ANNUAL REPORT 2019

International Institute of Molecular and Cell Biology in Warsaw

Director
Marta Miączyńska

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

Chair of the International Advisory Board
Walter Chazin

International Institute of Molecular and Cell Biology in Warsaw
4 Ks. Trojdena Street
02-109 Warsaw, Poland

(48 22) 59 70 700

(48 22) 59 70 715

secretariat@iimcb.gov.pl

www.iimcb.gov.pl

IIMCB_Poland

IIMCBinWarsaw

MIBMiKwWarszawie

IIMCB

IIMCBtv

Edited by **Agnieszka Wagner-Ziemka** and **Daria Goś**

Designed by **MÏCU™ Studio**

Photos by **Kryspin Andrzejewski** (pages 3a, 3b, 3d, 3e, 5, 8a, 8b, 9, 13, 17, 25, 29, 33, 36, 37, 40, 41, 45, 48, 49, 53, 57, 58, 61, 96a, 96c, 97b), **Cezary Balcerzak** (65), **Katarzyna Banasiak** (99), **Michał Bazała** (63, 64, 90/91), **Tomasz Bielecki** (52), **Patrycja Dołowy** (94c, 94d), **Małgorzata Figiel** (69a), **Daria Goś** (68b, 94b), **Magdalena Krupa** (62), **Kamil Kobyłecki** (65), **Aleksandra Kot-Horodyńska** (94a), **Katarzyna Marszałek** (68a), **Mariusz Marszałek** (12, 20, 24, 28, 32, 44, 48, 49, 92, 96b, 97a, 97c), **Roman Szczepanowski** (6), **Pankaj Thapa** (16), **Magdalena Wiśniewska-Krasińska/FNP** (8c, 21)

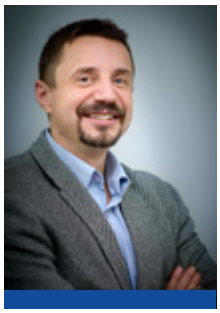
This report is part of the MOSaIC project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 810425

ISBN 978-83-944616-4-5

Directors

Marta Mączyńska

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

Director's note

The year 2019 was an intense and productive time at IIMCB, with several important developments in our research program, internal organization, international recognition, and arrangements for our new building.

In 2019, the IIMCB scientific community has grown. We welcomed Prof. Andrzej Dziembowski and his coworkers who joined our institute to form our 11th research group, the Laboratory of RNA Biology. Prof. Dziembowski won a competitive ERA Chair position to establish his new laboratory thanks to EU Horizon 2020 funds within the project entitled "MOlecular Signaling in Health and Disease - Interdisciplinary Centre of Excellence" (MOSaIC). He is an internationally renowned expert in the field of RNA metabolism and function, and his studies span multiple levels of biological organization, from molecules to model organisms, including the mouse. Some IIMCB groups have already been investigating certain aspects of RNA biology, and Prof. Dziembowski's expertise will greatly complement our research portfolio, creating additional synergy between laboratories.

IIMCB scientists reported the results of their studies in 57 publications in 2019. Among the most notable discoveries are those related to molecular mechanisms of human diseases, such as tuberous sclerosis or colorectal cancer, and atomic structures of DNA-modifying enzymes (see section on best paper awards in this Annual Report). The breadth of our findings reflects the diversity of interests of our scientists and their mastery of a wide range of research models and technologies.

An important landmark in the development of IIMCB is the right to confer PhD degree in biology that we obtained in 2019, following the introduction of the new Law on Higher Education and Science in Poland. This allowed us to establish, together with eight other research institutions, the Warsaw PhD School in Natural and BioMedical Sciences (Warsaw-4-PhD). This interdisciplinary school commenced its activities on October 1, 2019, to educate doctoral students in four scientific disciplines: biology, medicine, chemistry, and physics.

The recent growth of IIMCB to 260 staff members has required adjustments in our internal policies and regulations. They were reviewed and updated in 2019, thanks to the efforts of the entire administration team. Particularly noteworthy is our comprehensive Human Resources Strategy for 2019-2024, which defines the goals and principles of HR management for all employees. The realization of this strategy is supported by two institutional grants from the Polish National Agency for Academic Exchange (NAWA) to fund an integrated support program for foreigners at IIMCB and international promotion of the institute.

It was very satisfying to learn that our implementation of the Human Resources Strategy for Researchers at IIMCB was rated as excellent by experts appointed by the European Commission who visited us in October 2019. In their report, they praised our exemplary internal regulations and a very good work environment, collaborations, and top-down and bottom-up communication within the institute. The report of the experts stated, "The spirit and enthusiasm everybody (researchers, administration and technical staff) puts in being a part of this institution is one of their major strengths. (…) As a whole, the organization lives the culture it promotes." Reading such words would make every director proud of their team of co-workers!

This external opinion is well justified by numerous activities that were undertaken in 2019 by all of our groups of scientists. Our PhD students organized their first Young Scientists Conference on Molecular and Cell Biology, which was a great success and this initiative will be continued in 2020. Our postdocs have initiated several voluntary charity activities, demonstrating the social responsibility of our scientists. Our laboratory leaders continue to serve in various policy-making and advisory capacities in Poland, including Janusz Bujnicki in the Committee for Science Evaluation, Jacek Kuźnicki in the Council of National Science Center, and Marcin Nowotny in the Science Policy Committee, just to name a few. In 2019, several laboratory leaders became members of the Polish Academy of Sciences, Academia Europaea, and European Molecular Biology Organization.

At the international level, on January 1, 2020, IIMCB became the first Polish member of the EU-LIFE consortium. Together with IIMCB, the EU-LIFE alliance now has 14 members which are leading research institutes from 14 European countries. The mission of EU-LIFE is to support and strengthen European research excellence, share knowledge, and nurture talent. EU-LIFE is also a stakeholder in the EU policy dialog. As a result of a multi-step application process, the EU-LIFE Board of Directors considered that IIMCB was perfectly fulfilling the EU-LIFE eligibility and selection criteria. The Chair of EU-LIFE, Rene Medema from The Netherlands Cancer Institute, stated, "We are pleased to welcome a partner that actively shares our values, our commitment to excellence in research, and is engaged to act as an open and constructive member of the EU-LIFE alliance – and is enthusiastic about the collaborative possibilities offered by the alliance." IIMCB is honored to join EU-LIFE. Sharing and drawing on mutual experiences of the member institutes will further inspire our quest to achieve excellence in research and institutional organization.

Finally, 2019 opened a real perspective for our future expansion of activities. The Polish government made the decision to finance a new building for IIMCB and granted funds in the form of state bonds toward this goal. Having identified an appropriate site to construct a new building, we have begun land purchase negotiations. We hope to finalize this transaction in 2020. We are already working on a general concept of our new, larger building. In addition to laboratories, it will also house expanded and restructured core facilities that were included in the 2019 Polish Roadmap for Research Infrastructures. Although it will take a few years to complete, but we are well on our way toward realizing our long-standing goal to have a modern and spacious site for IIMCB!

IIMCB's success continues to be possible because of our dedicated staff, both scientists and administrative employees. I sincerely thank everyone at IIMCB for their hard and diligent work.

The 2019 Annual Report details our latest scientific discoveries, publications, ongoing projects and achievements of IIMCB as a community and an institution. We promise to maintain our high research and operational standards, so watch out for updates on IIMCB activities in 2020.

Marta Mączyńska

Warsaw, February 2020

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 IIMCB DEFINED IN THREE MAIN AREAS

Scientific quality

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

Institutional development and partnerships

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

Organizational culture

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

International Advisory Board

International Advisory Board meeting, 22.03.2019, IIMCB, Warsaw, Poland

2018-2021 TERM

Thomas Braun Max Planck Institute for Heart and Lung Research, Germany
Bernd Bukau University of Heidelberg, Germany
Jo Bury Vlaams Instituut voor Biotechnologie, Belgium
Walter Chazin (Chair) Vanderbilt University, USA
Aaron Ciechanover Technion - Israel Institute of Technology, Israel
Urszula Hibner Institut de Génétique Moléculaire de Montpellier, France
Artur Jarmołowski Adam Mickiewicz University, Poland
Peter Sicinski Harvard Medical School, USA
Lilianna Solnica-Krezel Washington University School of Medicine, USA
Anne Spang University of Basel, Switzerland
Angelo Azzi (Permanent Advisor) Tufts University, USA

2020-2023 TERM

Reinhard Jahn Max Planck Institute for Biophysical Chemistry, Germany
Caroline Kisker University of Würzburg, Germany
Barry Stoddard Fred Hutchinson Cancer Research Center, USA

Human Resources Strategy for Researchers (HRS4R)

HR EXCELLENCE IN RESEARCH

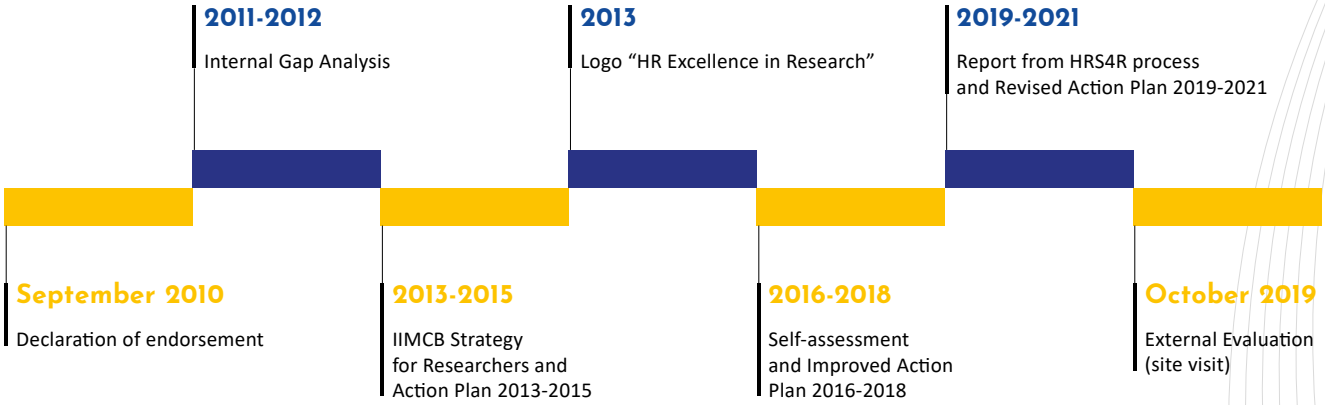
In 2013 International Institute of Molecular and Cell Biology in Warsaw was granted "HR Excellence in Research"- a prestigious logo awarded by the European Commission. It acknowledges progress in aligning research institutions' HRS4R policies with the principles set out in the European Charter for Researchers and Code of Conduct for the Recruitment of Researchers (Charter & Code). The "HR Excellence in Research" award obliges our Institute to continue improving human resources (HR) strategies alongside the principles set forth in the Charter & Code.

HR WORKING GROUP

Agnieszka Faliszewska, MSc HR Working Group Leader
Jacek Jaworski, PhD, Professor Representative of Directors and Lab Leaders
Małgorzata Figiel, PhD Representative of Postdoctoral Researchers
Elżbieta Purta, PhD Representative of Senior Researchers and Researchers
Gabriela Jędruszewska, MSc Representative of PhD Students
Katarzyna Fiedorowicz, MSc Head of Human Resources Unit
Katarzyna Marszałek, MSc Scientific Coordination Senior Specialist
Dorota Libiszowska, MSc Head of Grants Office
Daria Goś, MSc PR Senior Specialist (until December 2019)
Magdalena Krupa, MSc PR Senior Specialist (from December 2019)

HR EXCELLENCE IN RESEARCH

IIMCB in HRS4R process



SITE VISIT

October 2019 was the time for evaluation of implementation of HRS4R process by external experts. They met on site with representatives of all employee groups, both scientists and administration. The experts expressed their appreciation towards the IIMCB's efforts in implementing the principles of the Charter for Researchers and the Code of Conduct for the Recruitment of Researchers. They noted excellent working conditions and highly efficient internal communication at IIMCB. In their final report they wrote:

> According to their mission, the institution strives for excellent research - HR development lies at the heart of their strategy as a very important means to reaching this goal. The implementation of the HRS4R is excellent and perfectly embeds the HR strategy in the overall strategy of the institution. We congratulate the HR working group for their outstanding work.

> The institution offers a very good environment for researchers, not only through the core facilities but the spirit and enthusiasm everybody (researchers, administration and technical staff) puts in being part of this institution. This is one of their major strengths.

> Researchers feel very well supported by the administration departments, especially new researchers coming from abroad are very well taken care of. A completely new HR department has been built up, already trying to prepare for the planned growth during the next years.

> Internal collaboration, following the idea of the organisation as an "organism" (quoting the Director), is working on all levels and several mechanisms are in place to further develop it.

> As a whole, the organisation lives the culture it promotes.

> Bottom-up ideas from all levels (from PhD students to lab leaders) are heard and promoted by the hierarchy, made into institutional measures and implemented where possible. People feel that an open communication is possible at all times. PhD students are very much part of the institution.

> On the other hand, strategic plans are communicated top-down in a clear and transparent way, so that everybody feels informed.

> Input and feedback from the International Advisory Board is embedded in a useful and helpful way.

> Stress is put on building relationships and trust which last over time.

> A new appraisal and evaluation system is being introduced which is at the same time very "simple" and very thoughtful and inclusive (researchers and administrative staff are considered at the same time), their focus is on attitude and contribution to the institutional goals rather than on some formal criteria.

> The institution succeeds in creating synergies between the implementation of the HRS4R and other national and international projects. This provides additional leverage to the strategy.

Horizon 2020 ERA Chairs project at IIMCB

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

GOALS

Establishment of the ERA Chairs Laboratory headed by an outstanding, mid-career scientist with excellent leadership skills.

Structural improvements in science management and HR activities, according to best international standards.

GENERAL INFORMATION

Project Coordinator
Prof. Jacek Kuźnicki

Implementation period
2018-2023

Funding
2 498 887,50 EUR

Reference call
H2020 WIDESPREAD-03-2017

PROJECT WEBSITE

www.iimcb.gov.pl/pl/research/era-chairs-mosaic

ACTIVITIES IN 2019

RECRUITMENT OF THE ERA CHAIRS GROUP LEADER AND NEW GROUP MEMBERS

LEADER: MARCIN NOWOTNY

- the ERA Chairs Group Leader position was awarded to Prof. Andrzej Dziembowski
- Prof. Dziembowski was selected among 18 candidates from 11 countries, including top candidates from: Germany, Singapore and United Kingdom
- the ERA Chairs Group Leader joined IIMCB on 1.12.2019 as the Head of the new Laboratory of RNA Biology
- the new Group counts 19 staff members

R&I ACTIVITIES OF THE ERA CHAIRS RESEARCH GROUP

LEADER: ANDRZEJ DZIEMBOWSKI

Our ERA Chairs Laboratory of RNA Biology focuses on post-transcriptional regulation of gene expression. We ask questions: How processive ribonucleases through RNA degradation shape the transcriptomes of mammalian cells? How poly(A) and poly(U) polymerases regulate protein production?

In 2019 we have just started our activities at IIMCB. We:

- have been implementing the research grants: TEAM & TEAM-TECH Core Facility, FNP and MAESTRO & SONATINA, NCN
- brought in the Mouse Genome Engineering Facility which will foster research based on animal models at our Institute

EFFECTIVE SCIENCE MANAGEMENT
LEADER: URSZULA BIAŁEK-WYRZYKOWSKA

- IIMCB received the right to award PhD degrees in biology
- we established the Warsaw PhD School in Natural and BioMedical Sciences [Warsaw-4-PhD] in cooperation with 8 other scientific institutes
- we introduced regulations and started to formally recognize scientific degrees awarded by foreign academic institutions as equivalent to Polish ones
- we introduced the Open Access Policy and established a role of institutional Data Steward
- we actively support our researchers in implementation of ethical guidelines and best practices
- we verified the commercialisation potential of selected IIMCB inventions

IMPROVED HR MANAGEMENT
LEADER: KATARZYNA FIEDOROWICZ

We introduced:

- HR Strategy for IIMCB
- new evaluation system for scientists and administration
- professional support in recruitment processes
- comprehensive assistance to foreign employees and received funding from NAWA under Integr@IIMCB grant to strenghten multicultural integration at IIMCB
- system of trainings for IIMCB staff within which 150 participants were trained in management, career development and soft skills

DISSEMINATION AND COMMUNICATION
LEADER: DARIA GOŚ

We communicated MOSalC:

- at the MOSalC kick-off meeting, March 2019
- in more than 63 posts and special actions in Social Media:
  - > Facebook:
    16 466 total reach / 2 072 total engagements
  - > Twitter:
    8 700 total reach / 164 total reach
  - > LinkedIn:
    7 717 total reach / 520 total engagements
- on events posters, workshops agendas, roll-ups, presentations, in e-mail correspondence and at networking events such as R&I Info Days in Brussels

#MOSalC #ERAChairs #Horizon2020 #NewLaboratoryAtIIMCB #MOSalCdiary #DziembowskiLab

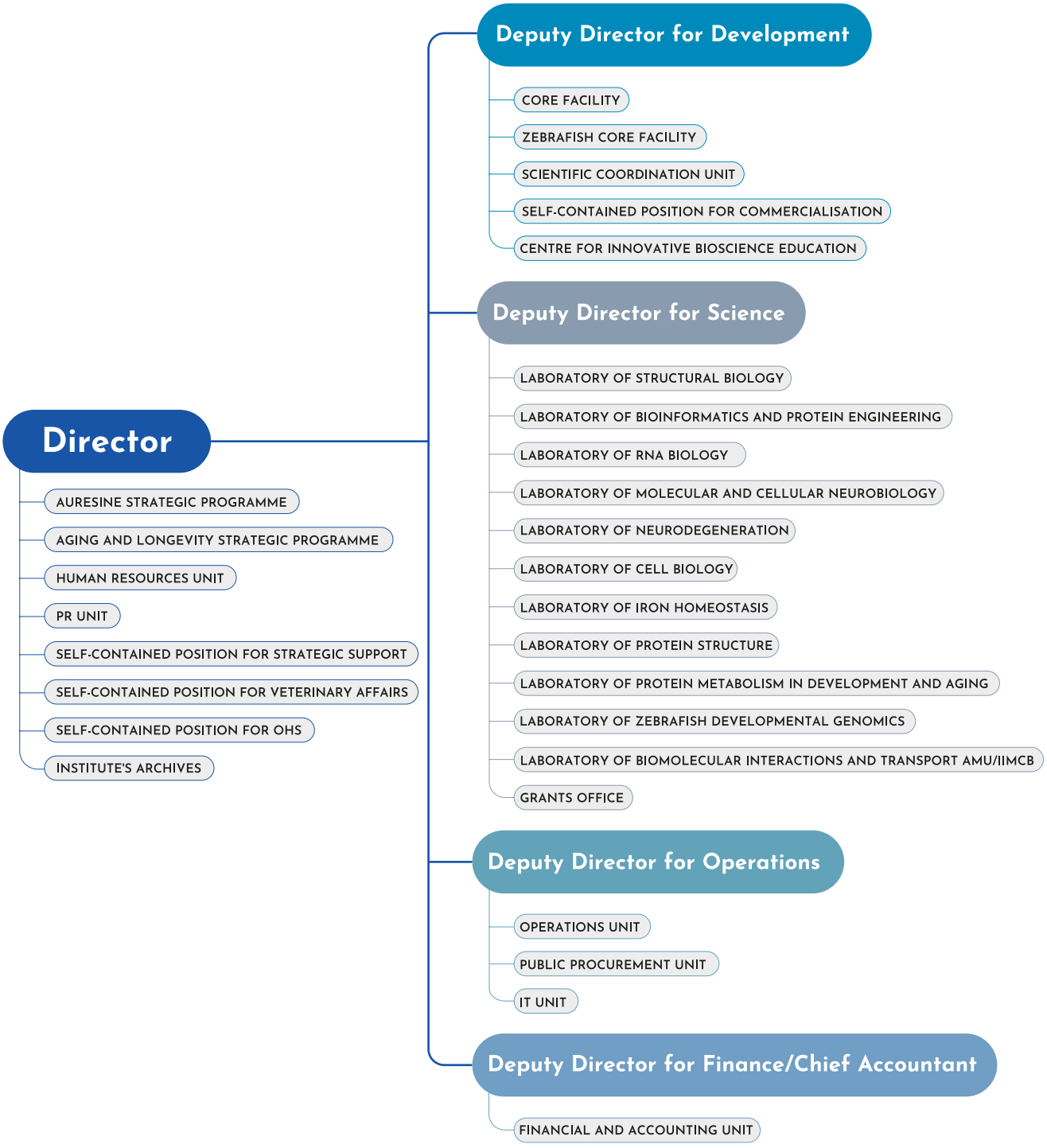
MANAGEMENT
LEADER: DOROTA LIBISZOWSKA

We ensured efficient management of MOSalC project by:

- supporting MOSalC team members in their daily work
- organizing quarterly Project Committee Meetings
- communicating MOSalC's progress to the EU Project Officer
- overseeing the MOSalC's finances
- ensuring that the ERA Chairs recruitment and the setup of the new Laboratory fulfilled MOSalC's requirements
- assisting Prof. Dziembowski in organizational matters at the beginning of his employment at IIMCB

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

Organizational structure



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
Charles Weige, PhD
Marek Wojciechowski, PhD

PhD Students
Igor Helbrecht, MSc
Magdalena Klimczak, MSc
Norbert Osiński, MSc
Michał Pastor, MSc
Abhishek Pateria, MSc
Dominik Rafalski, MSc
Anton Slyvka, MSc
Anna Stroynowska-Czerwińska, MSc
Katarzyna Szafran, MSc

Other co-worker
Anna Fedenko, MSc

Lab Technician
Agnieszka Olszewska (part-time)

Laboratory Support Specialist
Ewelina Borsuk, MSc (part-time)

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 EMPLOYMENT

2011-Present	Professor, Head of Laboratory of Structural in 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
2012	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

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

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

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

Czapinska H, Siwek W, Szczepanowski RH, Bujnicki JM, **Bochtler M,** Skowronek KJ. Crystal Structure and Directed Evolution of Specificity of NlaIV Restriction Endonuclease. ***J Mol Biol***, 2019; 431(11):2082-94

Mitkowski P, Jagielska E, Nowak E, Bujnicki JM, Stefaniak F, Niedziałek D, **Bochtler M,** Sabała I. Structural bases of peptidoglycan recognition by lysostaphin SH3b domain. ***Sci Rep***, 2019; 9(1):5965

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

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

Stroynowska-Czerwinska A, **Piasecka A**, **Bochtler M**. Specificity of MLL1 and TET3 CXXC domains towards naturally occurring cytosine modifications. ***Biochim Biophys Acta Gene Regul Mech***, 2018; 1861(12):1093-101

Piasecka A, **Czapinska H**, Vielberg MT, Szczepanowski RH, Kiefersauer R, Reed S, Groll M, **Bochtler M**. The Y. bercovieri Anbu crystal structure sheds light on the evolution of highly (pseudo)symmetric multimers. ***J Mol Biol***, 2018; 430(5):611-627

Bochtler M, Mizgalska D, Veillard F, Nowak ML, Houston J, Veith P, Reynolds EC, Potempa J. The Bacteroidetes Q-Rule: Pyroglutamate in Signal Peptidase I Substrates. ***Front Microbiol***, 2018; 9:230

Bennabi I, Quéguiner I, **Kolano A**, Boudier T, Mailly P, Verlhac MH, Terret ME. Shifting meiotic to mitotic spindle assembly in oocytes disrupts chromosome alignment. ***EMBO Rep***, 2018; 19(2):368-81

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

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

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

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

Wojciechowski M, **Rafalski D**, Kucharski R, **Misztal K**, Maleszka J, **Bochtler M**, Maleszka R. Insights into DNA hydroxymethylation in the honeybee from in-depth analyses of TET dioxygenase. ***Open Biol***, 2014; 4(8):140110

Kazrani AA, **Kowalska M**, **Czapinska H**, **Bochtler M**. Crystal structure of the 5hmC specific endonuclease PvuRts1I. ***Nucleic Acids Res***, 2014; 42(9):5929-36

Wojciechowski M, **Czapinska H**, **Bochtler M**. CpG underrepresentation and the bacterial CpG-specific DNA methyltransferase M.MpeI. ***Proc Natl Acad Sci USA***, 2013; 110(1):105-110

Siwek W, **Czapinska H**, **Bochtler M**, Bujnicki JM, **Skowronek K**. Crystal structure and mechanism of action of the N6-methyladenine-dependent type IIM restriction endonuclease R.DpnI. ***Nucleic Acids Res***, 2012; 40(15):7563-72

Antonczak AK, Simova Z, Yonemoto IT, **Bochtler M**, Piasecka A, **Czapinska H**, Brancale A, Tippmann EM. Importance of single molecular determinants in the fidelity of expanded genetic codes. ***Proc Natl Acad Sci USA***, 2011; 108(4):1320-5

Sokolowska M, **Czapinska H**, **Bochtler M**. Hpy188I-DNA pre- and post-cleavage complexes - snapshots of the GIYYIG nuclease mediated catalysis. ***Nucleic Acid Res***, 2011; 39(4):1554-64

Firczuk M, **Wojciechowski M**, **Czapinska H**, **Bochtler M**. DNA intercalation without flipping in the specific ThaI-DNA complex. ***Nucleic Acid Res***, 2011 39(2):744-54

Sokolowska M, **Czapinska H**, **Bochtler M**. Crystal structure of the ββα-Me type II restriction endonuclease Hpy99I with target DNA. ***Nucleic Acid Res***, 2009; 37(11):3799-810

Szczepanowski RH, Carpenter MA, **Czapinska H**, Zaremba M, Tamulaitis G, Siksnys V, Bhagwat AS, **Bochtler M**. Central base pair flipping and discrimination by PspGI. ***Nucleic Acids Res***, 2008; 36(19):6109-17

Tamulaitis G, Zaremba M, **Szczepanowski RH**, **Bochtler M**, Siksnys V. How PspGI, catalytic domain of EcoRII and Ecl18kI acquire specificities for different DNA targets. ***Nucleic Acids Res***, 2008; 36(19):6101-8

Kaus-Drobek M, **Czapinska H**, **Sokolowska M**, Tamulaitis G, **Szczepanowski RH**, Urbanke C, Siksnys V, **Bochtler M**. Restriction endonuclease MvaI is a monomer that recognizes its target sequence asymmetrically. ***Nucleic Acids Res***, 2007; 35(6):2035-46

Bochtler M, **Szczepanowski RH**, Tamulaitis G, Grazulis S, **Czapinska H**, Manakova E, Siksnys V. Nucleotide flips determine the specificity of the Ecl18kI restriction endonuclease. ***EMBO J***, 2006; 25(10):2219-29

Grazulis S, Manakova E, Rössle M, **Bochtler M**, Tamulaitiene G, Huber R, Siksnys V. Structure of the metal-independent restriction enzyme BfiI reveals fusion of a specific DNA-binding domain with a nonspecific nuclease. ***Proc Natl Acad Sci USA***, 2005; 102(44):15797-802

DESCRIPTION OF CURRENT RESEARCH

The prevalent DNA modification in eukaryotes is C5-cytosine methylation. Biophysically, this modification stabilizes double-stranded DNA. Evolution has built on and enhanced this tendency to utilize C5-methylation, which predominantly occurs in the symmetric CpG context for transcription control. Consistent with its biophysical effects, methylation in transcription control structures (e.g., promoters) represses transcription; methylation elsewhere (e.g., gene bodies) enhances transcription by suppressing aberrant initiation. Methylation can be introduced in one step by *de novo* and maintenance methyltransferases and propagated by feed-forward loops that link DNA methylation and repressive chromatin marks. Methylation is most easily lost passively as a result of DNA replication, but it can also be actively erased through a mechanism that utilizes TET catalyzed oxidation to prime DNA for base excision repair. The genetics and cell biology of DNA methylation are unique to eukaryotes, but the biochemistry of DNA methylation (and to some extent also DNA demethylation) is also conserved in prokaryotes. We seek to answer biochemical questions using more robust bacterial proteins and answer genetic/cell biological questions using zebrafish models and human genetic data (e.g., for malignancies with defects in demethylation machinery).

METHYLATION SENSING

In 2019, we continued our research on relatively well-behaved prokaryotic model proteins to study the specific recognition of 5-methylcytosine and its oxidized congeners in DNA.

The repertoire of 5mC and 5hmC binding proteins is relatively small, and most proteins that specifically bind DNA with these bases contain the same domains. Domains that bind fully methylated DNA in the context of CpG include zinc finger and MBD domains. In contrast, hemi-methylated DNA is typically bound by SRA domains. During the last year, we considerably broadened the repertoire of known domains that recognize 5mC and 5hmC.

SRA domains belong to the larger superfamily of PUA domains, which also comprises PUA domains in the strict sense, ASCH domains, EVE domains, and several other lesser known domain groups. To the extent that function was known, a clear division of labor appeared to be in place. SRA domains were associated with the binding of modified DNA, whereas other families within the PUA superfamily either were known to be involved in the binding or processing of modified RNA or had completely unknown functions. *In silico* screens that were performed in collaboration with Dr. Shuang-Yong Xu (New England Biolabs) showed that many PUA superfamily domains in bacteria are fused to endonucleolytic domains that are associated with DNA cleavage. Subsequent biochemical experiments demonstrated that the fusion proteins indeed cleaved modified DNA, although not with the same degree of specificity as SRA domains (Lutz et al., *Nucleic Acids Res,* 2019). We also crystallographically characterized a few prototype enzymes and solved their structures with and without DNA. The structures illustrate the mode of recognition of methyl- or hydroxymethyl modifications. Similar DNA modification-sensing domains exist in eukaryotes and have been implicated in malignancies, but their biochemical behavior requires further investigation.

NEco is the modification-sensing domain of EcoKMcrA, which is one of the earliest studied restriction endonucleases of *E. coli*. EcoKMcrA efficiently restricts DNA that contains 5mC or 5hmC, provided the modifications are present in the right context. Efficiency is much greater for fully methylated DNA than for hemi-methylated DNA. Our previous work indicated that the NEco modification-sensing domain was phylogenetically unrelated to other methylation-sensing domains. In 2019, we elucidated the binding mode of the domain to modified DNA and found that modification sensing is also locally very different from previous observations (Slyvka et al., *Nucleic Acids Res,* 2019). To date, NEco has been shown to be very good at discriminating (hydroxy)methylated from unmethylated DNA. Further research will likely expand the currently known and narrowly defined phylogenetic distribution (Fig. 1).

DEMETHYLATION

In contrast to methylation sensing, demethylation has no clear equivalent in prokaryotes and thus needs to be studied using eukaryotic models. Our research primarily focuses on the ways in which TET proteins identify their targets. Two of our collaborators, Dr. Tomasz Jurkowski (Cardiff University, United Kingdom) and Dr. Tim Hore (Otago University, New Zealand), provided strong biochemical evidence that the locus specificity of TETs is at least partially attributable to sequence specificity. We solved structures with preferred and discriminated substrates to better understand the mode of sequence recognition. Based on the lack of sequence-specific contacts between TETs and their target DNAs outside the CpG core recognition sequence, we initially hypothesized that DNA bending was responsible for sequence specificity. Our crystal structures do not support this hypothesis, however, and instead reveal an unexpected mechanism of sequence recognition that also explains similarities in preference of different TET paralogues.

We are also continuing our work on links between DNA reprogramming and DNA repair. Some of our experiments, such as investigating the role of NEIL1 and TDG in the excision of oxidized 5-methylcytosine bases, are consistent with the general paradigm that DNA reprogramming co-opts DNA repair. However, based on much circumstantial evidence and the work of others, we suspect that the converse may also be true and that DNA repair may use intermediates that are normally associated with reprogramming. We will test this hypothesis both biochemically and bioinformatically.

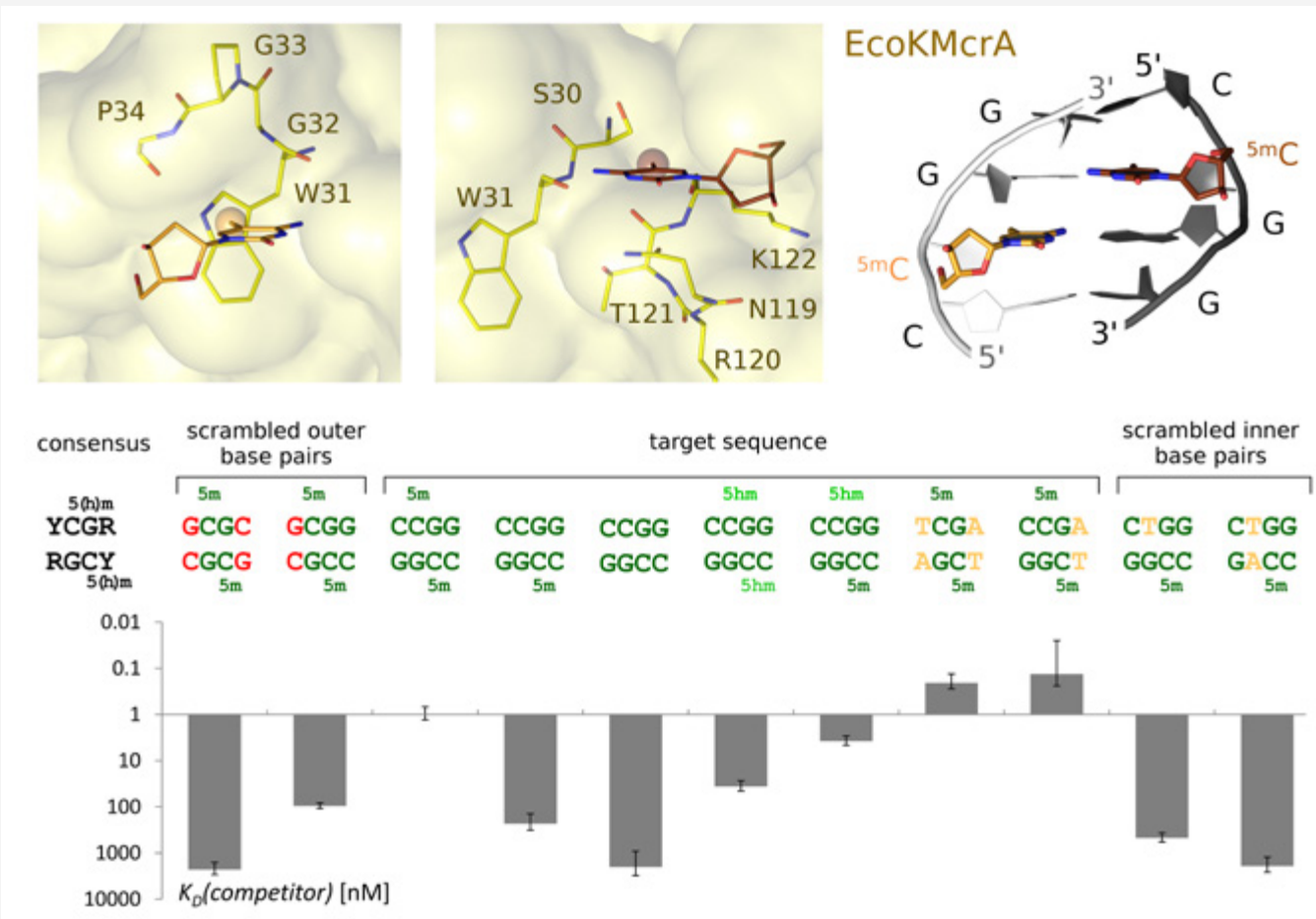


FIG. 1

Modification-dependent DNA binding by the N-terminal domain of EcoKMcrA endonuclease (NEco). (Top) Interaction between fully modified DNA with (hydroxy)methyl binding pockets of EcoKMcrA. (Bottom) Sequence and modification specificity of EcoKMcrA N-terminal domain determined by EMSA competition experiments (for experimental details, see Slyvka et al., *Nucleic Acids Res*, 2019).

