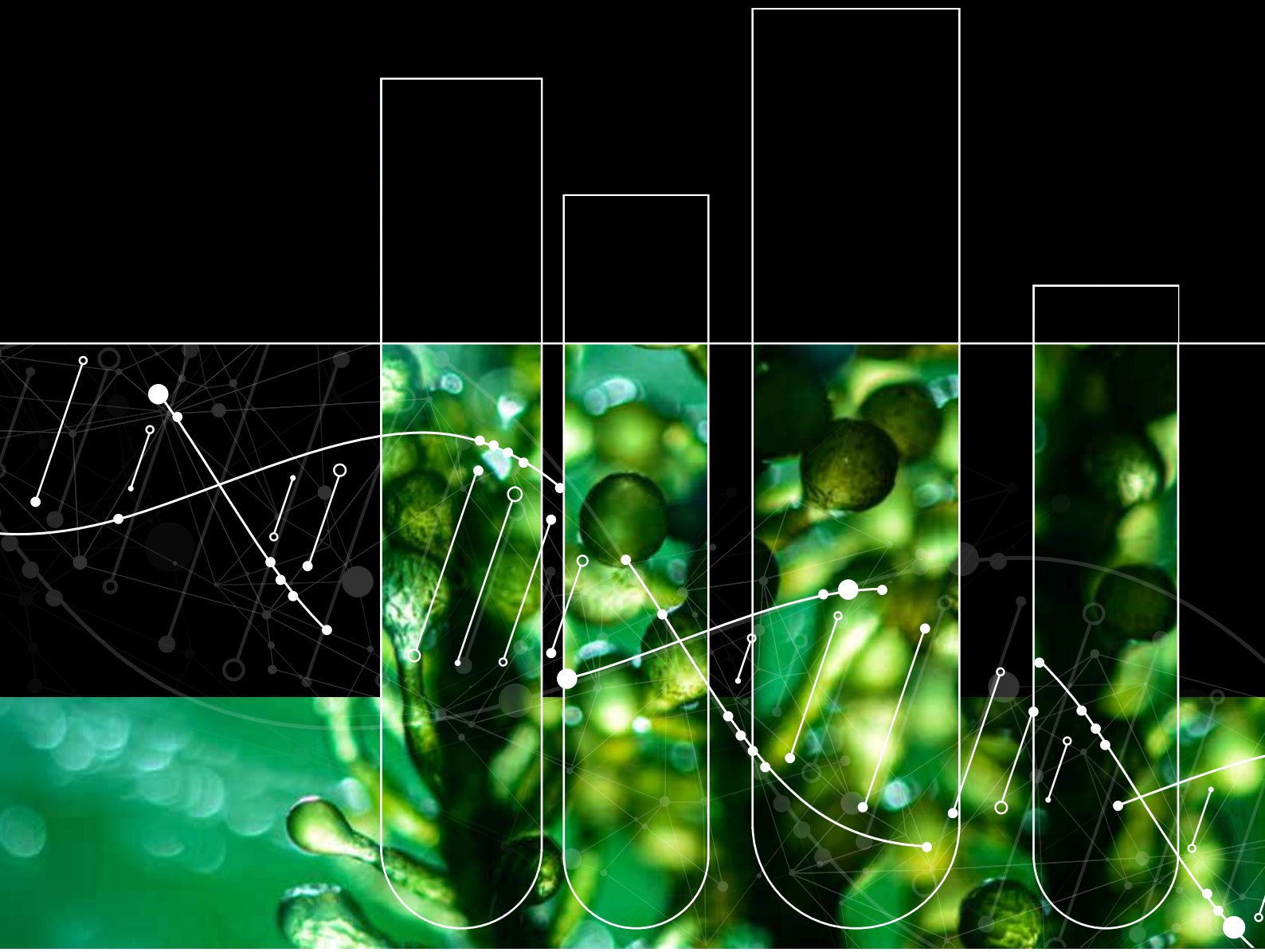

# Progress Report

## 2018-2020

Intercollegiate Faculty of Biotechnology
University of Gdańsk and Medical University of Gdańsk

University of Gdańsk

IFB

# Intercollegiate Faculty of Biotechnology
# University of Gdańsk and Medical University of Gdańsk

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Poland
www.biotech.ug.edu.pl

2018-2020

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**Research Group Profiles & Core Facility Descriptions**
Courtesy of the respective Research Groups and Core Facilities

**Text and Edition**
IFB Staff Members

University of Gdańsk

IFB

# Contents

# A Word from the Dean 2020-2024

**It is a great pleasure to present the latest edition of the Progress Report of the activities of the Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk. In September 2020, I was honored to become the new Dean of the Faculty. I would like to take this opportunity to thank Professor Anna Żaczek, Professor Mariusz Grinholc and Professor Rafał Sądej, who took up this challenge with me to continue the further development of our Faculty. Additionally, I would like to thank all IFB employees and students for their kind welcome.**

Unfortunately, we assumed our duties during a very difficult time due to the COVID-19 pandemic. While taking care of the health of employees and students of the Faculty, in a very short time, we had to put considerable effort into ensuring the highest quality of teaching. For this purpose, we introduced new guidelines for safe work for students and academic teachers. We also reorganized the timetable for all years of study, organized student internships and made every effort to carry out laboratory classes in person, despite the difficult pandemic situation.

Although the current report covers only 4 months of our work, we managed to make several important achievements. In September 2020, we established the Program Council, which is an advisory body contributing to the improvement of the quality of the study program. With the support of the Faculty of Biology, we took over and organized the functioning and administrative service of the Discipline Council of Biological Sciences. Moreover, at the request of the Ministry of Education and Science, we have coordinated many activities aimed at evaluating the scientific activity of the Faculty in the Discipline of Biological Sciences.

Despite many obligations and inconveniences in conducting research, such as the necessity of shift work and breaks in the implementation of research grants due to the quarantine of employees, the IFB has still been one of the most active national research centers involved in the fight against the COVID-19 pandemic in recent months. Faculty members helped to create and operate COVID-19 diagnostic points and provided the necessary equipment to increase testing efficiency. Our staff acted as advisors to ministerial and local government authorities and conducted an intensive educational campaign on the SARS-CoV-2 virus pandemic and vaccine research not only on radio and TV but also on social media. Our researchers have recently obtained funds for research related to SARS-CoV-2. Importantly, our Faculty received funding from the Ministry of Education and Science for the reconstruction and adaptation of the virological laboratory to the high safety standard (BSL3+) required for working with respiratory viruses, which will enable further scientific development.

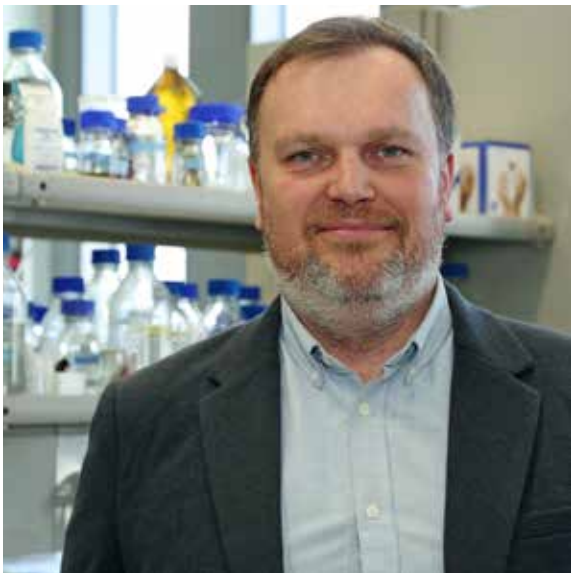
In closing, I wish all of us a quick and smooth return to normal life, all IFB researchers many scientific successes and satisfaction with their research and teaching, and all students persistence in pursuing the set goals.

Ewelina Król, IFB Dean 2020-2024

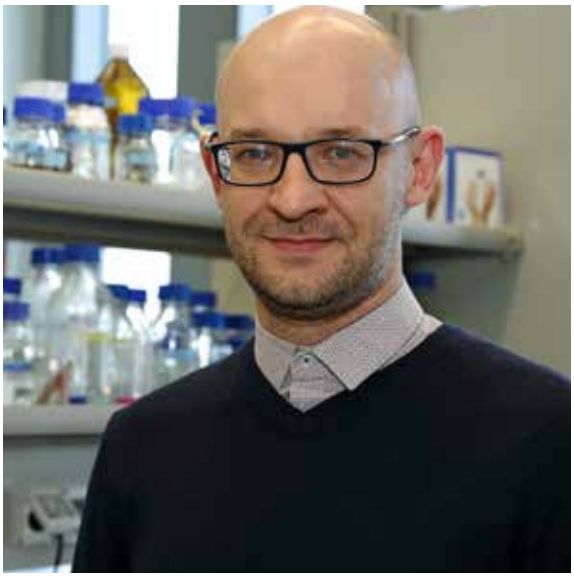
# Intercollegiate Faculty of Biotechnology University of Gdańsk and Medical University of Gdańsk (IFB)

## IFB Authorities (2020-2024)

**Dean**
Assoc. Prof. Ewelina Król



**Deputy Dean for Students Affairs and Education**
Assoc. Prof. Mariusz Grinholc



**Deputy Dean for Science**
Assoc. Prof. Rafał Sądej

**Deputy Dean for Internationalization and Development**
Assoc. Prof. Anna Żaczek

# A word from the Dean 2012-2020

**This progress report covers almost all of the last two years of the 2016–2020 dean cadency. This period was full of stress, unexpected circumstances and difficulties but also an exciting and successful time in the IFB's history. After we became accustomed to the new facility at the Oliva Campus and established a routine in our research and teaching, we had to prepare for and adapt to a new law that was launched by the Ministry of Science and Higher Education and the Polish Parliament.**

The so-called Constitution for Polish Science scrambled the previous rules that had provided a foundation for our Faculty's existence as an intercollegiate unit of two universities. Considerable effort was exerted to strike a new agreement between the universities to create new rules for the IFB. The new agreement regarding the IFB has been signed by the rectors of our universities and accepted by both senates. In Spring 2020, we were shocked by the COVID-19 pandemic. In a very short time, teaching at the IFB was reestablished in a fully online mode. This required hard work from the entire IFB community. Despite the adaptation to a new law and the COVID-19 pandemic, we were able to complete the conceptual work and preparation for and introduction of a new teaching system at the bachelor's level at the IFB. Under this new system, concepts in modern biology and biotechnology provide milestones to be achieved by students to gain knowledge and competencies. Our new innovative system was recognized by the Polish Accreditation Committee (PKA), which visited us in late 2019. We obtained the highest possible distinction of "perfection". We also successfully continued with our research. The first European Research Council (ERC) project was funded at the IFB, and many excellent data were published in prestigious journals. I would like to thank the IFB deans of cadency 2016–2020, professors Sylwia Jafra, Michał Obuchowski and Stanisław Ołdziej, for their accomplishments and dedication to their work. Additionally, I would like to thank all IFB employees for their tremendous work in research as well as in preparing for and introducing the new teaching system. I am sure that regardless of external conditions and political circumstances, the IFB is on the right course, constantly increasing the quality of research and teaching. I would also like to thank the students for their constant support and help in achieving our goals.

