochemical experiments. As in a real laboratory there is more than one way to get to the final result and some of the ways are blind paths. The game had its premiere on December 10, 2020 and the releasing of the game was preceded by planning and improving the plot and experiments based on research of board games market and 3 months of testing the prototype of the game with the groups of potential stakeholders. We have tested the gameplay and the reception of science experiments within the game. The experimental kit was also tested to check for safety according to the EU directives.

MACIEJ GELLER SCIENCE THEATRE

BioCEN has called into being Prof. Maciej Geller Science Theatre. Following the example of anatomical or alchemic theatres in the past, its idea is to merge features that are important in science and theatre, i.e. the scientific experiment, emotions and presence, distinguishing this kind of theatre from an ordinary spectacle or a science fair. The patron of the Science Theatre, the late Prof. Geller, was the co-creator of the Warsaw Science Festival - the first science festival in Poland, and he was one of the most recognizable Polish popularizers of science. Prof. Geller was awarded for his work several times, and that includes Professor Hugo Steinhaus Award in 2013. He inspired the present director of BioCEN with the idea of this kind of theatre. The first on-line science spectacle, based on the screenplay written by Dr. Patrycja Dołowy and Prof. Paweł Golik, was titled ”Linia boczna” (”The Sideline”) and directed by Paweł Passini. The screenplay is based on the lecture that concerned the genetic evolution of a human being, William Golding’s novella “Spadkobiercy” (”The Inheritors”), and also collected reflections of the participants who took part in the 10 on-line meetings with the scientists. Among the invited guests there were: Prof. Paweł Golik, Dr. Marta Witkowska, science journalist Tomasz Ulanowski, Prof. Magdalena Fikus, Dr. Wojciech Dragan, Dr. Mikołaj Golachowski, Prof. Krzysztof Dołowy, Dr. Tomasz Zajkowski, Dr. Urszula Zajączkowska. The spectacle ”Linia boczna” (”The Sideline”) is an attempt to take a closer look at the evolution of a human being. Just like Andreas Vesalius in the 16th century anatomical theatre, a couple of science commentators (members of the BioCEN team: Mikołaj Cup and Jan Malinowski) expose us to numerous possibilities of a scientific approach to a story that takes place in prehistoric times, about a meeting between *Homo sapiens* and *Homo neandertalensis.* Ilona Janyst and Paweł Janyst, professional theatre and tv actors, played in this spectacle. It was made with the use of VR technology. The premiere took place on the Youtube platform. The spectacle can be watched free of charge on the Vimeo platform in the VR mode, or it can be downloaded for special 3D VR goggles. This task was co-funded from the resources of the Ministry of Science and Higher Education “The Social Responsibility of Science”.

PROFESSIONAL TRAINING FOR TEACHERS AND EDUCATORS

One of our main goals is to improve the teaching skills of science educators who work at all levels of education. In 2020, BioCEN together with the Nencki Institute of Experimental Biology, Polish Academy of Sciences, in Warsaw organized an annual symposium for teachers and educators on-line on zoom platform.

19th Educational Symposium for Biology Teachers on-line

This annual symposium has become one of our most important events. Unlike in previous years, this year’s symposium could only be offered in the on-line form. On one hand, this year’s event lacked the important part in which scientists meet with teachers and exchange their experiences, and then teachers exchange their experiences among themselves. On the other hand, its accessibility increased enormously, thanks to this free and convenient form. 184 people took part in this year’s symposium, thus, three times as many when compared to the previous years.

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 the Nobel prizes in Physiology and Medicine.

The program of Symposium included workshops for teachers. Wiktor Niedzicki shared his secrets of presentations on-line and Anna Pietruszka-Dróżdż taught how to cope with stress related to one’s professional life, especially in times of the pandemic.

EXPERIMENTAL KITS AND OTHER SCIENTIFIC TOOLS

For those who are unable to attend our workshops, we provide alternatives. 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 a particular experiment either at school or at home. To date, 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”. As such, we also produce high-quality, and genuine BioCEN educational board games for schools:

- By the trails of evolution
- Dare to assemble your cell

AWARDS AND HONORABLE MENTIONS

BioCEN team was nominated for the title of “The Popularizer of the Year”, organized by PAP Nauka w Polsce and the Ministry of Education and Science and became the finalist of the competition in the category “Team”.