Laboratory of Bioinformatics and Protein Engineering

GROUP MEMBERS

Lab Leader
Janusz M. Bujnicki, PhD, Professor

Senior Researchers
Elżbieta Purta, PhD
Filip Stefaniak, PhD

Researcher
Michał Boniecki, PhD

Postdoctoral Researchers
Nithin Chandran, PhD
Pritha Ghosh, PhD
Sunandan Mukherjee, PhD
Almudena Ponce Salvatierra, PhD
Tales Rocha de Moura, PhD
Tomasz Wirecki, PhD

Research Assistant
Katarzyna Merdas, MSc

Research Technicians
Agata Bernat, MSc
Radosław Giziński, MSc
Małgorzata Kurkowska, MSc
Niloofar Shirvanizadeh, PhD
Ewa Skowronek, PhD (until April 2019)

PhD Students
Pietro Boccaletto, MSc (until September 2019)
Nagendar Goud Badepally, MSc
Kanchan Chauhan, MSc
Masoud Amiri Farsani, MSc
Sachin Gadakh, MSc (until February 2019)
Farhang Jaryani, PhD
Seyed Naeim Moafinejad, MSc
Iswarya Pandara Nayaka PJ, MSc
Ankita Rawat, MSc
Diana Toczydłowska-Socha, MSc (until February 2019)

Undergraduate Students
Michał Lechowski, BSc (until August 2019)
Agata Momot
Paweł Muzyka, BSc (until August 2019)
Dharm Skandh Jain, BSc (until September 2019)
Natalia Szulc, MSc
Jan Wójtowicz

Lab 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)
2006-Present	Associate Professor (extraordinarius), Bioinformatics Laboratory, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland (25% appointment)
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

2019-2022	Member, Committee for Science Evaluation, Ministry of Science and Higher Education
2018-Present	Member, Academia Europaea
2018-Present	Member, European Molecular Biology Organization
2017-Present	Member, European Science Advisors Forum
2016-Present	Corresponding Member, Polish Academy of Sciences
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
2013-2016	Member, Scientific Committee of the Innovative Medicines Initiative
2016-2017	Member, Council of the National Science Congress
2013-Present	Executive Editor, Nucleic Acids Research
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

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.

SELECTED PUBLICATIONS

IIMCB Best Papers Award

Czapinska H, Siwek W, Szczepanowski RH, **Bujnicki JM**, Bochtler M, Skowronek KJ. Crystal Structure and Directed Evolution of Specificity of NlaIV Restriction Endonuclease. ***J Mol Biol***, 2019; 431(11):2082-94

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)***, 2019; 85:102746

de Crécy-Lagard V, **Boccaletto P**, Mangleburg CG, Sharma P, Lowe TM, Leidel SA, **Bujnicki JM**. Matching tRNA modifications in humans to their known and predicted enzymes. ***Nucleic Acids Res***, 2019; 47(5):2143-59

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

Magnus M, Kappel K, Das R, **Bujnicki JM**. RNA 3D structure prediction guided by independent folding of homologous sequences. ***BMC Bioinformatics***, 2019; 20(1):512

Nowacka M, **Boccaletto P**, **Jankowska E**, **Jarzynka T**, **Bujnicki JM**, Dunin-Horkawicz S. RRMdb-an evolutionary-oriented database of RNA recognition motif sequences. ***Database (Oxford)***, 2019; doi: 10.1093/database/bay148

Nowacka M, Fernandes H, Kiliszek A, **Bernat A**, **Lach G**, **Bujnicki JM**. Specific interaction of zinc finger protein Com with RNA and the crystal structure of a self-complementary RNA duplex recognized by Com. ***PLoS One***, 2019; 14(4):e0214481

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). pii: BSR20180430

Radom M, **Machnicka MA**, **Krwawicz J**, **Bujnicki JM**, Formanowicz P. Petri net-based model of the human DNA base excision repair pathway. ***PLoS One***, 2019; 14(9):e0217913

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

Mazur M, Dymek B, Koralewski R, Sklepkiewicz P, Olejniczak S, Mazurkiewicz M, Piotrowicz M, Salamon M, Jędrzejczak K, Zagozdzon A, Czestkowski W, Matyszewski K, Borek B, Bartoszewicz A, Pluta E, Rymaszewska A, Mozga W, **Stefaniak F**, Dobrzański P, Dzwonek K, Golab J, Golebiowski A, Olczak J. Development of dual chitinase inhibitors as potential new treatment for respiratory system diseases. ***J Med Chem***, 2019; 62(15):7126-45

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

Nithin C, **Ghosh P**, **Bujnicki JM**. Bioinformatics tools and benchmarks for computational docking and 3D structure prediction of RNA-protein complexes. ***Genes***, 2018, 9(9):432

Foik IP, **Tuszynska I**, Feder M, **Purta E**, **Stefaniak F**, **Bujnicki JM**. Novel inhibitors of the rRNA ErmC′ methyltransferase to block resistance to macrolides, lincosamides, streptogramine B antibiotics. ***Eur J Med Chem***, 2018; 146:60-7

Boccaletto P, Machnicka MA, Purta E, Piatkowski P, Baginski B, Wirecki TK, de Crécy-Lagard V, Ross R, Limbach PA, Kotter A, Helm M, **Bujnicki JM**. MODOMICS: a database of RNA modification pathways: 2017 update. ***Nucleic Acids Res***, 2018; 46(D1):D303-D307

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

Piątkowski P, **Jabłońska J**, **Żyła A**, **Niedziałek D**, **Matelska D**, **Jankowska E**, **Waleń T**, **Dawson WK**, **Bujnicki JM**. SupeRNAlign: a new tool for flexible superposition of homologous RNA structures and inference of accurate structure-based sequence alignments. ***Nucleic Acids Res***, 2017; 45(16):e150

Dawson WK, **Maciejczyk M**, **Jankowska EJ**, **Bujnicki JM**. Coarse-grained modeling of RNA 3D structure. ***Methods***, 2016; 103:138-56

Machnicka MA, **Dunin-Horkawicz S**, de Crécy-Lagard V, **Bujnicki JM**. tRNAmodpred: a computational method for predicting posttranscriptional modifications in tRNAs. ***Methods***, 2016; 107:34-41

Glow D, **Kurkowska M**, **Czarnecka J**, **Szczepaniak K**, **Pianka D**, **Kappert V**, **Bujnicki JM**, **Skowronek KJ**. Identification of protein structural elements responsible for the diversity of sequence preferences among Mini-III RNases. ***Sci Rep***, 2016; 6:38612

Dawson WK, **Bujnicki JM**. Computational modeling of RNA 3D structures and interactions. ***Curr Opin Struct Biol***, 2016; 37:22-8

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

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

Matelska D, **Kurkowska M**, **Purta E**, **Bujnicki JM**, **Dunin-Horkawicz S**. Loss of conserved non-coding RNAs in genomes of bacterial endosymbionts. ***Genome Biol Evol***, 2016; 8(2):426-38

Magnus M, **Boniecki MJ**, **Dawson WK**, **Bujnicki JM**. SimRNAweb: a web server for RNA 3D structure modeling with optional restraints. ***Nucleic Acids Res***, 2016;44(W1):W315-W319

Piatkowski P, **Kasprzak JM**, **Kumar D**, **Magnus M**, **Chojnowski G**, **Bujnicki JM**. RNA 3D structure modeling by combination of templatebased method ModeRNA, template-free folding with SimRNA, and refinement with QRNAS. ***Methods Mol Biol***, 2016; 1490:217-35

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

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

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

Chojnowski G, **Walen T**, **Piatkowski P**, **Potrzebowski W**, **Bujnicki JM**. Brickworx builds recurrent RNA and DNA structural motifs into medium and low-resolution electron density maps. ***Acta Crystallogr Biol Crystallogr***, 2015; 71(Pt 3):697-705

Glow D, **Pianka D**, **Sulej A**, **Kozlowski L**, **Czarnecka J**, **Chojnowski G**, **Skowronek KJ**, **Bujnicki JM**. Sequence-specific cleavage of dsRNA by Mini-III RNase. ***Nucleic Acids Res***, 2015; 43(5):2864-73

Pietal M, **Bujnicki JM**, **Kozlowski LM**. GDFuzz3D: a method for protein 3D structure reconstruction from contact maps, based on a non-Euclidean distance function. ***Bioinformatics***, 2015; 31(21):3499-505

Tuszynska I, **Magnus M**, **Jonak K**, **Dawson W**, **Bujnicki JM**. NPDock: a web server for protein-nucleic acid docking. ***Nucleic Acids Res***, 2015; 43(W1):W425-W430

Byszewska M, **Smietanski M**, **Purta E**, **Bujnicki JM**. RNA methyltranserases involved in 5′ cap biosynthesis. ***RNA Biol***, 2014; 11(12):1597-607

Szczepaniak K, **Lach G**, **Bujnicki JM**, **Dunin-Horkawicz S**. Designability landscape reveals sequence features that define axial helix rotation in four-helical homo-oligomeric antiparallel coiled-coil structures. ***J Struct Biol***, 2014; 188(2):123-33

Walen T, **Chojnowski G**, **Gierski P**, **Bujnicki JM**. ClaRNA: a classifier of contacts in RNA 3D structures based on a comparative analysis of various classification schemes. ***Nucleic Acids Res***, 2014; 42:e151

Magnus M, **Matelska D**, **Lach G**, **Chojnowski G**, **Boniecki MJ**, **Purta E**, **Dawson W**, **Dunin-Horkawicz S**, **Bujnicki JM**. Computational modeling of RNA 3D structures, with the aid of experimental restraints. ***RNA Biol***, 2014; 11(5):522-36

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

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

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

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

DESCRIPTION OF CURRENT RESEARCH

Our group is involved in theoretical and experimental research on sequence structure-function relationships in proteins, nucleic acids, and macromolecular complexes. Theoretical research involves the development of computer software for the analysis of biological macromolecules. We are currently focusing on developing software for the structural prediction and modeling of RNA and RNA protein complexes. 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) and a database of RNA 3D motifs and their interactions (RNA Bricks; http://iimcb.genesilico.pl/rnabricks/).

Our suite of programs for the prediction and analysis of protein structures and macromolecular complexes includes the GeneSilico MetaServer (https://www.genesilico.pl/meta2/), methods for modeling large macromolecular complexes with the use of restraints that are derived from experimental data (PyRy3D, http://genesilico.pl/pyry3d/; MinkoFit3D, http://iimcb.genesilico.pl/minkofit3d/), and a method for discriminating models according to their agreement with experimental data (FILTREST3D; http://filtrest3d.genesilico.pl/). We also developed methods for predicting order/disorder in protein structures (http://iimcb.genesilico.pl/metadisorder/).

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 or crosslinking, mass spectrometry, circular dichroism, mutagenesis, etc. Most recently, our group began using cryo-EM. We have collected our first datasets and began developing software for interpreting the data.

RECENT HIGHLIGHTS

Matching tRNA modifications in humans to their known and predicted enzymes

he acquisition of post-transcriptional chemical modifications is an essential part of the maturation process, required to generate functional tRNA molecules. Modifications play different roles in controlling the stability, folding, and decoding properties of tRNAs and can be determinants or anti-determinants of other components of the translation apparatus, such as aminoacyl-tRNA synthetases. Additionally, tRNA modifications can be recognition elements of ribonucleases, leading to the generation of tRNA fragments that affect multiple cellular processes. However, very few modifications (e.g., m1G37, Ψ55, and t6A37) are present at a specific position of a particular tRNA in (almost) all known organisms. Most of them are specific to particular taxons, from species to kingdoms. For example, lysidine (k2C34) is a hallmark of bacteria, whereas archaeosine (G+15) is only found in archaea. Depending on the organism, the total number of genes that encode tRNA modification enzymes varies from 11 in some obligate symbionts to ~100 in humans, of which 50 are currently represented in MODOMICS, the main database of RNA modification pathways that was developed and is maintained by the Bujnicki group at IIMCB, in collaboration with various groups worldwide (http://modomics.genesilico.pl).

The near complete sets of tRNA modification genes are currently available for only one organism per domain of life: *Saccharomyces cerevisiae* for eukarya (where only one gene that is required for the formation of ncm5U out of cm5U is missing), *Escherichia coli* for bacteria (where only the genes for ho5U34 and Acp3U47 formation remain unidentified), and *Haloferax volcanii* for archaea (where a handful of genes are missing). Beyond these three organisms, the annotation of tRNA modification genes remains scarce because of difficulties in connecting various RNA modification enzymes, which often exhibit complex evolutionary relationships, with various modifications that are present in tRNAs. It has been difficult to identify enzymes that are responsible for many tRNA modifications and hence to determine the function of those tRNA modifications in many species, including humans.

Recently, an increasing number of mutations that cause genetic diseases have been mapped to human genes that encode tRNA modification enzymes, thus making a comprehensive list of these genes highly desirable. In a collaborative effort with Valerie de Crécy-Lagard and her team at the University of Florida, Sebastian Leidel and his team at the University of Bern (previously MPI Muenster and University of Muenster), and Todd Lowe at the University of California, Santa Cruz, we compiled a comprehensive list of known and predicted tRNA modifications in *Homo sapiens* with genes that are implicated in their biosynthesis. This analysis allowed the identification of remaining gaps in knowledge in the field of human tRNA modifications and will help guide future experiments. Furthermore, we have used publicly available datasets to determine the expression profiles and proteomic evidence of known and predicted modification enzymes. Our work will facilitate access to the current knowledge on human tRNA modification enzymes for a wider community of biologists. These results were published in *Nucleic Acids Research* (de Crécy-Lagard et al., 2019).

Ongoing work: new bioinformatics methods for predicting structures of RNA-protein complexes

RNA-protein (RNP) interactions play pivotal roles in various biological processes, such as protein synthesis, the regulation of gene expression, RNA splicing, transport, storage, and stabilization. To understand the functional and mechanistic details of these processes, it is essential to have information about the 3D structures of these complexes. The inherent flexibility of RNA molecules and transient nature of these complexes make it technically difficult to determine these structures experimentally. In addition to experimental work, our laboratory develops methods for theoretical predictions.

One of our recent developments is SimRNP, a variant of SimRNA that enables the modeling of interactions between RNA and other types of molecules, in this case proteins. The representation of RNA and statistical potential for RNA are essentially the same, whereas the representation of protein molecules is very similar to the coarse-grained model and statistical potential that are used in the REFINER program for protein folding (developed earlier by Michał Boniecki). Additionally, a statistical potential for RNA-protein interactions was introduced based on an analysis of high-resolution structures of RNA-protein complexes. A typical task for SimRNP is to predict the 3D structure of an RNP complex, starting from an unbound structure of the protein and RNA components or from an unbound protein structure and unfolded RNA sequence. Protein components are typically partially restrained to maintain the protein fold, but conformational changes are allowed to reflect induced fit upon RNA-protein binding.

If the structures of both components (RNA and protein) are available, then the RNP complex structure can be predicted by docking, in which conformational sampling can be separated from scoring of the conformations that are obtained. The existing docking methods can be broadly classified as rigid docking algorithms that do not account explicitly for conformational changes and flexible docking algorithms that attempt to account for conformational changes. Major challenges in RNP docking are molecular flexibility and computational complexity that are associated with flexible docking. Generating conformations that are similar to the bound conformation from the starting structures and discriminating them from others is a challenging task. We developed a prototypical meta-predictor for RNA-protein docking, which combines various existing methods for docking and scoring to obtain biologically, chemically, and physically relevant predictions. Such meta-predictions were successfully applied previously to model protein structures and protein-protein docking. RNP docking is performed using different methods, and the top-scoring docking poses from each of these are rescored using different scoring functions. If the scoring methods reach a consensus, then decoys that are obtained from different methods are clustered together. However, in the absence of consensus scoring, top models that are proposed by different methods are suggested as alternative solutions. The MetaRNPDock metaserver is available at http://genesilico.pl/metaRNPDock/ (co-authors: Nithin Chandran, Sunandan Mukherjee, Pietro Boccaletto, Michał J. Boniecki, and Janusz M. Bujnicki).

Laboratory of RNA Biology: ERA Chairs Research 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
Anna Hojka-Osińska, PhD
Paweł Krawczyk, PhD
Tomasz Kuliński, MD PhD
Monika Kusio-Kobiałka, PhD
Bartosz Tarkowski, PhD

Research Technicians
Aleksander Chlebowski, MSc
Jakub Gruchota, MSc
Marcin Szpila, MSc
Katarzyna Prokop, MSc

PhD Students
Aleksandra Bilska, MSc
Michał Brouze, MSc
Vladyslava Liudkovska, MSc
Karolina Wróbel, MSc

Undergraduate Students
Jan Brancewicz, BSc
Małgorzata Drabko
Konrad Szymański

Other co-workers
Kamil Kobyłecki

Lab Technician
Alina Zielińska, BSc (part-time)

Laboratory Support Specialist
Zofia Korbut-Mikołajczyk, MSc

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

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 EMPLOYMENT

2019-present	Professor, Head of the Laboratory of RNA Biology, 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

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 of the Order of Polonia Restituta
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 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

Płociński P, Macios M, Houghton J, Niemiec E, 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

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

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

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

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 non-coding 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

All publications with 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 (Fig.1). 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

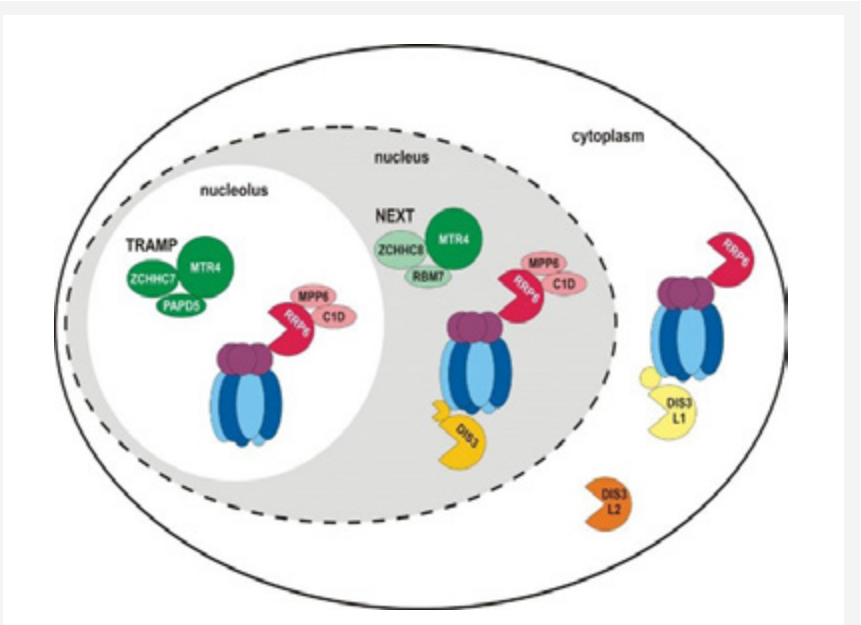


FIG. 1

Exosome complexes in humans (based on Tomecki et al., *EMBO J*, 2010; Lubas et al., *Mol Cell*, 2011; Lubas et al., *EMBO J*, 2013).

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 NON-CANONICAL 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 (Fig.2) (Warkocki et al., *Cell*, 2018). 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 Comm*, 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.

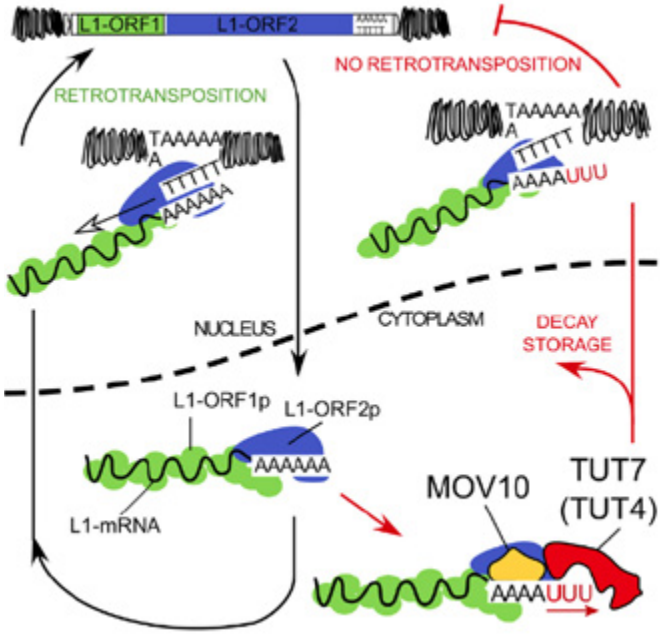


FIG. 2

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

OTHER ACTIVITIES

Mus musculus is a mouse model of choice for functional studies of human health and disease. Surprisingly, there is currently no facility that routinely generates mouse models by gene targeting approaches in Poland. During our work on the TENT5 and DIS3 protein families, we began efforts to implement CRISPR/Cas9 technology for the generation of transgenic mice. The method proved to be extremely efficient in our hands. Within the past 3 years, we have generated more than 20 different mouse lines with mutations in the locus (indels, point mutations, and tags). This prompted us to establish a core facility (https://crisprmice.eu) that has the potential to foster the usage of new mouse models in biomedical research in Poland.

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)
Małgorzata Urbańska, PhD
Justyna Zmorzyńska, PhD

Postdoctoral Researchers
Agnieszka Brzozowska, PhD
Aleksandra Janusz-Kamińska, PhD (until January 2020)
Andrii Kopach, PhD
Michalina Wężyk, PhD

Research Assistant
Marcelina Firkowska, PhD

Research Technicians
Kinga Kuchcińska, MSc
Katarzyna Machnicka, MSc
Aliaksandra Zdanovich

PhD Students
Magdalena Kędra, MSc
Alicja Kościelny, MSc (until August 2019)
Magdalena Mlostek, MSc
Karolina Ryczek, MSc
Aleksandra Tempes, MSc
Oliver Tkaczyk, MSc
Jan Węsławski, MSc
Juan Zeng, MSc

Lab Technician
Alina Zielińska, BSc (part-time)

Laboratory Support Specialist
Marcin Kozioł (part-time, until December 2019)
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

2018	TEAM, Foundation for Polish Science
2014	Master Award, Foundation for Polish Science
2011	Prime Minister Award for habilitation thesis
2009	2nd Division (Biological Sciences) of Polish Academy of Sciences Award for series of publications on MMP9 (together with teams of Prof. Kaczmarek and Dr. 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

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

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

Urbanska M, Kazmierska-Grebowska P, Kowalczyk T, Caban B, Nader K, Pijet B, Kalita K, **Gozdz A**, Devijvere H, Lechate B, Jaworski T, Grajkowska W, Sadowski K, Jozwiak S, Kotulska K, Konopacki J, Van Leuven F, van Vlieth E, Aronica E, **Jaworski J**. 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

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

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

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):1308-1327

Lipka J, Kapitein LC, **Jaworski J**, Hoogenraad CC. Microtubule-binding protein doublecortin-like kinase 1 (DCLK1) guides kinesin-3-mediated cargo transport to dendrites. ***EMBO J***, 2016; 35(3):302-18

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

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

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

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

Jaworski J, Kapitein LC, Montenegro Gouveia S, Dortland BR, Wulf PS, Grigoriev I, Camera P, Spangler SA, Di Stefano P, Demmers J, Krugers H, Defilippi P, Akhmanova A, Hoogenraad CC. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. ***Neuron***, 2009; 61:85-100

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

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

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

^no IIMCB affiliation

DESCRIPTION OF CURRENT RESEARCH

Mammalian/mechanistic target of rapamycin (mTOR) is a serine-threonine kinase that is involved in almost every aspect of mammalian cell function. It forms two protein complexes, initially identified as regulating translation (mTOR complex 1 [mTORC1]) or influencing the actin cytoskeleton (mTORC2). My postdoctoral work showed that the regulation of mTOR-dependent translation contributes to dendritogenesis (Jaworski et al., *J Neurosci*, 2005). This was subsequently confirmed by our recent work in which we identified the GluA2 subunit of glutamate receptors as a protein that is both translated in an mTORC1-dependent manner and vital for dendritogenesis (Koscielny et al., *Mol Neurobiol,* 2018). However, the list of cellular processes that involve both mTORCs has expanded, and new ways of regulating mTORC activity, novel mTOR partners, and mTOR effectors have been discovered. Nonetheless, their contribution to the neuronal functions of mTOR and neuropathology is still poorly understood. Therefore, 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 & *Sci Rep*, 2017; Malik et al., *J Biol Chem*, 2013).

Progress in meeting 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 GTPases 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**.

Our major success in 2019 falls within Main Research Objective 2, which focuses on mTOR-related disorders. One such disease is TSC. It is a multiorgan disease that is caused by mutations of the *TSC1* and *TSC2* genes, the products of which form a complex (TSC complex) that inhibits mTORC1 (Switon et al., *IUBMB Life,* 2016). The most common symptoms of TSC are epilepsy, autism, the formation of benign tumors in the brain, and so-called TSC-associated neuropsychiatric disorders (TANDs). We used zebrafish with the mutated *Tsc2* gene to study causal links between elementary neurodevelopment and epilepsy/TANDs. We found that the lack of Tsc2 in zebrafish resulted in heterotopias (Fig. 1A) and hyperactivation of the mTorC1 pathway in pallial regions, which are homologous to the mammalian cortex (Kedra et al., Proc Natl Acad Sci USA, 2020). We observed commissural thinning (Fig. 1B) that was responsible for brain dysconnectivity, recapitulating TSC pathology in humans (Kedra et al., Proc Natl Acad Sci USA, 2020). The mutants exhibited epileptogenesis that resulted in nonmotor seizures and an increase in anxiety-like behavior (i.e., one symptom of TANDs; Kedra et al., 2020; Fig. 1C,D), which were rescued by reducing tyrosine receptor kinase B (TrkB) signaling. TrkB inhibition also rescued brain dysconnectivity. These data provide a mechanistic link between brain anatomy and human TANDs and identify TrkB as a possible lead target in the search for TAND-specific drugs.

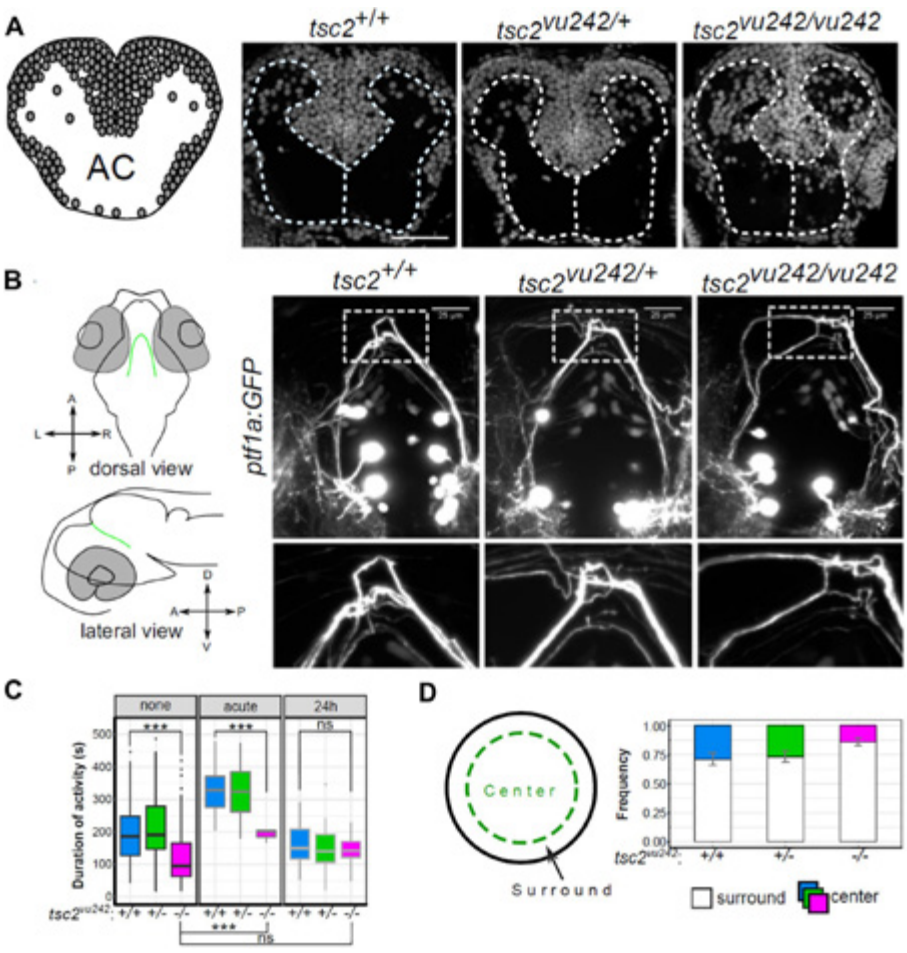


FIG. 1

Zebrafish model of Tuberous Sclerosis Complex (tsc2$^{vu242/vu242}$) can be used to study several disease symptoms. (A) Visualization of cell nuclei using DAPI shows that in TSC fish brain several cells are mislocalized causing disruption of white matter. AC - anterior commissure (B) ptf1a:GFP-positive neurons in the posterior tuberculum extend their axons dorsally and cross the brain midline through the posterior commissure (left panel). In the TSC fish these axonal tracts are disorganized and do not cross the midline as compact bundle (right panel). (C) TSC fish display absence seizure-like phenotype that can be "cured" with prolonged ethosuximide treatment used typically in clinic. (D) In open field test TSC fishes avoid center of the dish suggesting increased anxiety. For more details, see Kedra et al., *Proc Natl Acad Sci USA*, 2020. Photo: Justyna Zmorzyńska, PhD and Magdalena Kędra, MSc.

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 Wiweger, PhD

Postdoctoral Researchers
Evgeny Gasanov, PhD
Oksana Palchevska, PhD

Research Technician
Sergii Palchevskyi, PhD (part-time)

PhD Students
Justyna Jędrychowska, MSc
Rishikesh Kumar Gupta, MSc Tech.
Filip Maciąg, MSc Eng.
Iga Wasilewska, MSc

Trainees
Ewelina Latoszek
Joanna Oberska

Lab 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	Ordinary Member, Polish Academy of Sciences
2018-2022	Member, Council of the National Science Centre and Chair of International Commission
2017-2018	Deputy Chair, Council of Provosts, 2nd Division, Polish Academy of Sciences
2016-Present	Member, International Advisory Board, Małopolska Centre of Biotechnology, Jagiellonian University
2011-Present	Member, International Expert Council of the Research and Education Center, State Key Laboratory of Molecular and Cellular Biology, Ukraine
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
2002-Present	Head of Program Board, Centre for Innovative Bioscience Education
1993-2014	Member, Scientific Council, Nencki Institute of Experimental Biology, Polish Academy of Sciences
1996-1998 & 2000-2002	Vice-President, Biotechnology Committee, Polish Academy of Sciences
1989-1991	General Secretary, Polish Biochemical Society

HONORS, PRIZES AND AWARDS

2013	Award from the 2nd Division of Biological and Agricultural Sciences, Polish Academy of Sciences for series of works on β-catenin
2013	Crystal Brussels Sprout Award
2011	Konorski Award from the Polish Neuroscience Society and Committee on Neurobiology, Polish Academy of Sciences
2008	Officer's Cross of the Order of Polonia Restituta
2003	Prime Minister Award for scientific achievements
2001	Award from the Division of Biological Sciences, Polish Academy of Sciences, for work on calcium binding proteins
1998	Knight's Cross of the Order of Polonia Restituta

DOCTORATES DEFENDED UNDER LAB LEADER'S SUPERVISION

A. Filipek, J. Kordowska, U. Wojda, J. Hetman, M. Palczewska, M. Nowotny, K. Billing-Marczak, Ł. Bojarski, W. Michowski, K. Misztal, M. Figiel, K. Honarnejad, A. Jaworska, K. Gazda.

SELECTED PUBLICATIONS

IIMCB Best Papers Award

Majewski L, Maciąg 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 Jan 28;21(3). pii: E842

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 Jan 9;9(1). pii: E160

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 Nov 6;20(22). pii: E5539

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 Oct 15;8(10). pii: bio044347

Jędrychowska J, **Korzh V.** Kv2.1 voltage-gated 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

Wasilewska I, **Gupta RK**, **Palchevska O**, **Kuznicki J**. Identification of Zebrafish Calcium Toolkit Genes and their Expression in the Brain. ***Genes (Basel)***, 2019; Mar 18;10(3). pii: E230

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

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

Grzeczkowicz A, **Gruszczynska-Biegala J**, **Czeredys M**, Kwiatkowska A, Strawski M, Szklarczyk M, Kozbiał M, **Kuznicki J**, Granicka LH. Polyelectrolyte Membrane Scaffold Sustains Growth of Neuronal Cells. ***J Biomed Mater Res A***, 2019; 107(4):839-50

Minhas R, Paterek A, Łapiński M, **Bazała M, Korzh V,** Winata CL. A novel conserved enhancer at zebrafish zic3 and zic6 loci drives neural expression. ***Dev Dyn***, 2019; 248(9):837-49

Sokol AM, Uszczynska-Ratajczak B, Collins MM, **Bazala M**, Topf U, Lundegaard PR, Sugunan S, Guenther S, Kuenne C, Graumann J, Chan SSL, Stainier DYR, Chacinska A. Loss of the Mia40a oxidoreductase leads to hepato-pancreatic insufficiency in zebrafish. ***PLOS Genet***, 2018; 14(11):e1007743

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; 74:102-111

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

Brendel M, **Jaworska A**, Overhoff F, Blume T, Probst F, Gildehaus FJ, Bartenstein P, Haass C, Bohrmann B, Herms J, Willem M, Rominger A. Efficacy of chronic BACE1 inhibition in PS2APP mice depends on the regional Aβ deposition rate and plaque burden at treatment initiation. ***Theranostics***, 2018; 8(18):4957-68

De Assis GG, **Gasanov EV**, de Sousa MBC, Kozacz A, Murawska-Cialowicz E. Brain derived neutrophic factor, a link of aerobic metabolism to neuroplasticity. ***J Physiol Pharmacol***, 2018; 69(3):351-58

Korzh V. Development of brain ventricular system. ***Cell Mol Life Sci***, 2018; 75(3):375-83

Gazda K, **Kuznicki J**, **Wegierski T**. Knockdown of amyloid precursor protein increases calcium levels in the endoplasmic reticulum. ***Sci Rep***, 2017; 7(1):14512

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

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

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

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

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

Garcia-Lecea M, **Gasanov E, Jedrychowska J**, Kondrychyn I, Teh C, You M-S, **Korzh V**. Development of Circumventricular Organs in the Mirror of Zebrafish Enhancer-Trap Transgenics. ***Front Neuroanat***, 2017; 11:114

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

Majewski L, **Kuznicki J**. SOCE in neurons: Signaling or just refilling? ***BBA Mol Cell Res***, 2015; 1853(9):1940-52

Mills F, Bartlett TE, Dissing-Olesen L, **Wisniewska MB**, **Kuznicki J**, Macvicar BA, Wang YT, Bamji SX. Cognitive flexibility and long-term depression (LTD) are impaired following β-catenin stabilization in vivo. ***Proc Natl Acad Sci USA***, 2014; 111(23):8631-6

Czeredys M, **Gruszczynska-Biegala J**, Schacht T, Methner A, **Kuznicki J**. Expression of genes encoding the calcium signalosome in cellular and transgenic models of Huntington's disease. ***Front Mol Neurosci***, 2013; 6:42

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

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

Wojda U, **Kuznicki J**. Alzheimer's disease modeling: ups, downs, and perspectives for human induced pluripotent stem cells. ***J Alzheimers Dis***, 2013; 34(3):563-88

Nagalski A, Irimia M, Szewczyk L, Ferran JL, **Misztal K**, **Kuznicki J**, **Wisniewska MB**. Postnatal isoform switch and protein localization of LEF1 and TCF7L2 transcription factors in cortical, thalamic, and mesencephalic regions of the adult mouse brain. ***Brain Struct Funct***, 2013; 218(6):1531-49

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

Bialopiotrowicz E, **Szybinska A**, **Kuzniewska B**, Buizza L, Uberti D, **Kuznicki J**, **Wojda U**. Highly pathogenic Alzheimer's disease presenilin 1 P117R mutation causes a specific increase in p53 and p21 protein levels and cell cycle dysregulation in human lymphocytes. ***J Alzheimers Dis***, 2012; 32(2):397-415

Misztal K, **Wisniewska MB**, **Ambrozkiewicz M**, **Nagalski A**, **Kuznicki J**. WNT protein-independent constitutive nuclear localization of beta-catenin protein and its low degradation rate in thalamic neurons. ***J Biol Chem***, 2011; 286(36):31781-8

Gruszczynska-Biegala J, Pomorski P, **Wisniewska MB**, **Kuznicki J**. Differential roles for STIM1 and STIM2 in store-operated calcium entry in rat neurons. ***PLoS One***, 2011; 6(4):e19285

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

DESCRIPTION OF CURRENT RESEARCH

We are interested in the molecular mechanisms that are implicated 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 STIM1 is involved in a thapsigargin-induced SOCE-like process, whereas STIM2 is mostly active after the EGTA-driven depletion of extracellular Ca^{2+} (Gruszczynska-Biegala et al., *PLoS One*, 2011; Gruszczynska-Biegala and Kuznicki, *J Neurochem*, 2013). We searched for new partners of STIMs other than ORAI channels and found that endogenous STIMs associate with GluA subunits of AMPA receptors (Gruszczynska-Biegala et al., *Front Cell Neurosci*, 2016). STIM proteins also associate with NMDA receptors *in vitro*. The results suggest cross-talk between STIM proteins and 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 the cytosol and mitochondria, behavioral tests, and scRNA-Seq.