Igor Konieczny, IFB Dean 2012-2020

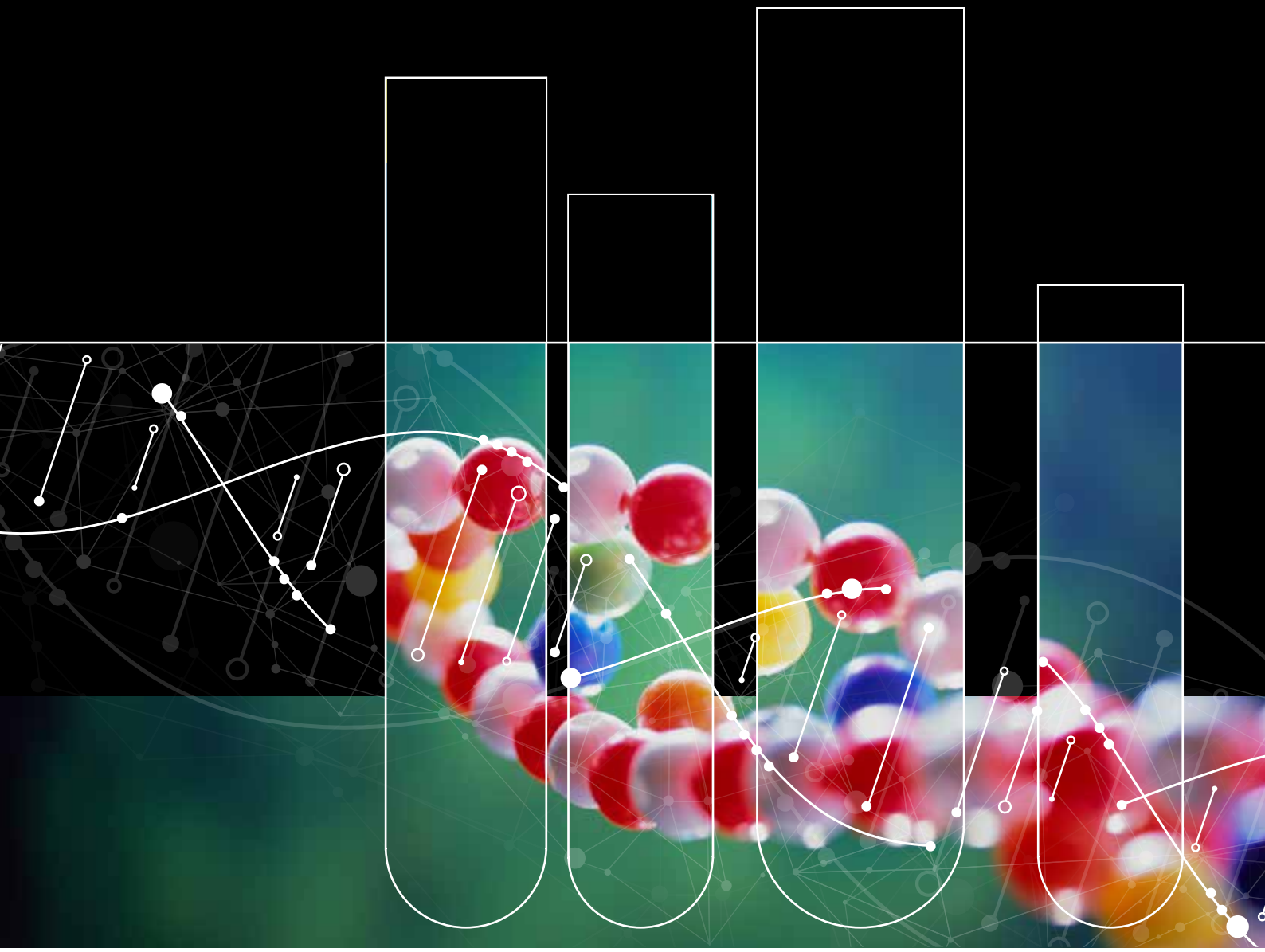
# Intercollegiate Faculty of Biotechnology
# University of Gdańsk and Medical University of Gdańsk (IFB)
## IFB Authorities (2016-2020)

**Dean**
Prof. Igor Konieczny

**Deputy Dean for Students Affairs and Education**
Assoc. Prof. Sylwia Jafra

**Deputy Dean for Science**
Prof. Michał Obuchowski

**Deputy Dean for Organisation and Development**
Assoc.Prof. Stanisław Ołdziej



# About IFB

# Structure of IFB

## Faculty Units at the University of Gdańsk

**Institute of Biotechnology**
Prof. Bogdan Banecki, Director
Wojciech Śledź PhD, Deputy-Director

- Research and Development Laboratory, Head: Prof. Ewa Łojkowska
- Laboratory of Physical Biochemistry, Head: Prof. Bogdan Banecki
- Laboratory of Biophysics, Head: Assoc. Prof. Jacek Piosik
- Laboratory of Experimental and Translational Immunology, Head: Assoc. Prof. Danuta Gutowska-Owsiak
- Laboratory of Biopolymers Structure, Head: Assoc. Prof. Stanisław Ołdziej
- Laboratory of Biomolecular Systems Simulations, Head: Assoc. Prof. Rajmund Kaźmierkiewicz
- Laboratory of Biologically Active Compounds, Head: Assoc. Prof. Aleksandra Królicka
- Laboratory of Protein Biochemistry, Head: Prof. Krzysztof Liberek
- Laboratory of Evolutionary Biochemistry, Head: Prof. Jarosław Marszałek
- Laboratory of Plant Biochemistry, Head: Prof. Antoni Banaś
- Laboratory of Molecular Biology, Head: Prof. Igor Konieczny
- Laboratory of Virus Molecular Biology, Head: Prof. Krystyna Bieńkowska-Szewczyk
- Laboratory of Structural Biology, Head Assoc. Prof. Michał Szymański
- Laboratory of Molecular Diagnostics, Head: Prof. Krzysztof Bielawski
- Laboratory of Biological Plant Protection, Head: Assoc. Prof. Sylwia Jafra
- Laboratory of Plant Protection and Biotechnology, Head: Prof. Ewa Łojkowska
- Laboratory of Recombinant Vaccines, Head: Prof. Bogusław Szewczyk
- Research and Technical Support Unit

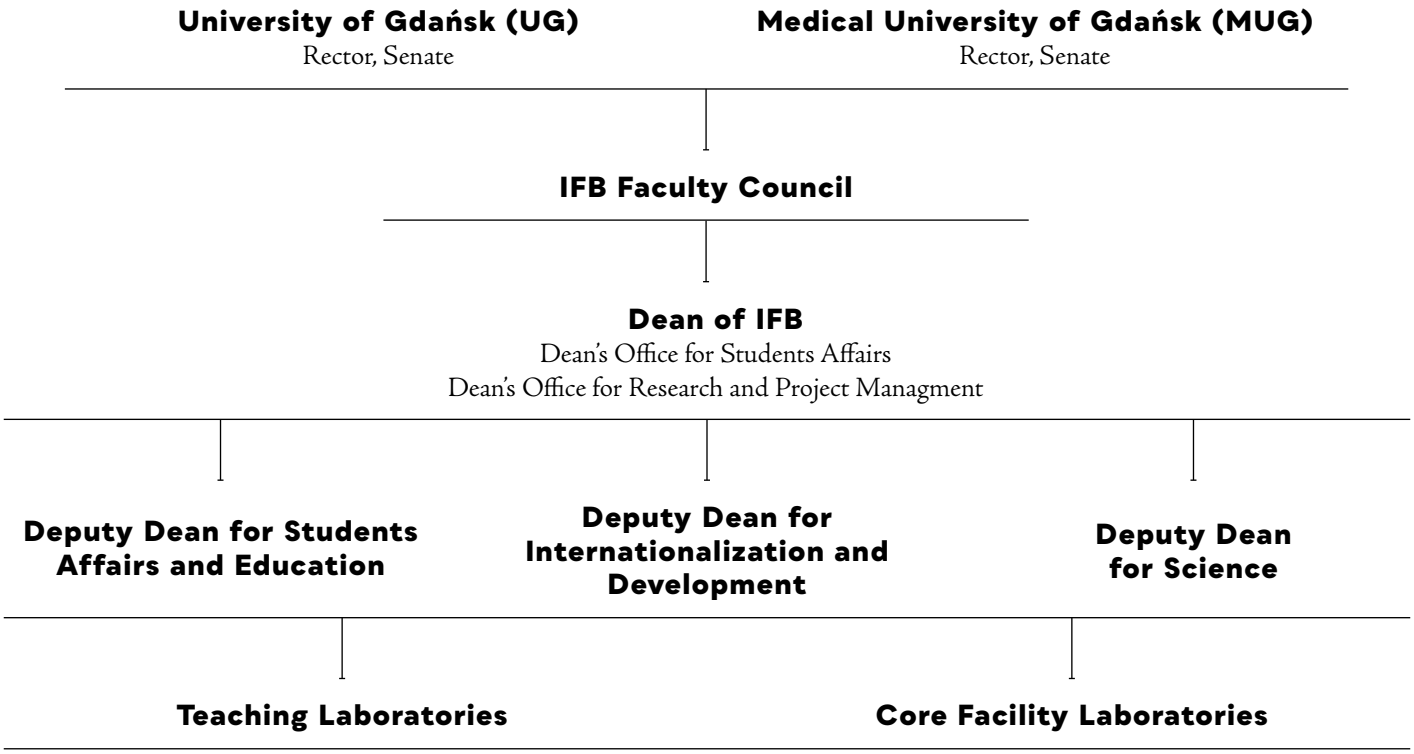
## Faculty Units at the Medical University of Gdańsk

**Institute of Medical Biotechnology and Experimental Oncology**
Prof. Jacek Bigda, Director

- Laboratory of Molecular Bacteriology, Head: Prof. Michał Obuchowski
- Laboratory of Cell Biology and Immunology, Head: Prof. Jacek Bigda
- Laboratory of Molecular Enzymology and Oncology, Head: Prof. Andrzej C. Składanowski
- Laboratory of Translational Oncology, Head: Assoc. Prof. Anna Żaczek,

## Dean's Offices and Faculty's Core Laboratories

- Teaching Laboratories, Head: Assoc. Prof. Mariusz Grinholc
- Core Facility Laboratories, Head: Assoc. Prof. Rafał Sądej
- Dean's Office for Students Affairs, Head: MSc Ewa Brzana
- Dean's Office for Research and Project Management, Head: MSc Patrycja Tucholska




## Faculty Units at the University of Gdańsk

**Institute of Biotechnology**

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Research and Development Laboratory
Laboratory of Physical Biochemistry
Laboratory of Biophysics
Laboratory of Experimental and Translational Immunology
Laboratory of Biopolymers Structure
Laboratory of Biomolecular Systems Simulations
Laboratory of Biologically Active Compounds
Laboratory of Protein Biochemistry
Laboratory of Evolutionary Biochemistry
Laboratory of Plant Biochemistry
Laboratory of Molecular Biology
Laboratory of Virus Molecular Biology
Laboratory of Structural Biology
Laboratory of Molecular Diagnostics
Laboratory of Biological Plant Protection
Laboratory of Plant Protection and Biotechnology
Laboratory of Recombinant Vaccines
Research and Technical Support Unit

## Faculty Units at the Medical University of Gdańsk

**Institute of Medical Biotechnology and Experimental Oncology**

•

Laboratory of Molecular Bacteriology
Laboratory of Cell Biology and Immunology
Laboratory of Molecular Enzymology and Oncology
Laboratory of Translational Oncology
Institute of Medical Biotechnology and Experimental Oncology

# Background

The Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk (IFB) was established in 1993 by the decision of the Senates of the University of Gdańsk (UG) and the Medical University of Gdańsk (MUG). The initiators of the Faculty were Prof. Anna Podhajska, Prof. Wacław Szybalski and Prof. Karol Taylor. The Faculty is a unique institution in Poland created by two universities, resulting in interdisciplinary research and teaching that combine biomedical and biomolecular issues and their biotechnological applications for health and quality of life. Since 1999, the IFB has been authorized to confer the degree of doctor, and since 2010, the scientific degree of habilitated doctors in the area of biological sciences – the discipline of biochemistry. Including PhD students, approximately 197 people participate in research and teaching at IFB.

# IFB International Scientific Advisory Board

In 2015, the decision was taken to establish the IFB International Scientific Advisory Board. According to Polish law, IFB as a research and teaching institution is evaluated every four years by two different government commissions. The international board is therefore not an obligation, nevertheless, it is part of the strategy for the further development of the faculty supported by distinguished scholars from different fields covering research topics conducted at IFB. The nominations to the IFB International Scientific Advisory Board were based on the experts' research excellence, management experience and extensive research expertise. These aspects are important to cover a broad spectrum of research work and other research-related activities at IFB and provide recommendations for further actions.

Members of the IFB International Scientific Advisory Board:

- Prof. Burkhard Brandt — Universitätsklinikum Schleswig Holstein, Institut für Klinische Chemie Kiel
- Prof. Bernd Bukau — Universität Heidelberg Center for Molecular Biology (ZMBH)
- Prof. Maarten Koornneef — Max Planck Institute for Plant Breeding Research
- Prof. Arvind H. Patel — MRC-University of Glasgow Centre for Virus Research,
- Prof. Dan Tawfik (†2021) — Weizmann Institute of Science

# IFB Employers' Expert Group

IFB Employers' Expert Group is an advisory body supporting the planning of the Faculty's development, particularly in the field of educational programmes. The Group consists of companies and institutions operating in the broadly defined biotechnological sector.

The aim of the cooperation with the members of the Group is to constantly improve the quality of education of biotechnology students and to foster their better position on the labour market after graduation. This is done, in particular, by obtaining opinions on the educational programme offered at the Faculty and its evaluation from the point of view of potential employers. Such activities resulted, among others, from the fact that the Faculty was awarded the title of the Best Course of Studies and was among 62 courses selected for co-financing for the best study programmes based on the National Qualification Framework and adapting studies to labour market requirements.