biocen.edu.pl

RESEARCH SUPPORT UNITS

Research Support Units

Self-contained position for strategic support

Chief Specialist
Ewa Jack-Górska

Human Resources Unit

Head
Katarzyna Fiedorowicz

Deputy Head
Agnieszka Faliszewska

Senior Specialists
Monika Nowicka
Beata Tkacz

Specialists
Monika Domańska-Paśko
Barbara Dusik
Aneta Walas
Adam Zieliński

Scientific Coordination Unit

Chief Specialist
Agnieszka Wagner-Ziemka

Senior Specialist
Katarzyna Marszałek

Specialist
Iwona Pilecka

PR Unit

Senior Specialists
Daria Goś
Magdalena Krupa

Grants Office

Head
Dorota Libiszowska

Deputy Head
Marcin Ogonowski

Senior Specialists
Alexia Danyłow
Justyna Mitan-Piłat
Katarzyna Nakielska
Agata Skaruz
Justyna Szopa

Financial and Accounting Unit

Head
Hanna Iwaniukowicz

Deputy Head
Agnieszka Kuna

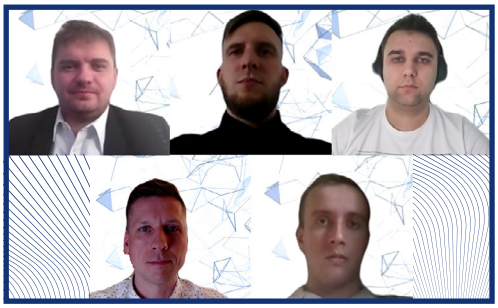
Senior Specialists
Małgorzata Bytner
Renata Knyziak

Specialist
Ewelina Duda

Public Procurement Unit

Senior Specialist
Jakub Wielgus

Specialists
Agnieszka Potęga
Agata Szulim



IT Unit

Head
Paweł Kobylarz

Senior Specialist
Piotr Świsłowski

Specialists
Łukasz Munio
Jakub Skaruz
Michał Taperek

Operations Unit

Specialists
Katarzyna Celińska-Zmorzyńska
Andrzej Cudny

Junior Specialists
Magdalena Dąbrowska
Mariola Sacharuk

Building Maintenance
Alicja Goldberg
Adam Kucharski

2020 TIMELINE

January 1

The IIMCB **joins the EU-LIFE consortium** as the **first Polish member**

January 9

Opening ceremony of the **Laboratory of RNA Biology - ERA Chairs Group** at the IIMCB

January 14

The IIMCB becomes part of the **Polish Roadmap for Research Infrastructures** which brings together 70 infrastructures with the highest potential for scientific excellence and significant research

February 3-5

The **21st plenary meeting of the European Commission's Group Chief Scientific Advisors** coordinated by J.M. Bujnicki at the IIMCB

February 28

M. Wiweger's prelection on **Rare Disease Day: Patients in Art, Science and Medicine**

March 9-13

Workshops for talented youth (mentees of the Polish Children's Fund) at IIMCB

March 13

M. Nowotny joins the **H2020 consortium** within the **EXSCALATE4CoV** project to combat the coronavirus pandemic

April 20

The IIMCB participates in organizing and launching **a new COVID-19 diagnostic laboratory** at the Central Clinical Hospital of the Warsaw Medical University

April 27

A. Dziembowski and W. Pokrzywa are **laureates of the GRIEG call co-funded from the Norwegian Financial Mechanism**

September 1

Start of A. Dziembowski's GRIEG project **"The impact of cytoplasmic polyadenylation on local translation in neurons"**

September 10-11 & October 5-6

PhD Students' Annual Report Session - online presentations by 46 students

September 22

Lectures "Before a new pastille is made" at the **Warsaw Festival of Science** by M. Czarnocki-Cieciura, K. Skowronek, F. Stefaniak and R. Szczepanowski

September 24

U. Białek-Wyrzykowska prelection at the **UNESCO Regional Consultation on Open Science for Eastern Europe**

October 6

The IIMCB takes over the presidency of the **Ochota Biocentre**

November 19-20

M. Miączyńska prelection at the **Polish-American Innovation Bridge & IX Meet-Up TOP 500 Innovators**

December 2

The IIMCB **Inter-lab Projects Report Session**

December 3

The IIMCB is a patron of the **"Get Re-Action" game, a BioCEN initiative**

December 15

The IIMCB is in the **PASIFIC Fellowship Programme**

December 20

J. Kuźnicki appointed **new President of the Council of the National Science Centre**

Contents

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