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 SAD 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., *IJMS*, 2020), and ORAI1 line (Maciag, Majewski et al., *BBA Mol Cell Res*, 2019; Majewski et. al., *IJMS*, 2019) has been reported. Strikingly, aged transgenic ORAI1 mice developed spontaneous seizure-like events that was observed only in females, suggesting a novel, sex-dependent role of ORAI1 in neural function (Maciag, Majewski et al., *BBA Mol Cell Res*, 2019). Furthermore, based on RNAseq gene expression profiling analysis and ddPCR of the hippocampus, we identified downregulation of the *Arx* gene. Loss-of-function mutations of *ARX* have been implicated in human cases of epilepsy (Majewski et al., *IJMS*, 2019).

Using quantitative PCR, we compared microRNA (miRNA) profiles in blood plasma from Alzheimer's disease patients with mild cognitive impairment (whose diagnoses were confirmed by cerebrospinal fluid [CSF] biomarkers), Alzheimer's disease patients, and non-demented, age-matched controls. We adhered to standardized blood and CSF 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.

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*. Mutations of *NPC2*, *SGSH*, *PPP3CA*, and *PTPN4* have been linked to neurological problems. Using CRISPR/Cas9 technology, we created zebrafish lines with genetic changes that mimic those that are found in patients. These zebrafish lines are being used to study Ca^{2+} homeostasis and its impact on the progression of neurodegeneration that is observed in patients with Niemann-Pick disease type C (mutation of *NPC2*), mucopolysaccharidosis type III A (*SGSH*), and child with mutated calcineurin (*PPP3CA*; Rydzanicz *et al*., *Eur J Hum Genet*, 2018). We also created zebrafish lines with the following calcium sensors under the *elavl3* promoter: G-CEPIAer, GEM-CEPIAer, and CAMPARI2. We use these lines to monitor [Ca^{2+}] in neurons in healthy and mutant zebrafish.

DEVELOPMENT OF HOLLOW ORGANS

Subunits of the voltage-gated potassium channels Kcnb1 (Kv2.1) and Kcng4 (Kv6.4) are expressed in several hollow organs (e.g., brain ventricular system [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 a opposite manner (Shen et al., *Sci Rep*, 2016). Deficiency of the BVS in humans causes epilepsy (Jedrychowska and Korzh, *Dev Dynam*, 2019). Formation of the BVS occurs during the early neural development of vertebrates (Korzh, *Cell Mol Life Sci*, 2018). Deficiency of the BVS has been linked to numerous neurodegenerative diseases. Formation of the BVS depends on many factors, including the ependyma (i.e., cells that line the BVS cavity) and circumventricular organs, including the choroid plexus (Garcia-Lecea et al., *Front Neuroanat*, 2017; Korzh and Kondrychyn, *Semin Cell Dev Biol*, 2019). To study the role of K^+ channels in the development of hollow organs, we generated a mutant of *kcnb1* and two mutants of *kcng4b* with deficiency of the BVS and ears. To distinguish the mechanism of action of KCNB1 mutations in humans, we initiated an analysis of developmental defects that are caused by mRNA of these mutants in the developing brain and ears in zebrafish.

Laboratory of Cell Biology

GROUP MEMBERS

Lab Leader
Marta Miączyńska, PhD, Professor

Senior Researchers
Magdalena Banach-Orłowska, PhD
Ewelina Szymańska, PhD
Daria Zdżalik-Bielecka, PhD

Postdoctoral Researchers
Jarosław Cendrowski, PhD
Kamil Jastrzębski, PhD
Krzysztof Kolmus, PhD
Lidia Wolińska-Nizioł, PhD

PhD Students
Marta Kaczmarek, MSc
Małgorzata Maksymowicz, MSc
Agata Poświata, MSc
Karolina Wojciechowska, MSc

Undergraduate Students
Purevsuren Erdenbat, BSc
Kamila Kozik, Eng. (until June 2019)
Karolina Romaniuk, Eng.
Małgorzata Świątek, Eng. (until September 2019)

Trainees
Kamila Kozik, Msc Eng.
Michał Mazur, Msc Eng.
Katarzyna Popiołek, Msc (August-October 2019)
Blair Stewig, BSc (Fulbright Student Researcher, until June 2019)

Lab Technician
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
1993	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
Jun 2014-Dec 2015	Deputy Director for Science, International Institute of Molecular and Cell Biology in Warsaw, Poland
Jun 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 training, European Molecular Biology Laboratory, Heidelberg, Germany
1993-1996	PhD studies, Institute of Microbiology and Genetics, University of Vienna, Austria
1990-1991	Exchange Student, University of Wolverhampton, UK

HONORS, PRIZES AND AWARDS

2020	Corresponding Member, Polish Academy of Sciences
2019	Member, Academia Europaea
2017	Member, European Molecular Biology Organization
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
2006-2010	International Research Scholar, Howard Hughes Medical Institute, USA
2006-2010	Partner Group grant, Max Planck Society, Germany
2001-2004	Postdoctoral Fellowship, Max Planck Society, Germany
1999-2000	Long-Term Postdoctoral Fellowship, Human Frontier Science Program Organization
1998-1999	Erwin Schrödinger Postdoctoral Fellowship, Austrian Science Fund
1993-1996	Bertha von Suttner PhD Scholarship, Austrian Ministry of Science
1990-1991	Studentship, 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.

SELECTED PUBLICATIONS

IIMCB Best Papers Award

Szymańska M, **Nowak P**, **Kolmus K**, Cybulska M, Goryca K, Derezińska-Wołek M, Szumera-Ciećkiewicz A, Brewińska-Olchowik M, Grochowska A, Piwocka K, Prochorec-Sobieszek M, Mikula M, **Miączyńska M.** Synthetic lethality between VPS4A and VPS4B triggers an inflammatory response in colorectal cancer. ***EMBO Mol Med***, 2020; 12(2): e10812

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

Banach-Orłowska M, **Jastrzębski K**, **Cendrowski J**, **Maksymowicz M**, **Wojciechowska K**, Korostyński M, Moreau D, Gruenberg J, **Miaczynska M**. The topology of lymphotoxin β receptor accumulated upon endolysosomal dysfunction dictates the NF-κB signaling outcome. ***J Cell Sci***, 2018; 131(22) pii: jcs218883

Budick-Harmelin N, **Miaczynska M**. Integration of the Endocytic System into the Network of Cellular Functions. ***Prog Mol Subcell Biol***, 2018; 57:39-63

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

Tudek B, **Zdżalik-Bielecka D**, Tudek A, Kosicki K, Fabisiewicz A, Speina E. Lipid peroxidation in face of DNA damage, DNA repair and other cellular processes. ***Free Radic Biol Med***, 2017; 107:77-89

Jastrzębski K, **Zdżalik-Bielecka D**, **Mamińska A**, Kalaidzidis Y, Hellberg C, **Miaczynska M**. Multiple routes of endocytic internalization of PDGFRβ contribute to PDGF-induced STAT3 signaling. ***J Cell Sci***, 2017; 130:577-89

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

Szymanska E, **Skowronek A**, **Miaczynska M**. Impaired dynamin 2 function leads to increased AP-1 transcriptional activity through the JNK/c-Jun pathway. ***Cell Signal***, 2016; 28:160-71

Cendrowski J, **Mamińska A**, **Miaczynska M**. Endocytic regulation of cytokine receptor signaling. ***Cytokine Growth Factor Rev***, 2016; 32:63-73

Song J, Mu Y, Li C, Bergh A, **Miaczynska M**, Heldin CH, Landström M. APPL proteins promote TGFβ-induced nuclear transport of the TGFβ type I receptor intracellular domain. ***Oncotarget***, 2016; 7:279-92

Mikula M, Skrzypczak M, Goryca K, Paczkowska K, Ledwon JK, Statkiewicz M, Kulecka M, Grzelak M, Dabrowska M, Kuklinska U, Karczmarski J, Rumienczyk I, **Jastrzebski K**, **Miaczynska M**, Ginalski K, Bomsztyk K, Ostrowski J. Genome-wide co-localization of active EGFR and downstream ERK pathway kinases mirrors mitogen-inducible RNA polymerase 2 genomic occupancy. ***Nucleic Acids Res***, 2016; 44:10150-64

Kalaidzidis I, **Miaczynska M**, **Brewińska-Olchowik M**, **Hupalowska A**, Ferguson C, Parton RG, Kalaidzidis Y, Zerial M. APPL endosomes are not obligatory endocytic intermediates but act as stable cargo sorting compartments. ***J Cell Biol***, 2015; 211:123-44

Toruń A, **Szymańska E**, Castanon I, **Wolińska-Nizioł L**, **Bartosik A**, **Jastrzębski K**, **Miętkowska M**, González-Gaitán M, **Miaczynska M**. Endocytic adaptor protein Tollip inhibits canonical Wnt signaling. ***PLoS One***, 2015; 10:e0130818

Banach-Orłowska M, **Szymanska E**, **Miaczynska M**. APPL1 endocytic adaptor as a fine tuner of Dvl2-induced transcription. ***FEBS Lett***, 2015; 589:532-9

Kolanczyk M, Krawitz P, Hecht J, **Hupalowska A**, **Miaczynska M**, Marschner K, Schlack C, Emerich D, Kobus K, Kornak U, Robinson PN, Plecko B, Grangl G, Uhrig S, Mundlos S, Horn D. Missense variant in CCDC22 causes X-linked recessive intellectual disability with features of Ritscher-Schinzel/3C syndrome. ***Eur J Hum Genet***, 2015; 23:633-8

Sadowski Ł, **Jastrzebski K**, Purta E, Hellberg C, **Miaczynska M**. Labeling of platelet-derived growth factor by reversible biotinylation to visualize its endocytosis by microscopy. ***Methods Enzymol***, 2014; 535:167-77

Miaczynska M. Effects of membrane trafficking on signaling by receptor tyrosine kinases. (Review) ***Cold Spring Harb Perspect Biol***, 2013; 5:a009035

Sadowski Ł, **Jastrzebski K**, Kalaidzidis Y, Heldin CH, Hellberg C, **Miaczynska M**. Dynamin Inhibitors Impair Endocytosis and Mitogenic Signaling of PDGF. ***Traffic***, 2013; 14:725-36

Pyrzynska B, **Banach-Orlowska M**, **Teperek-Tkacz M**, Miekus K, Drabik G, Majka M, **Miaczynska M**. Multifunctional protein APPL2 contributes to survival of human glioma cells. ***Mol Oncol***, 2013; 7:67-84

Winiarska M, Nowis D, Bil J, Glodkowska-Mrowka E, Muchowicz A, Wanczyk M, Bojarczuk K, Dwojak M, Firczuk M, Wilczek E, Wachowska M, Roszczenko K, **Miaczynska M**, Chlebowska J, Basak GW, Golab J. Prenyltransferases Regulate CD20 Protein Levels and Influence Anti-CD20 Monoclonal Antibody-mediated Activation of Complement-dependent Cytotoxicity. ***J Biol Chem***, 2012; 287:31983-93

Hupalowska A, **Pyrzynska B**, **Miaczynska M**. APPL1 regulates basal NF-κB activity by stabilizing NIK**.** ***J Cell Sci***, 2012; 125: 4090-102

Hupalowska A, **Miaczynska M**. The new faces of endocytosis in signaling. (Review) ***Traffic***, 2012; 13:9-18

Urbanska A, **Sadowski L**, Kalaidzidis Y, **Miaczynska M**. Biochemical Characterization of APPL Endosomes: The Role of Annexin A2 in APPL Membrane Recruitment. ***Traffic***, 2011; 12:1227-41

Pilecka I, **Sadowski L**, Kalaidzidis Y, **Miaczynska M**. Recruitment of APPL1 to ubiquitin-rich aggresomes in response to proteasomal impairment. ***Exp Cell Res***, 2011; 317:1093-107

Miaczynska M, Bar-Sagi D. Signaling endosomes: seeing is believing. (Review) ***Curr Opin Cell Biol***, 2010; 22:535-540

Banach-Orlowska M, **Pilecka I**, **Torun A**, **Pyrzynska B**, **Miaczynska M**. Functional characterization of the interactions between endosomal adaptor protein APPL1 and the NuRD corepressor complex. ***Biochem J***, 2009; 423:389–400

Pyrzynska B, **Pilecka I**, **Miaczynska M**. Endocytic proteins in the regulation of nuclear signaling, transcription and tumorigenesis. (Review) ***Mol Oncol***, 2009; 3: 321-338

Rashid S, **Pilecka I**, **Torun A**, **Olchowik M**, **Bielinska B**, **Miaczynska M**. Endosomal Adaptor Proteins APPL1 and APPL2 Are Novel Activators of beta-Catenin/TCF-mediated Transcription. ***J Biol Chem***, 2009; 284:18115-28

Sadowski L, **Pilecka I**, **Miaczynska M**. Signaling from endosomes: Location makes a difference. (Review) ***Exp Cell Res***, 2009; 315:1601-09

Ohya T, **^Miaczynska M**, Coskun U, Lommer B, Runge A, Drechsel D, Kalaidzidis Y, Zerial M. Reconstitution of Rab- and SNAREdependent membrane fusion by synthetic endosomes. ***Nature***, 2009; 459:1091-97

Miaczynska M, Stenmark H. Mechanisms and functions of endocytosis. ***J Cell Biol***, 2008; 80:7-11

Pilecka I, **Banach-Orlowska M**, **Miaczynska M**. Nuclear functions of endocytic proteins. ***Eur J Cell Biol***, 2007; 86:533-547

^Miaczynska M, Pelkmans L, Zerial M. Not just a sink: endosomes in control of signal transduction. (Review) ***Curr Opin Cell Biol***, 2004; 16:400-406

^Miaczynska M, Christoforidis S, Giner A, Shevchenko A, Uttenweiler-Joseph S, Habermann B, Wilm M, Parton RG, Zerial M. APPL proteins link Rab5 to signal transduction via an endosomal compartment. ***Cell***, 2004; 116:445-56

^ no IIMCB affiliation

DESCRIPTION OF CURRENT RESEARCH

We study the ways in which intracellular signal transduction and membrane trafficking in endocytosis are integrated at the molecular level. We focus on proteins that play well-known roles in endocytosis to investigate their involvement in various signaling pathways and their impact on patterns of gene expression. Our efforts initially focused on studying the endosomal and nuclear roles of APPL proteins. In recent years, we broadened our interests to other multifunctional proteins that act in endocytosis and signaling. The specific projects that are developed by our group seek to answer the following questions:

- What is the role of endosomal compartments in the trafficking and signaling of receptors for growth factors and cytokines?
- How does the endocytic trafficking of receptors impinge on the patterns of gene expression in different signaling pathways?
- What are the consequences of endosomal dysfunction in the cell and in the context of oncogenesis?

Endocytosis was first 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 (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). Moreover, several endocytic proteins can undergo nucleocytoplasmic shuttling and interact with nuclear molecules that are involved in transcription or chromatin remodeling, changing their localization or activity, and thus may directly modulate the levels or specificity of gene transcription (Pilecka et al., *Eur J Cell Biol*, 2007). Importantly, some such dual-function endocytic and nuclear proteins 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 identified four components of endosomal sorting complexes required for transport (ESCRTs) as novel inhibitors of nuclear factor-κB (NF-κB) signaling (Mamińska et al., *Sci Signal*, 2016). We found that the depletion of several ESCRT subunits in the absence of cytokine stimulation potently activated NF-κB signaling in cultured human cells, zebrafish embryos, and fat bodies in flies. These effects depended on cytokine receptors, such as lymphotoxin β receptor (LTβR) and tumor necrosis factor receptor 1 (TNFR1). We demonstrated that upon the depletion of ESCRT subunits, both receptors became concentrated on and signaled from endosomes. The endosomal accumulation of LTβR induced its ligand-independent oligomerization and inflammatory NF-κB signaling. We proposed that ESCRTs constitutively control the distribution of cytokine receptors in their ligand-free state to restrict their signaling.

As a follow-up of this work, we further investigated the mechanisms of intracellular trafficking and inflammatory signaling by LTβR (Banach-Orłowska et al., *J Cell Sci*, 2018). 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. Most recently, we focused on the ligand-induced trafficking and signaling of LTβR (Banach-Orłowska et al., *Cell Commun Signal*, 2019). 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. We proposed that drugs that modulate cholesterol levels could potentially improve the efficacy of LTβR-based therapies for autoimmune diseases and cancer.

In another recently published study, we uncovered an exciting connection between two ESCRT accessory proteins (VPS4A and VPS4B) and cancer (Szymańska et al., *EMBO Mol Med*, 2020). In humans, these ubiquitous ATPases are encoded by *VPS4A* and *VPS4B* paralogous genes. Together with other ESCRT subunits, VPS4 proteins participate in the remodeling of biological membranes that occurs during endocytosis and other intracellular processes, such as cytokinesis and exosome release. Thus, VPS4A and VPS4B are of importance for cellular homeostasis, and as enzymes they may represent convenient drug targets. By first exploring the publicly available Cancer Genome Atlas, we found that the *VPS4B* gene is frequently lost in many cancer types, notably colorectal cancer, along with a larger part of chromosome 18. Our clinical collaborators at the Maria Skłodowska-Curie Institute-Oncology Centre in Warsaw confirmed lower levels of VPS4B protein in tumor samples from colorectal cancer patients compared with healthy colon tissue. Using cancer cell lines that were grown *in vitro* and *in vivo*, we found that *VPS4A* and *VPS4B* paralogs were synthetically lethal, in which their simultaneous depletion (*VPS4A*+*VPS4B*) caused cell death, whereas the loss of any single paralog (*VPS4A* or *VPS4B*) was well tolerated. Our study demonstrated that VPS4B-deficient cancer cells are selectively vulnerable to perturbations of VPS4A activity. Importantly, we also discovered that dying cells that lacked both VPS4A and VPS4B proteins induced a strong inflammatory response that could evoke an anti-tumor reaction in the organism, thus supporting a positive therapeutic outcome (Fig. 1). In summary, we provided a rationale for future work to develop a VPS4 inhibitor as a putative precision therapy for patients with VPS4B-deficient cancers, such as colorectal cancer.

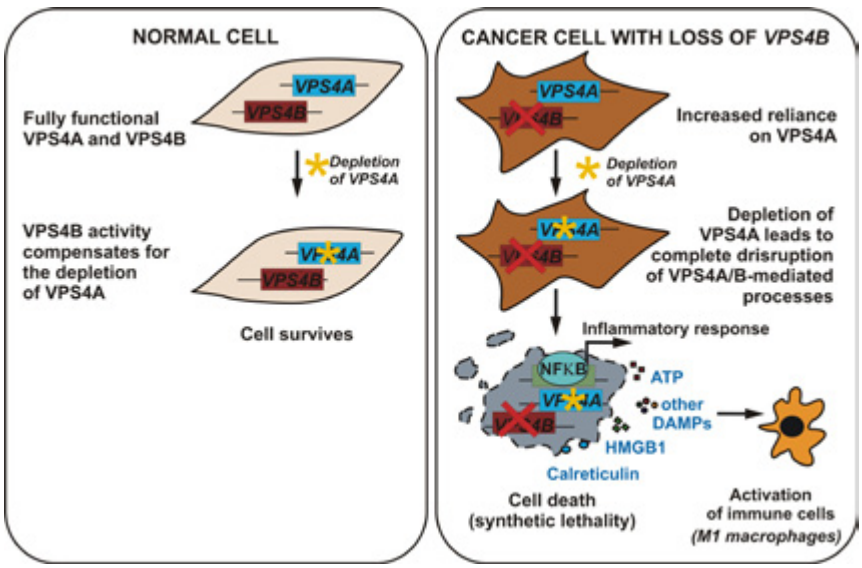


FIG. 1

Model of synthetic lethal interaction between VPS4A and VPS4B. (Left) In normal cells, both VPS4A and VPS4B act redundantly in several essential intracellular processes. The single depletion of either paralog (e.g., VPS4A) is well tolerated because the unperturbed expression of the other paralog alone (e.g., VPS4B) can compensate for its downregulated counterpart. (Right) Cells that have lost VPS4B expression (e.g., because of oncogenic genome rearrangements) rely exclusively on VPS4A activity. The inactivation of VPS4A in these cells leads to synthetic lethality, accompanied by the strong induction of an inflammatory response and the release of immunogenic DAMPs. Immunomodulatory molecules that are released by dying VPS4A+VPS4B-deficient cancer cells can elicit paracrine effects on primary immune cells, such as reprogramming of macrophages toward the M1 anti-tumor phenotype.

Author: Ewelina Szymańska (after Szymańska et al., *EMBO Mol Med*, 2020)

Laboratory of Iron Homeostasis

GROUP MEMBERS

Lab Leader
Katarzyna Mleczko-Sanecka, PhD

Postdoctoral Researcher
Sandhya, PhD

PhD Students
Gabriela Jędruszewska, MSc
Pratik Kumar Mandal, MSc
Patryk Ślusarczyk, MSc

Lab Technician
Marta Niklewicz (part-time)

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

LAB LEADER

Katarzyna Mleczko-Sanecka, PhD

CURRICULUM VITAE

DEGREES

2011 PhD in Biology, European Molecular Biology Laboratory and Heidelberg University, Heidelberg, Germany

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

PROFESSIONAL EXPERIENCE

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

2011-2015 Postdoctoral research with Prof. Martina Muckenthaler and Prof. Matthias W. Hentze, Molecular Medicine Partnership Unit, European Molecular Biology Laboratory and Heidelberg University, Heidelberg, Germany

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

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

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

2005 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

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, 2011-2009 Travel Grant 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

Pasricha SR, Lim PJ, Duarte TL, Casu C, Oosterhuis D, **Mleczko-Sanecka K**, Suciu M, Da Silva AR, Al-Hourani K, Arezes J, McHugh K, Gooding S, Frost JN, Wray K, Santos A, Porto G, Repapi E, Gray N, Draper SJ, Ashley N, Soilleux E, Olinga P, Muckenthaler MU, Hughes JR, Rivella S, Milne TA, Armitage AE, Drakesmith H. Hepcidin is regulated by promoter-associated histone acetylation and HDAC3. ***Nat Commun***, 2017; 8(1):403

Mleczko-Sanecka K, da Silva AR, Call D, Neves J, Schmeer N, Damm G, Seehofer D, Muckenthaler MU. Imatinib and spironolactone suppresshepcidin expression. ***Haematologica***, 2017; 102(7):1173-84

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

^Mleczko-Sanecka K, Roche F, da Silva AR, Call D, D'Alessio F, Ragab A, Lapinski PE, Ummanni R, Korf U, Oakes C, Damm G, D'Alessandro LA, Klingmüller U, King PD, Boutros M, Hentze MW, Muckenthaler MU. Unbiased RNAi screen for hepcidin regulators links hepcidin suppression to proliferative Ras/RAF and nutrient-dependent mTOR signaling. ***Blood***, 2014; 123(10):1574-85 (Article with a comment: Arosio P. New signaling pathways for hepcidin regulation. ***Blood***, 2014; 123(10):1433-4)

Sonnweber T, Nachbaur D, Schroll A, Nairz M, Seifert M, Demetz E, Haschka D, Mitterstiller AM, Kleinsasser A, Burtscher M, Trübsbach S, Murphy AT, Wroblewski V, Witcher DR, **^Mleczko-Sanecka K**, Vecchi C, Muckenthaler MU, Pietrangelo A, Theurl I, Weiss G. Hypoxia induced downregulation of hepcidin is mediated by platelet derived growth factor BB. ***Gut***, 2014; 63(12):1951-9

Vujić Spasić M, Sparla R, **^Mleczko-Sanecka K**, Migas MC, Breitkopf-Heinlein K, Dooley S, Vaulont S, Fleming RE, Muckenthaler MU. Smad6 and Smad7 are co-regulated with hepcidin in mouse models of iron overload. ***Biochim Biophys Acta***, 2013; 1832(1):76-84

^Mleczko-Sanecka K, Casanovas G, Ragab A, Breitkopf K, Müller A, Boutros M, Dooley S, Hentze MW, Muckenthaler MU. SMAD7 controls iron metabolism as a potent inhibitor of hepcidin expression. ***Blood***, 2010; 115(13):2657-65

Casanovas G, **^Mleczko-Sanecka K**, Altamura S, Hentze MW, Muckenthaler MU. Bone morphogenetic protein (BMP)-responsive elements located in the proximal and distal hepcidin promoter are critical for its response to HJV/BMP/SMAD. ***J Mol Med***, 2009; 87(5):471-80

Jozkowicz A, Was H, Taha H, Kotlinowski J, **^Mleczko K**, Cisowski J, Weigel G, Dulak J. 15d-PGJ2 upregulates synthesis of IL-8 in endothelial cells through induction of oxidative stress. ***Antioxid Redox Signal***, 2008; 10(12):2035-46

Funovics P, Brostjan C, Nigisch A, Fila A, Grochot A, **^Mleczko K**, Was H, Weigel G, Dulak J, Jozkowicz A. Effects of 15d-PGJ(2) on VEGF-induced angiogenic activities and expression of VEGF receptors in endothelial cells. ***Prostaglandins Other Lipid Mediat***, 2006; 79(3-4):230-44

^ 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, an excess of 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. Broadening our knowledge of the genetic control of iron homeostasis is important for human health. The major objective of research in the Laboratory of Iron Homeostasis is to better understand the processes that impact systemic and cellular iron levels and identify new players in iron-regulatory pathways.

At the systemic level, more than 90% of daily iron needs are met by internal iron recycling from senescent erythrocytes by splenic macrophages. The iron pool in the body is largely preserved. Because iron excretion is unregulated, iron acquisition in the intestine and its release from splenic macrophage stores must be tightly controlled. These tasks are chiefly fulfilled by the master iron-regulatory hormone hepcidin. 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. Follow-up work of our unbiased screens (i) revealed that SMAD7 is an important hepcidin inhibitor, (ii) linked hepcidin control to proliferative signaling, and (iii) aided in the identification of two commonly prescribed drugs, the antihypertensive drug spironolactone and antineoplastic drug imatinib, as hepcidin-suppressing agents in cultured cells and mice (Mleczko-Sanecka et al., *Blood*, 2010; *Gut*, 2014; *Haematologica*, 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. Thus, 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 elusive how different cell types in the liver may contribute to Bmp6 regulation. To address these issues, we first employed two immortalized cell lines that were derived from murine LSECs. We found that both LSEC models efficiently took up non-transferrin bound iron (NTBI), FeNTA, and iron citrate but not iron-bound transferrin. Iron accumulation led to oxidative stress but did not induce Bmp6 mRNA expression. Physiologically, liver hepatocytes are the first cells that acquire NTBI. We speculated that communication between LSECs and hepatocytes may contribute to the control of Bmp6 transcription. We established a system in which LSEC models or primary liver cultures that contain LSECs are co-cultured with hepatocytes (i.e., Hepa1-6 cells or primary

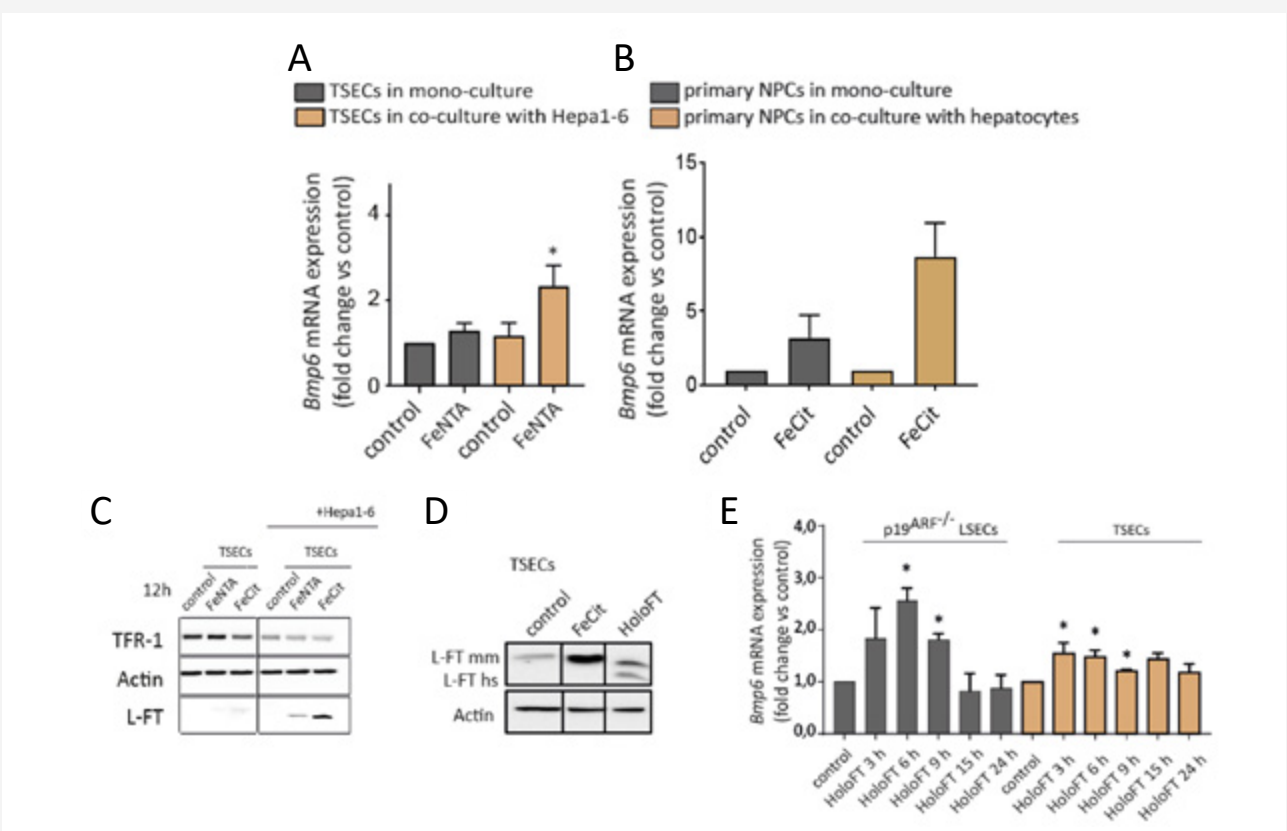


FIG. 1

(A) Unlike in TSECs that were cultured alone, TSECs that were maintained in culture together with hepatocytic Hepa1-6 cells induced Bmp6 mRNA expression in response to iron supplementation. (B) 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. (C) Upon iron treatments, ferritin levels are more elevated in TSECs after co-culture with Hepa1-6 cells. (D) Human (hs) ferritin was internalized into murine TSEC cells. (E) Supporting the hypothesis that ferritin is secreted from hepatocytes to stimulate LSEC Bmp6 expression, Bmp6 mRNA levels in LSEC models were responsive to ferritin treatment.

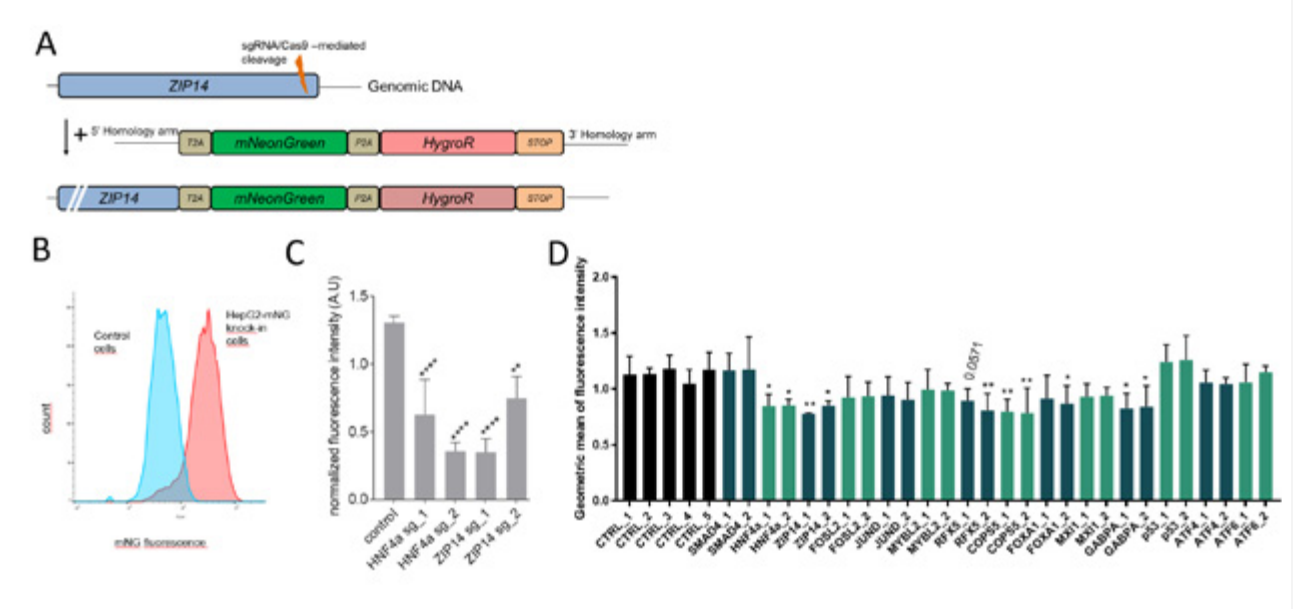


FIG. 2

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 decreased upon CRIPSR knockout of the ZIP14 regulator HNF4a and ZIP14 itself. (D) CRISPR-mediated knockout in engineered fluorescent cells aided the identification of new ZIP14 regulators.

murine hepatocytes), treated with NTBI, and then separated using 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, B). 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 showed that one candidate molecule that may perform this function was extracellular ferritin. Upon iron treatment, this protein may shuffle between hepatocytes and LSECs (Fig. 1C, D) and it stimulated Bmp6 expression in LSEC models (Fig. 1E). Our future work will utilize primary liver cell cultures and mice to improve our understanding of ferritin-dependent and possibly ferritin-independent intercellular communication that is required for iron sensing in the liver.

Iron levels increase when iron challenge persists or when hepcidin responses are dysregulated, ultimately leading to the excessive saturation of transferrin and generation of NTBI. This form of "free iron" is highly toxic and currently considered the main contributor to iron-overload disorders. Liver hepatocytes are the primary cell type that acquires NTBI, which can lead to impairments in liver function and a higher risk of aggressive hepatocellular carcinoma. Hepatic iron accumulation is a hallmark of hereditary hemochromatosis and some severe anemias (e.g., thalassemias) and accompanies several other common liver diseases. 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 are seeking to identify signaling mechanisms that control or alter the hepatic expression of ZIP14 (which is encoded by SLC39A14), the key metal transporter that is responsible for NTBI uptake in the liver. ZIP14 is considered an attractive therapeutic target to prevent or limit liver iron loading. The identification of druggable ZIP14 regulators may contribute to new pharmacological interventions and shed light on underdiagnosed iron-related side effects of some pharmaceutical compounds, which we demonstrated recently for hepcidin. Insights into regulatory mechanisms of ZIP14 may also help identify genes that modify the severity of iron overload and can serve as diagnostic markers to predict which patients are at risk of developing overt clinical symptoms. 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, a comprehensive characterization of the ZIP14 regulatory network has medical relevance to our understanding of Mn homeostasis.