Members of IFB Employers' Expert Group are representatives of companies and institutions such as:

- Adamed Company
- The Institute of Biotechnology and Antibiotics
- Saint John Paul II Secondary School in Gdynia
- KAWA.SKA Company
- The Polpharma Scientific Foundation
- Polpharma Biologics Company
- Pomeranian Special Economic Zone / Gdańsk Science and Technology Park
- Pomeranian Science and Technology Park in Gdynia
- Innovation and Implementation Company "IMPULS"
- I Academic Secondary School in Gdynia

# Achievements

IFB is a leading research and teaching institution that since 2002 has had the status of the European Centre of Excellence in Biosafety and Molecular Biomedicine (since EU FP5) and is ranked highly by the Ministry of Science and Higher Education (MNiSW) regarding scientific effectiveness. In 2017, in a parametric assessment, IFB was granted the highest-level category, A+.

The quality of teaching at IFB is the highest in Poland. The Polish Accreditation Committee awarded the Faculty a distinction for teaching quality (2011), and the Ministry of Science and Higher Education granted the BIOTECHNOLOGY specialty at IFB the title of Best Major (2012). The Central Council of Science and Higher Education recognized the set of learning outcomes prepared by IFB for the specialty of Biotechnology as a model. In 2020, the Polish Accreditation Committee (PKA) awarded the Biotechnology study programme at IFB the Certificate of Educational Excellence in the category "Excellent programme - excellence in education". These are the only distinctions of that kind granted in Poland in the area of biological sciences.

Faculty members perform important functions in international societies and scientific commissions; for example, Prof. Ewa Łojkowska is President of the Polish Academy of Sciences Committee on Biotechnology, Vice-President of the ScanBalt Association, President of the Polish Jury for the L'Oréal-UNESCO for Women in Science award and a member of the International Selection Committee of the Award L'OREAL-UNESCO For Women in Science International Rising Talents. Prof. Krystyna Bieńkowska-Szewczyk was appointed to the Ministerial Advisory Group on COVID-19. Prof. Krzysztof Bielawski, as Vice-Rector for Innovation and Liaison with Business and the Community, and Prof. Jacek Bigda, as Vice-Rector of Development, are directly involved in governing the University of Gdansk and the Medical University of Gdansk, respectively. IFB staff members are also laureates of prestigious programmes and awards (ERC Starting Grant, EMBO YIP, HHMI, EUPHRESCO ERANET, InfectEra, STRATEGMED2, Polish-Norwegian Research Programme, Polish-South Africa Programme, Polish-Chinese Programme, Polish-French Polonium Programme, and Polish national programmes such as LIDER, TOP 500 Innovators, MISTRZ, START, HOMING PLUS, TEAM, and First TEAM).

Publications by IFB staff have received numerous awards and distinctions for the best work conducted in Polish laboratories, granted by the Committee of Microbiology of the Polish Academy of Science, the Polish Genetic Society or the Polish Biochemical Society. In 2018, a publication by the research group of Prof. Igor Konieczny in Nucleic Acid Research was recognized by the Committee of the Cell Molecular Biology of Polish

Academy of Science (PAN). A publication by Dr. Aleksandra Markiewicz received the Prof. Wacław Szybalski Foundation Award from the Biotechnology Committee of the Polish Academy of Science.

We have recently succeeded in increasing the quality of publications, with a consequently growing percentage of Q1 publications (Fig. 1). In 2018-2020, IFB researchers published 181 papers, of which almost all were published in indexing journals and approximately 50% with international collaborators (Fig. 2). The results of our research have been published in high-impact journals such as *Science, Science Translational Medicine, Trends in Biotechnology, Nucleic Acids Research, Journal of Experimental Medicine, JNCI-Journal of the National Cancer Institute, Genome Research, Journal of Allergy and Clinical Immunology Current Biology, Current Biology, Industrial Crops and Products, Trends in Biochemical Sciences, Frontiers in Immunology, Journal of Virology, Plant Physiology, Journal of Molecular Biology, The EMBO Journal, FEBS Journal, and the Journal of Experimental & Clinical Cancer Research.*

IFB researchers have received various awards, including the EMBO Best Poster Award for Assoc. Prof. Michał Szymański, a COST Action grant for Training School for Dr. Katarzyna Węgrzyn, the Krzysztof Celestyn Mrongowiusz award for the best academic teacher at the University of Gdansk for Assoc.Prof. Sylwia Jafra, and the "Primum Cooperatio" award, given by the Pracodawcy Pomorza for the best collaboration of research and business, for prof. Bogdan Banecki. The Prime Minister Award for an outstanding PhD thesis was given to Agnieszka Kłosowska and Agata Motyka-Pomagaruk, while Assoc.Prof. Ewelina Król received the "L'Oréal-UNESCO For Women in Science" award in the category of habilitation theses. A prestigious START stipend from the Foundation for Polish Science was obtained by Igor Obuchowski and Dr. Anna Supernat. The team led by Prof. Ewa Łojkowska was given a special award from the President of Patent Office of RP in X Student-inventor competition.

## PUBLICATIONS AT IFB IN THE YEARS 2018-2020

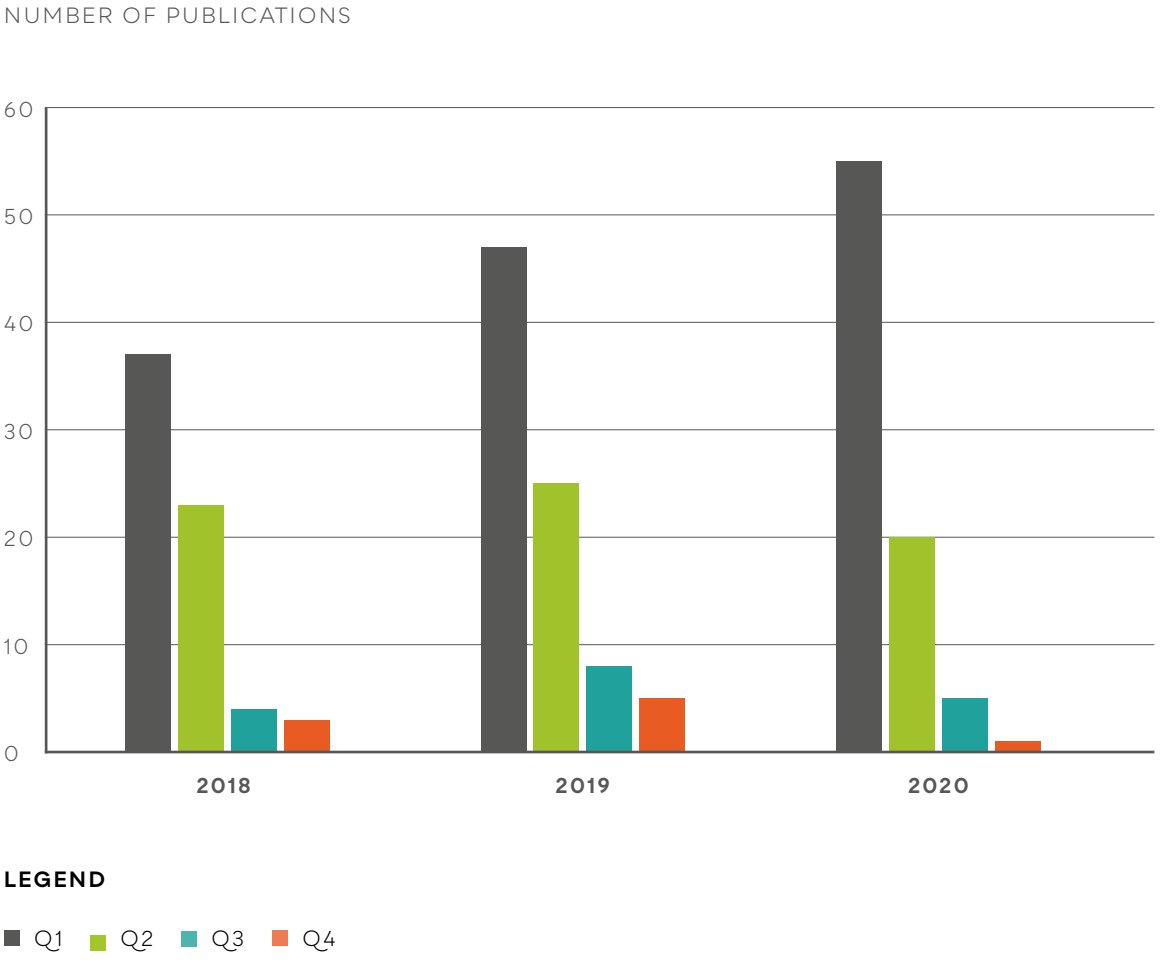



FIGURE 1. Number of publications with IFB affiliation in the years 2018-2020 divided into quadrilles. Scientific journals are classified into quadrilles according to the Journal Citation Reports — Clarivate Analytics

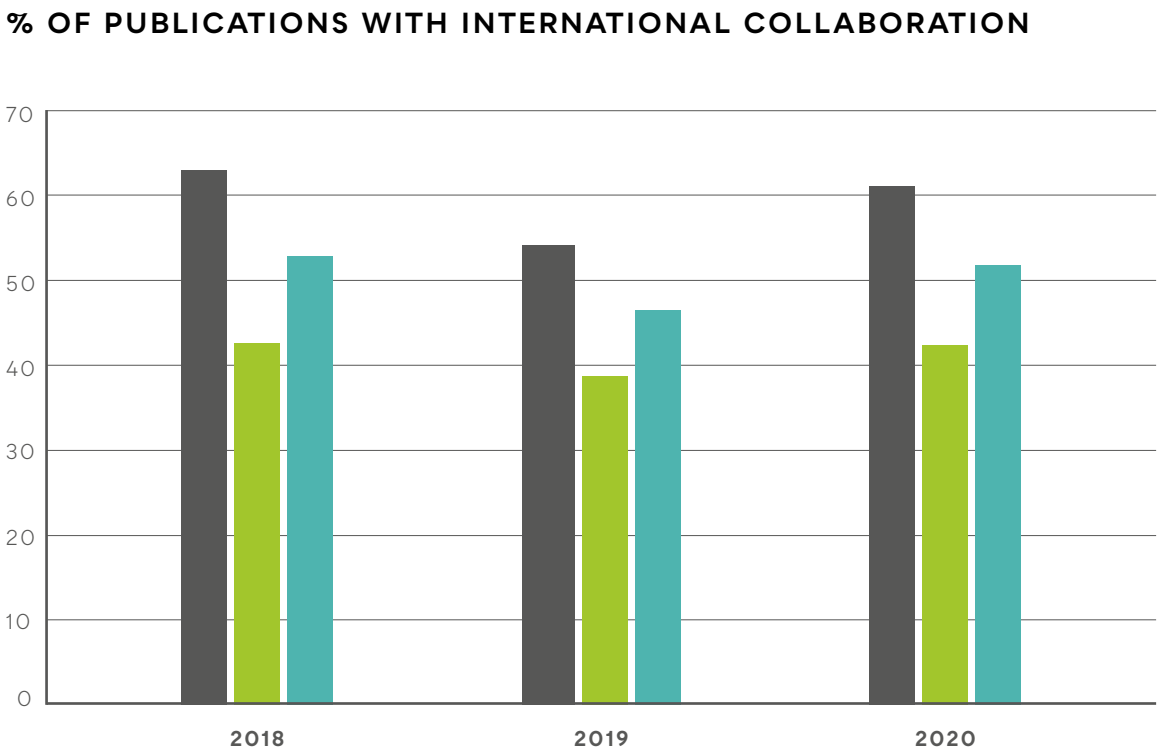



FIGURE 2. Percentage of publications with IFB affiliation (for medical – MUG, for biological – UG part separately, and for the whole faculty – IFB) and international collaboration in 2018-2020 [based on Scopus].

The Intercollegiate Faculty of Biotechnology is widely collaborating at the national and international levels. This collaboration resulted in publications with 70 institutions in Poland and abroad, including prestigious foreign institutions such as the University of Oxford, University of Texas, Heidelberg University, University of Washington, University of Missouri, Università degli Studi di Roma Tor Vergata, University of Bremen, University of Wisconsin-Madison, Wellcome Sanger Institute, Lawrence Berkeley National Laboratory, Karolinska University, Princeton University, and Cornell University.

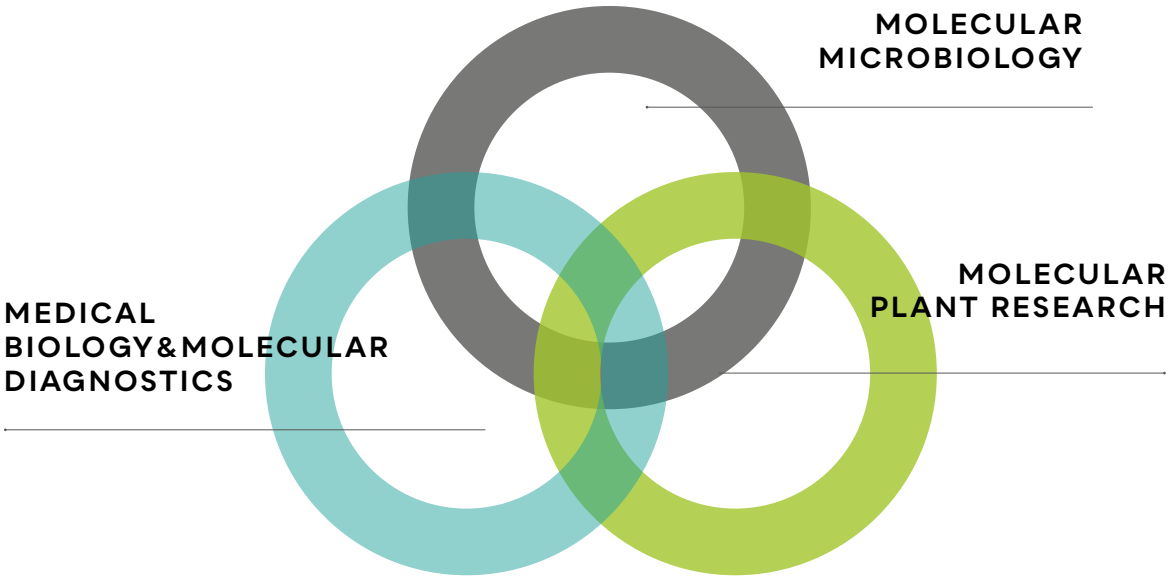
# Research Potential

IFB comprises 20 teams involved in research activities. The basic and applied research areas at IFBs cover virology, molecular microbiology, medical biology and molecular diagnostics, and molecular plant biology. These areas are the basis of biotechnology development. The relationships and synergy between these research areas give added value in the form of common thematic areas. Maintaining three main research areas increases the number of interactions between research teams, improves their effectiveness and broadens competence.

At the IFB, approximately 88 research projects supported by external funding are conducted simultaneously. Thirty-one of these projects concern molecular microbiology. In these projects, various microorganisms are used as models to analyse basic cell processes. The main research topics include protein aggregation and disaggregation; the role of molecular chaperones; proteolysis; DNA replication; plant, animal and human pathogens; infection mechanisms; cell response to viral infections; and pathogen diagnostics.

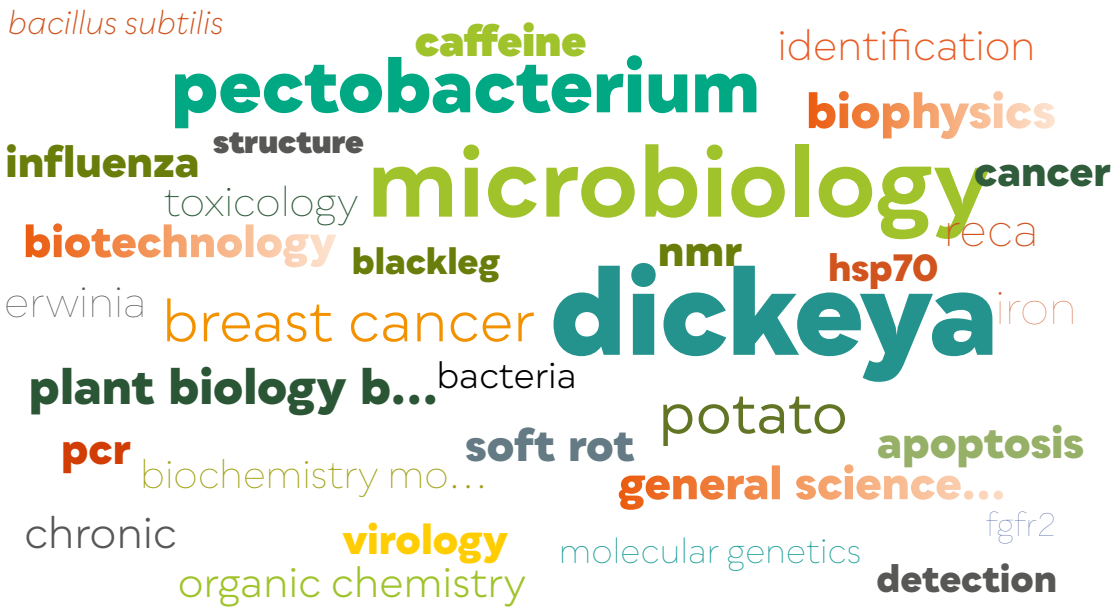
Thirty-eight projects concern the area of medical biology and molecular diagnostics. We conduct research concerning recombinant and edible vaccines, markers used in neurodegenerative disease diagnostics, and nanobiotechnology for treating burns. Cancer research, including studies on cancer biology, therapy response and resistance, prognostic and predictive biomarkers, liquid biopsy, and new immune-modulating substances and protease

inhibitors in anticancer treatment, is being dynamically developed. Recently, new research lines have been initiated: immunology research focused on allergic and inflammatory reactions and structural biology research focused on the replication of mitochondrial DNA and its impact on health problems.




A third area, molecular plant research (19 projects), is dedicated to the diagnostics and infection mechanisms of plant diseases and to the identification of genes and lipid metabolic pathways in plant cells. Research aimed at the identification of plant nutraceuticals is also carried out.

Since 2018, when the Constitution for Science introduced comprehensive reform of Polish higher education and science, two scientific disciplines are led at the Faculty: biological sciences and medical sciences. Within the biological sciences, microbiology, including virology and plant research, is the main research topic, while the medical sciences focus on molecular and translational studies of cancer as well as immunology and the use of bacterial spores for medical applications.

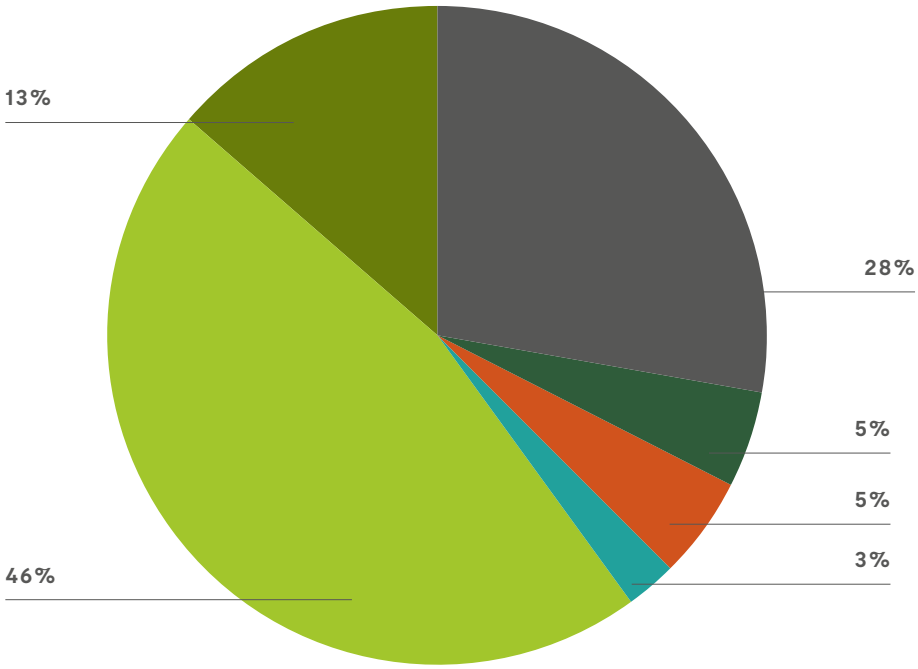



In recent years, we also improved our expertise in mass spectrometry and initiated the development of state-of-the-art technology for the analysis of biomolecular interactions by using atomic force microscopy (AFM). We are developing an AFM microscopy facility for studying biological systems. Recently, we started to develop imaging flow cytometry (Amnis® ImageStream®XMArk II, Luminex) and single-cell RNA sequencing (Chromium Controller, 10x Genomics).

In 2018-2020 we invested approximately 1 500 000 EUR in upgrading our equipment and obtaining new equipment. For example, in this period, we obtained a Western blot near-infrared detection system (LI-COR), a Guava easyCyte flow cytometer (Merck), a J-1500 circular dichroism spectrophotometer (Jasco), a T200 plasmon resonance analyser (Biacore), a 24-node computation cluster (Intel), a Synergy H1 Hybrid Multi-Mode Reader (BioTeK), a CytoFLEX flow cytometer (Beckman Coulter), an IX83 Research Inverted Microscope with DP74 20 Mpix and a CAM-XM10 camera (Olympus), an IRIS TM FluoroSpot/ELISpot Reader (Mabtech), a Panalytical NanoSight NS300 Instrument (Malvern Panalytical), a TOMOCUBE HT-2H microscope (Tomocube) and a Typhoon RGB Biomolecular Imager (Amersham). The Faculty continually increases research potential by obtaining new, modern research equipment.

The IFB continuously increases the budget for research obtained from various external sources, reaching almost 6 M EUR in 2020. In 2020, over 65% of the Faculty budget came from competitive grants from national and international agencies (Fig. 3), and international EMBO and ERC grants secured almost 5% of IFB funding.

## IFB BUDGET | 2020

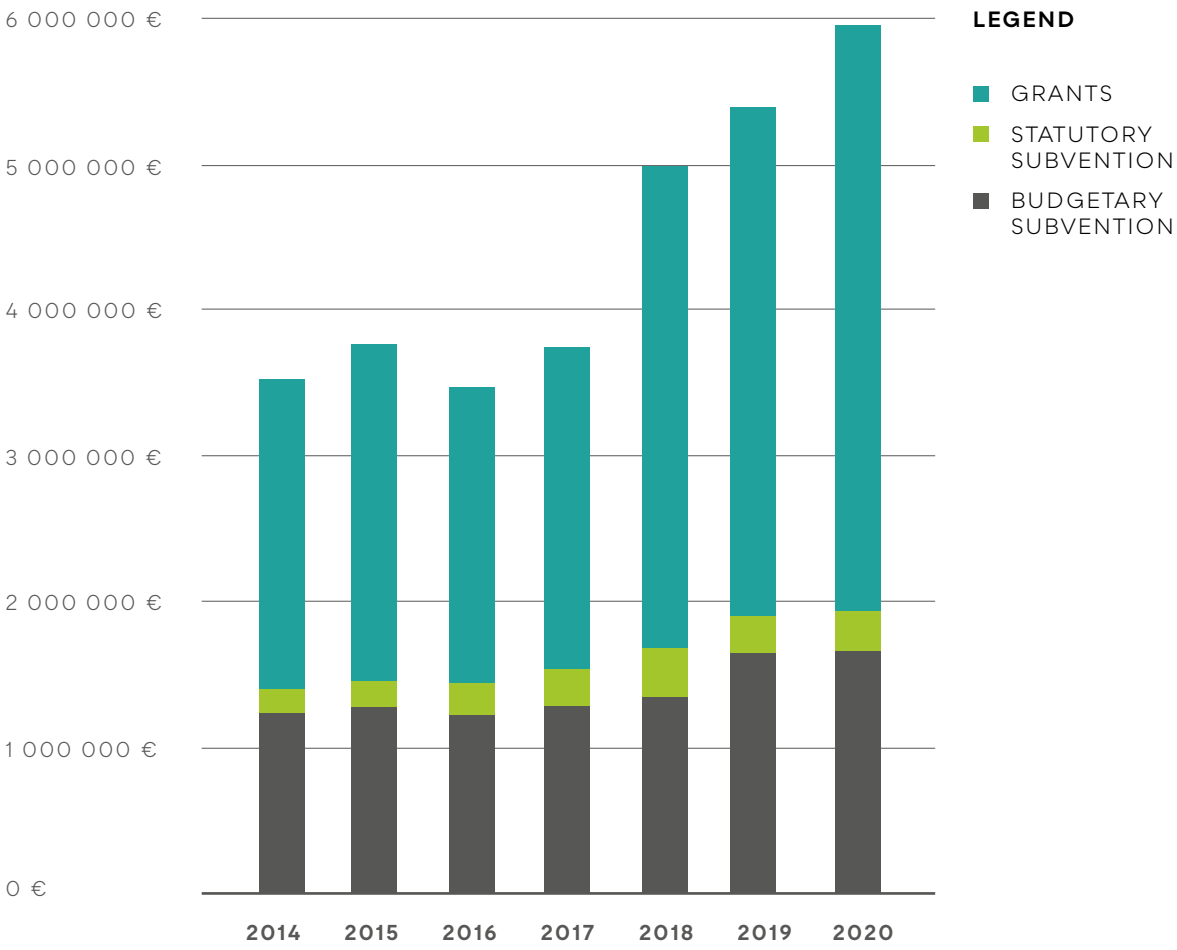



**LEGEND**

- NATIONAL SCIENCE CENTRE | NCN
- BUDGETARY SUBVENTION
- FOUNDATION FOR POLISH SCIENCE | FNP
- NATIONAL CENTRE FOR RESEARCH AND DEVELOPMENT | NCBiR
- STATUTORY SUBVENTION
- EMBO + ERC

Possibilities for new collaborations for IFB research groups were prepared at the end of 2017 by establishing two international research agendas in the field of biotechnology. Two research centres within the framework of the International Research Agenda Programme from The Foundation for Polish Science were established at the University of Gdańsk and Medical University of Gdańsk. First, the International Centre for Cancer Vaccine Science was established in cooperation with the University of Edinburgh (UK), and 3P-Medicine – Preventive, Personalized, Precision was established in cooperation with the University of Uppsala (Sweden).