To decipher the ZIP14 regulome, we aim to apply state-of-the-art 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 CRISPR-based depletion of the newly identified ZIP14 regulator, HNF4α, as well as ZIP14 itself, efficiently reduced the fluorescent signal, reflecting the response of endogenous ZIP14 mRNA (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 ChIP-seq 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 our engineered fluorescent cell line, thus validating three of the genes (COPS5, RFX5, and GABPA; Fig. 2D). Our further work will help us to better understand the involvement of these and other genes in ZIP14 regulation.

Laboratory of Protein Structure

GROUP MEMBERS

Lab Leader
Marcin Nowotny, PhD, DSc Habil

Senior Researchers
Karolina Górecka, PhD (until July 2019)
Elżbieta Nowak, PhD, DSc Habil

Researcher
Vineet Gaur, PhD (until April 2019)

Postdoctoral Researchers
Mariusz Czarnocki-Cieciura, PhD
Małgorzata Figiel, PhD
Filip Gołębiowski, PhD (until May 2019)
Malwina Hyjek-Składanowska, PhD
Zuzanna Kaczmarska, PhD
Shivlee Nirwal, PhD
Zbigniew Pietras, PhD (until March 2019)
Michał Rażew, PhD (until March 2020)

Research Technicians
Aleksandra Jakielaszek, MSc (until February 2020)
Agnieszka Napiórkowska, MSc
Sergii Palchevskyi, PhD (part-time)
Justyna Studnicka, MSc
Aleksandra Szlachcic, BSc (until January 2020)
Weronika Zajko, MSc

PhD Students
Sebastian Chamera, MSc
Prabaharan Chandrasekaran, MSc
Anuradha Chaudhary, MSc
Marta Gapińska, MSc
Deepshikha Malik, MSc
Marzena Nowacka, MSc

Technician
Iwona Ptasiewicz (part-time)

Laboratory Support Specialist
Kinga Adamska, MSc
Agnieszka Konkol, MSc (January 2019-March 2020)

LAB LEADER

Marcin Nowotny, PhD, DSc Habil

CURRICULUM VITAE

DEGREES

2013	DSc Habil in Molecular Biology, Institute of Biochemistry and Biophysics, Warsaw, Poland
2002	PhD *magna cum laude* in Biochemistry, under the supervision of Prof. 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

2019	Member, European Molecular Biology Organization
2019	Member, Academia Europaea
2019	Chair, Scientific Policy Committee, Ministry of Science and Higher Education, Poland
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 Europaea Burgen Scholar
2013	Knight's Cross of the Order of Polonia Restituta
2012	Polish Prime Minister 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 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

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

Gaur V, **Ziajko W**, **Nirwal S**, **Szlachcic A**, **Gapińska M**, **Nowotny M**. Recognition and processing of branched DNA substrates by Slx1-Slx4 nuclease. ***Nucleic Acids Res***, 2019; 47:11681-90

Hyjek M, Figiel M, Nowotny M. RNases H: Structure and mechanism. ***DNA Repair (Amst.)***, 2019; 102672

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

Figiel M, Krepl M, Park S, Poznański J, **Skowronek K**, Gołąb A, Ha T, Šponer J, **Nowotny M**. Mechanism of polypurine tract primer generation by HIV-1 reverse transcriptase. ***J Biol Chem***, 2018; 293(1):191-202

Figiel M, **Nowotny M**. Structural Studies of RNases H2 as an Example of Crystal Structure Determination of Protein-Nucleic Acid Complexes. ***Methods Enzymol***, 2017; 592:123-43

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

Stracy M, **Jaciuk M**, Uphoff S, Kapanidis AN, **Nowotny M**, Sherratt DJ, Zawadzki P. Single-molecule imaging of UvrA and UvrB recruitment to DNA lesions in living Escherichia coli. ***Nat Commun,*** 2016; 7:12568

Nowotny M, **Gaur V**. Structure and mechanism of nucleases regulated by SLX4. ***Curr Opin Struct Biol***, 2016; 36:97-105

Gaur V, Wyatt HDM, **Komorowska W**, Szczepanowski RH, de Sanctis D, **Gorecka KM**, West SC, **Nowotny M**. Structural and Mechanistic Analysis of the Slx1-Slx4 Endonuclease. ***Cell Rep,*** 2015, 10(9):1467-76

Miętus M, **Nowak E**, **Jaciuk M**, **Kustosz P**, **Studnicka J**, **Nowotny M**. Crystal structure of the catalytic core of Rad2: insights into the mechanism of substrate binding. ***Nucleic Acids Res***, 2014; 42(16):10762-75

Figiel M, **Nowotny M**. Crystal structure of RNase H3-substrate complex reveals parallel evolution of RNA/DNA hybrid recognition. ***Nucleic Acids Res***, 2014; 42(14):9285-94

Nowak E, Miller JT, Bona MK, **Studnicka J**, **Szczepanowski RH**, **Jurkowski J**, Le Grice SFJ§, **Nowotny M**§. Ty3 reverse transcriptase complexed with an RNA-DNA hybrid shows structural and functional asymmetry. ***Nat Struct Mol Biol,*** 2014; 21(4):389-96

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

Górecka KM, **Komorowska W**, **Nowotny M**. Crystal structure of RuvC resolvase in complex with Holliday junction substrate. ***Nucleic Acids Res,*** 2013; 41(21):9945-55

Nowak E, Potrzebowski W, Konarev P, Rausch J, Bona M, Svergun DI, Bujnicki JM, Le Grice SF, **Nowotny M**. Structural analysis of monomeric retroviral reverse transcriptase in complex with an RNA/DNA hybrid. ***Nucleic Acids Res,*** 2013; 41(6):3874-87

Figiel M, Chon H, Cerritelli SM, **Cybulska M**, Crouch RJ, **Nowotny M**. The structural and biochemical characterization of human RNase H2 complex reveals the molecular basis for substrate recognition and Aicardi-Goutieres syndrome defects. ***J Biol Chem,*** 2011; 286(12):10540-50

Jaciuk M, **Nowak E**, Skowronek K, **Tanska A**, **Nowotny M**. Structure of UvrA nucleotide excision repair protein in complex with modified DNA. ***Nat Struct Mol Biol,*** 2011; 18(2):191-7

Rychlik MP, Chon H, Cerritelli SM, Klimek P, Crouch RJ, **Nowotny M**. Crystal structures of RNase H2 in complex with nucleic acid reveal the mechanism of RHAN-DNA junction recognition and cleavage. ***Mol Cell,*** 2010; 40(4):658-70

Nowotny M, Yang W. Structural and functional modules in RNA interference (review). ***Curr Opin Struct Biol,*** 2009; 19(3):286-93

Nowotny M. Retroviral integrase superfamily: the structural perspective (review). ***EMBO Rep,*** 2009; 10(2):144-51

^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

* equally contributing,
§ corresponding authors
^ no IIMCB affiliation

DESCRIPTION OF CURRENT RESEARCH

Our laboratory focuses on structural and biochemical studies of nucleic acid processing enzymes using protein crystallography as a primary method. The key results that have been recently generated by our group concern DNA processing by RuvC (Holliday junction resolvase) and Slx1-Slx4 (DNA repair nuclease).

1. RUVC: THE BACTERIAL HOLLIDAY JUNCTION RESOLVASE

Holliday junctions (HJs) are four-way DNA structures that are formed by strand exchange between two helices. They are intermediates in homologous recombination, a process that is used to repair dangerous DNA lesions, such as double-strand breaks. Once the repair process is completed, HJs need to be removed because they are highly mutagenic and can prevent chromosome segregation. One way to achieve their removal is through the action of specialized structure-specific (selective) nucleases called resolvases. RuvC is a canonical bacterial resolvase. It functions as a dimer and introduces two symmetric cuts in the HJ that lead to the clean separation of its two halves.

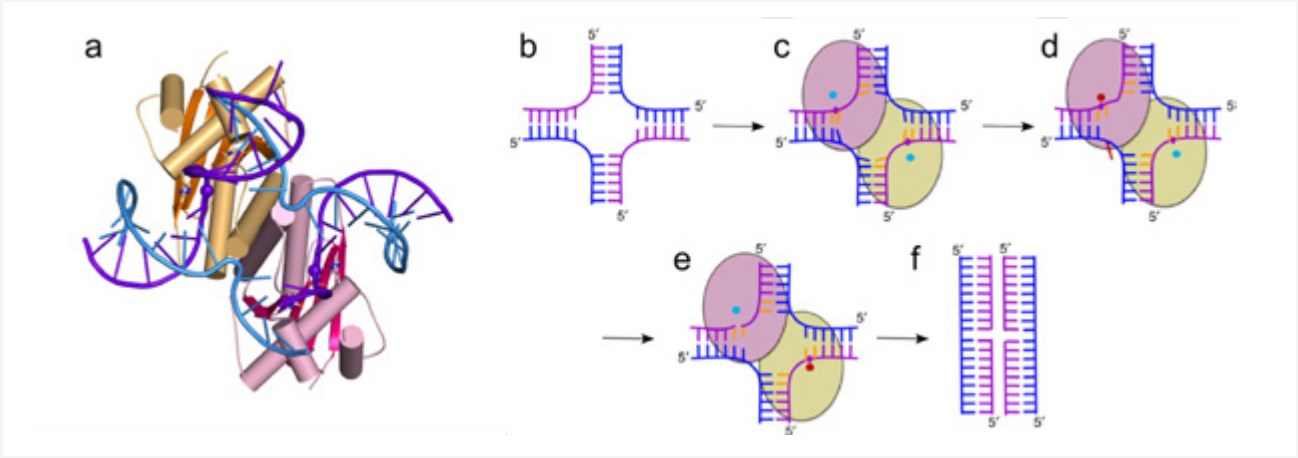


FIG. 1

Structure and mechanism of RuvC. (a) Crystal structure of RuvC in complex with a Holliday junction. The two protomers are shown in pink and orange. The DNA is shown in blue and purple with cleaved phosphates indicated with spheres. Schematic of the reaction. (b) Holliday junction. Cleaved and non-cleaved DNA strands are shown in purple and blue ladder-like representations, respectively. (c) Binding of the HJ DNA. The subunits of the dimer are shown as yellow green and pink ovals. The consensus sequence is shown in orange. The scissile phosphate is marked as a purple circle. Cyan circles show active sites in an inactive configuration. (d) Flipping of the adenine (red) opposite the scissile base. The active site in the catalytic configuration is shown as a red circle. (e) The second cut. (f) Resolution products.

It is structurally related to RNases H. We solved the first crystal structure of RuvC in complex with a HJ substrate, which at the time was the first enzyme-substrate complex structure of a cellular resolvase (Fig. 1a, Górecka et al., *Nucleic Acids Res,* 2013). It was initially solved at 3.8 Å resolution. In the structure, the HJ is present in a tetrahedral conformation which, at the time, was observed for the first time. The two phosphate groups that are symmetrically located 1 nt from the HJ exchange point interact with two active sites of the RuvC dimer. This mode of HJ binding was novel and different from phage enzymes for which complex crystal structures were available.

Recently, we solved an improved 3.4 Å structure of the RuvC-HJ complex. Using this structure in combination with molecular dynamics and biochemical experiments, we showed how RuvC recognizes its cognate sequence and how the two HJ cuts are coordinated. We discovered that the enzyme introduces structural tension in the DNA and that the two scissile phosphates are displaced from the active sites. The tension is released by flipping the base opposite to the scissile phosphate. This is easier for an adenine-thymine base pair, which explains the enzyme's preference for cleavage after a thymine residue (Fig. 1b-f). This mechanism also explains how the two cuts of the HJ are coordinated. The first cut is relatively slow, whereas the second cut is very fast, which ensures complete resolution. The results we obtained for RuvC are an example of indirect readout of a nucleic acid sequence through the conformational probing of its dynamic properties. We have described another example of such probing for HIV-1 reverse transcriptase (RT). This enzyme uses this mechanism to protect a specific sequence of the original viral RNA, termed polypurine tract, from degradation so that it can serve as a primer for DNA synthesis (Figiel et al., *J Biol Chem*, 2018). Studies of the mechanisms of HIV-1 RT and RuvC have been performed in collaboration with Jiří Šponer and Miroslav Krepl (Institute of Biophysics, CAS).

2. SLX1-SLX4 COMPLEX: DNA REPAIR NUCLEASE AND A COMPONENT OF THE EUKARYOTIC HOLLIDAY JUNCTION RESOLVING COMPLEX

Slx1 is a nuclease that comprises a catalytic GIY-YIG domain and a C-terminal RING zinc-binding domain. Slx1 cleaves various different branched DNA structures to function in DNA repair and recombination. It associates with Slx4 scaffold protein, which coordinates the action of multiple proteins. For example, Slx1, together with Slx4 and Mus81-Eme1 nuclease, constitutes one of the major HJ resolution pathways in higher eukaryotes. Slx1 has two key biochemical features. First, it is only active upon binding to Slx4. Second, it can cut various branched DNA substrates in the vicinity of the junction point.

We determined the first structures of Slx1 and its complex with the binding domain from Slx4, called the C-terminal conserved domain (CCD), using proteins from *C. glabrata* (Gaur et al., *Cell Rep,* 2015). Fungal Slx1 forms a homodimer where the active site is blocked. This explains why Slx1 alone is inactive. We showed that the CCD domain binds between the nuclease and RING domains, and this is mutually exclusive with homodimerization. The interaction between Slx1 and Slx4^{CCD} exposes the active site of Slx1 and activates the nuclease. This mechanism ensures that the promiscuous and potentially dangerous Slx1 nuclease is only active when bound to the Slx4 platform, which regulates its activity and coordinates it with other proteins. To our knowledge, this is the first time that inhibitory homodimerization has been described as a mechanism of nuclease regulation. The work on Slx1-Slx4 was performed in collaboration with Dr. Stephen West (The Crick Institute, UK).

Recently, we also solved crystal structures of the Slx1-Slx4 complex with non-catalytically bound DNA (Gaur et al., *Nucleic Acid Res*, 2019). Based on these crystal structures, modeling, and biochemical studies, we proposed a mechanism whereby Slx1 bends the DNA and identifies the branch point in the nucleic acid as a flexible discontinuity (Fig. 2). This way, the Slx1-Slx4 complex is able to cleave branched DNA substrates of various structures, which explains its promiscuous activity. Furthermore, our model also explains the observed positioning of the cuts on the 3′ side of the branch point.

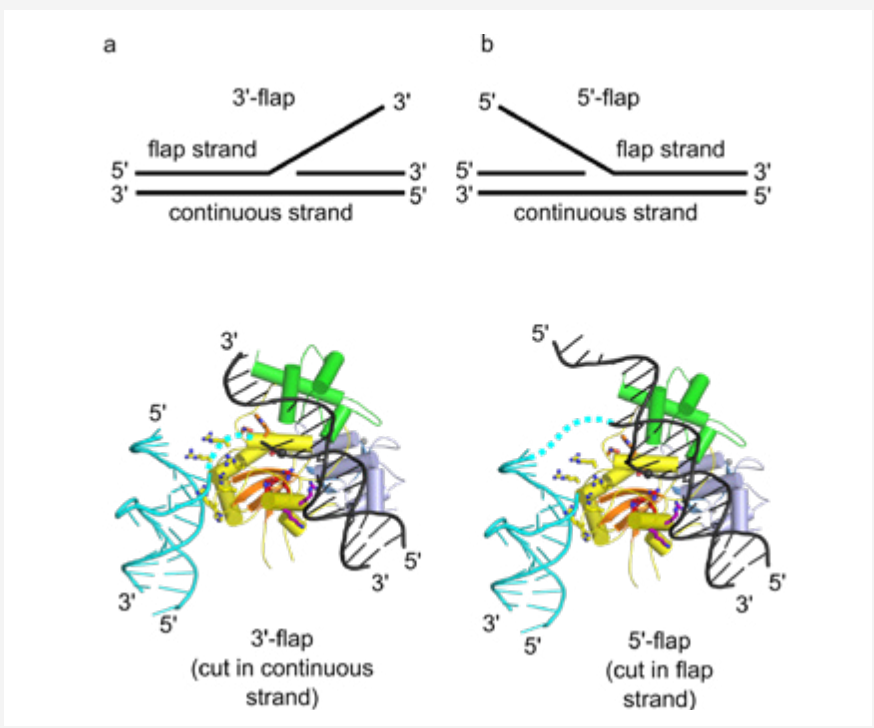


FIG. 2

Models of Slx1–Slx4CCD3 interactions with two types of branched DNA. Upper panels show the schematics of the DNA flap substrates with terminology of the strands. Lower panels show structural models of Slx1–Slx4CCD3 interacting with respective DNAs in configuration conducive to cleavage. Slx1 GIY-YIG domain is shown in yellow with β-strands in orange, and the RING domain is shown in light blue. Slx4CCD3 is shown in green. The modeled DNA is based on the structure of R.Eco29kI restrictase (PDB ID: 3NIC) and is shown in black with the scissile phosphate shown as a sphere. A fragment of the DNA that is observed in the Slx1–Slx4CCD3-DNA structure is shown in cyan. The possible link between the two DNA double helices is shown as a dashed cyan line. Residues of the active sites are shown as red sticks.

Laboratory of Protein Metabolism in Development and Aging

GROUP MEMBERS

Lab Leader
Wojciech Pokrzywa, PhD, DSc Habil

Postdoctoral Researchers
Michał Chojnacki, PhD
Nilesh Shanmugam, PhD

Research Technician
Małgorzata Piechota, PhD

PhD Students
Katarzyna Banasiak, MSc
Aniruddha Das, MSc
Pratik Kumar Mandal, MSc
Pankaj Thapa, MSc

Trainees
Anna Kociołek
Agata Kowalska
Konrad Kowalski
Anwesha Sarkar
Natalia Szulc, MSc

Lab Technician
Marta Niklewicz, MSc (part-time)

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

LAB LEADER

Wojciech Pokrzywa, PhD, DSc Habil

CURRICULUM VITAE

DEGREES

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

PROFESSIONAL EXPERIENCE

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

HONORS, PRIZES AND AWARDS

2018	FIRST TEAM, Foundation for Polish Science
2018	EMBO Installation Grant
2017	OPUS, National Science Centre
2005	PhD Fellowship, FNRS-Fund for Scientific Research, Belgium
2004	ERASMUS Scholarship

SELECTED PUBLICATIONS

Thapa P, **Shanmugam N**, **Pokrzywa W**. Ubiquitin Signaling Regulates RNA Biogenesis, Processing, and Metabolism. ***Bioessays***, 2020; 42(1):e1900171

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

Balaji V, **Pokrzywa W***, Hoppe T. Ubiquitylation pathways in insulin signaling and organismal homeostasis. ***Bioessays***, 2018; 40(5):e1700223

Pokrzywa W, Hoppe T. CHIPped balance of proteostasis and longevity. ***Oncotarget***, 2017; 8(57):96472-3

Pokrzywa W, Lorenz R, Hoppe T. Chaperone-directed ubiquitylation maintains proteostasis at the expense of longevity. ***Worm***, 2017; 6(2):e1371403

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

Tawo R, **^Pokrzywa W***, Kevei E, Akyuz ME, Balaji V, Adrian S, Hoehfeld J, Hoppe T. The ubiquitin ligase CHIP integrates proteostasis and aging by regulation of insulin receptor turnover. ***Cell***, 2017; 169(3):470-82

Ackermann L, Schell M, **^Pokrzywa W**, Kevei E, Gartner A, Schumacher B, Hoppe T. E4 ligase-specific ubiquitylation hubs coordinate DNA double-strand break repair and apoptosis. ***Nat Struct Mol Biol***, 2016; 23(11):995-1002

Frumkin A, Dror S, **^Pokrzywa W**, Bar-Lavan Y, Karady I, Hoppe T, Ben-Zvi A. Challenging muscle homeostasis uncovers novel chaperone interactions in Caenorhabditis elegans. ***Front Mol Biosci***, 2014; 1:21

Bonizec M, Hérissant L, **^Pokrzywa W**, Geng F, Wenzel S, Howard GC, Rodriguez P, Krause S, Tansey WP, Hoppe T, Dargemont C. The ubiquitin-selective chaperone Cdc48/p97 associates with Ubx3 to modulate monoubiquitylation of histone H2B. ***Nucleic Acids Res***, 2014; 42(17):10975-86

Segref A, Kevei E, **^Pokrzywa W**, Schmeisser K, Mansfeld J, Livnat-Levanon N, Ensenauer R, Glickman MH, Ristow M, Hoppe T. Pathogenesis of human mitochondrial diseases is modulated by reduced activity of the ubiquitin/ proteasome system. ***Cell Metab***, 2014; 19(4):642-52

^Pokrzywa W, Hoppe T. Chaperoning myosin assembly in muscle formation and aging. ***Worm***, 2013; 2(3): e25644

Gazda L, **^Pokrzywa W***, Hellerschmied D, Loewe T, Forné I, Mueller-Planitz F, Hoppe T, Clausen T. The myosin chaperone UNC-45 is organized in tandem modules to support myofilaments formation in C. elegans. ***Cell***, 2013; 153:183-95

^ no IIMCB affiliation
* co-first authorship

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 the 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 the 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. As such, impairments in proteostasis are a major hallmark of aging and associated with dementia, neurodegeneration, type 2 diabetes, cystic fibrosis, cancer, and cardiovascular disease (Labbadia and Morimoto, *Annu Rev Biochem*, 2015). One of the central nodes of the eukaryotic proteostasis network is the interaction between molecular chaperones and proteolytic machinery. To maintain the cellular proteome, molecular chaperones and ubiquitin-dependent degradation pathways coordinate protein refolding and the removal of terminally damaged proteins. Irreversibly affected proteins are recognized by chaperone-assisted E3 ubiquitin ligases, which target them for degradation by the ubiquitin-proteasome system (UPS) or autophagy (Fig. 1). Our studies concentrate on the basic understanding of the spatiotemporal regulation of protein quality control activity and processing of its substrates. In our research, we use a combination of biochemical, microscopic, and molecular genetic techniques and tissue-specific approaches in *C. elegans*.

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, which is 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, which is 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. Scott et al., (*Cell*, 2016) reported that two distinctive E3s could reciprocally monitor each other for the simultaneous and joint regulation of substrate ubiquitylation. 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.

A combination of E3 ligases could support the formation of alternative ubiquitylation structures in different physiological processes, which probably allows them to improve substrate recognition and ubiquitylation process. 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 the regulation of many biological processes, including gene expression, signaling, protein synthesis, and lipid metabolism. SAM is synthesized in the cytosol of every cell from L-methionine and adenosine triphosphate in a reaction that is catalyzed by methionine adenosyltransferase (Fontecave et al., *Trends Biochem*, 2004). Despite fundamental roles of the SAM cycle in a broad range of biological processes, the mechanisms of its regulation are still enigmatic. Our preliminary

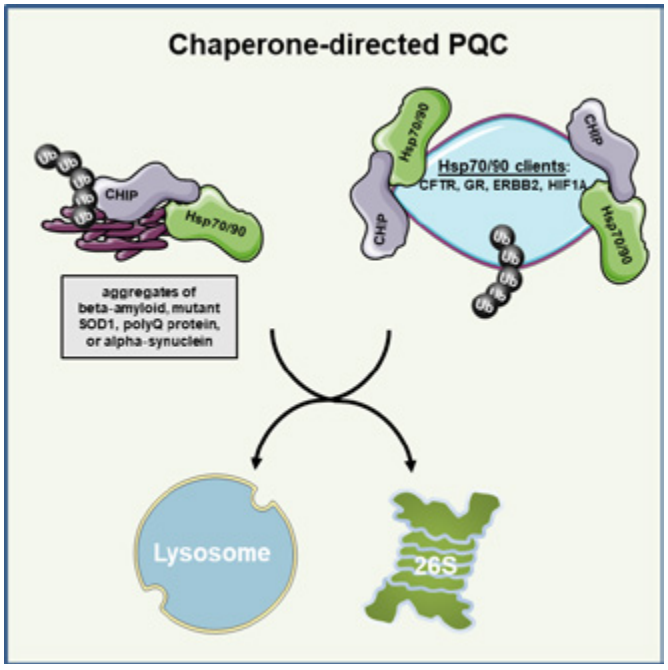


FIG. 1

The illustration represents an important component of the protein homeostasis system, the quality control ubiquitin ligase CHIP. The primary function of CHIP is polyubiquitylat ion 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

studies suggest that the UPS regulates SAM cycle activity. We aim to understand the ways in which the UPS modulates methionine metabolism, methylation potential of the cell, and epigenetic memory using *C. elegans* as an animal model. Our research may have broad implications for understanding the regulation of methionine metabolism in both health and disease.

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 *Caenorhabditis elegans* UNC-45, a protein that is essential for the organization of striated muscle filaments (Price et al., *J Cell Sci*, 2002). Moreover, UNC-45 homologs exist in vertebrates, indicating a 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 (Janiesch et al., *Nat Cell Biol*, 2007).

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 the impaired myofibers to support their repair (Fig. 2). However, little is known about the coordination of protein homeostasis pathways upon mechanical stress. Therefore, the long-term objective of this project 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.

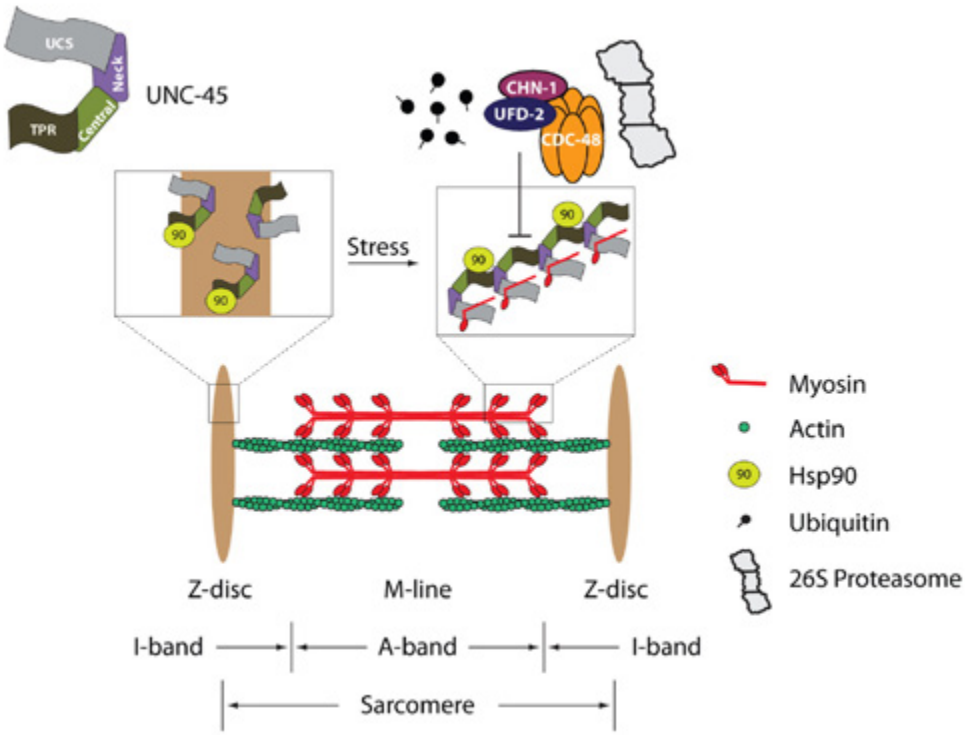


FIG. 2

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.

Laboratory of Zebrafish Developmental Genomics Max Planck/IIMCB Research Group

GROUP MEMBERS

Lab Leader
Cecilia Lanny Winata, PhD

Senior Researcher
Michał Pawlak, PhD (until January 2020)

Postdoctoral Researchers
Katarzyna Nieścierowicz, PhD
Leszek Pryszcz, PhD
Agata Sulej, PhD
Rohit Suratekar, PhD
Shikha Vashist, PhD
Barbara Uszczyńska-Ratajczak, PhD

Research Technicians
Christina Navarrete Hernandez, BSc (until February 2020)
Adrianna Pakuła, MSc
Aleksandra Paterek MSc Eng. (part-time, until July 2019)

PhD Students
Monika Kamińska, MSc Eng.
Maciej Łapiński, BSc
Maciej Migdał, MSc
Karim Abu Nahia, MSc
Sreedevi Sugunan, MSc
Eugeniusz Tralle, MSc

Undergraduate Students
Angelika Brzozowska, BSc (until July 2019)
Tomasz Obrębski, BSc

Lab Technician
Agnieszka Olszewska (part-time)

Laboratory Support Specialist
Marcin Kozioł (part-time, until December 2019)
Agnieszka Konkol, MSc

LAB LEADER

Cecilia Lanny Winata, PhD

CURRICULUM VITAE

DEGREES

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

PROFESSIONAL EXPERIENCE

2014-Present	Professor, Head, Zebrafish Developmental Genomics Laboratory, Max Planck/International Institute of Molecular and Cell Biology Research Group in Warsaw, Poland
2013-2014	Research Associate, Genome Institute of Singapore (with 2013 research visit to laboratory of Prof. Peter Alestrom, Norwegian School of Veterinary Sciences, Oslo, Norway)
2009-2013	Postdoctoral Fellow with Dr. Sinnakaruppan Mathavan, Genome Institute of Singapore
2004-2019	Doctoral research with Prof. Gong Zhiyuan and Prof. Vladimir Korzh, Department of Biological Sciences, National University of Singapore

HONORS, PRIZES AND AWARDS

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

SELECTED PUBLICATIONS

IIMCB Best Papers Award

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):167-8

Minhas R, Paterek A, Łapiński M, Bazała M, Korzh V, Winata CL. A novel conserved enhancer at zebrafish zic3 and zic6 loci drives neural expression. ***Dev Dyn***, 2019; 248(9):837-49

Pawlak M, **Kedzierska KZ**, **Migdal M**, Nahia KA, Ramilowski JA, Bugajski L, Hashimoto K, **Marconi A**, Piwocka K, Carninci P, **Winata CL**. Dynamics of cardiomyocyte transcriptome and chromatin landscape demarcates key events of heart development. ***Genome Res***, 2019 Mar;29(3):506-519

Pawlak M, **Niescierowicz K**, **Winata CL**. Decoding the Heart through Next Generation Sequencing Approaches. ***Genes (Basel)***, 2018;9(6). pii: E289

Winata CL, **Łapiński M**, **Pryszcz L**, Vaz C, Bin Ismail MH, Nama S, Hajan HS, Lee SGP, Korzh V, Sampath P, Tanavde V, Mathavan S. Cytoplasmic polyadenylation-mediated translational control of maternal mRNAs directs maternal-to-zygotic transition. ***Development***, 2018; 145(1). pii: dev159566

Winata CL, Korzh V. The translational regulation of maternal mRNAs in time and space. ***FEBS Lett***, 2018; 592(17):3007-23

Korzh V, Kondrychyn I, **Winata C**. The Zebrafish as a New Model System for Experimental Biology. ***Cytol Genet***, 2018; 52(6):406-15

Winata CL, Korzh V. Zebrafish Zic Genes Mediate Developmental Signaling. ***Adv Exp Med Biol***, 2018; 1046:157-77

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

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

Tan HH, Onichtchouk D, **Winata C**. DANIO-CODE: Toward an encyclopedia of DNA elements in Zebrafish. ***Zebrafish***, 2016; 13(1): 54-60

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

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

Utami KH, **^Winata CL**, Hillmer AM, Aksoy I, Long HT, Liany H,Chew EG, Mathavan S, Tay SK, Korzh V, Sarda P, Davila S, Cacheux V. Impaired development of neural-crest cell-derived organs and intellectual disability caused by MED13L haploinsufficiency. ***Hum Mutat***, 2014; 35(11):1311-20

Aanes H, **^Winata C**, Moen LF, Ostrup O, Mathavan S, Collas P, Rognes, T, Alestrom P. Normalization of RNA-sequencing data from samples with varying mRNA levels. ***PLoS One***, 2014; 9(2): e89158

^Winata CL, Kondrychyn I, Kumar V, Srinivasan KG, Orlov Y, Ravishankar A, Prabhakar S, Stanton LW, Korzh V, Mathavan S. Genome wide analysis reveals Zic3 interaction with distal regulatory elements of stage specific developmental genes in zebrafish. ***PLoS Genet***, 2013; 9(10): e1003852

Aanes H*, **^Winata CL***, Lin CH, Chen JP, Srinivasan KG, Lee SG, Lim AY, Hajan HS, Collas P, Bourque G, Gong Z, Korzh V, Alestrom P, Mathavan S. Zebrafish mRNA sequencing deciphers novelties in transcriptome dynamics during maternal to zygotic transition. ***Genome Res***, 2011; 21(8): 1328-38

Lindeman LC, Andersen IS, Reiner AH, Li N, Aanes H, Ostrup O, **^Winata C**, Mathavan S, Muller F, Alestrom P, Collas P. Prepatterning of developmental gene expression by modified histones before zygotic genome activation. ***Dev Cell***, 2011; 21(6):993-1004

Korzh S, **^Winata CL**, Zheng W, Yang S, Yin A, Ingham P, Korzh V, Gong Z. The interaction of epithelial Ihha and mesenchymal Fgf10 in zebrafish esophageal and swimbladder development. ***Dev Biol***, 2011; 359(2):262-76

Yin A, Korzh S, **^Winata CL**, Korzh V, Gong Z. Wnt signaling is required for early development of zebrafish swimbladder. ***PLoS One***, 2011; 6(3): e18431

Lindeman LC, **^Winata CL**, Aanes H, Mathavan S, Alestrom P, Collas P. Chromatin states of developmentally-regulated genes revealed by DNA and histone methylation patterns in zebrafish embryos. ***Int J Dev Biol***, 2010; 54(5):803-13

^Winata CL, Korzh S, Kondrychyn I, Korzh V, Gong Z. The role of vasculature and blood circulation in zebrafish swimbladder development. ***BMC Dev Biol***, 2010; 10:3

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

Ung CY, Lam SH, Hlaing MM, **^Winata CL**, Korzh S, Mathavan S, Gong Z. Mercury-induced hepatotoxicity in zebrafish: in vivo mechanistic insights from transcriptome analysis, phenotype anchoring and targeted gene expression validation. ***BMC Genomics***, 2010; 11:212

^Winata CL, Korzh S, Kondrychyn I, Zheng W, Korzh V, Gong Z. Development of zebrafish swimbladder: the requirement of Hedgehog signaling in specification and organization of the three tissue layers. ***Dev Biol***, 2009; 331(2):222-36

Korzh S, Pan X, Garcia-Lecea M, **^Winata CL**, Pan X, Wohland T, Korzh V, Gong Z. Requirement of vasculogenesis and blood circulation in late stages of liver growth in zebrafish. ***BMC Dev Biol***, 2008; 8:84

Lam SH*, **^Winata CL***, Tong Y, Korzh S, Lim WS, Korzh V, Spitsbergen J, Mathavan S, Miller LD, Liu ET, Gong Z. Transcriptome kinetics of arsenicinduced adaptive response in zebrafish liver. ***Physiol Genomics***, 2006; 27(3):351-61

Lam SH, Mathavan S, Tong Y, Hu J, **^Winata CL**, Lee S, Miller LD, Liu ET, and Gong Z. Preliminary microarray analyses of gene expression in zebrafish treated with xenobiotic and bioactive compounds. ***Mar Biotechnol***, 2004; 6:S468-S474

*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 at 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. We investigate gene regulation at two different levels: transcriptional and post-transcriptional.

Elucidating the genome-wide regulatory landscape of heart development

At the level of transcriptional regulation, 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. We also seek to understand how the disruption of this regulation results in congenital malformations, which would ultimately provide critical insight into human diseases. We posed our questions within the context of heart development because of the importance of this organ for life and its relatively well studied morphology, physiology, and development. Key factors regulating the development and functioning of the heart have been established. However, still unknown are precisely how these factors are regulated, how they interact with each other and with epigenetic factors, and how the regulatory landscape dynamically changes throughout development in order to orchestrate different phases of organogenesis. Understanding such regulation would contribute to the identification of non-genic risk factors and elucidation of the complex pathophysiology of congenital heart defects.