**IFB BUDGET 2014-2020**




# Excellence Initiative – Research University

The quality of the Faculty is also based on the reputation of the home universities, both of which were included in the elite group of twenty best Polish universities qualified for the "Excellence Initiative – Research University" programme. The Medical University of Gdańsk gained the status of a Research University, becoming the first medical research university in Poland. The staff of IFB employed at MUG is actively involved in the initiative. Of the three priority research areas, i) oncology, ii) cardiology and cardiovascular medicine, and iii) biochemistry, genetics and molecular biology, we are involved in two. Assoc.Prof. Anna Żaczek is coordinating the topic of precision oncology. Dr. Anna Supernat is coordinating artificial intelligence within oncology, while Assoc. Prof. Rafał Sądej is coordinating technological developments for bioanalysis and therapy including cell-based, 3D and artificial engineered models. Assoc.Prof. Patrycja Koszałka is coordinating *in vivo* studies on disease mechanisms and therapy within biochemistry, genetics and molecular biology research.

New structures have been created at MUG to promote the framework of the "Excellence Initiative – Research University" programme. The Centre of Biostatistics and Bioinformatics Analysis, created and led by Dr. Anna Supernat, offers a wide range of services related to the broadly understood biostatistical and bioinformatic analysis as well as big data analysis. The Laboratory of Single Cell Analysis coordinated by Prof. Anna Żaczek offered an advanced platform to study the genotype, phenotype and function of single cells, including the micromanipulation system (TransferMan, Eppendorf), Parsortix isolation system (Angle), imaging flow cytometer (Amnis® ImageStream®XMArk II, Luminex) and system for single-cell RNA sequencing (Chromium Controller, 10x Genomics).

# Intercollegiate Biotechnology Doctoral School

In 2019, according to the Constitution for Science, the Intercollegiate Biotechnology Doctoral School of University of Gdańsk and Medical University of Gdańsk (IBDS) was established. It consists of doctoral students within both biological and medical disciplines and is led by Prof. Igor Konieczny. At the moment, we have 91 students associated with IBDS and Life Sciences and Mathematics Interdisciplinary Doctoral Studies (LiSMIDoS).


POLSKIE CENTRUM AKREDYTACJI
POLISH CENTRE FOR ACCREDITATION
CERTYFIKAT AKREDYTACJI
LABORATORIUM BADAWCZEGO
ACCREDITATION CERTIFICATE OF TESTING LABORATORY
Nr AB 1760
UNIWERSYTET GDAŃSKI

# Research and Development Laboratory of IFB & MUG

A research group from the Laboratory of Plant Protection and Biotechnology has been providing professional molecular diagnostic services for seed potato breeding companies since 2011. To confirm the high competencies and the quality of the conducted research, the PN-EN ISO/IEC 17025: 2005 + Ap1: 2007 quality management system was introduced, thanks to funding from the EU MOBI4Health project (FP7-REG-POT-2012-2013-1). Later, the Research and Development Laboratory of IFB&MUG (RDL) led by Prof. Ewa Łojkowska was established by the University of Gdańsk Rector's decision. Financing from the STARBIOS2 project (EU Horizon 2020), which supported a structural transformation to attain responsible biosciences, allowed RDL to receive accreditation in 2020 from the Polish Center for Accreditation (No AB 1760). Our primary services rely on the detection and identification of phytopathogens from the genera Dickeya and Pectobacterium in latently infected seed potatoes with the use of a multiplex PCR method that was developed, patented (Pat. 223540) and published (Potrykus et al., Annals of Applied Biology; 2014). In addition, various kinds of microbiological assays are offered, including analyses of the antibacterial properties of different agents. The staff of the RDL conducted studies in collaboration with the group of Prof. Paweł Pohl from Wroclaw University of Science and Technology (WUST) that resulted in the development of several inventions intended for the eradication of bacteria or degradation of biologically active contaminants using cold atmospheric-pressure plasma. We investigated potential applications of cold plasma for different approaches and finally obtained three patents: Pat. 236055, Pat. 236377, and P. 431823. Two other are pending.

PCA
AB 1760

Research & Development Laboratory
Intercollegiate Faculty of Biotechnology
University of Gdańsk and Medical University of Gdańsk

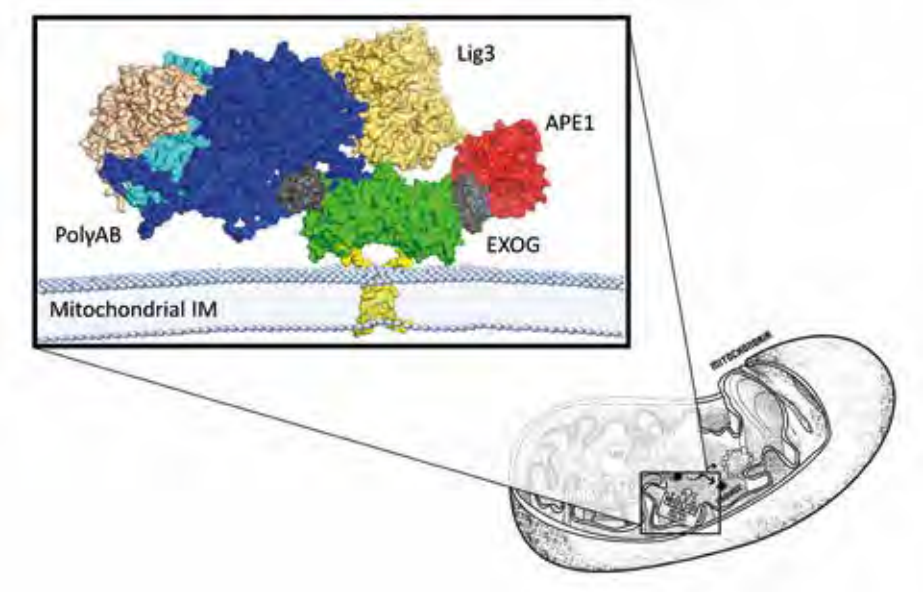
www.rd-lab.ug.edu.pl

# European Research Council Starting Grant

In 2019 Assoc. Prof. Michał R. Szymański received a prestigious European Research Council grant in the amount of 1.5 million Euro. The research conducted as part of the grant will offer insight into the fundamental principles of human mitochondrial DNA repair, which will in the future result in a greater understanding of broadly defined mitochondrial diseases.

European Research Council
Established by the European Commission

www.erc.europa.eu




The grant by the European Research Council (ERC) was awarded as part of the ERC Starting Grant competition. The project entitled Dissecting the mechanism of DNA repair in human mitochondria (MitoRepairosome) will be implemented over a period of five years. A European Research Council grant is an exceptional distinction. The ERC's mission is to finance pioneering and ground-breaking scientific research, and the only criterion applied to assess the ideas are scientific excellence and their leading motto: high risk – high gain. Mitochondrial DNA (mtDNA) repair occurs in the mitochondria, and the main enzymes responsible for the process have been identified. However, the spatial organization of the mitochondrial repair complex that repairs mtDNA is not well known. The aim of the project is to provide fundamental mechanistic insights into the assembly, composition, activities and structures of human mitochondrial repairosomes.

# Research Projects
# Selected granted research projects 2018-2020

| N° | Project | Project Leader | Start | Funding Source* | Programme | Euro |
|---|---|---|---|---|---|---|
| 1. | Dissecting the mechanism of DNA repair in human mitochondria | Szymański Michał | 2020 | ERC | Starting Grant | **1 428 900 €** |
| 2. | Smart antigen provision for efficient induction of allergen tolerance | Gutowska-Owsiak Danuta | 2020 | NCN | SONATA BIS 9 | **841 456 €** |
| 3. | Condition-dependent protease activation for targeted proteolysis in the regulation of DNA replication | Konieczny Igor | 2018 | FNP | TEAM | **763 415,4 €** |
| 4. | Hsp70/J-protein chaperones substrate binding cycle: molecular mechanisms and functional consequences | Marszałek Jarosław | 2018 | FNP | TEAM | **760 348,6 €** |
| 5. | New approaches to characterize TH17/Treg cell balance contributing to anti-tumour immune responses | Chen Jane | 2019 | NCN | SHENG 1 | **603 628,08 €** |
| 6. | FGFR2 role in ER/PR relationship – molecular mechanism and predicative significance in patients with luminal A breast cancer | Sądej Rafał | 2019 | NCN | SONATA BIS 8 | **549 780 €** |
| 7. | Hsp70 and its cochaperones in protein recovery from aggregates | Liberek Krzysztof | 2020 | NCN | OPUS 18 | **539 088 €** |
| 8. | Mammalian cell delivery of stable therapeutic mRNA packaged into virus-like particles | Szewczyk Bogusław | 2020 | NCN | OPUS17 | **504 820,8 €** |
| 9. | Emergence of the two-component small heat shock protein system – a fortified first line of defense against stress in Enterobacterales | Liberek Krzysztof | 2020 | NCN | OPUS 17 | **468 864 €** |
| 10. | Highly Efficient Enrichment, Single-cell Analysis, and Drug Screening of Circulating Tumor Cells for Personalized Medicine (HESCAP) | Żaczek Anna | 2019 | NCBiR | Polish/Chinese Joint Research Call | **440 000 €** |
| 11. | Targeting mitochondrial DNA repair for novel anti-cancer therapies. | Szymański Michał | 2018 | FNP | FIRST TEAM | **440 000 €** |
| 12. | Ecological biochemistry of Pectobacterium – decoding of molecular interactions between bacteria and plants. | Waleron Małgorzata | 2020 | NCN | OPUS 18 | **427 944 €** |
| 13. | Development and study of photodynamic and Ga loaded high efficient and dual-functional antimicrobial agents. | Grinholc Mariusz | 2019 | NCN | SHENG 1 | **385 616 €** |
| 14. | Molecular and clinical characterization of keratins-based phenotypes in prostate cancer | Bednarz-Knoll Natalia | 2018 | NCN | SONATA | **338 826,4 €** |
| 15. | FGFR2 role in autophagy regulation in breast cancer – predicative significance | Sądej Rafał | 2018 | NCN | OPUS | **329 824 €** |
| 16. | Immunogenic properties of hepatitis C virus NS3 protein epitopes fused to hepatitis B small surface antigen | Grzyb Katarzyna | 2018 | NCN | OPUS | **310 288 €** |
| 17. | Substrate specificity of LPCATs from photosynthetic oleaginous microalgae in forward and reverse reactions and characterization of their functions in acyl editing of phosphatidylcholine | Banaś Antoni | 2019 | NCN | SHENG 1 | **296 384 €** |
| 18. | Host receptors involved in bacteriophage adsorption to Dickeya solani and Pectobacterium parmentieri cells and the ecological costs of phage resistance in vitro and in planta | Czajkowski Robert | 2018 | NCN | OPUS13 | **286 352 €** |
| 19. | CD73 (ecto-5'-nucleotidase) function in breast cancer progression and promotion, and its assessment as a therapeutic target | Koszałka Patrycja | 2018 | NCN | OPUS | **282 972,8 €** |
| 20. | Comprehensive analysis of the interactions between plant-beneficial strain P482 of Pseudomonas donghuensis and the mono- (maize) and dicot plants | Jafra Sylwia | 2018 | NCN | OPUS13 | **275 220 €** |