To gain a comprehensive view of the gene regulatory network in 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 induction of the expression of distinct TFs, resulting in their different properties. Parallel studies in these two cell types will 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. In collaboration with K. Piwocka (Nencki Institute, Poland) and P. Carninci (RIKEN Center for Integrative Medical Sciences, Japan), we performed RNA-seq and ATAC-seq to profile the transcriptome and chromatin accessibility across three developmental stages Fig.1. 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 for DNA sequence motifs that belong to the family of corresponding TFs. 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 the regions with differential chromatin accessibility in the mutants were highly conserved non-coding elements that represent putative enhancers that drive heart development.

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. These include in silico TF footprinting analysis of ATAC-seq data and Boolean modeling of the cardiac transcriptional regulatory network based on genomic and epigenomic data. Such modeling-based approaches will enable us to better define and characterize principles of molecular interactions and project these principles to predicting the outcomes of genetic perturbations. This provides a system where 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 potentially refute many unnecessary or implausible hypotheses, thus saving time and resources by addressing specific questions and pinpointing crucial links or correlations in the system for targeted validation. Ultimately, we seek to characterize the contribution of the dynamic transcriptional regulatory landscape to heart development and identify novel elements (both genic and non-genic) 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 are set apart early from CMs in the course of heart development through induction of the expression of core TFs, such as Tbx2, Tbx3, Tbx18, and Isl1, which prevents 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. Despite the knowledge of key genetic factors that are required for pacemaker cell specification, the molecular mechanisms that regulate their development are still insufficiently understood. Important questions remain unanswered with regard to the ways in which the underlying molecular mechanism translates into the proper functioning of pacemaker cells and the consequences of their dysregulation. 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. Importantly, zebrafish have the potential to allow large-scale pharmaceutical screening to discover new therapies for heart disease, particularly those that affect the pacemaker. In collaboration with Vladimir Korzh (IIMCB), we utilized the transgenic lines ET33mi59B, ET33mi28, and ET31, which express green fluorescent protein in subpopulations of pacemaker cells, to characterize the morphology of the zebrafish pacemaker and isolate pacemaker cells for further genomic analyses to elucidate gene regulatory networks in pacemaker development. 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. However, in addition to these genes, we also identified genes that are normally expressed in the working myocardium, epicardium, and endocardium. These observations reflect the heterogeneity of cell types that comprise the pacemaker region, thus posing additional challenges to data interpretation. We are currently focusing our analyses at the single cell level through the application of a Drop-seq-based method to characterize the diversity of cell types that constitute the pacemaker and assign molecular identities to each specific cell population. The 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, a detailed knowledge of the distinct cell types that constitute the pacemaker and a thorough understanding of their nature is an essential step to understand 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 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 mode of post-transcriptional gene regulation, such as RNA modification, would nicely fit into the scenario that occurs at the earliest stages of embryonic development, during which the embryo contains an abundant supply of maternal mRNAs and zygotic transcription is still absent. Surprisingly, RNA editing has been seldom considered in the context of embryonic development. In collaboration with the Bochtler laboratory (IIMCB), we aim to elucidate the biological function of A-to-I RNA editing in early embryonic development using zebrafish as a model organism.

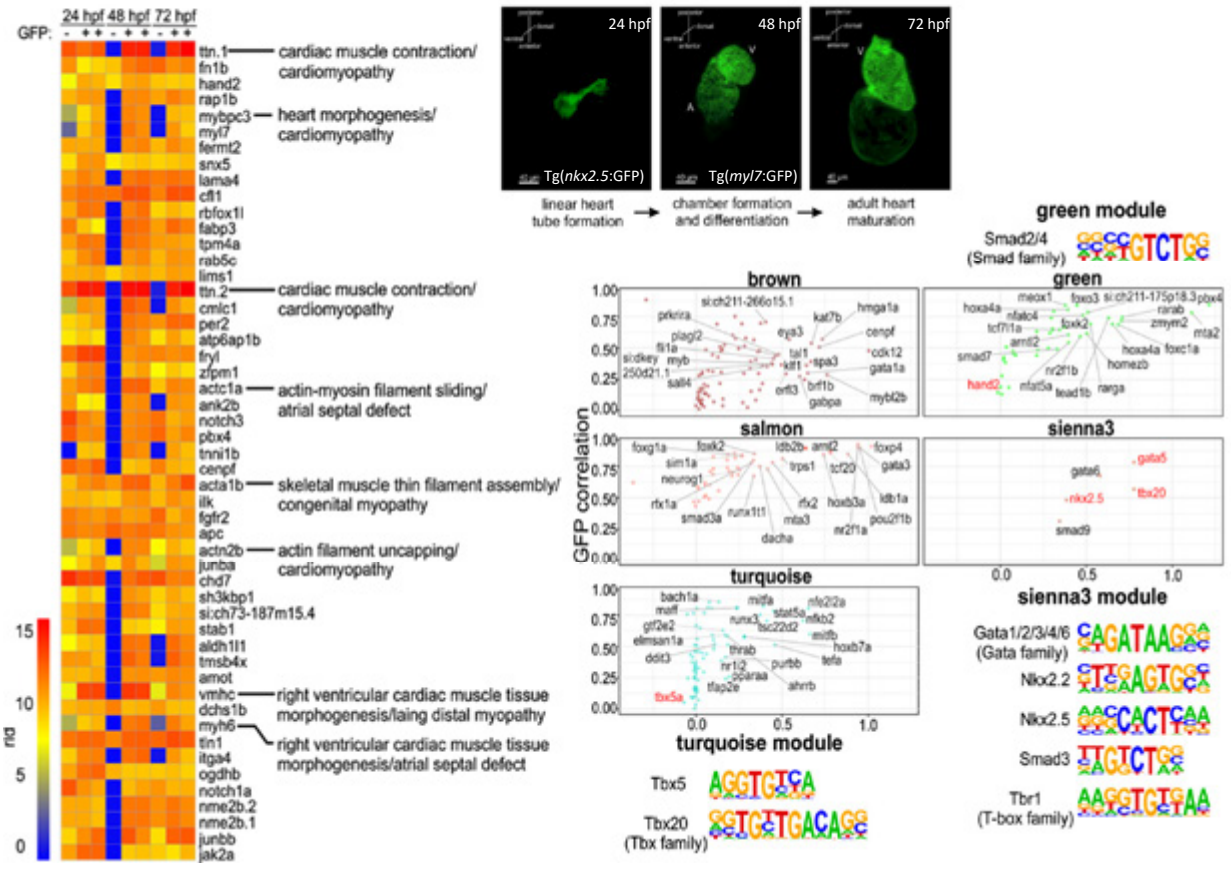


FIG. 1

Cardiomyocyte transcriptome landscape during heart development and coexpression regulatory networks showing module gene connectivity plot of selected TFs (Pawlak et al., *Genome Res*, 2019).

Laboratory of Biomolecular Interactions and Transport AMU/IIMCB in Poznań

GROUP MEMBERS

Lab Leader
Jan Brezovsky, PhD

Postdoctoral Researchers
Nikhil Agrawal, PhD (AMU)
Cedrix Dongmo Foumthuim, PhD (until November 2019)

PhD Students
Nishita Mandal, MSc (AMU)
Dheeraj Kumar Sarkar, MSc (AMU)
Carlos Eduardo Sequeiros Borja, MSc
Bartłomiej Surpeta, MSc
Aravind Selvaram Thirunavukkarasu, MSc

Undergraduate Students
Wiktor Szymkowski, BSc (AMU)

Research Technician & 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

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	5th place at national competition Chemistry Prize of Jean-Marie Lehnen
2011	Dean's prize for outstanding PhD research, Masaryk University, Brno, Czech Republic
2007	Research grant from Masaryk University, Brno, Czech Republic

SELECTED PUBLICATIONS

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). pii: E2713

Jurcik A, Bednar D, Byska J, Marques SM, Furmanova K, Daniel L, Kokkonen P, **Brezovsky J**, Strnad O, Stourac J, Pavelka A, Manak M, Damborsky J, Kozlikova B. CAVER Analyst 2.0: Analysis and Visualization of Channels and Tunnels in Protein Structures and Molecular Dynamics Trajectories. ***Bioinformatics***, 2018; 34(20):3586-88

Grulich M, **Brezovsky J**, Stepanek V, Palyzova A, Maresova H, Zahradnik J, Kyslikova E, Kyslik P. In-silico driven engineering of enantioselectivity of a penicillin G acylase towards active pharmaceutical ingredients. ***J Mol Catal B Enzym***, 2016; 133, Supplement 1:S53-S59

^Brezovsky J*, Babkova P*, Degtjarik O, Fortova A, Gora A, Iermak I, Rezacova P, Dvorak P, Smatanova IK, Prokop Z, Chaloupkova R, Damborsky J. Engineering a de novo transport tunnel. ***ACS Catal,*** 2016; 6(11):7597-610

Bendl J, Musil M, Stourac J, Zendulka J, Damborsky J, **^Brezovsky J**#. PredictSNP2: A unified platform for accurately evaluating SNP effects by exploiting the different characteristics of variants in distinct genomic regions. ***PLoS Comput Biol,*** 2016; 12(5):e1004962

Bendl J, Stourac J, Sebestova E, Vavra O, Musil M, **^Brezovsky J**#, Damborsky J#. HotSpot Wizard 2.0: automated design of site-specific mutations and smart libraries in protein engineering. ***Nucleic Acids Res,*** 2016; 44:W479-487

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

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 disease-related mutations. ***PLoS Comput Biol,*** 2014; 10(1):e1003440

Prokop Z*, Sato Y*, **^Brezovsky J***, Mozga T, Chaloupkova R, Koudelakova T, Jerabek P, Stepankova V, Natsume R, Leeuwen JGE, Janssen DB, Florian J, Nagata Y, Senda T, Damborsky J. Enantioselectivity f Haloalkane Dehalogenases and its Modulation by Surface Loop Engineering. ***Angew Chem Int Ed Engl,*** 2010; 49(35):6111-15

* 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 investigate the 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 given moment, living systems contain several thousand small organic molecules, both endogenous and exogenous, comprising the metabolome. To exert their function, the hosts of molecules need to arrive at their sites of action, mostly represented by protein surfaces and internal cavities. The transport of the metabolome is largely governed by protein tunnels and channels. Such tunnels and channels secure the transport of ligands between different regions and connect inner protein cavities with the protein surface, connect two or more different cavities, or connect even different cellular environments, such as in membrane proteins. The presence of very sophisticated transport processes markedly contributes to the symbiotic co-existence of individual chemical species within a single compartment or whole cell without the presence of overly disruptive interference. Protein channels facilitate the regulated and very selective transport of ions and ligands across a membrane between different cellular compartments. The role of channels in the function of various proteins has been the focus of intense research for years. Their importance is illustrated by many diseases that are caused by channel mutations. Such channel pathologies can severely impair the function of many physiological systems, manifested as various diseases, including epilepsy, hypertension, cystic fibrosis, diabetes, and cancer. To counteract these malfunctions, many inhibitors or activators that affect transport through these channels have been identified.

Tunnels connect buried functional sites to the bulk solvent, enabling the access of substrates and release of products. Moreover, the tunnels are responsible for many additional functions that are essential for the proper actions of proteins that are exposed to interference from individual species that are present in the metabolome of the living cell. The tunnels enable the access of preferred substrates and deny access to non-preferred substrates. The tunnels can prevent damage to enzymes that contain transition metals through ligation and damage to the cell that is caused by the release of toxic intermediates to the cellular environment. The tunnels also enable reactions that require the absence of water and allow the temporal and spatial synchronization of reactions. Most enzymes likely possess tunnels. In fact, the presence of tunnels was already described for enzymes from six Enzyme Commission classes and four structural classes of proteins. In many cases, tunnels are transient, meaning they cannot be readily identified from static crystal structures. Therefore, we can expect the discovery of tunnels in many other protein families. Recognizing the importance of transport processes for enzymatic catalysis, many protein engineering studies have successfully modified tunnels to improve enzymatic activity, specificity, enantioselectivity, and stability. Tunnels were established as critical functional factors in enzyme catalysis relatively recently, and their role in cellular biochemistry and tunnel mutations in disease etiology has been largely overlooked. However, many enzymes that are known to contain tunnels have been associated with the development of various ailments, including cancer, neurodegenerative disorders, autoimmune diseases, and inflammation. Inhibitors of some of these enzymes have been shown to bind to tunnels exclusively, thus confirming the proposed role of tunnels in disease etiology and treatment.

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

Enabling routine and reliable analysis of transient transport tunnels in proteins

The primary goal of this project is to enable large-scale studies of 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. In the next step, we will utilize the developed and thoroughly validated method for the detection of transient tunnels in all proteins with buried functional cavities with available 3D structures. The biologically relevant tunnels that are identified in these proteins will extend our fundamental knowledge about the properties that determine the transport component of protein function. We believe that the results of this large-scale analysis will stimulate further investigations of transient tunnels and their gates and enable the targeting of transient tunnels in protein engineering and drug discovery efforts (Fig. 1).

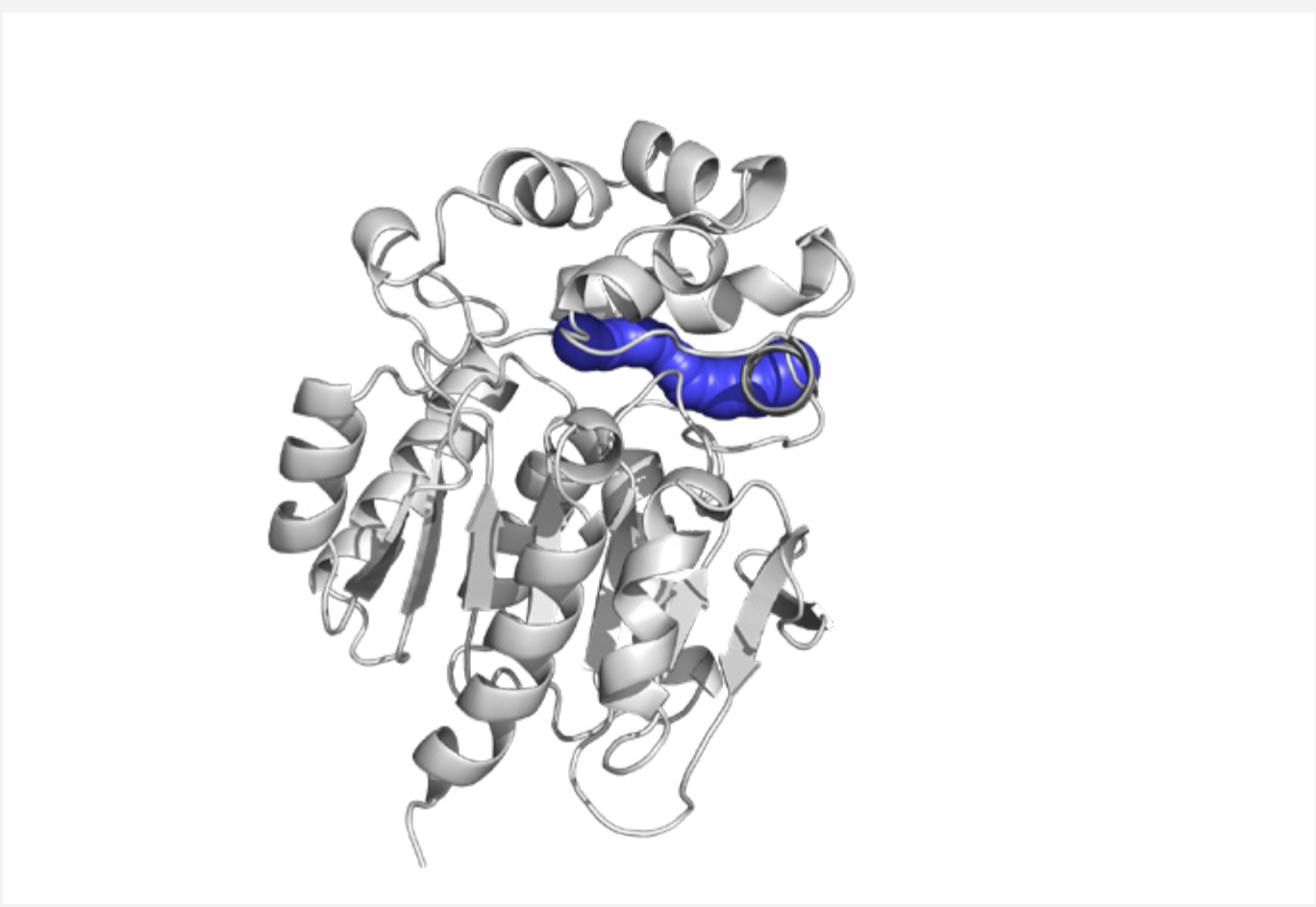


FIG. 1

A prospective transient tunnel (blue spheres) that forms a "back-door" to the active site of haloalkane dehalogenase DhaA (white cartoon) as identified from analyses of massive molecular dynamics simulations, capturing a total of 5 μs of simulation time.

Understanding molecular origins of mechanisms that govern functions of enzymes with buried actives sites

The primary goal of this research is to unveil molecular bases of largely unexplored factors that notably affect the biological function of enzymes with buried active sites (i.e., substrate inhibition, cooperativity, and interference between molecules of substrates and products during their simultaneous transport via the tunnels). We are working on comprehensive kinetics models of ligand transport in enzymes that will enable us to perform detailed analyses of structure-dynamics-function relationships that govern the transport of multiple ligands via the tunnels, revealing roles of direct interactions among ligands and allosteric effects on the tunnels that are mediated by protein-ligand interactions. We expect to obtain novel insights into the origins of substrate inhibition and cooperativity (i.e., phenomena that are necessary for the proper *in vivo* functions of many enzymes). The knowledge that is obtained can then be exploited to target these properties in research that seeks to engineer better enzymes and develop novel inhibitors. Finally, the findings on mutual interference among different ligands and effects on their transport will facilitate more accurate studies of enzyme-drug association/dissociation processes, thus paving the way toward the optimization of drug residence times.

STRATEGIC PROGRAMMES

Study on Aging and Longevity

Project Coordinator
Małgorzata Mossakowska, PhD, DSc Habil

Project Assistant
Aleksandra Szybalska, MSc

RESEARCH FOCUS

A study on aging and longevity was launched in 1999 at the International Institute of Molecular and Cell Biology in Warsaw (IIMCB) by a pilot study concerning Polish centenarians (PolStu99). Data obtained in PolStu99 project formed the basis for further research commissioned by the Committee for Scientific Research (project: called "Genetic and Environmental Factors of Longevity of Polish Centenarians" - PolStu2001)

The PolSenior project, which was performed in 2007-2012, 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 90 articles have been published from this effort. The results of the PolSenior project served as the basis for recommendations in regards to public health and social policies for the elderly population that should be developed at both the national and local levels.

In 2019, the PolSenior Study Group published the following articles:

- Puzianowska-Kuznicka et al., *J Nutr Health Aging*, 2019
- Pac et al., *J Nutr Health Aging*, 2019
- Królczyk et al., *Aging Clin Exp Res*, 2019
- Bulska-Będkowska et al., *J Clin Med*, 2019
- Olczak et al., *Exp Gerontol*, 2019
- Wyskida et al., *Exp Gerontol*, 2019
- Deskur-Śmielecka et al., *BMC Geriatr*, 2019

The PolSenior Study Group continued its activities as a member of the NCD Risk Factor Collaboration (NCD-RisC). In 2019, the PolSenior project data were included in the pooled analyses of trends in cholesterol ratios in a population of 82.1 million participants from Asian and Western countries (NCD-RisC group, *Int J Epidemiol*, 2019) and the rise in rural Body Mass Index as a main driver of the global obesity epidemic in 112 million adults (NCD-RisC group, *Nature*, 2019). In 2019, cooperation on the PolSenior2 project continued, led by the Medical University of Gdańsk and financed by the Ministry of Health was continued. The methodology of the PolSenior2 project is based on the previous study that was coordinated by IIMCB. Fieldwork was performed from October 2018 to the end of 2019. The PolSenior2 project will enable not only the insightful characterization of older Poles, but also comparisons with data that were gathered from the original PolSenior project, and observations of epidemiological trends over 10 years. Researchers who have been involved in implementing the PolSenior project will 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.

Moreover, based on data that were obtained from the PLGen project ("Polish Reference Genome for Genomic Diagnostics and Personalized Medicine"), a paper that describes the diverse status of longevity in a 102-year-old married couple was published (Skubiszewska et al., *Geriatria*, 2019).

As a result of cooperation with the Polish Association Supporting People with Inflammatory Bowel Disease ("J-elita") and Jagiellonian University Medical College and with support from the European Federation of Crohn's and Ulcerative Colitis Associations (EFCCA), a study of indirect costs associated with inflammatory bowel disease (IBD) was conducted in 12 European Union countries and Argentina from October 2018 to October 2019. The aim of the study was to obtain information on work-related productivity impairment of patients with IBD and their informal carers. Of 3828 patients (≥ 18 years of age), 3243 completed self-report questionnaires. A project on productivity losses among parents of children with IBD was launched in Poland by the same consortium.

A full list of relevant publications is available at www.iimcb.gov.pl/en/research/publications/33-polsenior-project.

Auresine

Project Coordinator
Izabela Sabała, PhD, DSc Habil

Senior Researcher
Elżbieta Jagielska, PhD

Postdoctoral Researcher
Piotr Małecki, PhD

PhD Students
Paweł Mitkowski, MSc
Alicja Wysocka, MSc

Undergraduate students
Piotr Bartosz (until September 2019)
Paulina Brodacka
Łukasz Łężniak
Karolina Trochimiak (until September 2019)

Project Assistant
Weronika Augustyniak, MSc

RESEARCH FOCUS

Our research focuses on bacteriolytic enzymes that selectively and efficiently eliminate staphylococcal cells from various environments. Together with our industrial partners, we develop commercial applications for such enzymes, especially for a patented one, Auresine®, and its derivatives. Bacteriolytic enzymes can be used 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. Our basic research helps us to address very practical issues, such as the development of resistance, tolerance to environmental conditions, and protein stability. The structural and biochemical characterization of enzymes broadens our knowledge of the regulation of their activity and enzyme specificity and provides a scientific basis for structure-designed enzyme engineering.

MAIN ACHIEVEMENTS IN 2019

We are conducting the TEAM-TECH project, "INFECTLESS New generation of antibacterial wound dressing" (Foundation for Polish Science program), which is focused on development of new-generation wound dressings that are functionalized with bacteriolytic enzymes.

We were also performing activities that are supported by a grant from the International Academic Partnerships program, funded by the Polish National Agency for Academic Exchange (NAWA). Thanks to the MolSpec project, "Molecular basis of enzyme specificity and applications," we can intensify our collaborations with Trinity College Dublin (Ireland), the Applied Molecular Biosciences Unit of the Department of Life Sciences FCT-NOVA (Portugal), and the Fraunhofer Institute for Silicate Research (Germany). This project has also given us the opportunity to initiate new collaborations with Tubingen University and Norwegian University of Natural Sciences.

We are continuing our collaborations with business partners to test the implementation of Auresine® in industry, with a global supplier of R&D chemicals for the worldwide distribution of Auresine® (Merck-Sigma-Aldrich, catalog no. SAE0083-1MG) and a global supplier of veterinary products that is interested in implementing our enzymes in their innovative products.

Our latest results are summarized in two articles that were published in 2019:

- **Mitkowski P, Jagielska E**, Nowak E, Bujnicki JM, Stefaniak F, Niedziałek D, Bochtler M, **Sabała I.** Structural bases of peptidoglycan recognition by lysostaphin SH3b domain. *Sci Rep* 2019; 9(1):5965
- 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

Our research has been presented at prestigious international meetings:

- The Bacterial Cell Envelope, Tubingen, Germany
- AMR Conference, Berlin, Germany
- The Great Wall Symposium, Paris, France
- Emerging Antimicrobials & Diagnostics, Amsterdam, The Netherlands

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)

Two of our Master students, Karolina Trochimiak and Piotr Bartosz, successfully completed their experimental work and defended their Master thesis at Warsaw University of Technology.

Dr. Izabela Sabała was awarded habilitation with distinction for structural and biochemical characterisation of bacteriolytic enzymes from M23 family and their biotechnological applications (Resolution no 167/2019 of IBB PAS Scientific Council).

Auresine eliminates *Staphylococcus* from infected wounds without harming the skin's natural microflora.

CORE FACILITIES

Core Facility

Head
Alicja Żylicz, PhD, Professor (until August 2019)
Krzysztof Skowronek, PhD, DSc Habil (since September 2019)

Deputy Head
Roman Szczepanowski, PhD

Staff Scientists
Matylda Macias, PhD (part-time)
Katarzyna Misztal, PhD
Tomasz Węgierski, PhD (part-time)

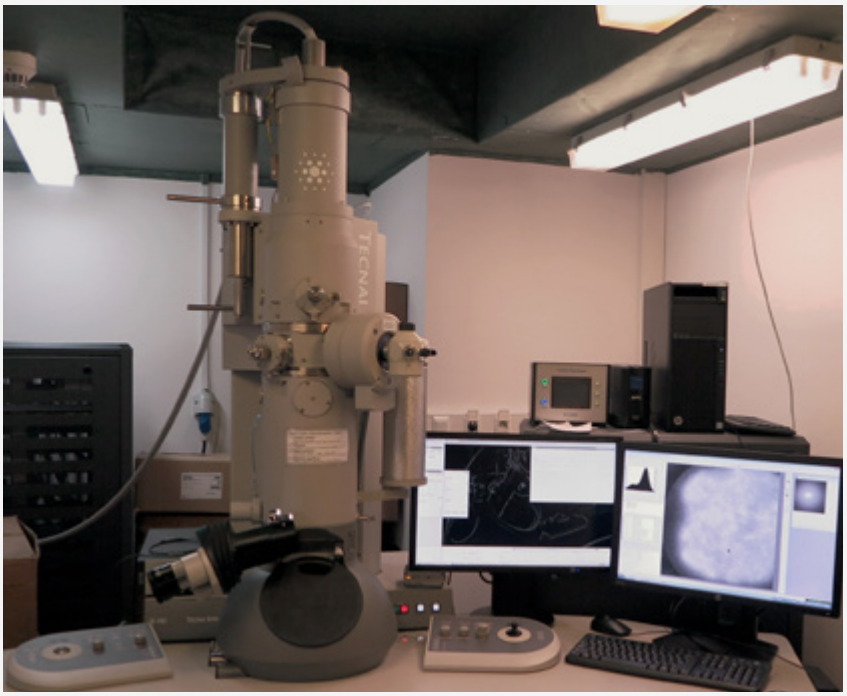
The IIMCB Core Facility was established as a shared research resource that provides access to a broad range of cutting-edge research technology platforms that are extensively used in such diverse areas as structural biology, bioinformatics, and molecular and cell biology. The Core Facility is managed 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 CCD camera. The crystals are analyzed using an X-ray generator (Proteum Bruker) that is equipped with a CCD detector (Platinum 135) and cryostream cooler (Oxford Cryosystems series 700). This facility allows the collection of a complete set of diffraction data within a few hours. The Structural Biology Unit also shares an FEI Tecnai T12 Transmission Electron Microscope with the Bioimaging Unit for Cryo-EM analyses of protein complexes. The system is combined with the TemCam F-Series camera and mostly used for structural biology and the analysis 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 oftentimes cannot be crystallized. The T12 microscope can be used to investigate polymers, thin films, fibers, ceramics, powders, and single crystals. 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 the deposition of layers of carbon on grids. As part of our Cryo-TEM workflow, we have a Vitrobot FEI, which offers fully automated cryo-fixation (vitrification) under constant physical and mechanical conditions. This ensures high-quality cryo-fixation results and high sample preparation throughput prior to cryo-TEM observations.

The Molecular Bioanalytics Unit is equipped with analytical instruments for in-depth analyses of proteins and nucleic acids using different methods. Interactions between molecules are studied using several complementary techniques: microcalorimetry (VP-DSC and VP-ITC), surface plasmon resonance (SPR) and analytical ultracentrifugation, and AUC (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 AUC. The Molecular Bioanalytics Unit is also equipped with a wide selection of spectrometers, including spectrophotometers, spectrofluorometers, a CD spectropolarimeter, and an FT-IR spectrometer. The list of instruments has recently been broadened by a new Biacore

S200 SPR, the most sensitive equipment of this class, which replaced the Biacore 3000. We also offer access to an UPLC system equipped with UV/VIS and fluorescence detectors and a selection of reverse-phase and size-exclusion chromatography columns for precise qualitative and quantitative analyses of proteins, nucleic acids, and small molecules.

The Mass Spectrometry Unit has two mass spectrometers: MALDI-TOF-TOF (ultrafleXtreem, Bruker) and 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 use of MS analysis for RNA targets makes this facility a unique entity nationwide.



FEI Tecnai T12 Transmission Electron Microscope with 120 kV accelerating voltage. Used for both negatively stained specimens in standard transmission-EM and cryo-EM samples that after cryopreservation in FEI Vitrobot station can be directly visualized in their native state.

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 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). Other 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. Image analysis in 2D and 3D is possible using dedicated software, such as Imaris and Harmony. The Bioimaging and High-Throughput Screening Unit also has a BD FACSAria II for cell sorting and BD FACSCalibur for the quantitative analysis of suspension cells. The FEI Tecnai T12 TEM 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 EM and LM 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, SEM, AFM, and LM 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 *Arabidopsis thaliana*. The purchase of the NGS instrument was supported by the Polish Ministry of Science and Higher Education equipment grant for the scientific consortium of IIMCB and the Museum and Institute of Zoology, Polish Academy of Sciences. 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. The Core Facility is also available to scientists from other institutions. IIMCB emphasizes cooperation with rapidly developing biotech companies in Poland. Within the framework of bilateral agreements, IIMCB laboratories collaborated with biotech companies, such as Adamed, Celon Pharma, Fermentas, BioVectis, Glia, Polfa, OncoArendi Therapeutics, and Helix Immuno-Oncology.

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 Core Technologies for Life Sciences (CTLS) network. We represent Poland on the Management Committee of the COST Action "MOBIEU" ("Between Atom and Cell: Integrating Molecular Biophysics Approaches for Biology and Healthcare"). We are participating in the organizing committee of the next Core Technologies for Life Sciences Association Congress in Lisbon in 2020.

Zebrafish Core Facility

The Zebrafish Core Facility (ZCF) was established in 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 051). The facility was established to introduce a new vertebrate model to researchers at the International Institute of Molecular and Cell Biology in Warsaw (IIMCB). Moreover, as a first in Poland, the ZCF joined the prestigious European Society for Fish Models in Biology and Medicine (EuFishBioMed) and is registered in the Zebrafish Model Organism Database (ZFIN).

Zebrafish is a small (3-5 cm) tropical freshwater fish. Because of its high genetic similarity to humans, a very short reproduction cycle, and the generation of transparent embryos, zebrafish is an excellent model for biomedical research. Moreover, access to experimental manipulations, extensive collections of mutant/transgenic animals, and low maintenance costs make zebrafish an attractive alternative to mammalian *in vivo* models that can be used to implement the "3R" principles (reduction, replacement, and refinement). In 2013, approximately 6,000 fish (30 lines) were kept in the ZCF in 300 tanks. Currently, our zebrafish collection consists of more than 16,000 fish, including wildtype lines and more than 120 genetically modified lines (see examples in Table 1). 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 that are involved in the mammalian/mechanistic target of the rapamycin signaling pathway, 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. Currently, six research groups at IIMCB use zebrafish and equipment resources of the ZCF. In 2019, the ZCF also served external users, including research groups from the Centre of New Technologies at the University of Warsaw, Medical University of Warsaw, Warsaw University of Life Sciences, and University of Warmia and Mazury in Olsztyn. Additionally, because of our international reputation and scientific collaborations, every year we export fish 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. Additionally, ZCF users have at their disposal a laboratory that is dedicated to behavioral testing. The room is equipped with two 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. Zebrafish diagnostic and health services are conducted by an IIMCB 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 possess appropriate qualifications to work with an 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 seven members, including head of the facility, five animal caretakers, and a technician. Zebrafish Core Facility personnel provide training courses to new users of the facility, including practical elements of handling, husbandry, breeding, fin clipping, microinjections, and behavioral testing.

Table 1. Example of zebrafish lines currently kept in the ZCF stock (note that the usage of some lines is limited by MTAs):

WILDTYPE LINES	MUTANT LINES				TRANSGENIC LINES
Name	Name	Affected genomic region	Allele	Molecular change	Name
AB	albino	slc45a2	unknown	unknown	Tg(ath5:gap43-GFP)
TL	casper	(roy x nacre)	unknown	unknown	Tg(cmlc2c:GFP)
ABTL	fmr1	fmr1	hu2787	point mutation	Tg(cmlc2:mRFP)
TU	gata5	gata5	tm236a	point mutation	Tg(CMV:GFP-map1lc3b)
	gba1	gba1	sh391	small deletion	Tg(fabp10a:dsRed)
	hand2	hand2	Hanc99	insertion	Tg(fli:eGFP)
	nacre	mitfa	unknown	unknown	Tg(gata1:dsRed)
	pink1	pink1	sh397	point mutation	Tg(gata1:dsRed;globin:GFP)
	CR2:stim2b	stim2b		insertion	Tg(-14.8gata4:GFP)
	tbx5	tbx5	Hstm21	point mutation	Tg(hand2:GFP)
	tet1	tet1	g.74453	deletion	Tg(mnx1:TagRFP-T)
	tet2	tet2	g.23316	deletion	Tg(myl7:eGFP)
	tet3	tet3	g.52494	deletion	Tg(nkx2.5:eGFP)
	tsc2	tsc2	vu242	point mutation	Tg(ptf1a:GFP)
	mtor(ztor)	mtor(ztor)	xu015	transgenic insertion	Tg(vas:eGFP)

ZEBRAFISH STOCK COLLECTION OVER THE YEARS

2013

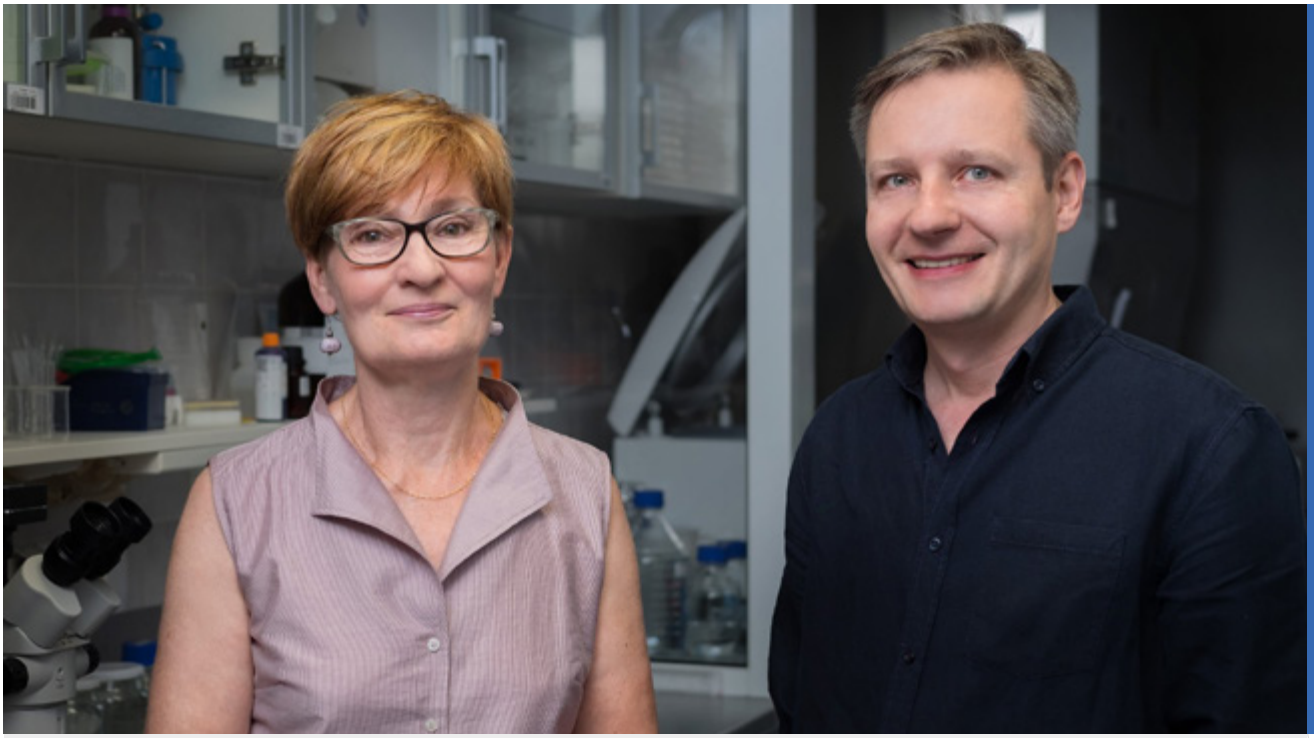
- 300 tanks (6 racks)
- 2 individual aquatic systems
- 6 000 fish
- 30 lines

2019

- 1,200 tanks (23 racks)
- 8 individual aquatic systems
- >16 000 fish
- >120 lines

Zebrafish Core Facility at IIMCB

zcf-team@iimcb.gov.pl https://www.iimcb.gov.pl/en/equipment-facilities/zebrafish-core-facility



Mouse Genome Engineering Facility

Scientific founder and coordinator of the molecular biology part
Andrzej Dziembowski, PhD, Professor

Scientific founder and coordinator of the embryology part
Ewa Borsuk, PhD, Professor

Staff
Olga Gewartowska, PhD
Jakub Gruchota, MSc
Michał Brouze, MSc
Marcin Szpila, MSc

Mouse Genome Engineering Facility

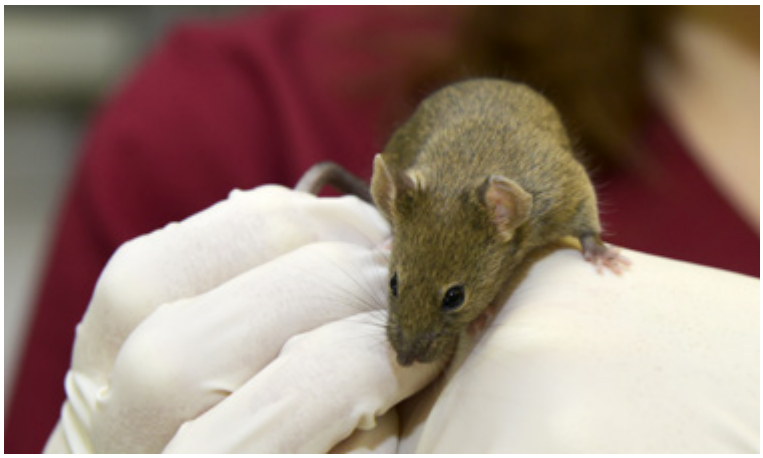
SERVICES

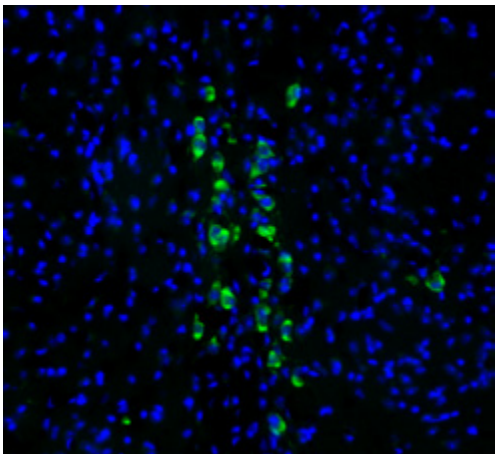
The Mouse Genome Engineering Facility provides customized transgenic mouse models that are generated using CRISPR/Cas9 methodology. The facility is exceptionally efficient in generating knock-in mouse lines with large inserts. We offer many types of genetic modifications: knockout, indels, floxed exons, 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

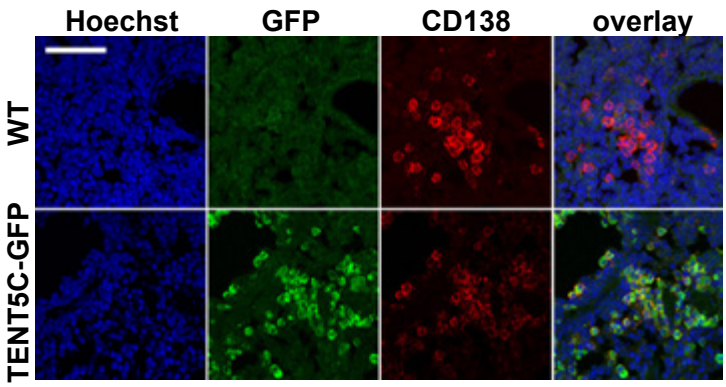
EXPERIENCE

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.