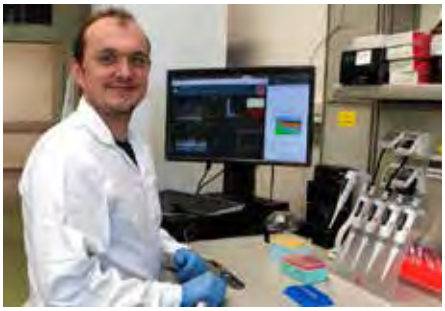
| N° | Project | Project Leader | Start | Funding Source* | Programme | Euro |
|---|---|---|---|---|---|---|
| 21. | Assessment of the antibacterial properties of post-plasma solutions generated with the use of cold atmospheric pressure plasmas against economically important phytopathogens and impact of the obtained liquids on the growth of crops and vegetables | Wojciech Śledź | 2019 | NCN | OPUS 17 | **262 974,8 €** |
| 22. | The role of acyl-CoA:lysophosphatidylethanolamine acyltransferases (LPEATs) in plants | Banaś Antoni | 2018 | NCN | OPUS 13 | **261 140 €** |
| 23. | Spcoal interaction between B. subtilis and D. solani | Obuchowski Michał | 2019 | NCN | OPUS | **244 596 €** |
| 24. | Analysis of interaction between Tumor Educated Platelets and ovarian cancer cells | Supernat Anna | 2019 | NCN | SONATA | **229 699,36 €** |
| 25. | Towards safe anti-SARS-CoV-2 coronavirus vaccine – modifications of the spike protein leading to the elimination of ADE effect | Król Ewelina | 2020 | NCN | Express call to fund research on COVID-19 | **219 912 €** |
| 26. | The influence of antimicrobial photoinactivation on virulence of Staphylococcus aureus strains colonizing atopic dermatitis patients: in vitro and in vivo studies | Nakonieczna Joanna | 2018 | NCN | OPUS | **208 846 €** |
| 27. | The impact of model biological membranes on the structure and oligomerization process of human cystatin C | Czaplewska Paulina | 2019 | NCN | HARMONIA 10 | **185 570 €** |
| 28. | Structural-functional analysis of nucleoprotein complexes of plasmid Rep proteins and ssDNA DUE origin region. | Węgrzyn Katarzyna | 2018 | NCN | SONATA | **183 260 €** |
| 29. | Antiviral activity of interferon induced transmembrane proteins (IFITM) as a novel therapeutic strategy to control viral infections – evaluation in vitro and in vivo | Chmielewska Alicja | 2018 | FNP | POWROTY | **175 991,86 €** |
| 30. | Application of cold atmospheric pressure plasmas generated in contact with a flowing for direct degradation of antibiotics and limitation of multi-drug resistance in the natural environment | Wojciech Śledź | 2020 | NCN | SONATA 15 | **175 208,88 €** |
| 31. | Evaluation of photoinactivation potential in the eradication of Streptococcus agalactiae carrier-state in the urogenital system: in vitro and in vivo studies. | Grinholc Mariusz | 2018 | NCN | OPUS 12 | **147 752 €** |
| 32. | Structural basis for DNA repair in human mitochondria | Szymański Michał | 2019 | EMBO | Installation Grant | **141 863,7 €** |
| 33. | Elucidating the role and mechanism of regulatory network of genes encoding dioxygenases in terms of plant adaptation to land conditions. | Łojkowska Ewa | 2020 | NCN | PRELUDIUM BIS 1 | **117 216 €** |
| 34. | Gain-of-function complement C2 mutant as a supporter of anti-CD20 therapy in murine lymphoma model | Urban Aleksandra | 2019 | NCN | PRELUDIUM | **46 200 €** |
| 35. | Participation of cellular proteins in the inhibition of antigen presentation dependent on UL49.5 protein of bovine herpesvirus BHV-1 | Wąchalska Magda | 2020 | NCN | ETIUDA 8 | **30 657,44 €** |
| 36. | Hijacking the ubiquitin-proteasome system by the UL49.5 protein of bovine herpesvirus 1 as an immune evasion strategy – elucidating the mechanismof TAP transporter degradation | Wąchalska Magda | 2020 | NCN | PRELUDIUM | **30 624 €** |
| 37. | Analysis of the role of pseudorabies virus-encoded microRNAs on virus infection using RNA inhibitors | Hoffmann Weronika | 2018 | NCN | PRELUDIUM13 | **26 400 €** |

**LEGEND**

**ERC** – EUROPEAN RESEARCH COUNCIL
**FNP** – THE FOUNDATION FOR POLISH SCIENCE
**NCBIR** – THE NATIONAL CENTRE FOR RESEARCH AND DEVELOPMENT
**NCN** – NATIONAL SCIENCE CENTRE

# IFB researchers combating COVID-19

photo. Michał Łepecki



## Participation of IFB employees in counteracting the SARS-CoV-2 pandemic

The SARS-CoV-2 outbreak started in December 2019 in China. At that time, we did not realize that the virus would cause a global pandemic by as early as March 2020. As a consequence of this rapid viral spread, teaching at the IFB had to shift to online learning in a very short time. Despite many inconveniences, such as the necessity of shift work and breaks in the implementation of research grants due to the quarantine of employees, the IFB has been one of the most active national research centers involved in the fight against the COVID-19 pandemic. The scientific experience of our employees found practical application in the medical service activities focused on the improvement of the situation during the pandemic disaster.

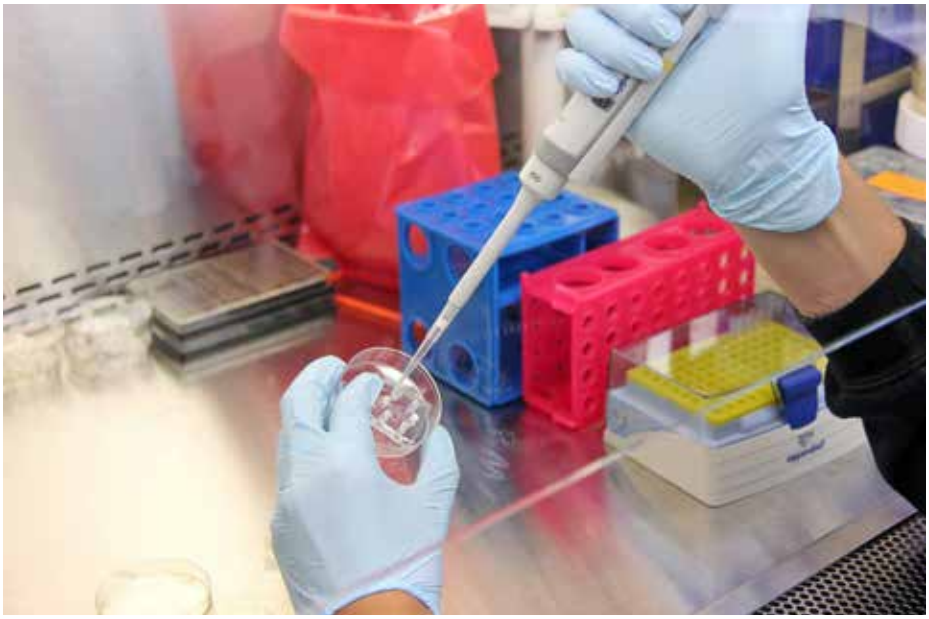
We increased the scale of diagnostic activities for the detection of SARS-CoV-2 and accelerated testing by comparing and verifying the quality of tests from various producers, including tests made in Poland. We created and operated COVID-19 diagnostic points and provided the necessary equipment to increase testing efficiency. Additionally, we played a crucial role in the establishment of the Medical Diagnostic Laboratory at the 7$^{th}$ Naval Hospital in Gdańsk for the detection of SARS-CoV-2 infections.

We have also organized a drive-through diagnostic point at the University of Gdańsk. Due to our previous experience in the dangerous pathogens field, many of our employees have worked at diagnostic points as volunteers. In addition, we provided expert advice to Invicta Medical Diagnostic Laboratories to increase the SARS-CoV-2 detection potential in the region.

Moreover, our staff acted as advisors to ministerial and local government authorities. **Prof. Krystyna Bieńkowska-Szewczyk** was an advisor to the Marshal's Office of the Pomeranian Voivodeship in areas related to COVID-19 diagnostics. Her commitment contributed to regional regulations that were introduced during the pandemic to ensure the safety of Pomeranian residents. In 2020, immediately after the outbreak of the pandemic in Poland, **Prof. Bieńkowska-Szewczyk** was appointed as an expert to the advisory team of the Ministry of Science and Higher Education for matters related to the prevention and combating of COVID-19.

As one of the strongest virology research centers in Poland, we have been engaged in activities for doctors and patients by conducting webinars addressed to the medical community and education for members of society outside the academic world (**Prof. Bieńkowska-Szewczyk, Prof. Szewczyk, Prof. Król, Dr. Chmielewska, Dr. Rąbalski**). We have been providing the most important and up-to-date information on coronavirus and vaccine research through numerous mass media outlets. To target younger generations, we have increased activities on the internet. We cocreated videos that were broadcast on social media and various websites.

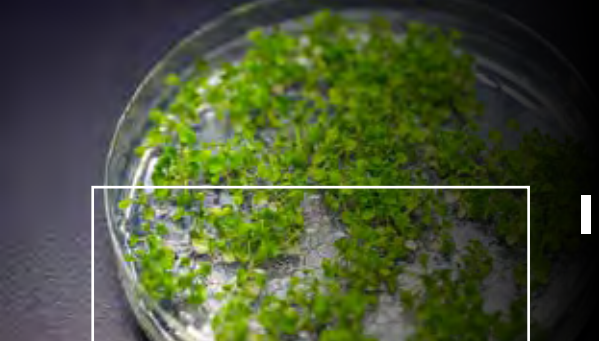
Finally, we have to stress that our researchers were intensely engaged in work on the diagnosis, prevention and treatment of symptoms caused by SARS-CoV-2. **Dr. Łukasz Rąbalski**, as a first in Poland, determined the genetic sequence of the SARS-CoV-2 virus isolated directly from a patient sample. Genetic analyses (sequencing) to detect SARS-CoV-2 virus variants have been carried out at the Department of Recombinant Vaccines



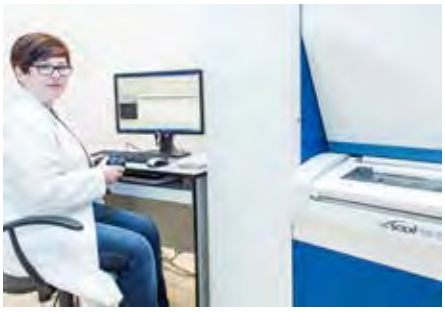
since the beginning of the pandemic. We also detected the presence of the new coronavirus in farmed mink. As an advisor to the Chief Sanitary Inspector, we investigated genetic variants of the SARS-CoV-2 virus spreading in Polish miners in Silesia.

Last but not least, our researchers have also obtained funds for research related to SARS-CoV-2. **Prof. Ewelina Król** received funding for the development of a potential anti-SARS-CoV-2 vaccine, **Prof. Danuta Gutowska-Owsiak** obtained a grant for the application of artificial exosomes as a novel COVID-19 vaccine approach, **Dr. Rąbalski** obtained a grant for the study of infections accompanying SARS-CoV-2 infection, and **Dr. Chmielewska** obtained a grant for research related to interferon-induced proteins.


In December 2020, the Ministry of Education and Science awarded **Prof. Bieńkowska-Szewczyk** a 4 million PLN subsidy for the reconstruction and adaptation of the virological laboratory at the IFB to the high safety standard required for working with respiratory viruses.



# IFB Core Facility Laboratories



- ## Laboratory of Biomolecular Analysis

  This laboratory allows insight into molecular structure and interactions within biological systems. It is already equipped with spectrofluorometers and fluorometers: an FP-8500 (Jasco), a J-1500 circular dichroism spectrophotometer (Jasco), an EnVision advanced microplate reader (Perkin-Elmer), a Biacore T200 plasmon resonance analyser, an anaerobic chamber (COY Lab Products), ultracentrifuges (Beckman), a BioScope Resolve atomic force microscope with a scanning head and MultiMode8 Lockout Specs (Bruker), an MS-NEX high-speed atomic force microscope (RIBM) and a Monolith NT.115 apparatus for microscale thermophoresis (NanoTemper Technologies).

- ## Laboratory of Mass Spectrometry

  This unit was created as part of the MOBI4Health FP7 project. It is equipped with a MALDI-TOF/TOF 5800 spectrometer and two electrospray ionization spectrometers, a QTrap 6500 and Triple TOF 5600+, with the possibility of working with the Eksigent Micro LC 200 chromatographic system and the CESI 8000 Plus electrophoretic system. All instruments allow extensive work in the field of proteomics, quantitative and qualitative analysis of proteins, and the registration of native and intact spectra. We specialize in SWATH MS quantitative analysis. Laboratory staff facilitate research work, starting with experimental design and the preparation of grant projects, as well as the recording of spectra and interpretation of results. They also engage in education by organizing courses and regular classes for students in the field of mass spectrometry.

- ## Isotope Laboratory Type III

  The laboratory contains the full equipment indispensable for conducting research with radioisotopes such as 3H, 14C, 32P, 33P, 55Fe and 35S. Two scintillation counters (LC 6000TA Beckmann and MicroBeta LumiJET) are available in the laboratory.

- ## Laboratory of Genetic Analysis

  Here, the equipment includes new genetic analysis devices such as a MassARRAY® Analyzer 4, a Light Cycler 480 real-time thermocycler (Roche), an xCELLigence DP real-time cell analyser, a MagnaLyser homogenizer, a MagnaPure 2.0 apparatus for the automatic isolation of nucleic acids (Roche), a Tape Station 2200 apparatus for the capillary electrophoresis of nucleic acids and an epMotion 5070 automatic pipetting station (Eppendorf).

- ## Laboratory of Imaging and Data Analysis

  IFB has three confocal microscopes (Nikon PCM-2000, Leica DMI6000 CS SP8 and Leica HCS LSI), a TOMOCUBE HT-2 microscope and several fluorescence microscopes. The Leica TCS SP8 confocal microscope is equipped with a white light laser, which can perfectly match the excitation wavelength ranging between 470 and 670 nm of any fluorophore. Up to eight excitation lines can be used simultaneously. This microscope is equipped with five spectral detectors (350-800 nm) working independently. The Leica TCS LSI macroconfocal microscope is the first superzoom

confocal microscope that offers high resolution plus a large 16x16 mm field of view for in vivo imaging. Both Leica microscopes were equipped with a special incubation chamber for live cell imaging. A Nikon PCM 2000 fluorescent confocal microscope was equipped with 3 lasers and an ultrasensitive colour camera (Hamamatsu). TOMOCUBE HT-2 holotomographic microscope with 3D fluorescence imaging capability uses holotomographic technology to measure the 3D and 4D refractive indexes using a dynamic micromirror device (DMD). These innovations allow the quantitative, label-free imaging of cells. HT-2 combines holotomographic imaging with the added ability to perform fluorescence microscopy.

- ## Laboratory of In Vitro Plant Cultures

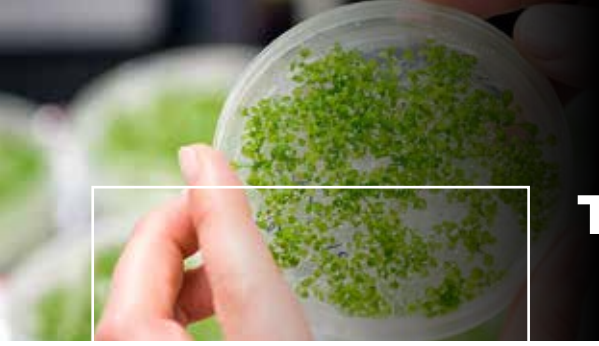
The infrastructure consists of ten growth chambers that serve as a controlled environment for the growth of in vitro-cultured plants. The growth chambers contain various in vitro-cultured endangered plants (e.g., Droseracae species), ferns, ornamental plants, hairy root cultures, cell suspensions, plants containing bioactive compounds and GM plants. These cultures are used for breeding and reintroduction purposes and transformations are important for obtaining biologically active secondary metabolites.

- ## Laboratory for Highly Infectious Pathogens

The IFB core facility established at the Institute of Biotechnology possesses two well-equipped Biosafety Level 2 plus (BSL2+) laboratories dedicated to working with dangerous animal and human viruses and bacterial pathogens (for example, influenza virus, herpesviruses, Zika virus or Mycobacterium tuberculosis). Both laboratories allow the safe manipulation of such pathogens and are equipped with several laminar class II and III cabinets. Safety is achieved by strictly controlled access to the laboratory as well as by a pass-through autoclave and a multistep pressure barrier between the working space and the rest of the building. Thanks to a 4 million PLN grant funded in 2020 by the Ministry of Education and Science, one of the laboratories will be reconstructed to increase the level of biosafety to BSL3+ to enable safe work with airborne viral pathogens, including SARS-CoV-2.

- ## Laboratory of Single-Cell Analysis

This recently established unit focuses on the isolation and profiling of single cells using the following equipment: (1) an Amnis ImageStreamX Mk II Imaging Flow Cytometer System, which combines the capabilities of flow cytometry and the imaging of (immuno)fluorescently stained cells (3 lasers: 405 nm, 488 nm and 642 nm; MultiMag with 20x, 40x, 60x objectives); (2) Parsortix , a microfluidics-based system for the isolation of cells (e.g., circulating tumour cells from the blood of cancer patients) based on their size and deformability, where captured cells can be recovered and subjected to further downstream profiling; (3) a 10x Genomics Chromium Controller, a high-throughput single-cell sequencing device allowing the parallel transcriptomic profiling of thousands of cells in a sample; (4) a TransferMan micromanipulator system (Eppendorf) attached to a fluorescence microscope (Zeiss Axiovert 200), used for the isolation of single cells from suspension, followed by further Smart-Seq2-based transcriptomic profiling by qPCR or sequencing.



# Teaching Programmes

## Team for developing a new education programme at IFB UG and MUG

Assoc. Prof. Sylwia Jafra,
Assoc. Prof. Stanisław Ołdziej,
Assoc. Prof. Robert Czajkowski
Assoc. Prof. Patrycja Koszałka
Dr. Andrea Lipińska
Dr. Katarzyna Węgrzyn
Dr. Wioletta Żmudzińska
Adviser: Prof. Jarosław Marszałek

- **The Teaching Concept**

  The concept of education at IFB assumes an interdisciplinary study programme in biotechnology for health and improvement in quality of life. Education is provided by IFB in cooperation with other UG and MUG units and is based on the highest teaching standards in international cooperation. The concept-based learning at IFB was initiated by prof. Igor Konieczny and his team. Students are involved in the implementation of research projects, which has been the main principle for the 25-year history of IFB. The curriculum for the first degree of studies was based on "concept-based learning". It goes beyond the simple communication of information developed for traditional education and focuses on understanding and relating facts to a coherent and logical whole, combining theory and practice. In the study programmes (1st and 2nd degree), we consider the idea of "Responsible Research and Innovation" (RRI) – the concept of responsible research and innovation assuming the inclusion of society in research, the openness of science, ethically conducted research and adaptation to the needs of society.

- **First-Level Degree**

  The first-degree study programme is divided into six semesters corresponding to 6 modules to implement the related curriculum content. Each module carries out 30 ECTS. The content of the individual modules is integrated and covers topics ranging from basic to more complex and from theoretical to practical aspects of biotechnology. Modules M01-4 and M06 cover biomolecular issues and content related to the functioning of unicellular and multicellular organisms. The contents of the M05 module are dedicated to the discipline of medical sciences. Modules are organized into blocks of consistent content that integrates knowledge, practical skills and social competencies. In each block, there are Foundations (lectures) and Methodology – the classes combine theoretical knowledge with practice (seminars, diploma seminar, tutorials) and practical classes (laboratory exercises). Students also carry out individual classes (individual laboratory training, workshops).

- **Second-Level Degree**

  The implementation of the second-degree study programme assumes the full involvement of students in scientific and research work. The programme is based on a traditional class schedule divided into lectures, laboratories, seminars and diploma seminars. Master's project – Master's thesis is held in the research and development laboratories of IFB. The programme content is closely related to the research carried out at the Faculty in the disciplines of biological sciences and medical sciences. It covers issues in the field of molecular biotechnology, medical biotechnology, experimental oncology, plant and industrial biotechnology, molecular diagnostics in medicine, and the protection of plants and the environment. The study programme is focused on the scientific work of students during the implementation of Master's thesis projects.

- **Innovative Education Programme in the Field of Biotechnology**
- **Three-Year Undergraduate Studies**
- **Thematic Modules**
- **Concept-Based Learning Brief history of Biotechnology**

| | |
|---|---|
| **01**<br>**Basics of Biotechnology**<br>  | Introduces students to knowledge and practical issues: (i) the philosophy of science, the history of scientific discoveries, the scientific method, the techniques used in scientific research in the field of life sciences; (ii) model organisms and biotechnology; (iii) application of science in biotechnology, (iv) basics of cell organization. |
| **02**<br>**Biomolucles**<br>  | It provides knowledge and practical issues on: (i) the structure, properties and functions of biomolecules that make up more complex biological systems, cellular compartments; (ii) cell organization including molecular processes related to the synthesis of biomolecules and the network of interactions between biomolecules, their transport and role in the process of gene expression. |
| **03**<br>**Unicellular organisms**<br>  | It provides knowledge and practical examples regarding: (i) structure and functioning of unicellular organisms; (ii) the molecular processes of unicellular organisms underlying their functioning; (iii) the life processes of microorganisms in a given environment. |
| **04**<br>**Multicellular organisms**<br>  | It provides knowledge and practical examples regarding: (i) the genetic basis of the functioning of multicellular organisms; (ii) the specialization of cells into different types and the way they are organized into higher-order structures; (iii) the functioning of plant organisms and (iv) the functioning of the human body to maintain homeostasis. |
| **05**<br>**Biotechnology in medicine**<br>  | It provides knowledge and practical issues on: (i) disturbances in homeostasis at the cellular and human body level; (ii) mechanisms regulating the cell's response to damage (at the cell and organism level); (iii) the role of the environment and pathogens on the processes related to pathological changes in the human body, including the basics of the diagnosis of these changes; medical applications of biotechnology; (iv) the medical applications of biotechnology in the service of humans; (iv) intellectual property, entrepreneurship and social responsibility law. |
| **06**<br>**Biotechnology in industry and agriculture**<br>  | It provides knowledge and practical issues on: (i) practical aspects of microbiology, synthetic biology, agricultural and industrial biotechnology; (ii) learning about and discussing the latest trends and challenges in biotechnology. |

# Biotechnology Summer Schools

## Brief history of Biotechnology Summer Schools at IFB

**Biotechnology Summer Schools (BSS) have been organized annually since 1994. The idea of BSS came from Professor Anna J. Podhajska, IFB co-founder, brilliant scholar and science activist who implied that students and young scientists should actively participate in obtaining knowledge and establishing contacts with scientists from all over the world, not only in formal conditions but also outside the University.**

The participants of BSS include students of biotechnology as well as students of related and other disciplines who are particularly interested in the issues discussed. Scientific topics of BSS vary each year, however BSS is a combination of learning and spending quality time in the natural surroundings and many entertaining activities, fancy-dress party, workshops, sports, field games or regional trips are always on the agenda.

So far Biotechnology Summer Schools were honoured with the presence of many eminent scientists such as professors: Ewa and Ernest Bartnik, Stanisław Bielecki, Klaus Halhlbrock, Waleria Hryniewicz, Robert Huber (Nobel Prize winner in Chemistry in 1988), Berndt Jastorf, Adam Jaworski, Roman Kaliszan, Władysław Kunicki Goldfinger, Andrzej Legocki, Janusz Limon, Mirosław Matuszyński, Jerzy Paszkowski, Andrzej Płuciennicak, Richard P. Sinden, Piotr Stępien, Wadaw Szybalski, Tomasz Twardowski, Jacques H. Weil, Robert Wells, Brigitte Wittman-Liebold, Maciej Zenktler, Maciej Żylicz.

www.bss.ug.edu.pl

### XXIV Biotechnology Summer School | 2018

The XXIV Biotechnology Summer School was organized under the umbrella of the Horizon 2020 Project "STARBIOS2: Structural Transformation to Attain Responsible Biosciences" and dedicated to the concept of Responsible Research and Innovation (RRI). The event took place between September 4 and 8 2018 in the conference centre of Hotel Orle on Sobieszewo Island, a beautiful seaside area of Gdańsk with a status of ecological island and unique nature reserve areas.

The goal of the XXIV BSS was to reflect on the social responsibility of science and focus on the relation between bioscience research and RRI aspects to raise the awareness of young researchers on RRI issues and how they can impact their professional work and scientific practice in the future.

The programme included lectures and workshop sessions dedicated to both bioscience issues and RRI components targeted in the STARBIOS2 project: societal engagement, ethics, education, gender and open access. Speakers and workshop leaders from STAR-BIOS2 partner institutions as well as from an outside consortium presented and investigated RRI through a wide range of topics, involving 72 participants from both European and non-European countries.

The programme consisted of 7 lectures given by Prof. Vittorio Colizzi and Prof. Carla Montesano (University of Rome Tor Vergata, Italy), Dr. Lorna Henderson (NIHR Oxford Biomedical Research Centre University Hospitals NHS Foundation Trust, UK), Dr. Vasiliki Kiparoglou (Oxford University Hospitals, Biomedical Research Centre, UK), Dr. Phoebe Friesen (Ethox Centre, University of Oxford, UK), Dr. Elena Bužan (University of Primorska, Slovenia), and Prof. Doris Elster (Bremen University, Germany).