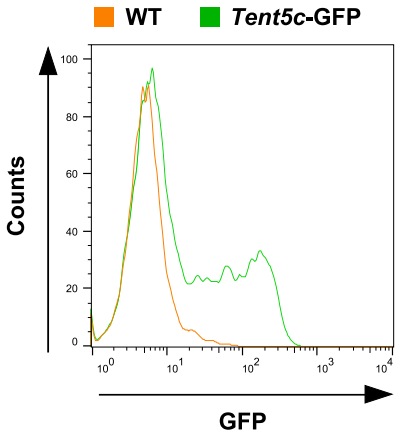




Immunohistochemical staining for FLAG (green) of TENT5A-3xFLAG mouse midbrain. DNA counterstained with Hoechst (blue) (Bartosz Tarkowski, unpublished)



Immunohistochemical staining for plasma cell marker CD138 and GFP in mouse spleen (Bilska et al., *Nat Commun*, 2020)



Flow cytometry analysis of TENT5C-GFP expression in plasma cells activated with LPS and IL-4 for 10 days (Bilska et al., *Nat Commun*, 2020)

FIG. 1

Examples of results that were obtained using knock-in mouse lines generated by the Facility.

SELECTED CLIENTS

University of Warsaw

University of Cambridge

Narodowy Instytut Onkologii

University of Warmia and Mazury in Olsztyn

Jagiellonian University in Kraków

UMCS Maria Curie-Skłodowska University in Lublin

Linköping University

Selected publications that utilized our generated mouse lines:

Mroczek S, Chlebowska J, Kulinski 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. *Nature Communications*, 2017; 8:619

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

SCIENTIFIC REPRESENTATION

Scientific Representation

PHD STUDENTS COUNCIL

The group of PhD Students at IIMCB is represented by Justyna Jędrychowska and Maciej Migdał. They are supported by Lab Representatives: Agata Poświata, Marta Gapińska, Karim Abu Nahia, Iswarya Pandara Nayaka, Anton Slyvka, Jan Węcławski, Gabriela Jędruszewska, Katarzyna Banasiak, Paweł Mitkowski and Aravind Selvaram. The PhD Students of 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) and Warsaw PhD School in Natural and BioMedical Sciences (Warsaw-4-PhD). Our representatives in those schools are Justyna Jędrychowska (SMM), Katarzyna Banasiak and Anna Stroynowska - Czerwińska (IBB), Jan Węsławski (Nencki Institute) and Monika Kwiatkowska (Warsaw-4-PhD).

In 2019 PhD Students organized the following scientific and social events:

- In April we gathered more than 150 early career researchers for a one day Young Scientists Conference. During the event we had talks by four invited speakers: Peter Cherepanov, Thomas Carell, Holger Stark and Jacek Kolanowski. Most importantly, it was also an opportunity for students to present their scientific work, there were 11 talks and a poster session. The conference was received warmly by the students community and the Institute which encouraged us to organize yet another conference, this time aiming at the international community - International Young Scientists Conference 2020.

- In September PhD Students gathered for the yearly annual report session and PhD Students Council elections. This year for the first time the event was held outside the Institute. We travelled to Sromowce Wyżne, a small town in Polish Pieniny mountains. In total 43 students attended the meeting, where each of us had an opportunity to present an oral presentation. Moreover this year we also changed the format such that the first year students had a chance to give us short introductory presentations. By vote Jan Węsławski and Sebastian Chamera won the best presentation awards, we also presented Gabriela Jędruszewska with a best student award for her outstanding work in PhD Council and contribution to our community.

- In November PhD Students Council together with the HR Logo group organized Halloween Horror Movie Night event. We met to immerse ourselves into Hallowen tradition and learn more about American culture over horror movies and pizza.

POSTDOCTORAL COUNCIL

The group of postdocs at IIMCB is represented by Dr. Małgorzata Figiel and Dr. Almudena Ponce Salvatierra. The group currently includes 36 postdocs from at least six different countries. Our responsibilities include the management of our own projects, other projects of more junior researchers and ensuring continuous access to experimental facilities in Poland and abroad. Our goals are to excel at the scientific level and create a community within IIMCB.

In 2019 four members of our group obtained independent grants.

- Dr. Oksana Palchevska received funding for studies on the oxidative status of STIM2 protein and its interaction partners in the mouse brain (NCN MINIATURA grant).

- Dr. Tomasz Kuliński obtained funding for studies on the nuclear RNA-degrading enzyme and how its dysfunction leads to mitotic defects, creating a possible therapeutic strategy for multiple myeloma (NCN SONATINA grant).

- Dr. Almudena Ponce Salvatierra received funding for her project, “DNA catalysis: bridging the gap,” to study deoxyribozymes, which are synthetic single-stranded DNA molecules that are able to catalyze many different chemical reactions for various substrates (NCN SONATA grant).

- Dr. Barbara Uszczyńska-Ratajczak obtained funding for her project on identifying novel long non-coding RNAs in *Danio rerio* (NCN OPUS grant).

Overall, six postdocs managed their own research projects in 2019, with a gross budget of 5.1 million PLN.

In 2019, the postdoc community at IIMCB proposed two additional initiatives: organization of the first Women in Science Symposium and creation of a volunteer group for charity actions at IIMCB.

The First Women in Science Symposium aims to increase the awareness of young female scientists about their own potential and opportunities. During the 2-day symposium, highly qualified PhD students and postdocs (mainly from the natural sciences) will come together with successful, renowned women with diverse professional backgrounds (including academia, industry, science journalism, and

politics) to take advantage of their experiences and discuss different career options. The symposium will provide an interactive environment and networking possibilities between the participants and speakers. The program will include numerous lectures, one or two workshops, and a networking dinner. Because of its international character, the symposium will be held entirely in English. The meeting is planned for March 4-5, 2021.

The volunteer group for charity actions was formed in May 2019, with the goal of inspiring people at IIMCB to commit their time and resources to those in need. By bringing together members of different groups at IIMCB, this initiative also creates an opportunity to socialize with other colleagues. The actions that have been organized to date include donations of winter clothes for people in need, walking dogs at an animal shelter, picking up litter at the Kampinos National Park, and cleaning in a nearby residential home.

SENIOR RESEARCHERS COUNCIL

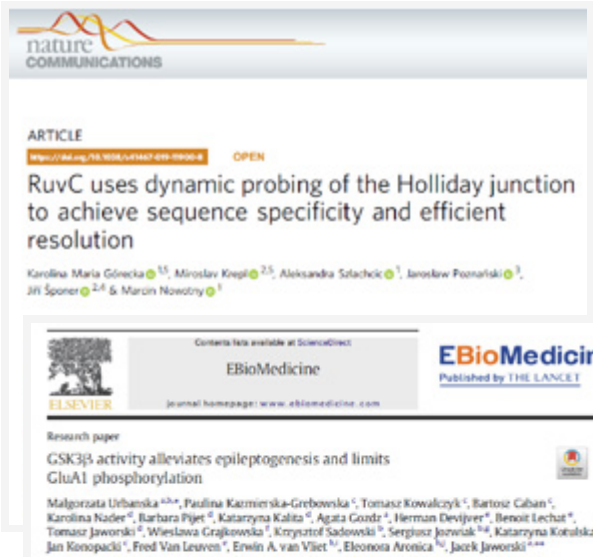
The group of researchers and senior researchers was established in 2019 and currently comprises 23 members. In 2019, the group was represented by Dr. Justyna Zmorzyńska and Dr. Honorata Czapińska. The key responsibilities of our members, in addition to designing and performing scientific projects, are to preserve the expertise of IIMCB, educate younger members of the laboratories, and assist lab leaders in organizational matters. Our goal is to obtain scientific results and gain experience in small-scale management to be better prepared to start our own research groups or become professional core facility staff. In 2019, two of our members (Dr. H. Czapińska and Dr. I. Sabała) received a habilitation degree (DSc Habil).

Currently, more than half of our members performs their own research projects with a gross budget of approximately 15.1 million PLN. In 2019:

- Dr. M. Czeredys received funding to study the link between Ca^{2+} signaling and Huntington's disease pathology in mice and induced pluripotent stem cell-derived neurons from Huntington's disease patients (NCN OPUS grant for 1.85 million PLN).
- DSc Habil I. Sabała and Dr. E. Jagielska filed a patent application to the Polish Patent Office (P.431445, "Recombinant polypeptide with potential therapeutic, antiseptic, antibacterial and anti-inflammatory properties, its compositions and uses").

Last year our group co-authored 25 publications, with an average of one publication per researcher. In 10 of these publications, our members were either first or corresponding author (see examples below).

In addition to scientific activities, our group is also involved in educational, science-promoting, and monitoring activities. In 2019, the group presented 19 external lectures, of which Dr. V. Korzh presented six. Dr. M. Boniecki reached the finals of the 8th edition of the FameLab Poland competition and won the Special Prize of the Minister of Science and Higher Education. Dr. D. Zdżalik-Bielecka received a Fellowship from the Kosciuszko Foundation for a research stay at the Ludwig Institute for Cancer Research/University of California San Diego (UCSD). DSc Habil I. Sabała is an Editorial Board Member of Scientific Reports and an EU Expert evaluator of Marie Sklodowska-Curie Programs. Dr. M. Wiweger Poland gives lectures during regular PolLASA courses. Completion of such courses is required for scientists to start animal studies in Poland. She is also one of three ministerial experts who can be appointed for the control of experiments with *D. rerio.* Dr. Ł. Majewski is responsible for the IIMCB mouse facility, and Dr. M. Macias is the coordinator of the occupational health and safety activities at IIMCB.


nature COMMUNICATIONS

ARTICLE

OPEN

RuvC uses dynamic probing of the Holliday junction to achieve sequence specificity and efficient resolution


Crystal Structure and Directed Evolution of Specificity of NlaIV Restriction Endonuclease

Honorata Czapinska[1], Wojciech Siwek[1,4], Roman H. Szczepanowski[1], Janusz M. Bujnicki[1,2], Matthias Bochtler[1,3] and Krzysztof J. Skowronek[1]

EBioMedicine

Published by THE LANCET

Research paper

GSK3β activity alleviates epileptogenesis and limits GluA1 phosphorylation

BBA - Molecular Cell Research

Behavioral and electrophysiological changes in female mice overexpressing ORAI1 in neurons

DO SCIENCE!

Do Science! (http://doscience.iimcb.gov.pl/) is an informal science club that was formed by PhD students and postdocs from IIMCB and is maintained by young scientists of the Biocentrum Ochota Campus. The Do Science! team seeks to create opportunities for young scientists to meet, discuss, and learn from the most successful scientists from Poland and abroad in an informal atmosphere where lectures are followed by short career advice sessions and long discussions in a relaxed setting. The Do Science! team also manages the Do Science! SciEvents calendar that aggregates all scientific events that occur on campus.

During 6 years of its activities, Do Science! has organized meetings with more than 50 scientists from very diverse fields of biology from all over the world. Do Science! was the inspiration for creating RNA Club Warsaw (https://www.facebook.com/RNAClubWarsaw/) and Do Science! Poznań (https://www.facebook.com/DoSciencePoznan/).

Do Science! organized the following meetings in 2019

November 21, 2019

Uwe Ohler, Berlin Institute for Medical Systems Biology, Germany

Gene regulation/scientific careers

Uwe Ohler is a Professor at the Max Delbrück Center at the Berlin Institute for Medical Systems Biology. He is a prominent scientist in the field of computational biology, with experience on gene regulatory networks. Dr. Ohler's lab focuses on many aspects of gene regulation, including transcription regulation by DNA sequences and chromatin, chromatin dynamics during differentiation and development, RNA regulatory mechanisms, localization and translation, and the integrative modeling of regulatory networks that link these different levels of gene regulation. During Do Science!, Dr. Ohler answered many of our questions about scientific careers.

RNA Club/Do Science! June 17, 2019

Matthew Disney, The Scripps Research Institute, USA

Targeted therapeutics

Matthew Disney is a Professor in the Department of Chemistry at The Scripps Research Institute (Florida, USA). The Disney group develops rational approaches to design selective therapeutics from only genome sequences. They work on general approaches to provide lead targeted therapeutics and precise medicines that target RNAs that cause disease, including rare neuromuscular diseases (e.g., muscular dystrophy), neurodegenerative diseases (e.g., Alzheimer's disease and amyotrophic lateral sclerosis), difficult-to-treat cancers (e.g., breast, pancreatic, and prostate, among others), and infectious diseases that can emerge through seasonal exposure. They have been developing a proprietary platform, Inforna, over the past 13 years that merges chemoinformatics and RNA structures to identify lead compounds that target RNAs of interest. This platform can be used to target RNAs that cause neuromuscular, neurodegenerative, and infectious diseases and difficult-to-treat cancers in preclinical animal models. During the meeting, Prof. Disney spoke about combining fundamental research and bringing new drugs to the pharmaceutical market.

RNA Club/Do Science! June 10, 2019

Rhiju Das, Stanford University School of Medicine, USA

Directions of the evolution of scientific approaches

Rhiju Das is an Associate Professor of Biochemistry at Stanford University School of Medicine (California, USA). His computational biochemistry lab seeks to predictively understand the ways in which RNA molecules code complex biological machinery. The lab's computer algorithms have consistently achieved leading predictions in worldwide structure prediction trials. Complementing these computer methods, Dr. Das is designing high-throughput "multidimensional chemical mapping" experiments to uncover three-dimensional structures and conformational changes in non-coding RNAs within their biological milieu, leading to discoveries of RNA regulons and influenza packaging signals that are critical for mammalian development and viral infection. To identify novel molecules of biomedical interest, Dr. Das leads the Eterna massive open laboratory, which couples a 100,000-player videogame with the lab's massively parallel experimental tools and deep learning, the first such platform in citizen science. Dr. Das' research has been recognized by the Burroughs-Wellcome Career Award at the Interface of Science, a W.M. Keck Medical Research Program award, and the OpenEye/American Chemical Society Outstanding Junior Faculty Award. Dr. Das mentors students from the biochemistry, biophysics, biomedical informatics, chemistry, and learning sciences PhD programs. During the RNA Club/Do Science! meeting, he discussed possible directions of the evolution of scientific approaches.

May 5, 2019

Martin Bommer, Bayer, Germany

Modular cloning

Martin Bommer currently works for Bayer. He is a former postdoctoral fellow in the laboratory of Prof. Udo Heinemann at the Max Delbrück Center for Molecular Medicine, Berlin. He previously worked with Prof. Holger Dobbek at the Institute of Biology in Berlin and at the BESSY II synchrotron. He has expertise in X-ray crystallography and various biochemical techniques, including cloning, protein purification, and protein characterization. Dr. Bommer discussed his current research on modular cloning and provided career advice.

April 17, 2019

Patrick Osmer, Ohio State University, USA

Astronomy and parallels in instrumentation and data analysis between astronomy and life sciences

Patrick Osmer is a Professor in the Department of Astronomy at Ohio State University. Prof. Osmer is an astronomer who works on the nature and evolution of high- and low-redshift quasars, focusing on large-scale photometric and spectroscopic surveys. He is the former Director of the Cerro Tololo Inter-American Observatory and former Chair of the Department of Astronomy at Ohio State University. Prof. Osmer is the first astronomer who has been hosted by Do Science! He discussed how similar computational and experimental approaches can be applied in both astronomy and RNA structure prediction.

March 6, 2019

Ben Luisi, University of Cambridge, UK

RNA metabolism and transport

Ben Luisi is a Professor of structural biology in the Department of Biochemistry at the University of Cambridge. His research group studies the regulation of RNA metabolism and membrane transporters in bacteria. He was elected as an EMBO member and Faculty of 1000. He has received numerous Wellcome Trust grants and holds an ERC Advanced Grant. He established a modern platform for CryoEM and CryoET in the Department of Biochemistry at the University of Cambridge. Prof. Luisi was a PhD student of Dr. Max Perutz (Nobel laureate in Chemistry in 1962) in whose laboratory he worked on the structure of hemoglobin in LMB MRC in Cambridge together with Kiyoshi Nagai. He also studied protein-DNA interactions under the direction of Dr. Paul Sigler at Yale University. During his lecture, Prof. Luisi discussed developments in the field of RNA biology and described some of his recent discoveries.

RNA Club Warsaw

The RNA Club Warsaw is an initiative that seeks to enhance scientific discussions and inspire collaborations between groups that work on RNA biology at the Ochota Campus. In 2019, we arranged several events, including meetings, a workshop, and special talks by invited speakers. The first RNA Club Warsaw meeting consisted of talks that were given by junior scientists, presenting challenging research cases with the opportunity to consult with experienced researchers in the field. The second meeting was a rehearsal before the 2019 RNA Meeting in Cracow, where researchers from the Ochota Campus, who were chosen to give talks at RNA 2019, gave their talks and received valuable feedback from the audience. For the third year in a row, the RNA Club Warsaw was awarded a $1,500 grant from the RNA Salon Selection Committee (RNA Society) and Lexogen to support planned activities.

RNA CLUB MEETS #NGSCHOOL

RNA Club meets #NGSchool is a continuation of our collaboration with the #NGSchool. In 2019, we organized one workshop on the basics of Python and R programming languages for biologists. The workshop was aimed at beginners with no prior programming experience. It covered the very basics of both languages, along with some useful packages for biologists.

May 6, 2019

RNA CLUB MEETS #NGSCHOOL WORKSHOP - BASICS OF R AND PYTHON

- **Maja Kuzman** (University of Zagreb)
- **Katarzyna Kędzierska** (University of Oxford)

RNA CLUB & DO SCIENCE!

This year, we have also invited expert speakers in areas of RNA research from abroad. For this initiative, we cooperated with Do Science! and adopted a special format for the event (i.e., an hour presentation from the speaker, followed by career advice and less formal discussions). The first invited speaker was Dr. Rhiju Das from Stanford University (USA). Dr. Das focuses on computational work, mainly 3D structure determination and high-throughput chemical mapping experiments. The second invited speaker was Dr. Matthew Disney from The Scripps Research Institute (USA). The main focus of the Disney Group is to develop rational approaches to design selective therapeutics from only genome sequences.

June 10, 2019

RNA CLUB & DO SCIENCE! WITH RHIJU DAS

February 7, 2019

RNA CLUB WARSAW MEETING I

- **Aleksandra Kwaśnik** (University of Warsaw)
- **Natalia Gumińska** (University of Warsaw)
- **Katarzyna Eysmont** (Centre of New Technologies University of Warsaw)
- **Diana Toczydłowska-Socha** (IIMCB)

June 4, 2019

RNA CLUB WARSAW MEETING II

- **Katarzyna Eysmont** (Centre of New Technologies University of Warsaw)
- **Vladyslava Liudkovska** (Institute of Biochemistry and Biophysics, Polish Academy of Sciences)
- **Paweł Krawczyk** (Institute of Biochemistry and Biophysics, Polish Academy of Sciences)
- **Sebastian Sacharowski** (Institute of Biochemistry and Biophysics, Polish Academy of Sciences)
- **Filip Stefaniak** (IIMCB)

June 17, 2019

RNA CLUB & DO SCIENCE! WITH MATTHEW DISNEY

FACTS & FIGURES

HR Data

260 Staff

165	Female	**48**	Foreigners
95	Male	**16**	Nations

11	Lab Leaders	**9**	Core Facilities Staff
2	Project Coordinators	**21**	Research Technicians
20	Senior Researchers	**14**	Lab Technicians & Support Specialists
2	Researchers	**39**	Administration
40	Postdoctoral Researchers	**19**	Others
2	Project Assistants		
59	PhD Students		
13	Undergraduate Students		
9	Trainees		

Best Papers Award 2019

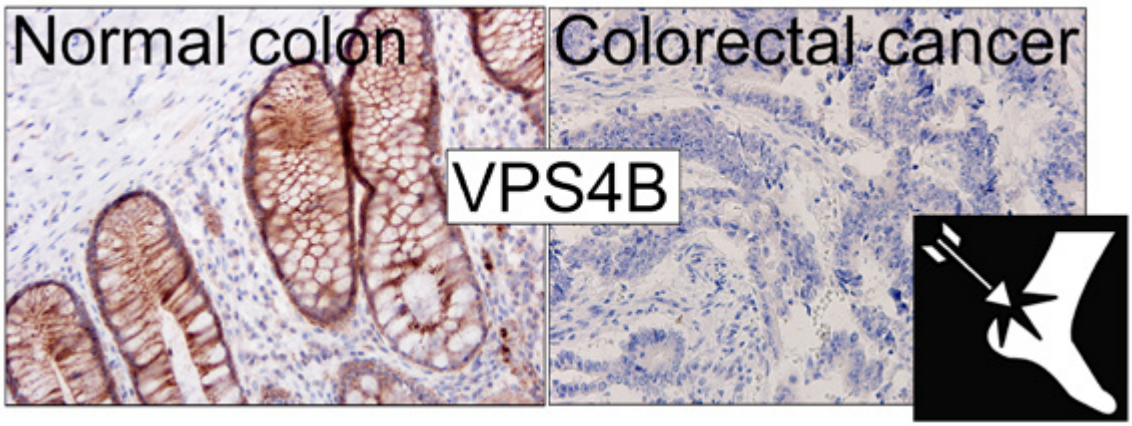
The best papers are selected by the Institute's Lab Leaders based on contents and significance, but not the bibliometric data. A full list and the pdf files of all the papers submitted for the Award (together with the supporting statements) are sent to each PI. They look through all of them and choose 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 winning papers' list is approved. The financial prizes are divided among the best papers' authors with IIMCB affiliation listed below.

1st Place

Szymańska M, Nowak P, Kolmus K, Cybulska M, Goryca K, Derezińska-Wołek M, Szumera-Ciećkiewicz A, Brewińska-Olchowik M, Grochowska A, Piwocka K, Prochorec-Sobieszek M, Mikula M, **Miączyńska M**. Synthetic lethality between VPS4A and VPS4B triggers an inflammatory response in colorectal cancer. ***EMBO Mol Med***, 2020; 12(2):e10812 (accepted in 2019)

IIMCB researchers from the Laboratory of Cell Biology identified a novel druggable "Achilles' heel" of colorectal cancer cells. They uncovered a synthetic lethality interaction between VPS4A and VPS4B paralogs that encode multifunctional ATPases. They further proposed the VPS4A activity as a promising target for therapy of patients with VPS4B-deficient tumors. The study was performed with help of researchers and clinicians from the Maria Skłodowska-Curie Institute-Oncology Centre and the Nencki Institute of Experimental Biology.

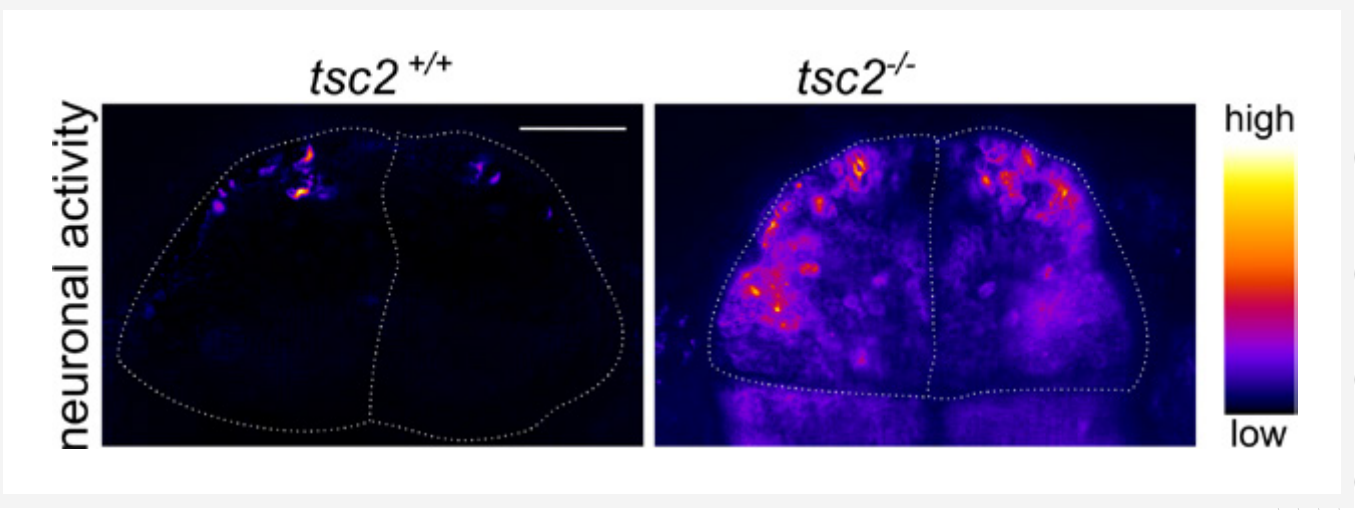
Specifically, the researchers showed that the VPS4B gene was frequently deleted in many cancer types, including in colorectal cancer, which was reflected by low VPS4B mRNA and protein levels in colorectal cancer samples from patients. They further identified the VPS4A gene as a synthetic lethal partner for VPS4B. They demonstrated that the perturbation of VPS4A protein in a tumor cell with loss or low level of VPS4B induced the death of cells grown in vitro and in mice xenografted tumors. Moreover, the study revealed that upon concomitant depletion of VPS4A and VPS4B proteins, dying cancer cells secreted immunomodulatory molecules that mediated inflammatory and anti-tumor responses. These results identify a novel pair of druggable targets for personalized oncology.



2nd Place

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 (accepted in 2019)

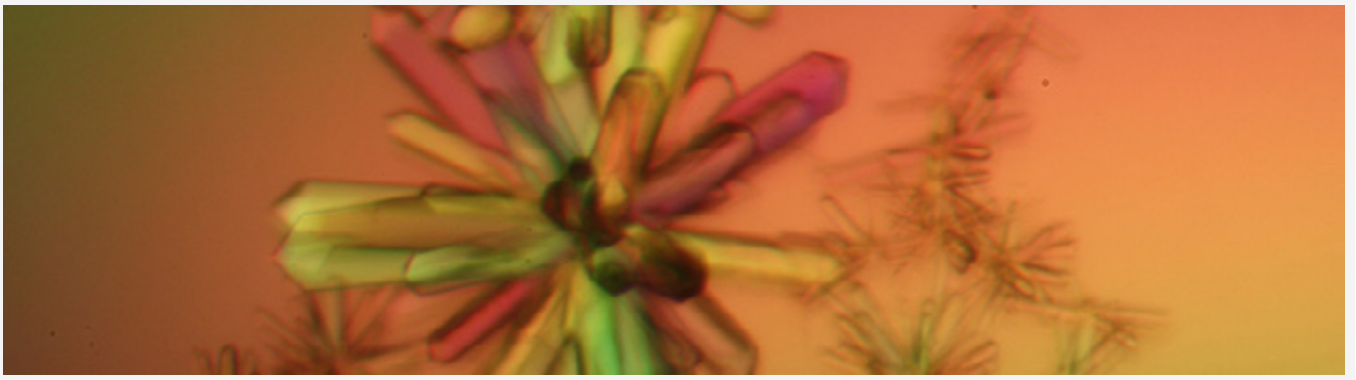
Tuberous Sclerosis Complex (TSC) is a hereditary disease, caused by mutations in TSC1 or TSC2, that presents with early brain malformations, childhood epilepsy, and TSC-associated neuropsychiatric disorders (TANDs). Among these symptoms TANDs still remain poorly investigated and understood on a molecular and anatomical levels. In this manuscript, researchers from the Laboratory of Molecular and Cellular Neurobiology performed an in-depth study of a zebrafish model of TSC and reported that Tsc2-deficient zebrafish recapitulated symptoms seen in TSC patients on anatomical and behavioral levels, including aberrant brain morphology, thinning of brain connections, epileptogenesis, and increased anxiety-like behavior. Moreover, IIMCB researchers were able to functionally connect changes in hemisphere connectivity with aberrant regulation of anxiety, providing a link between brain anatomy and emotion. Last but not least, quite unexpectedly Kedra et al. discovered a new therapeutic target, neurotrophin receptor, TrkB- targeting of which could potentially improve the effectiveness of existing therapies and the quality of life of TSC patients.



3rd Place

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

This publication is a result of a collaboration between the scientists from the Laboratory of Protein Structure and Institute of Biophysics of the Czech Academy of Sciences. It describes the mechanism of RuvC Holiday junction resolvase and how the protein uses dynamic probing of the Holliday junction to achieve sequence specificity and efficient resolution. The bacterial protein RuvC is a canonical resolvase that introduces two symmetric cuts into the HJ. For complete resolution of the HJ, the two cuts need to be tightly coordinated. They are also specific for cognate DNA sequences. Using a combination of structural biology, biochemistry, and a computational approach, it was shown that correct positioning of the substrate for cleavage requires conformational changes within the bound DNA. These changes involve rare high-energy states with protein-assisted base flipping that are readily accessible for the cognate DNA sequence but not for non-cognate sequences. These conformational changes and the relief of protein-induced structural tension of the DNA facilitate coordination between the two cuts. The unique DNA cleavage mechanism of RuvC shows the importance of high-energy conformational states in nucleic acid readouts. This highly interdisciplinary work also demonstrated the power of combining experimental and computational know-how of the two collaborating groups.



Publications in 2019

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	**Górecka KM**, Krepl M, Poznański J, Šponer J, **Nowotny M.**	RuvC uses dynamic probing of the Holliday junction substrate to achieve sequence specificity and efficient resolution.	Nat Commun. 2019; 10(1):4102 doi: 10.1038/s41467-019-11900-8	13.811	MULTIDISCIPLINARY SCIENCES	Q1
2	**Nowotny M.**	Crosslink and shield: protecting abasic sites from error-prone repair.	Nat Struct Mol Biol. 2019; 26(7):530-532 doi: 10.1038/s41594-019-0264-4	12.650	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
3	**Pawlak M, Kedzierska KZ, Migdal M, Nahia KA,** Ramilowski JA, Bugajski L, Hashimoto K, **Marconi A,** Piwocka K, Carninci P, **Winata CL.**	Dynamics of cardiomyocyte transcriptome and chromatin landscape demarcates key events of heart development.	Genome Res. 2019; 29(3):506-519 doi: 10.1101/gr.244491.118	11.638	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
4	de Crecy-Lagard V, **Boccaletto P**, Mangleburg C, Sharma P, Lowe T, Leidel S, **Bujnicki JM**.	Matching tRNA modifications in humans to their known and predicted enzymes.	Nucleic Acids Res. 2019; 47(5):2143-2159 doi: 10.1093/nar/gkz011	10.727	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
5	**Gaur V, Ziajko W, Nirwal S, Szlachcic A, Gapińska M, Nowotny M**	Recognition and processing of branched DNA substrates by Slx1-Slx4 nuclease.	Nucleic Acids Res. 2019; 47(22):11681-11690 doi: 10.1093/nar/gkz842	10.727	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
6	**Slyvka A,** Zagorskaitė E, **Czapinska H,** Sasnauskas G, **Bochtler M.**	Crystal structure of the EcoKMcrA N-terminal domain (NEco): recognition of modified cytosine bases without flipping.	Nucleic Acids Res. 2019; 47(22):11943-11955 doi: 10.1093/nar/gkz101	10.727	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
7	de Assis GG, **Gasanov EV.**	BDNF and Cortisol integrative system - Plasticity vs. degeneration: Implications of the Val66Met polymorphism.	Front Neuroendocrinol. 2019; 55:100784 doi: 10.1016/j.yfrne.2019.100784	9.421	ENDOCRINOLOGY & METABOLISM	Q1
8	**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; 39:377-387 doi: 10.1016/j.ebiom.2018.11.040	6.486	MEDICINE, RESEARCH & EXPERIMENTAL	Q1
9	**Maciąg F, Majewski Ł,** Boguszewski P.M, **Gupta R.K, Wasilewska I,** Wojtas B, **Kuznicki J.**	Behavioral and electrophysiological changes in female mice overexpressing ORAI1 in neurons.	Biochim Biophys Acta Mol Cell Res. 2019; 1866(7):1137-1150 doi: 10.1016/j.bbamcr.2019.01.007	5.281	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
10	**Tarkowski B, Kuchcinska K, Blazejczyk M, Jaworski J.**	Pathological mTOR mutations impact cortical development.	Hum Mol Genet. 2019; 28(13):2107-2119 doi: 10.1093/hmg/ddz042	5.281	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
11	**Firkowska M, Macias M, Jaworski J.**	ESCRT Proteins Control the Dendritic Morphology of Developing and Mature Hippocampal Neurons.	Mol Neurobiol. 2019; 56(7):4866-4879 doi: 10.1007/s12035-018-1418-9	4.643	NEUROSCIENCES	Q1
12	**Banach-Orłowska M, Wyszyńska R,** Pyrzyńska B, **Maksymowicz M,** Gołąb J, **Miączyńska M.**	Cholesterol restricts lymphotoxin β receptortriggered NF-κB signaling.	Cell Commun Signal, 2019; 17:171 doi: 10.1186/s12964-019-0460-1	4.603	CELL BIOLOGY	Q1
13	**Klimczak M,** Biecek P, **Zylicz A, Zylicz M.**	Heat shock proteins create a signature to predict the clinical outcome in breast cancer.	Sci Rep. 2019; 9(1):7507 doi: 10.1038/s41598-019-43556-1	4.525	MULTIDISCIPLINARY SCIENCES	Q1

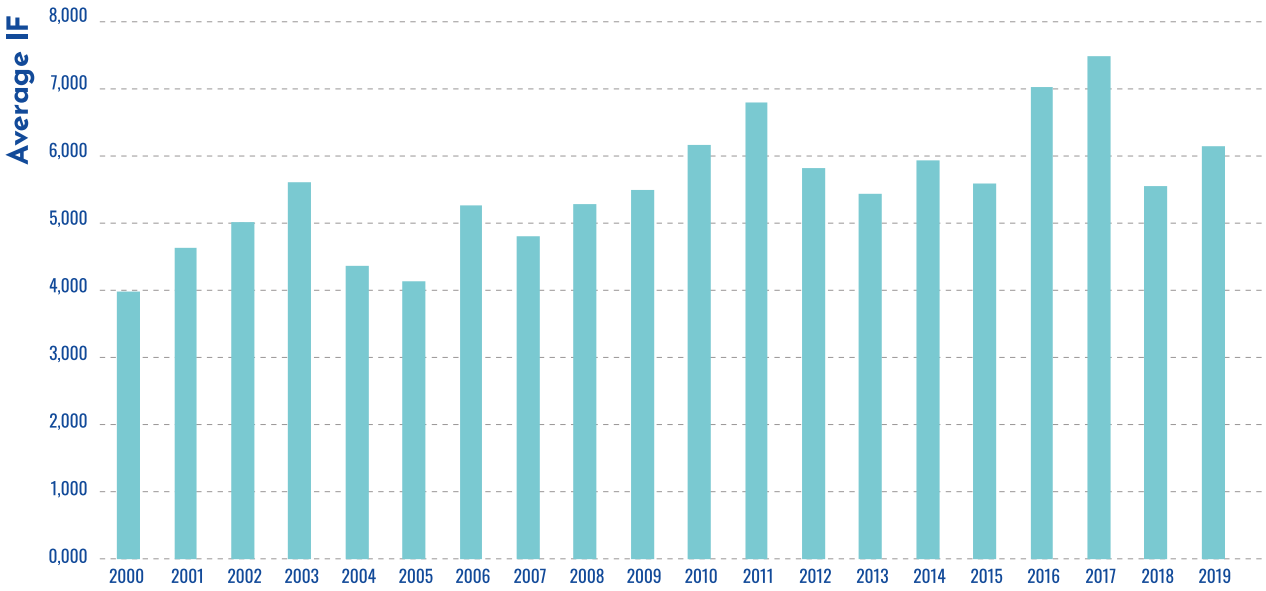
14	**Mitkowski P, Jagielska E, Nowak E, Bujnicki JM, Stefaniak F, Niedziałek D, Bochtler M, Sabała I.**	Structural bases of peptidoglycan recognition by lysostaphin SH3b domain.	Sci Rep. 2019; 9(1):5965 doi: 10.1038/s41598-019-42435-z	4.525	MULTIDISCIPLINARY SCIENCES	Q1
15	**Czapinska H, Siwek W, Szczepanowski RH, Bujnicki JM, Bochtler M, Skowronek KJ.**	Crystal structure and directed evolution of specificity of NlaIV restriction endonuclease.	J Mol Biol. 2019; 431(11):2082-2094 doi: 10.1016/j.jmb.2019.04.010	4.514	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
16	**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). pii: E5539 doi: 10.3390/ijms20225539	4.331	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q2
17	**Samluk L,** Urbanska M, **Kisielewska K,** Mohanraj K, Kim MJ, **Machnicka K, Liszewska E, Jaworski J, Chacinska A.**	Cytosolic translational responses differ under conditions of severe short-term and long-term mitochondrial stress.	Mol Biol Cell. 2019; 30(15):1864-1877 doi: 10.1091/mbc.E18-10-0628	4.084	CELL BIOLOGY	Q2
18	**Nowacka M, Boccaletto P,** Jankowska E, Jarzynka T, **Bujnicki JM,** Dunin-Horkawicz S.	RRMdb – an evolutionary-oriented database of RNA recognition motif sequences.	Database. 2019 Jan 1; doi: 10.1093/database/bay148	3.793	MATHEMATICAL & COMPUTATIONAL BIOLOGY	Q1
19	**Wasilewska I, Gupta RK, Palchevska O, Kuźnicki J.**	Identification of Zebrafish Calcium Toolkit Genes and their Expression in the Brain.	Genes (Basel). 2019; 10(3). pii: E230 doi: 10.3390/genes10030230	3.484	GENETICS & HEREDITY	Q2
20	**Hyjek M, Figiel M, Nowotny M.**	RNases H: Structure and mechanism.	DNA Repair (Amst). 2019; 84:102672 doi: 10.1016/j.dnarep.2019.102672	3.388	GENETICS & HEREDITY	Q2
21	**Olszewski MB, Pruszko M,** Snaar-Jagalska E, **Zylicz A, Zylicz M.**	Diverse and cancer type-specific roles of the p53 R248Q gain-of-function mutation in cancer migration and invasiveness.	Int. J. Oncol. 2019; 54:1168-1182 doi: 10.3892/ijo.2019.4723	3.356	ONCOLOGY	Q2
22	**Nowacka M,** Fernandes H, Kiliszek A, **Bernat A, Lach G, Bujnicki JM.**	Specific interaction of zinc finger protein Com with RNA and the crystal structure of a self-complementary RNA duplex recognized by Com.	PLoS One. 2019; 14(4):e0214481 doi: 10.1371/journal.pone.0214481	3.337	MULTIDISCIPLINARY SCIENCES	Q2
23	Radom M, **Machnicka MA, Krwawicz J, Bujnicki JM,** Formanowicz P	Petri net-based model of the human DNA base excision repair pathway.	PLoS One. 2019; 14(9):e0217913 doi: 10.1371/journal.pone.0217913	3.337	MULTIDISCIPLINARY SCIENCES	Q2
24	Grzeczkowicz A, **Gruszczynska-Biegala J, Czeredys M,** Kwiatkowska A, Strawski M, Szklarczyk M, Kozbiał M, Kuźnicki J, Granicka LH.	Polyelectrolyte Membrane Scaffold Sustains Growth of Neuronal Cells.	J Biomed Mater Res A. 2019; 107(4) :839-850 doi: 10.1002/jbm.a.36599	3.317	ENGINEERING, BIOMEDICAL	Q2
25	Puzianowska-Kuznicka M, Kuryłowicz A, Walkiewicz D, Borkowska J, Owczarz M, Olszanecka-Glinianowicz M, Wieczorowska-Tobis K, Skalska A, **Szybalska A, Mossakowska M.**	Obesity Paradox in Caucasian Seniors: Results of the PolSenior Study.	J Nutr Health Aging. 2019; 23(9):796-804 doi: 10.1007/s12603-019-1257-z	3.215	GERIATRICS & GERONTOLOGY	Q3
26	**Magnus M,** Kappel K, Das R, **Bujnicki JM**	RNA 3D structure prediction guided by independent folding of homologous sequences.	BMC Bioinformatics. 2019; 20(1):512 doi: 10.1186/s12859-019-3120-y	2.970	MATHEMATICAL & COMPUTATIONAL BIOLOGY	Q1
27	**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) doi: 10.1042/BSR20180430	2.939	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q3
28	**Jędrychowska J, Korzh V.**	Kv2.1 voltage-gated potassium channels in developmental perspective.	Dev Dyn, 2019; 248(12):1180-1194 doi: 10.1002/dvdy.114	2.624	DEVELOPMENTAL BIOLOGY	Q2
29	**Minhas R, Paterek A, Łapiński M, Bazała M, Korzh V, Winata CL.**	A novel conserved enhancer at zebrafish zic3 and zic6 loci drives neural expression.	Dev Dyn. 2019; 248(9):837-884 doi: 10.1002/dvdy.69	2.624	DEVELOPMENTAL BIOLOGY	Q2
30	**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). pii: bio044347 doi: 10.1242/bio.044347	2.170	BIOLOGY	Q2
31	**Stasiewicz J, Mukherjee S, Nithin C, Bujnicki JM.**	QRNAS: software tool for refinement of nucleic acid structures.	BMC Struct Biol. 2019; 19(1):5 doi: 10.1186/s12900-019-0103-1	1.647	BIOPHYSICS	Q4
32	**Sulej AA.**	Improving selectivity of DNA-RNA binding zinc finger using directed evolution.	BMC Res Notes. 2019; 12(1):792 doi: 10.1186/s13104-019-4833-8			

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

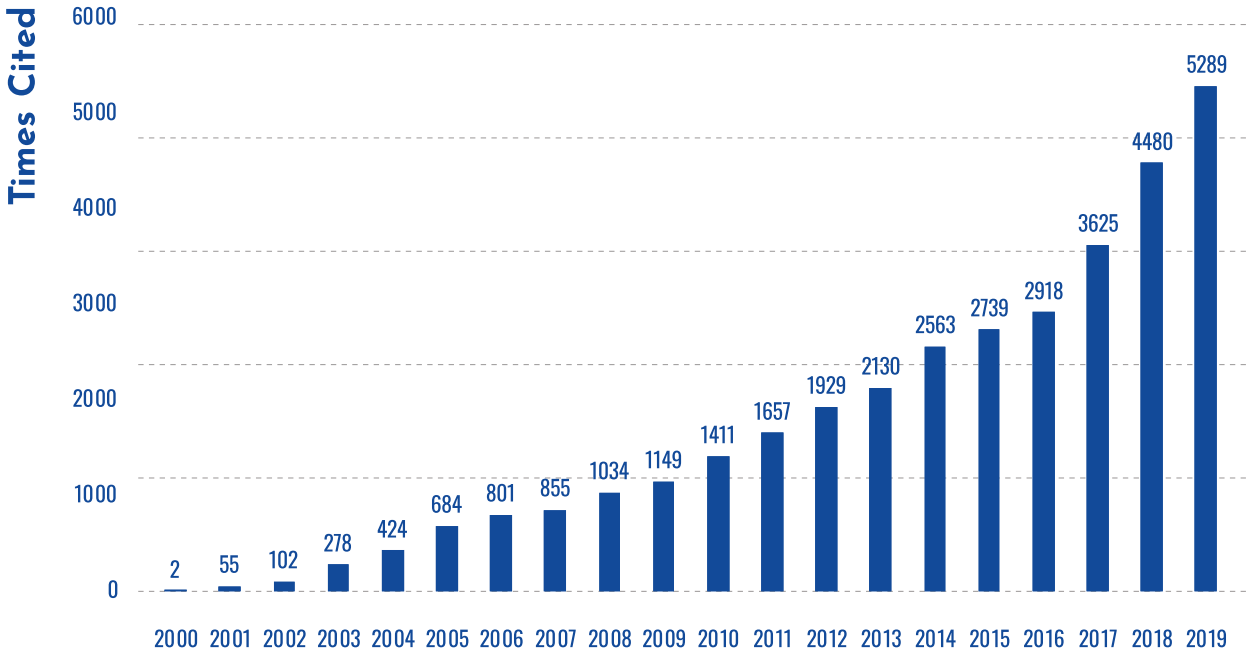
No	Authors	Title	Journal	5 Year IF	Journal Category	Quartile in Category
1	NCD Risk Factor Collaboration **(Mossakowska M)**	Rising rural body-mass index is the main driver of the global obesity epidemic in adults.	Nature. 2019; 569(7755):260-264 doi: 10.1038/s41586-019-1171-x	45.819	MULTIDISCIPLINARY SCIENCES	Q1
2	Kotrys AV, Cysewski D, Czarnomska SD, **Pietras Z,** Borowski LS, Dziembowski A, Szczesny RJ.	Quantitative proteomics revealed C6orf203/MTRES1 as a factor preventing stress-induced transcription deficiency in human mitochondria.	Nucleic Acids Res. 2019; 47(14):7502-7517 doi: 10.1093/nar/gkz542	10.727	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
3	Lutz T, Flodman K, Copelas A, **Czapinska H,** Mabuchi M, Fomenkov A, He X, **Bochtler M,** Xu S.	A protein architecture guided screen for modification dependent restriction endonucleases.	Nucleic Acids Research, 2019; 47(18):9761-9776 doi: 10.1093/nar/gkz755	10.727	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
4	Negri A, Jąkalski M, Szczuka A, **Pryszcz LP,** Mruk I.	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.	Nucleic Acids Res. 2019; 47(18):9542-9556 doi: 10.1093/nar/gkz665	10.727	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
5	The RNAcentral Consortium **(Bujnicki JM, Boccaletto P)**	RNAcentral: a hub of information for non-coding RNA sequences.	Nucleic Acids Res. 2019; 47(D1):D221-D229 doi: 10.1093/nar/gky1034	10.727	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
6	Tamulaitiene G, Manakova E, Jovaisaite V, Tamulaitis G, Grazulis S, **Bochtler M,** Siksnys V.	Unique mechanism of target recognition by PfoI restriction endonuclease of the CCGG-family.	Nucleic Acids Res. 2019; 47(2):997-1010 doi: 10.1093/nar/gky1137	10.727	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
7	NCD Risk Factor Collaboration **(Mossakowska M, Slusarczyk 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. 2019. pii: dyz099 doi: 10.1093/ije/dyz099	9.758	PUBLIC, ENVIRONMENTAL & OCCUPATIONAL HEALTH	Q1
8	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 doi: 10.1371/journal.pbio.3000253	9.311	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
9	Ahuja G, Bartsch D, Yao W, Geissen S, Frank S, Aguirre A, Russ N, Messling JE, **Dodzian J,** Lagerborg KA, Vargas NE, Muck JS, Brodesser S, Baldus S, Sachinidis A, Hescheler J, Dieterich C, Trifunovic A, Papantonis A, Petrascheck M, Klinke A, Jain M, Valenzano DR, Kurian L.	Loss of genomic integrity induced by lysosphingolipid imbalance drives ageing in the heart.	EMBO Rep. 2019; 20(4). pii: e47407 doi: 10.15252/embr.201847407	8.957	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q1
10	Mazur M, Dymek B, Koralewski R, Sklepkiewicz P, Olejniczak S, Mazurkiewicz M, Piotrowicz M, Salamon M, Jędrzejczak K, Zagozdzon A, Czestkowski W, Matyszewski K, Borek B, Bartoszewicz A, Pluta E, Rymaszewska A, Mozga W, **Stefaniak F,** Dobrzański P, Dzwonek K, Golab J, Golebiowski A, Olczak J.	Development of Dual Chitinase Inhibitors as Potential New Treatment for Respiratory System Diseases.	J Med Chem. 2019; 62(15):7126-7145 doi: 10.1021/acs.jmedchem.9b00681	6.060	CHEMISTRY, MEDICINAL	Q1
11	Bulska-Będkowska W, Chełmecka E, Owczarek AJ, Mizia-Stec K, Witek A, **Szybalska A,** Grodzicki T, Olszanecka-Glinianowicz M, Chudek J.	CA125 as a Marker of Heart Failure in the Older Women: Population-Based Analysis.	J Clin Med. 2019; 8(5). ! 607 doi: 10.3390/ jcm8050607	5.688	MEDICINE, GENERAL & INTERNAL	Q1
12	McIntyre J, Sobolewska A, Fedorowicz M, McLenigan MP, **Macias M,** Woodgate R, Sledziewska-Gojska E.	DNA polymerase ι is acetylated in response to SN2 alkylating agents.	Sci Rep. 2019; 9(1):4789 doi: 10.1038/s41598-019-41249-3	4.525	MULTIDISCIPLINARY SCIENCES	Q1
13	Hamann L, Ruiz-Moreno JS, Szwed M, **Mossakowska M**, Lundvall L, Schumann RR, Opitz B, Puzianowska-Kuznicka M.	STING SNP R293Q Is Associated with a Decreased Risk of Aging-Related Diseases.	Gerontology. 2019; 65(2):145-154 doi: 10.1159/000492972	4.086	GERIATRICS & GERONTOLOGY	Q2

14	Rydzanicz M, Wachowska M, Cook EC, Lisowski P, Kuźniewska B, Szymańska K, Diecke S, Prigione A, Szczałuba K, **Szybińska A,** Koppolu A, Murcia Pienkowski V, Kosińska J, **Wiweger M,** Kostrzewa G, Brzozowska M, Domańska-Pakieła D, Jurkiewicz E, Stawiński P, Gromadka A, Zielenkiewicz P, Demkow U, Dziembowska M, **Kuźnicki J,** Creamer TP, Płoski R.	Novel calcineurin A (PPP3CA) variant associated with epilepsy, constitutive enzyme activation and downregulation of protein expression.	Eur J Hum Genet. 2019; 27:61-69 doi: 10.1038/s41431-018-0254-8	3.907	BIOCHEMISTRY & MOLECULAR BIOLOGY	Q2
15	Olczak E, Kuryłowicz A, Wicik Z, Kołodziej P, Cąkała-Jakimowicz M, Buyanovskaya O, **Ślusarczyk P, Mossakowska M,** Puzianowska-Kuźnicka M.	Glucocorticoid receptor (NR3C1) gene polymorphisms are associated with age and blood parameters in Polish Caucasian nonagenarians and centenarians.	Exp Gerontol. 2019; 116:20-24 doi: 10.1016/j.exger.2018.12.006	3.597	GERIATRICS & GERONTOLOGY	Q2
16	Wyskida M, Owczarek AJ, Chełmecka E, Szczerbowska I, **Mossakowska M,** Grodzicki T, Puzianowska-Kuźnicka M, Olszanecka-Glinianowicz M, Chudek J.	Parathyroid hormone response to different vitamin D levels in population-based old and very-old Polish cohorts.	Exp Gerontol. 2019; 127:110735 doi: 10.1016/j.exger.2019.110735	3.597	GERIATRICS & GERONTOLOGY	Q2
17	Bizzarri M, Cassanelli S, Bartolini L, **Pryszcz LP,** Dušková M, Sychrová H, Solieri L.	Interplay of Chimeric Mating-Type Loci Impairs Fertility Rescue and Accounts for Intra-Strain Variability in Zygosaccharomyces rouxii Interspecies Hybrid ATCC42981.	Front. Genet. 2019; 10:137 doi: 10.3389/fgene.2019.00137	3.517	GENETICS & HEREDITY	Q2
18	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 doi: 10.1152/ajprenal.00126.2019	3.487	UROLOGY & NEPHROLOGY	Q1
19	Deskur-Śmielecka E, Chudek J, Neumann-Podczaska A, **Mossakowska M,** Wizner B, Wieczorowska-Tobis K.	Use of renal risk drugs in a nation-wide Polish older adult population: an analysis of PolSenior database.	BMC Geriatr. 2019; 19(1):70 doi: 10.1186/s12877-019-1075-5	3.458	GERONTOLOGY	Q1
20	Pac A, Tobiasz-Adamczyk B, Błędowski P, Skalska A, **Szybalska A,** Zdrojewski T, Więcek A, Chudek J, Michel JP, Grodzicki T.	Influence of Sociodemographic, Behavioral and Other Health-Related Factors on Healthy Ageing Based on Three Operative Definitions.	J Nutr Health Aging. 2019; 23(9):862-869 doi: 10.1007/s12603-019-1243-5	3.215	GERIATRICS & GERONTOLOGY	Q3
21	Kampinga HH, Andreasson C, Barducci A, Cheetham ME, Cyr D, Emanuelsson C, Genevaux P, Gestwicki JE, Goloubinoff P, Huerta-Cepas J, Kirstein J, Liberek K, Mayer MP, Nagata K, Nillegoda NB, Pulido P, Ramos C, De Los Rios P, Rospert S, Rosenzweig R, Sahi C, Taipale M, Tomiczek B, Ushioda R, Young JC, Zimmermann R, **Zylicz A, Zylicz M,** Craig EA, Marszalek J.	Function, evolution, and structure of J-domain proteins.	Cell Stress Chaperones. 2019; 24(1):7-15 doi: 10.1007/s12192-018-0948-4	2.944	CELL BIOLOGY	Q3
22	Pawełkowicz M, **Pryszcz L,** Skarzyńska A, Wóycicki RK, Posyniak K, Rymuszka J, Przybecki Z, Pląder W.	Comparative transcriptome analysis reveals new molecular pathways for cucumber genes related to sex determination.	Plant Reprod. 2019; 32(2):193-216 doi: 10.1007/s00497-019-00362-z	2.606	PLANT SCIENCES	Q1
23	Królczyk J, Piotrowicz K, Chudek J, Puzianowska-Kuźnicka M, **Mossakowska M, Szybalska A,** Grodzicki T, Skalska A, Gąsowski J.	Clinical examination of peripheral arterial disease and ankle-brachial index in a nationwide cohort of older subjects: practical implications.	Aging Clin Exp Res. 2019; 31(10):1443-1449 doi: 10.1007/s40520-018-1095-6	1.934	GERIATRICS & GERONTOLOGY	Q3
24	Hodorová V, Lichancová H, Zubenko S, Sienkiewicz K, Penir SMU, Afanasyev P, Boceck D, Bonnin S, Hakobyan S, Smyczynska U, Zhivkoplias E, Zlatohurska M, Tralle E, Frolova A, **Pryszcz LP,** Brejová B, Vinař T, Nosek J.	Genome Sequence of the Yeast Saprochaete ingens CBS 517.90.	Microbiol Resour Announc. 2019; 8(50). pii: e01366-19 doi: 10.1128/MRA.01366-19			
25	Lichancová H, Hodorová V, Sienkiewicz K, Penir SMU, Afanasyev P, Boceck D, Bonnin S, Hakobyan S, Krawczyk PS, Smyczynska U, Zhivkoplias E, Zlatohurska M, Odrzywolski A, Tralle E, Frolova A, **Pryszcz LP,** Brejová B, Vinar T.	Genome Sequence of Flavor-Producing Yeast Saprochaete suaveolens NRRL Y-17571	Microbiol Resour Announc. 2019; 8(9). pii: e00094-19 doi: 10.1128/MRA.00094-19			

Average IF of journals with IIMCB's publications 2000-2019



Sum of Times Cited per Year

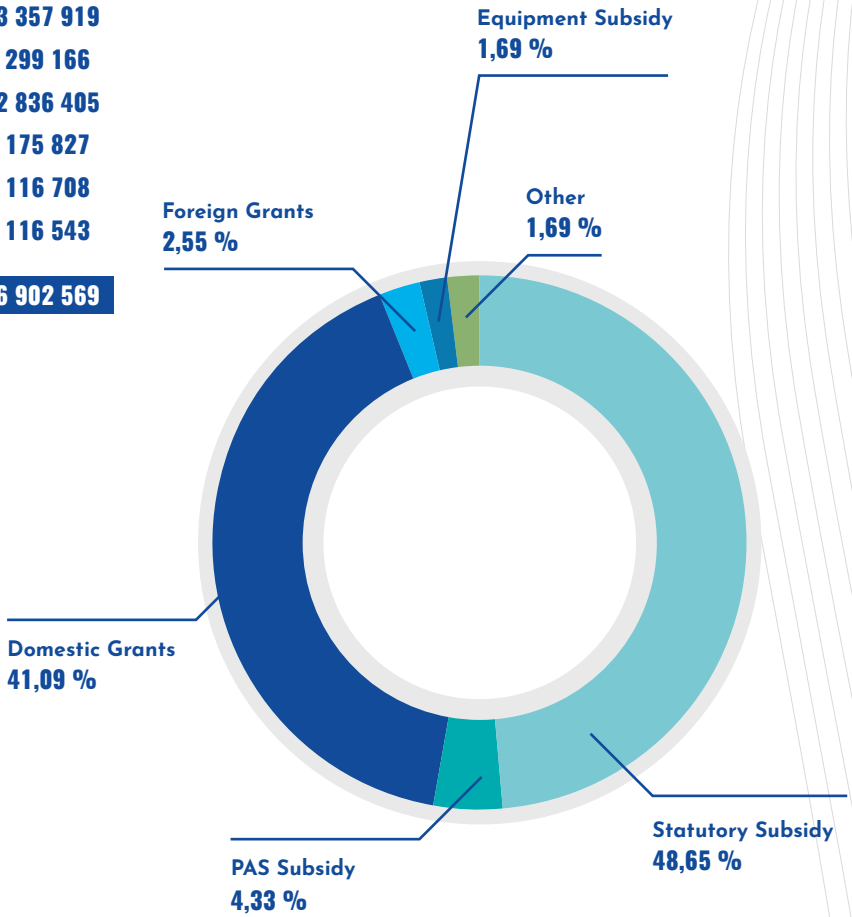


Diversity of Funding

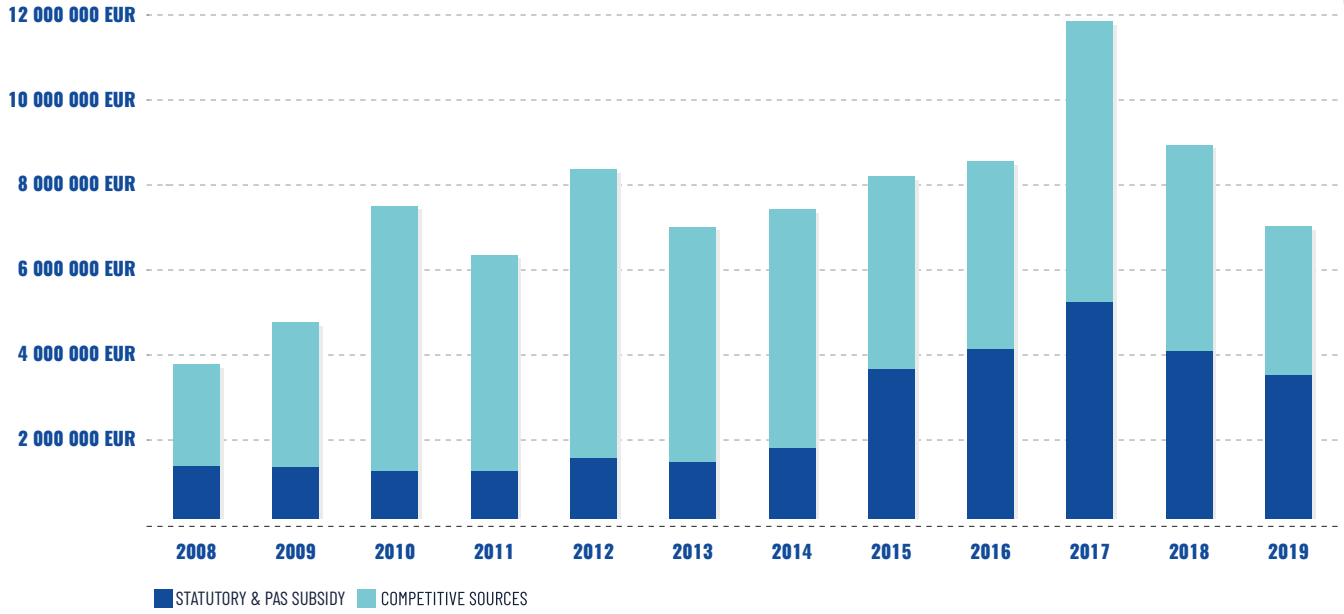
Sources of Funding in 2019

	PLN	EUR*
Statutory Subsidy	14 299 700	3 357 919
PAS Subsidy	1 274 000	299 166
Domestic Grants	12 078 829	2 836 405
Foreign Grants	748 761	175 827
Equipment Subsidy	497 000	116 708
Other	496 300	116 543
Total	29 394 590	6 902 569

** 1 EUR - 4,2585 @ 31st Dec'2019*



Annual Income 2008-2019



Grants

TOTAL NUMBER OF GRANTS IMPLEMENTED IN 2019: 57
TOTAL FUNDING FROM GRANTS IMPLEMENTED IN 2019: 93 716 535 PLN

NATIONAL SCIENCE CENTRE

37 PROJECTS 46 965 751 PLN

MAESTRO

"Integrative modeling and structure determination of macromolecular complexes comprising RNA and proteins" (UMO-2017/26/A NZ1/01083); 3 500 000 PLN; 2018-2023; **J.M. Bujnicki**

"Structural and mechanistic studies of bacterial DNA repair" (UMO-2017/26/A/NZ1/01098); 4 228 500 PLN; 2018-2023; **M. Nowotny**

"Molecular mechanisms of pro-survival processes in breast cancer" (2012/06/A/NZ1/00089); 3 000 000 PLN; 2013-2019; **M. Żylicz**

"Transgenic mice with elevated basal level of calcium ions in neurons as a model of aged-induced neurodegeneration of sporadic Alzheimer's disease" (2011/02/A/NZ3/00144); 2 989 800 PLN; 2012-2019; **J. Kuźnicki**

SYMFONIA

"Mitochondrial RNA decay and surveillance – comprehensive interdisciplinary studies" (2014/12/W/NZ1/00463); 2 953 248 PLN (total grant budget: 6 879 968 PLN); 2014-2019; **M. Nowotny**

HARMONIA

"Structural biology of mixed lineage leukemia (MLL) proteins" (2014/14/M/NZ5/00558); 1 255 000 PLN; 2015-2019; **M. Bochtler**

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**; project partner: Vilnius University, Lithuania

OPUS

"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**

"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**

"Approaching integrative genomics to identify molecular drivers of congenital heart disease" (2018/29/B/NZ2/01010); 1 880 050 PLN; 2019-2022; **M. Pawlak**

"Role of TBC1D5 phosphorylation in neurodevelopment and TSC-related cell pathology" (2017/27/B/NZ3/01358); 1 795 700 PLN; 2018-2021; **J. Jaworski**

"Development of new methods for designing RNA molecules that fold into desired spatial structures and their use for development of new functional RNAs and for prediction of noncoding RNAs in transcriptome sequences" (2017/25/B/NZ2/01294): 1 494 250 PLN; 2018-2021; **J.M. Bujnicki**

"Enabling routine and reliable analysis of transport tunnels in proteins" (2017/25/B/NZ1/01307); 1 375 050 PLN; 2018-2021; **J. Brezovsky**

"Exploring Baltic Sea cyanobacteria for small-molecule inhibitors of microRNA function" (2017/25/B/NZ9/00202); 27 000 PLN (total grant budget: 1 410 100 PLN); 2018-2021; **F. Stefaniak** (partner); Coordinator: University of Warmia and Mazury in Olsztyn

"mTOR kinase impact on cellular functions of selected molecular motors" (2016/21/B/NZ3/03639); 1 336 250 PLN; 2017-2020; **J. Jaworski**

"Finding novel determinants of the brain ventricular system" (2016/21/B/NZ3/00354); 1 294 885 PLN; 2017-2020; **V. Korzh**

"Biochemical and structural studies of retroviral reverse transcriptases evolution" (2016/21/B/NZ1/02757); 1 145 000 PLN; 2017-2020; **E. Nowak**

"The role of E3 ligase complexes in integration of protein homeostasis and aging" (2016/23/B/NZ3/00753); 1 116 875 PLN; 2017-2020; **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; 2017-2020; **M. Boniecki**

"Role of STIM2 isoforms in regulation of neuronal calcium channels in *Danio rerio*" (2016/23/B/NZ3/03142); 2 085 031 PLN; 2017-2020; **J. Kuźnicki**

"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); 2016-2010; **C.L. Winata** (partner); Coordinator: Institute of Mother and Child

"New 5-hydroxymethylcytosine binding proteins" (2014/13/B/NZ1/03991); 1 283 750 PLN; 2015-2019; **M. Bochtler**

SONATA

"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; 2017-2020; **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. Zdżalik-Bielecka**

"The role of Zebrafish mTOR in neuronal development in vivo as a key regulator of neuronal circuit formation" (2015/17/D/NZ3/03735); 689 000 PLN; 2016-2019; **J. Zmorzyńska**

"Functional Characterization of Non-Collagenous Extracellular Matrix Proteins as a Potential Molecular Target and Novel Biomarker of Liver Fibrosis" (2014/15/D/NZ5/03421); 541 875 PLN; 2015-2019; **M. Pawlak**

"Role of CacyBP/SIP in the dysregulation of beta-catenin ubiquitination in YAC128 mouse model of Huntington's disease" (2014/15/D/NZ3/05181); 650 000 PLN; 2015-2019; **M. Czeredys**

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**

"Is endocytosis disrupted in tuberous sclerosis complex? Novel studies on human neural stem cells" (2016/23/N/NZ3/00108); 100 000 PLN; 2017-2019; **A. Kościelny**

MINIATURA

"Study of the function and regulation of the key molecular chaperone UNC-45 in the development of CIM myopathy" (2019/03/X/NZ3/00824); 49 401 PLN; 2019-2020; **M. Piechota**

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

"Photoswitchable Ligands for Riboswitches" (2018/02/XNZ1/01468); 21 670 PLN; 2018-2019; **F. Stefaniak**

FOUNDATION FOR POLISH SCIENCE

8 PROJECTS 25 699 907 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; 2018-2021; **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; 2018-2021; **M. Bochtler**

SG OP 4.4. **TEAM** "Structural and biochemical studies of the mechanism of LINE-1 retrotransposition and hepadnaviral replication" (POIR.04.04.00-00-20E7/16-00); 3 690 834 PLN; 2017-2020; **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; 2017-2020; **M. Miączyńska**

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-TECH** "INFECTLESS New generation of antibacterial wound dressing" (POIR.04.04.00-00-3D8D/16-00); 3 463 780 PLN; 2017-2020; **I. Sabała**

SG OP 4.4. **FIRST TEAM** "The regulation of methionine metabolism by the ubiquitin-proteasome system: CHIPed supervision of the methylation potential" (POIR.04.04.00-00-5EAB/18-00); 1 999 823 PLN; 2018-2021; **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; 2017-2020; **C.L. Winata**

EU FRAMEWORK PROGRAMMES & COST

6 PROJECTS 14 578 677 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**

VERTIGO STARTS epiMimesis "Epizode V: Shifting Identities"; 2018-2019 (supporting role as a producer) **J.M. Bujnicki**; Coordinator: University of the Arts in Poznań

FP 7

Collaborative Project EPISTOP "Long-term, prospective study evaluating clinical and molecular biomarkers of epileptogenesis in a genetic model of epilepsy – tuberous sclerosis complex" (602391); 774 818 EUR; matching funds 829 113 PLN; (total grant budget: 13 019 934,80 EUR); 2013-2019; **J. Jaworski** (partner); Coordinator: Children's Memorial Health Institute

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**

IONCHAN-IMMUNRESPON "Ion Channels and Immune Response toward a global understanding of immune cell physiology and for new therapeutic approaches" (BM1406); 2015-2019; **J. Kuźnicki, Ł. Majewski**

NATIONAL CENTRE FOR RESEARCH AND DEVELOPMENT

1 PROJECT 3 088 120 PLN

STRATEGMED (acronym 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; 2019-2020; **A. Skaruz**

International Academic Partnerships "Molecular basis of enzyme specificity and applications" (PPI/APM/2018/1/00034/U/001); 2 000 000 PLN; 2018-2020; **M. Bochtler, I. Sabała**

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

1 PROJECT 630 000 PLN

EMBO Installation Grant (3913); 150 000 EUR, 2018-2021; **W. Pokrzywa**

MINISTRY OF SCIENCE AND HIGHER EDUCATION

1 PROJECT 199 980 PLN

Diamond Grant "Elucidating the mechanism of translational control of maternal transcripts through cytoplasmic polyadenylation" (DI2014 008644); 199 980 PLN; 2015-2019; **M. Łapiński**

SCIENTIFIC MEETINGS & LECTURES

ERA Chairs Research Symposium

March 21, 2019

The event started with the **MOSaIC kick-off meeting** and the presentation of **IIMCB ERA Chairs H2020** project by its coordinator, **Jacek Kuźnicki**. The meeting was followed by the ERA Chairs Research Symposium. Four top candidates for the ERA Chairs Group Leader position gave research seminars concerning their scientific interests and achievements: **Curtis A. Davey** (Singapore), **Andrzej Dziembowski** (Poland), **Tomasz Jurkowski** (Germany) and **Gracjan Michlewski** (United Kingdom).

Young Scientists Conference on Molecular and Cell Biology

April 11, 2019

Event organized by IIMCB PhD students and staff members, was dedicated to PhD, master and undergraduate students interested in molecular and cell biology. Invited keynote speakers: **Peter Cherepanov** (The Francis Crick Institute, United Kingdom), **Thomas Carell** (Ludwig-Maximilians-Universität München, Germany), **Holger Stark** (Max Planck Institute for Biophysical Chemistry, Germany) and **Jacek Kolanowski** (Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poland). Keynote lectures were followed by talks given by **11 young scientists** - MSc and PhD students. **58 students presented their scientific posters.** The event was attended by more than 150 people and became a great platform for early-stage researchers to share their knowledge and experience in molecular and cell biology field.

Excellent science - how model organisms support scientific research

October 9-10, 2019

The conference for a group of 50 **teachers and science communicators** was organized by **Centre for Innovative Bioscience Education** (BioCEN) and IIMCB. The program included lectures presenting the **role of model organisms** (nematode, mouse, zebrafish, yeast and daphnia) in research, medicine, ecology and education (**Joanna Dodzian**, IIMCB "Zebrafish – a small fish, big challenges"). **Wiktor Niedzicki**, Polish journalist and science communicator, shared the secrets of an effective presentation providing valuable tips during the workshop entitled "Present science well". **David Price** from Science Made Simple, during his class entitled "Getting Your Science Noticed" showed how to use performance techniques and have fun in science communication.

Regular IIMCB Seminars

Liliane Brunner Halbach (Business Development Consultant, IIMCB, Warsaw, Poland) *Introducing SPARK Poland, enhancing translational research in academia and call for projects*. 18.12.2019

Anna Malik (Nencki Institute of Experimental Biology PAS, Warsaw, Poland and Max Delbrück Center for Molecular Medicine, Germany) *Let's sort it out: VPS10P proteins as sorting receptors in neurons and astrocytes*. 12.12.2019

Aleksandra Pękowska (Dioscuri Center for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology PAS, Warsaw, Poland) *Dynamics of chromatin topology in differentiation and in transcriptional regulation*. 05.12.2019

Anja Zeigerer (Institute for Diabetes and Cancer, Helmholtz Center, Munich, Germany) *Coupling intracellular transport to metabolic control.* 14.11.2019

Richard Moriggl (University of Veterinary Medicine and Medical University, Vienna, Austria) *Beasty Driver Mutations in JAK-STAT and How to Target STAT3 and STAT5 with Novel Approaches and Agents.* 07.11.2019

Savani Anbalagan (Centre of New Technologies, University of Warsaw, Poland) *Role of Glia in Neurovascular Developmental Morphogenesis.* 31.10.2019

Tales Rocha De Moura (Division of Structural Biotechnology, KTH Royal Institute of Technology, Stockholm, Sweden) *Structural Biology Studies: From Soluble Proteins to Membrane Channels*. 28.10.2019

Bożena Kamińska-Kaczmarek (Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland) *Dissecting immune cell heterogeneity of brain tumors*. 18.10.2019

Tomasz Trombik (Department of Biophysics, Faculty of Biotechnology, University of Wrocław, Poland) *Multiple faces of ABCA1 protein: from immunology to antibiotic resistance*. 17.10.2019

Piotr Szwedziak (Laboratory of Structural Cell Biology, Centre of New Technologies, Warsaw University, Poland) *Bidirectional contraction of a contractile injection system*. 10.10.2019

Gintautas Tamulaitis (Institute of Biotechnology, Vilnius University, Ukraine) *Type III CRISPR-Cas immunity*. 07.10.2019

John Mattick (University of Oxford, UK) *The central role of RNA in cell and developmental biology*. 03.10.2019

Steven Fong (Republic Polytechnic, Singapore) *The Singapore Food Story: A career detour.* 5.09.2019

Grzegorz Sumara (Dioscuri Center for Metabolic Diseases, Nencki Institute of Experimental Biology PAS, Warsaw, Poland) *Signaling Cascades in Metabolic Diseases*. 19.09.2019

Radosław Nowak (Department of Cancer Biology, Dana-Farber Cancer Institute Harvard Medical School, Boston, MA, USA) *Targeted protein degradation: Plasticity of inter-protein contacts confers selectivity*. 05.09.2019

Marcin Tabaka (Broad Institute, MIT and Harvard, Cambridge, MA, USA) *Reconstruction of developmental landscapes from single-cell gene expression profiles.* 27.08.2019

Allan V Kalueff (ZENEREI Institute and The International Zebrafish Neuroscience Research Consortium, Slidell, LA, USA) *How zebrafish models are reshaping modern translational neuroscience*. 26.08.2019

Panagiotis Alexiou (Central European Institute of Technology Masaryk University, Brno, Czech Republic) *Hunting for Genomic patterns via Deep Learning*. 17.07.2019

Alexander Wlodawer (Macromolecular Crystallography Laboratory, National Cancer Institute, Frederick, MD, USA) *Structure and mechanism of a cancer drug L-asparaginase - half century in a search for answers*. 12.07.2019

David Eisenberg (Howard Hughes Medical Institute, University of California, Los Angeles, CA, USA) *The expanded amylome: structure, energetics, and function*. 12.07.2019

Thorsten Hoppe (Institute for Genetics and Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Germany) *Ubiquitin Tips the Balance: Impact of Food Perception on Proteostasis & Aging*. 04.07.2019

Paul Hurd (School of Biological and Chemical Sciences Queen Mary University of London, UK) *The epigenetic basis of nutrition-mediated caste identity in the honeybee*. 27.06.2019

Marek Postuła (1st Faculty of Medicine, Department of Experimental and Clinical Pharmacology, Medical University of Warsaw) *Fulbright grants for Polish citizens – what should you know?* 26.06.2019

Matthew D. Disney (The Scripps Research Institute, Department of Chemistry Florida Campus, Jupiter, FL, USA) *Translating RNA Sequence into Lead Small Molecule Medicines.* 17.06.2019

Lars Larsson (Department of Physiology and Pharmacology Karolinska Institute, Stockholm, Sweden) *Critical illness myopathy: Mechanisms and interventions*. 17.06.2019

Rhiju Das (Physics Stanford University School of Medicine, Stanford, CA, USA) *Computer modeling and design of 3D RNA structure/function*. 10.06.2019

Roman Szczęsny (Mitochondrial Research Group, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland) *Post-transcriptional shaping of the human mitochondrial transcriptome*. 06.06.2019

Martin Bommer (The Max Delbrück Center for Molecular Medicine, Berlin, Germany) *Modular Cloning is here ... to make your life easier*. 30.05.2019

Wladek Minor (Department of Molecular Physiology and Biological Physics University of Virginia, Charlottesville, VA, USA) *Reproducibility of biophysical and biomedical experiments.* 23.05.2019

Simon Reed (Division of Cancer and Genetics School of Medicine, Cardiff University, UK) *How chromatin is remodelled during Global Genome-NER: origins of DNA repair*. 09.05.2019

Jakub Urbański (Biotech Innovations, Warsaw, Poland) *To eat a cookie and to have a cookie - how to combine patents and publishing.* 04.04.2019

Anton Slyvka (Laboratory of Structural Biology, IIMCB, Warsaw, Poland) *Detecting and processing enzymatically modified cytosines*. 28.03.2019

Michał Opas (University of Toronto, Canada) Intralumenal *Calcium Homeostasis of the Endoplasmic Reticulum and the Cell Fate Choice during Differentiation of the Derivative Cell Types.* 18.03.2019

Kamil Jastrzębski, **Matylda Macias**, **Katarzyna Misztal**, **Tomasz Węgierski** (Core Facility, IIMCB, Warsaw, Poland) *IIMCB Core Facilities: equipment, techniques and experts' support*. 07.03.2019

Joanna Dodzian, **Krzysztof Skowronek**, **Roman Szczepanowski** (Zebrafish Core Facility and Core Facility, IIMCB, Warsaw, Poland) *IIMCB Core Facilities: equipment, techniques and experts' support*. 28.02.2019

Katarzyna Kołtowska (Department of Immunology, Genetics and Pathology Uppsala University, Sweden) *A transcriptional journey through the lymphatic vessel development*. 21.02.2019

Ruth Palmer (University of Gothenburg, Sweden) *Regulating ALK activity in cancer-lessons from model organisms*. 14.02.2019

Magdalena Kędra (Laboratory of Molecular and Cellular Neurobiology, IIMCB, Warsaw, Poland) *White matter disruption and altered commissural tract connectivity is related to increased epileptogenesis and anxiety in Zebrafish model of Tuberous Sclerosis Complex*. 07.02.2019

Małgorzata Maksymowicz (Laboratory of Cell Biology, IIMCB, Warsaw, Poland) *Cellular trafficking and signaling of lymphotoxin* β *receptor*. 31.01.2019

Marek Cieplak (Institute of Physics, PAS, Warsaw, Poland) *Structural changes in proteins at fluid-fluid interfaces.* 17.01.2019

Jarosław Stolarski (Institute of Paleobiology PAS, Warsaw, Poland) *Emergence of coral reefs: deciphering history of scleractinian coral biomineralization from modern and fossil skeletons*. 10.01.201

Annual Report Session

May 31, 2019, Przypki, Poland

Best Presentations Award

Katarzyna Nieścierowicz (Laboratory of Zebrafish Developmental Genomics) *Adar, first league player in body planning and development.*

Aniruddha Das (Laboratory of Protein Metabolism in Development and Aging) *Cooperation of ubiquitin ligases in the service of protein homeostasis.*

Diana Toczydłowska-Socha (Laboratory of Bioinformatics and Protein Engineering) *Human RNA cap1 methyltransferase CMTr1 cooperates with RNA helicase DHX15 to modify highly structured 5' termini of RNAs.*

Ewa Liszewska (Laboratory of Molecular and Cellular Neurobiology) *Modeling neurodevelopmental disorders with the use of induced pluripotent stem cells.*

Daria Zdżalik-Bielecka (Laboratory of Cell Biology) *GAS6 N'AXL: endocytic trafficking, signaling and role in cancer.*

Abhishek Pateria (Laboratory of Structural Biology) *CRISPR tools for targeted RNA knockdown in zebrafish.*

Gabriela Jędruszewska (Laboratory of Iron Homeostasis) *Communication with iron-loaded hepatocytes contributes to the transcriptional control of Bmp6 in the liver endothelium.*

Evgeny Gasanov (Laboratory of Neurodegeneration) *Kv2.1 potassium voltage-gated channels in vertebrates development and disease.*

Carlos Eduardo Sequeiros Borja (Laboratory of Biomolecular Interactions and Transport) *Structural insights into phosphoregulation of endocytosis via Adaptor Protein complex 2.*

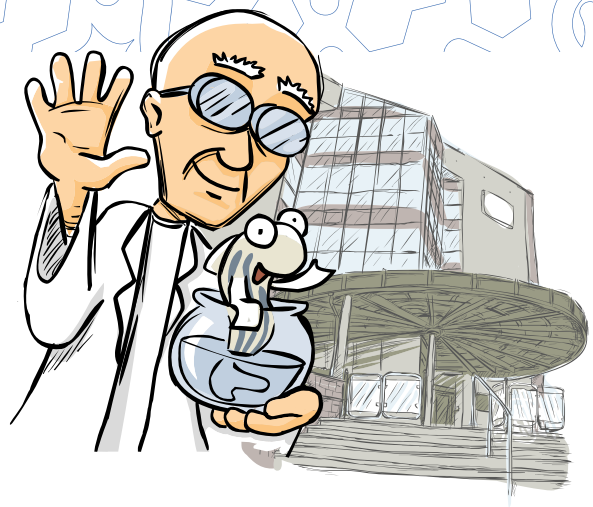
Mariusz Czarnocki-Cieciura (Laboratory of Protein Structure) *Cryo-electron miscroscopy at IIMCB*.

Michał Boniecki (Laboratory of Bioinformatics and Protein Engineering) *Let's do that in non-canonical way.*

Jacek Jaworski (Laboratory of Molecular and Cellular Neurobiology) *Popular Science lecture in Polish: Can you make a brain out of skin?*

EDUCATION

Be Healthy as a Fish educational program



Mice and rats are still the most common choices for modeling human diseases, but zebrafish (*Danio rerio*) are becoming increasingly popular. In response to this growing potential, IIMCB introduced the *Be Healthy as a Fish* campaign in 2014. This program aims to educate school children on how zebrafish can be used as a model organism to help scientists understand the way the human body works.

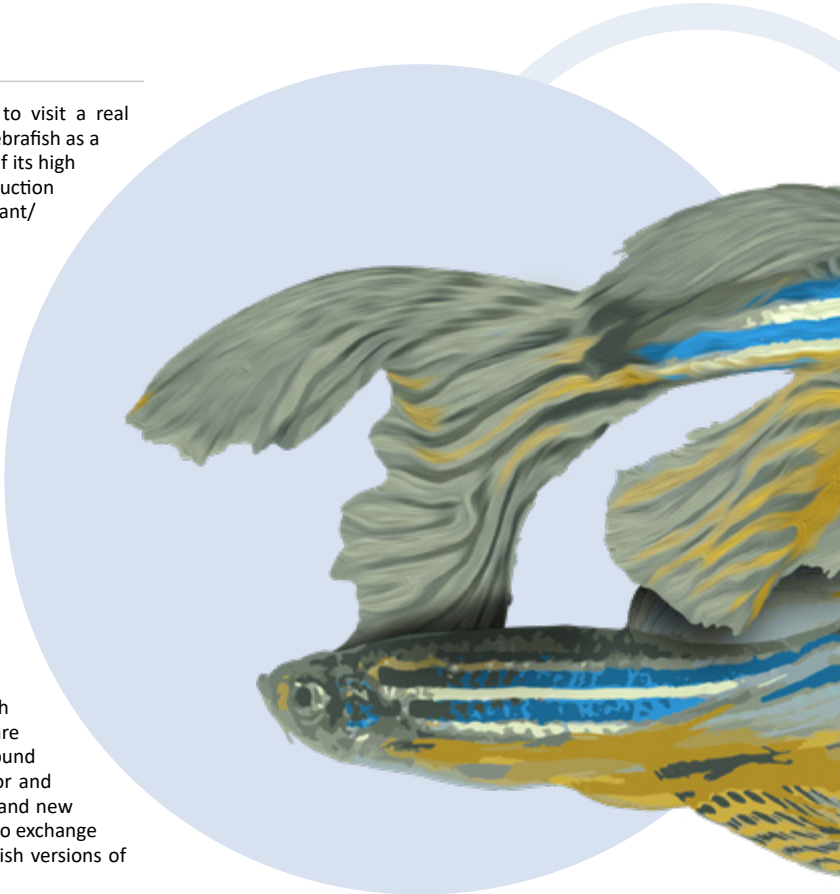
The main character of the *Be Healthy as a Fish* educational campaign is a zebrafish that helps scientists find the causes of human diseases and discover new therapies and medicines. Interactive workshops that utilize modern research equipment are part of the educational campaign, which also includes a short animated movie and an illustrated booklet.

WORKSHOP

1035 PARTICIPANTS

For the young target audience, the workshop is an opportunity to visit a real scientific institution. During the classes, children are introduced to zebrafish as a model organism that is used in basic and applied research because of its high genetic similarity to humans, transparent embryos, very short reproduction cycle, access to experimental manipulations, large collection of mutant/transgenic lines, and low maintenance cost.

Participants of the workshop learn how development of the human body is similar to zebrafish. They learn that scientists can better understand human embryogenesis and functions of the human body by observing zebrafish. By explaining the concept of gene homology, they learn the importance of animals in the multi-stage process of discovering new drugs and therapies.



MOVIE

5700 VIEWS

The movie seeks to familiarize viewers with IIMCB's facilities and scientific interests and show the everyday work lives of scientists. This 6-min movie is mostly animated. However, part of it shows real images of various locations within the institute (e.g., laboratories, fish facility, and a lecture hall where scientific seminars and workshops are held). The storyline of the animation consists of a humorous tour around the institute that is guided by two cartoon characters: the Professor and a zebrafish. The viewers are informed that science has no borders, and new discoveries result from joint efforts of scientists around the world who exchange information during seminars and conferences. Both Polish and English versions of the movie are available on the IIMCB YouTube channel.

BOOKLET

3000 DOWNLOADS

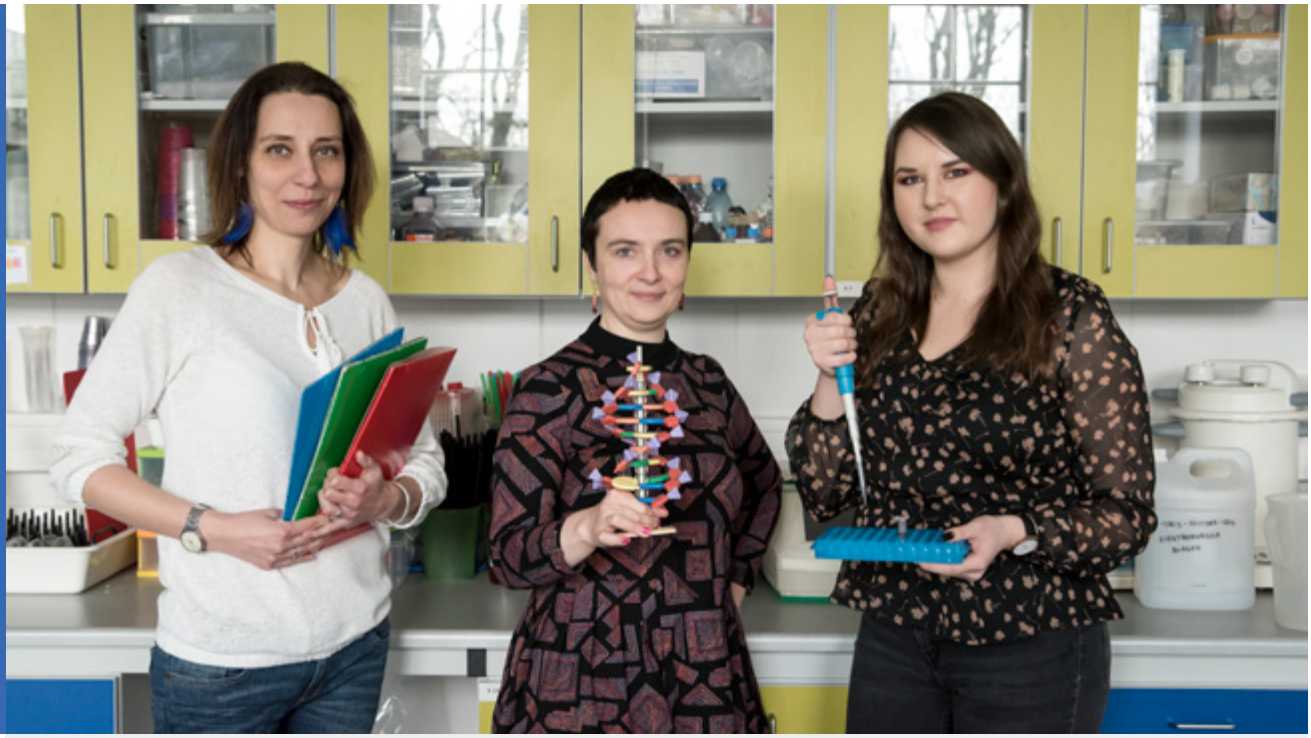
The book is distributed to all of the participants of the workshop as an invitation to broaden their knowledge beyond the topics that are discussed in their classes. The content of the book was written so that it can be regarded as an independent story from which people who do not participate in the workshops can benefit. The *Be Healthy as a Fish book* is interesting additional material for teachers to support discussions about the evolution of life, cell biology, heredity, and anatomical similarities between humans and animals. The book is also a good starting point for discussions about why scientists need animals, what we can learn from observing their physiology, and how this affects discoveries of new medicines.

ACHIEVEMENTS

3 PUBLICATIONS
1 POSTER PRIZE
2 LECTURES AT INTERNATIONAL EVENTS

- Goś D. “Be Healthy as a Fish” educational program - Presenting how zebrafish can improve our understanding of human diseases. Dev Biol. 2020 Jan 15;457(2):169-171. doi: 10.1016/j.ydbio.2019.01.012
- Goś D, Szymańska E, Białek-Wyrzykowska U, Wiweger M, Kuźnicki J. Be Healthy as a Fish Educational Program at the International Institute of Molecular and Cell Biology in Warsaw, Poland. Zebrafish. 2016 Aug;13(4):266-71. doi: 10.1089/zeb.2015.1195
- Presentations on the *Be Healthy as a Fish* campaign at the 9th European Zebrafish Meeting, 2015, Oslo, Norway, and at the 7th European Forum for Marketing of Scientific and Research Organizations, 2016, Warsaw, Poland
- Poster prize at the 6th European Forum for Marketing of Scientific and Research Organizations, 2015, Warsaw, Poland
- Invitation for the co-author of the *Be Healthy as a Fish* educational program to chair the Education Session at the 10th European Zebrafish Meeting, 2017, Budapest, Hungary
- Workshops for Ukrainian students as part of the International Biology School in cooperation with the Minor Academy of Sciences of Ukraine, 2017, Warsaw, Poland
- Presentation on the *Be Healthy as a Fish* program at “Artful Zebrafish Exhibition: Earn your Stripes” at the Festival of Mind 2016 in Sheffield, UK
- Promotion of the zebrafish model at Biologists’ Night 2018 at the Faculty of Biology, University of Warsaw

Children’s drawings by Nadia Kalita.

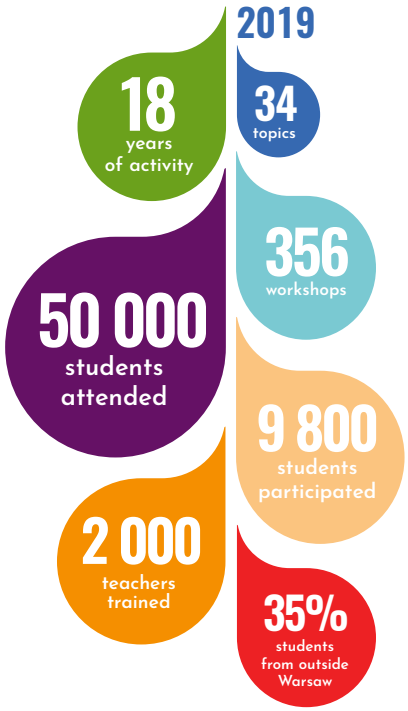


Centre for Innovative Bioscience Education

Head
Patrycja Dołowy, PhD

Laboratory Manager
Aleksandra Olszańska, BEng

Project Manager
Aleksandra Kot-Horodyńska, MSc (until November 2019)
Katarzyna Tomaszewska, MSc



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 the city, especially in schools in small towns and villages. Our activities are based on sound scientific results. Learning and applying the scientific method are keys to understanding both science and today's world as a remedy to exposure to inaccurate information that influences almost every area of modern life, including public and social relations, the environment, health, security, and politics.

BioCEN receives financial support from the International Institute of Molecular and Cell Biology (IIMCB) in Warsaw, which has been BioCEN's Strategic Sponsor since 2015. In addition to IIMCB's support, BioCEN is subsidized by the Nencki Institute of Experimental Biology (Polish Academy of Sciences), Institute of Biochemistry and Biophysics (Polish Academy of Sciences), 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. Our laboratory workshops are based on contemporary scientific interests, such as molecular and cellular biology, histology, immunology, biochemistry, biotechnology, microbiology, biophysics, plant physiology, bionics/bioengineering, environmental sciences, and medical sciences. Over the last 18 years, approximately 50 000 students have taken advantage of the workshops that are offered by BioCEN. During courses that are organized for biology teachers, 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.

In 2019, we introduced seven new workshops to our main program. Three workshops were tailored to younger primary school children (age 7-11): two about ecology and the environment and one about forensic science. Two new workshops were tailored to 7th and 8th grade students: sewage plant science and forensic science. Two workshops were tailored to high school students: enzymology and forensic science. We also introduced four special topics: one for groups of small children about nature's structures (during the Summer in the City - Warsaw City Hall programme), one for primary school students about dirt and cleanliness (in cooperation with Domestos brand), and two commercial workshops for adults on cell biology and forensic DNA.

In 2019, BioCEN held 356 workshops based on 34 different topics. Nearly 9 800 students participated. Creating and conducting the workshops were possible because of programs that were coordinated by Patrycja Dołowy and Aleksandra Kot-Horodyńska, co-funded by the Department of Education in Warsaw City Hall, Ministry of Science and Higher Education, Polish Academy of Sciences, and Domestos brand.

Approximately 35% of the students who participated in our workshops were from outside Warsaw (voivodeships: Podlaskie, Kujawsko-Pomorskie, Wielkopolskie, Zachodnio-Pomorskie, Lubelskie, Podkarpackie, Świętokrzyskie, and Mazowieckie). Nonetheless, because of the location of BioCEN, access is somewhat limited to students from different parts of Poland. As such, the BioCEN team is ready to organize and implement laboratory workshops outside its headquarters. This year, we organized nine workshops in schools, cultural, social, and refugee centers in Lubelskie, Podlaskie, Świętokrzyskie, and Mazowieckie regions. We believe that this outreach of our workshops effectively increases the awareness of life sciences and scientific skills among a wider population and will be an important component of our program.

WORKSHOP FOR GIFTED CHILDREN

The Polish Children's Fund is an independent, non-governmental organization and one of its major objectives is to help exceptionally gifted students develop their academic interests and artistic talents. To support this mission in 2019, BioCEN organized training for talented youth where children had an opportunity to conduct experiments and gain new knowledge in the environmental and genetic microbiology fields.

FLYING SCIENCE CAFES AND WORKSHOPS FOR REFUGEES

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 Lubelskie and Podlaskie regions of Poland. Workshops concerned DNA structure, biophysics and optics.

THE EXEMPLARY BATHROOM – PROGRAM FOR PRIMARY SCHOOLS

Together with Domestos brand, BioCEN introduced this program for primary school pupils in Mazowieckie and Świętokrzyskie voivodeships. It includes 3-h laboratory workshops on the microbiology of dirt and cleanliness.

LABORATORY WORKSHOPS FOR BUSINESS

In 2019, BioCEN organized two laboratory workshops for Sisley Polska and prepared one workshop for Sephora Polska that was dedicated to groups of employees. Workshops for adults were based on cell biology. BioCEN has also conducted a workshop for girls on the beauty of life sciences and experiments in collaboration with Dwie Siostry Publishing House. During the workshop, young girls (6-12 years old) learned about isolating DNA from fruits and observed biological structures under a microscope. The event was related to the release of a popular science book for children by Dwie Siostry Publishing House and International Women's Day celebrations.

INTERNATIONAL COOPERATION

BioCEN participated in the Helmholtz Horizons Symposium in Berlin in November 2019. The mission of the Helmholtz Association is to answer key questions about challenges of our times and how science can contribute to answering these questions by combining cutting-edge research with outstanding in-house technologies.

BioCEN participated in Horizon 2020 Training in the Copernicus Centre in Warsaw in October 2019. The goal of this 2-day meeting was to initiate future collaborations in the field of science communication and open-schooling education.

The head of BioCEN, Patrycja Dołowy, participated in the International Learning Adventures Conference in the Copernicus Centre in November 2019. During the conference the following questions were raised: how to cope with pollution and global warming and how to harness the potential of science and technology for social benefit; will the skills that are gained in the online realm of coding and programming truly suffice?; how important for learning is contact with physical objects and constructing knowledge by physically experiencing the world?

PROFESSIONAL TRAINING FOR TEACHERS AND EDUCATORS

One of our main goals is to improve teaching skills of science educators who work at all levels of education. In 2019, BioCEN together with science institutes organized two conferences for teachers and educators.

Excellent Science: **How Model Organisms Support Scientific Research.** The conference for teachers and science popularizers was organized by BioCEN, the BioEducation Foundation, and IIMCB in Warsaw and held on October 9-10, 2019. The event was attended by 50 participants. The program included lectures on the use of model organisms (nematode, mouse, zebrafish, yeast, and daphna) in research, medicine, ecology, and education and workshops. Wiktor Niedzicki shared his secrets of presentations and gave valuable tips during the workshop entitled "Present Science Well". David Price from Science Made Simple demonstrated how to use performance techniques and have fun in science communication during classes entitled "Getting Your Science Noticed". The event was co-financed by the Polish Ministry of Science and Higher Education.

18th Educational Symposium for Biology Teachers. This annual symposium has become one of our most important events. The most recent was held on November 30, 2019. 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 those related to the Nobel prizes in Physiology and Medicine. In 2019 we also proposed a workshop on communication for teachers, conducted by Anna Pietruszka-Dróżdż, a trainer from Synergis Co. The event was attended by 60 participants. The symposium was organized in cooperation with the Nencki Institute of Experimental Biology, Polish Academy of Sciences, in Warsaw.

Workshop for teachers in the BioCEN laboratory in Warsaw. In November 2019, BioCEN organized a workshop for teachers from the Wielkopolskie district. During the workshop, participants performed experimental tasks individually and analyzed the results. The goal was to learn about experimental methods in biology and how to apply this knowledge in biology classes at schools.

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 (each kit comes in small and large versions):

- 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, genuine BioCEN educational board games:

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

Especially for teachers, we have created lesson scripts and experimental protocols that are available on our new website: https://biocen.edu.pl/scenariusze-volvox/. Some of the scripts and protocols have been updated based on the latest research results.

EVENTS

In June 2019, together with the Lublin Cultural Centre, BioCEN organized a workshop based on science theater for high school students. In the future, both organizations will produce a "scientific performance".

23rd Festival of Science in Warsaw

As in previous years, BioCEN participated in the Festival of Science in Warsaw. Head of BioCEN, Patrycja Dołowy, gave a lecture in the Biology Department of Warsaw University on the influence of Charles Darwin's Theory on art and science.

NOMINATIONS FOR BIOCEN

In 2019, BioCEN was nominated to receive the Słoneczniki Award for the best initiative for children in Warsaw in two categories: life sciences and logic.

The head of BioCEN, Patrycja Dołowy, and the Laboratory Manager of BioCEN, Aleksandra Olszańska, received an award from the Children's Friends Society in Turek for their sensitivity and care for children.

BIOCEN ANIMATORS AND CO-WORKERS

Important members of the BioCEN team include animators and co-workers without whom the educational activities would not be possible. In 2019, the following individuals collaborated with BioCEN: Kryspin Andrzejewski (animator), Tamara Aleksandrzak-Piekarczyk (laboratory support, author of workshops), Kalina Burnat-Kuijpers (member of BioEducation Foundation board - since November 2019), Mikołaj Cup (animator), Patrycja Dołowy (president of the BioEducation Foundation board, animator, author of workshops), Tuguldur Enkhbaatar (animator), Maciej Grochowski (animator), Andrzej Gruza (animator), Piotr Horodyński (designer, author of workshops), Weronika Iwaniuk (animator), Rafał Jabłuszewski (animator), Katarzyna Jędrzejowska (animator), Magdalena Karpińska (animator), Izabela Kern-Zdanowicz (laboratory support, author of workshops), Marcin Kleibert (animator), Aleksandra Kot-Horodyńska (project manager, member of BioEducation Foundation board - untill November 2019, author of workshops), Maciej Kotliński (member of BioEducation Foundation board, animator), Kinga Lipka (animator), Katarzyna Łepeta (laboratory support, author of workshops), Aleksandra Kowalczyk (animator), Paweł Morga (animator), Anna Osinka (laboratory support), Aleksandra Olszańska (laboratory coordinator, animator), Aleksandra Owczarek (laboratory support), Małgorzata Orłowska (animator), Michał Oziębło (laboratory support), Teresa Ozimek (laboratory support, author of workshops), Kamil Synoradzki (animator), Jan Maurycy Święcicki (animator), and Michał Wielądek (animator).

RESEARCH SUPPORT UNITS

Research Support Units

Grants Office

Alexia Danyłow, Specialist
Dorota Libiszowska, Head
Agata Skaruz, Specialist
Marcin Ogonowski, Deputy Head
Justyna Szopa, Specialist
Katarzyna Nakielska, Specialist (not in the picture)

HR Unit

Katarzyna Fiedorowicz, Head
Adam Zieliński, Specialist
Beata Tkacz, Senior Specialist
Monika Domańska-Paśko, Specialist
Barbara Dusik, Specialist
Agnieszka Faliszewska, Senior Specialist
Monika Nowicka, Senior Specialist

Financial and Accounting Unit

Małgorzata Bytner, Senior Specialist
Hanna Iwaniukowicz, Deputy Director for Finance
Agnieszka Kuna, Head
Renata Knyziak, Senior Specialist
Ewelina Duda, Specialist (not in the picture)

Administration

Agnieszka Potęga, Specialist

Andrzej Cudny, Specialist

Ewa Jack-Górska, Senior Specialist

Katarzyna Celińska-Zmorzyńska, Specialist

Anna Zolnik, Deputy Director for Operations

Alicja Goldberg, Building Maintenance

Agata Szulim, Specialist

Mariola Sacharuk, Junior Specialist

Adam Kucharski, Building Maintenance

Daria Goś, Senior Specialist (not in the picture)

Magdalena Krupa, Senior Specialist (not in the picture)

Scientific Coordination Unit

Katarzyna Marszałek, Senior Specialist

Agnieszka Wagner-Ziemka, Chief Specialist

IT Unit

Michał Taperek, Specialist

Łukasz Munio, Specialist

Jakub Skaruz, Specialist

Piotr Świsłowski, Senior Specialist

Marcin Gwis, Senior Specialist (not in the picture)

Contents

Young Scientists Conference on Molecular and Cell Biology

April 11, 2019

International Institute of Molecular and Cell Biology in Warsaw, Poland

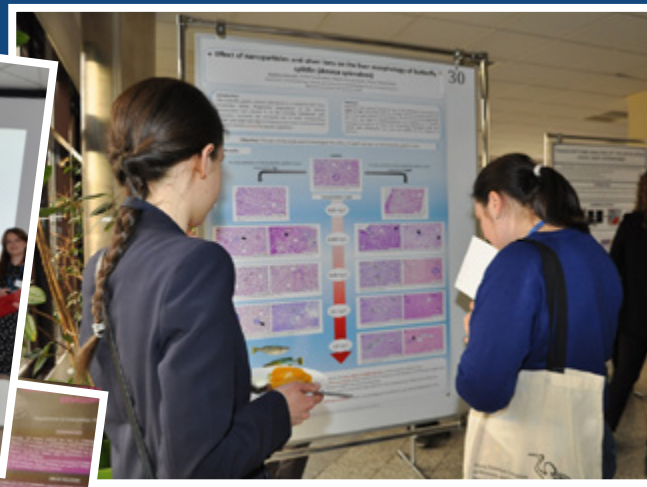
4 keynote speakers:

Peter Cherepanow (The Francis Crick Institute, United Kingdom)

Thomas Carell (Ludwig-Maximilians-Universität München, Germany)

Holger Stark (Max Planck Institute for Biophysical Chemistry, Germany)

Jacek Kolanowski (Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poland)



more than **150** participants

talks given by **11** exceptional young scientists

42 average speaker's h-index

poster session with **58** student-submitted posters

2020

September, 10-11

International Young Scientists Conference on Molecular and Cell Biology

Cell Biology - Cancer & Ageing & Diseases - Development & Stem Cell Biology

Methods & Trends in Biology - Structural Biology

IIMCBinWarsaw

iimcb.gov.pl

NAWA POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE

International Institute of Molecular and Cell Biology in Warsaw
4 Ks. Trojdena Street, 02-109 Warsaw, Poland

www.iimcb.gov.pl