Apart from traditional informal events such as field games or fancy dress parties attended by BSS participants, 5 interdisciplinary workshops were carried out by the invited leaders. Two workshop sessions dedicated to diversity management were led by Dr. Magdalena Żadkowska and Assoc. Prof. Natasza Kosakowska-Berezecka from UG; science and media relation workshop led by Dariusz Aksamit from The Spokesmen of Science from Warsaw, Poland. The session on "Societal engagement and knowledge transfer – issues and challenges" was carried out by Prof. Doris Elster from Bremen University, Germany, and "RRI and ongoing changes in scientific research and innovation field workshops" run by Claudia Colonnello (Laboratory of Citizenship Sciences, Italy), Andrea Declich and Daniele Mezzana (University of Rome Tor Vergata, Italy).

## **XXV Biotechnology Summer School** | 2019

The 25th jubilee edition of BSS took place on 3rd-7th September 2019 in Ostrzyce, at the heart of a picturesque area in Kashubian Lake District. This location (and early September weather) offered excellent possibilities for outdoor activities such as boat trips, kayaking, walking, and traditional field games, all of which helped to integrate participants. The general topic of the event was "Translational Research", and the overall goal was to make the audience familiar with the process of bringing an invention from the bench to the bedside. Invited speakers aimed to talk about filling the gap between scientific concepts, target identification, or mechanistic studies and stages preceding the release of products into the market.

The overall programme was divided into three topic sessions:

1. Models and markers in oncology, with Prof. Jacek Jassem (Medical University of Gdańsk), Prof. Ruben Pio and Dr. Daniel Ajona (Navarra University, Spain) and Dr. Sven Peterssen (Tessa Therapeutics, Singapore) as speakers.
2. Diagnostics, genomics, and rare diseases, with speakers from Radboud Medical Centre, The Netherlands (Dr. Elena Volokhina), Genomics Core Facility University of Bergen, Norway (Dr. Tomasz Stokowy), and MNM Diagnostics, Poland (Dr. Paweł Zawadzki)
3. Big Pharma issues, where two speakers, Dr. Aleksandra Stańczak from Celon Pharma, Poland, and Dr. Ewa McGrowder, the Research Study Coordinator at the Institute of Cancer Research, UK, talked about the coordination of preclinical studies and the management of clinical research.

Additionally, four workshops were led by invited guests, who revealed more practical details about the interpretation of diagnostic biomarkers in cancer (Prof. Ruben Pio), signatures of mutational processes in tumorigenesis (Dr. Tomasz Stokowy), handling of plastic, and possible threads related to endocrine disruption (Dr. Aleksanda Rutkowska and Dr. Aleksandra Konieczna, DetoxED, Poland) as well as a class about scientific communication led by MSc Mikołaj Fedorowicz, PAN, Poland.

**Unfortunately, the XXVI edition of the Biotechnology Summer School was not held in 2020 due to the COVID-19 pandemic.**

XXV Biotechnology Summer School

www.bss.ug.edu.pl

# Responsible Research and Innovation Projects at IFB

## STARBIOS2: Structural Transformation to Attain Responsible BIOsciences | 2016–2020

www.starbios2.eu

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement N° 709517

A consortium of 12 international partners worked on improving RRI embedment in bioscience research institutions within the framework of the EU Horizon 2020 Framework Programme for Research and Innovation by implementing **pilot project STARBIOS2**. The objective of the project's Action Plan was to provide a set of actions for facilitating structural change in the area of Responsible Research and Innovation (RRI) in partner institutions, including IFB at UG. The actions from the 5 key areas of RRI, i.e., societal engagement, gender, education, open access, and ethics, **were targeted at IFB needs**; nevertheless, in cases when overall university-wide regulations were affected, the effects of implemented actions were expected to concern other UG faculties.

The results of the STARBIOS2 project are expected to serve as a basis for the further development of the RRI strategy within UG and acting as a trigger to introduce changes that go beyond the area of bioscience, as well as offer guidelines and model solutions for other organizations intending to induce the RRI strategy in biosciences.

The project leader of the project at UG was **Prof. Krzysztof Bielawski from IFB**, Vice-Rector for Innovation and Liaison with Business and the Community.

**Some activities performed within STARBIOS2 at IFB:**

- Organization of Biotechnology Summer School entirely dedicated to Responsible Research and Innovation concept, 2018
- "Diversity Team Management" workshop for IFB researchers, 2018
- Introduction of RRI-oriented courses at the IFB at the BA, MA and PhD levels since 2020/2021
- Publication of the report "Women in science. Diversity management and gender equality in social responsibility of University of Gdańsk", 2020

## ResBios - RESponsible research and innovation grounding practices in BIOSciences | 2020-2023

www.resbios.eu

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement N° 872146

This EU-funded project is coordinated by the University of Rome Tor Vergata, Rome. Over the next three years, ResBios will draw on the expertise of 12 partners from 11 countries to bring sustainable institutional changes into biosciences research. Responsible Research and Innovation (RRI) provides a framework for bringing research closer to the needs and values of society, indicating the biosciences sector's vital role in this process. Based on the STARBIOS2 project's experience, UG will act as a mentor in supporting the implementation of structural changes in five key RRI areas in the selected "learning" research organizations. The project leader of the project at UG is **Prof. Krzysztof Bielawski** from IFB, Vice-Rector for Innovation and Liaison with Business and the Community.

### **MINDtheGEPs - Modifying Institutions by Developing Gender Equality Plans** | 2021-2025

The project aims to promote gender equality through the implementation of Gender Equality Plans (GEPs) in different types of research organizations in five European countries that have not reached the 25% score for the proportion of women in leadership positions in higher education. At the University of Gdańsk, the main objective of the project will be to create and implement GEPs. The planned activities aim at increasing the participation of women in decision-making and management bodies of the university, appointing a gender equality officer, and conducting open and targeted training for specific groups of employees, as well as monitoring recruitment and promotion processes for scientific and administrative positions from the perspective of equal opportunities.

The project leader at UG is **Prof. Ewa Łojkowska from IFB**, who will head a multidisciplinary team formed by representatives of several UG units.

MINDtheGEPs gender equality in research

www.mindthegeps.eu

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement N° 101006543

## Other projects

### **BSN_Powerhouse** | 2019-2021

**BSN_Powerhouse** is an extension of the Baltic Science Network (BSN), an overall coordination framework to develop and implement science policy in a macroregional dimension and to ensure a better representation of macroregional interests at the EU level. While BSN has provided science ministries, governmental/funding agencies, and university networks with a general platform to devise joint strategic approaches, BSN_Powerhouse moves further toward implementation. **The thematic focus is on the identified priority research fields of Photon and Neutron Science, Life Sciences and the Welfare State.**

Interreg Baltic Sea Region

www.interreg-baltic.eu

Baltic Science Network

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FOUND

EUSBSR EU STRATEGY FOR THE BALTIC SEA REGION

The IFB has been involved in implementing the **BARI Mobility Programme for Research Internship** for MA/BA and PhD students as one of the BSN_Powerhouse instruments for increasing the interconnectedness of research infrastructure in the Baltic Sea Region. Through this internship programme, BSN_Powerhouse tests out a new decentralized and flexible funding mode that can serve as a model for multilevel governance and funding. Three IFB students were granted scholarships and participated in the BARI programme in 2019-2021.

BARI
The Baltic Science Network Mobility Programme for Research Internships

www.baltic-science.org/bari

# Science and Society at IFB

**During the last three years at the Intercollegiate Faculty of Biotechnology, we have conducted several events to disseminate knowledge and popularize scientific achievements in biotechnology among primary and high school pupils.**

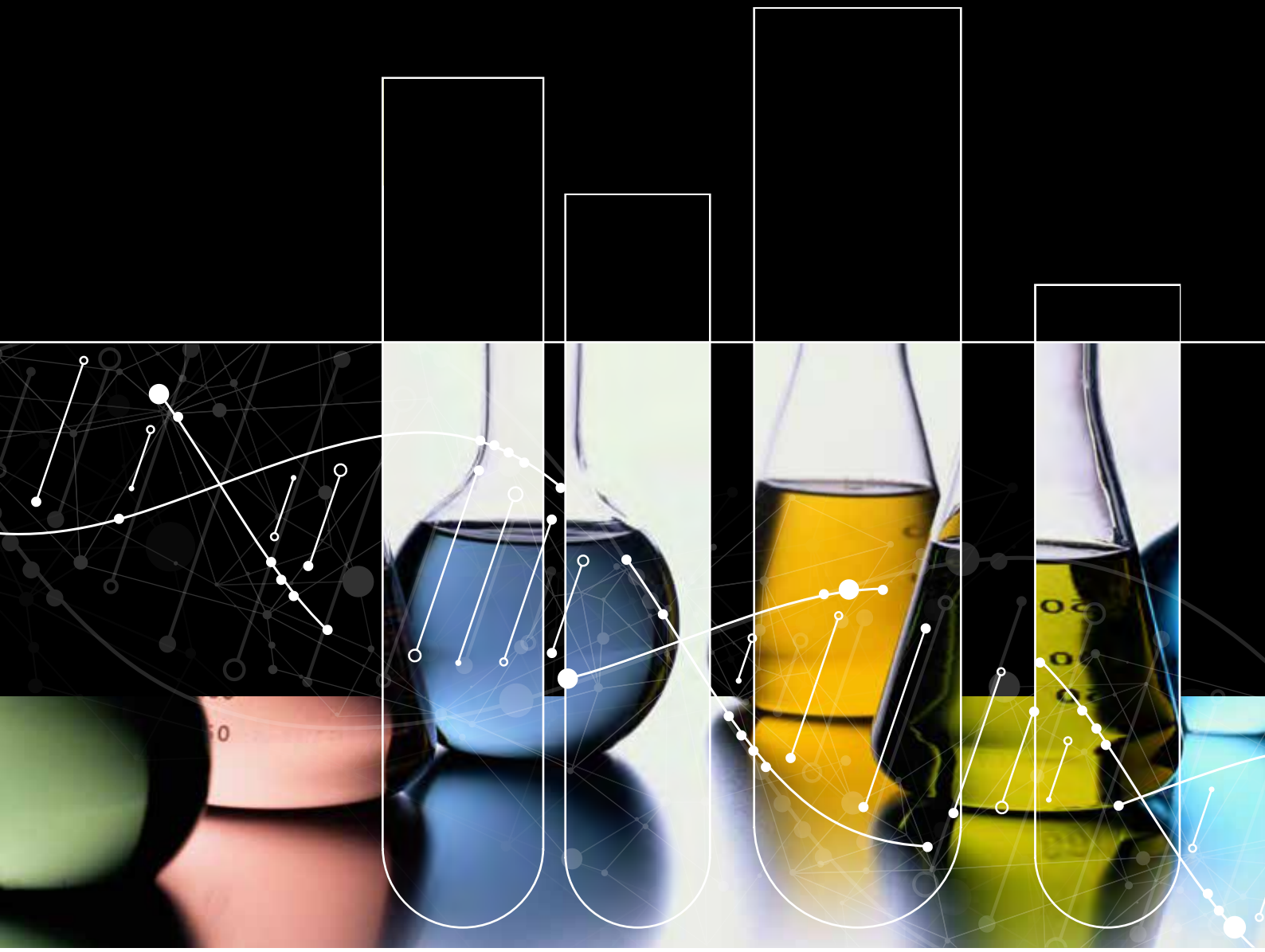
Each year, we have organized numerous scientific workshops, open-door days, science events, lectures and classes not only for children and youth to become more familiar with important scientific issues but also for the entire Pomeranian community. In these days, the laboratory doors were opened to all those who wanted to visit our well-equipped scientific laboratories and lecture halls and become familiar with the interdisciplinary research carried out at the Faculty that combines biomedical and biomolecular issues and their application in biotechnology for health and improving the quality of life. It is important to emphasize that the researchers, PhD students and students mostly from BIO-MED student clubs, were actively involved in the organization of these events to present interesting issues related to science in a very accessible and attractive way.

Participants had the opportunity to see, among others, glowing bacteria and bacteria on various coloured media, cultures of environmental bacteria that we encounter every day, antibiograms, cells and tissues of our body under the microscope, the secret world of viruses (fluorescent viruses in living cells under the microscope), in vitro plant cultures, and fluorescent DNA. Additionally, they could taste unusual products of molecular cuisine and edible microorganisms, and they could obtain the answers for very important and interesting questions such as the following: How much life is in a drop of water? How is the rainbow created? What is the secret of non-Newtonian fluids? What plants are used in biotechnology? How do scientists use molecular modelling? They could also determine how to prepare their own cosmetics at home and learn how to fight dangerous bacteria using natural and commercially available antibiotics. The youngest children could design their new microorganisms or create a DNA necklace.

IFB is a cofounder of the educational initiative Pomeranian InnovaBio Programme, in which the students and pupils of high schools gain practical skills and are actively involved in the research and development projects commissioned by entrepreneurs in the life science sector.

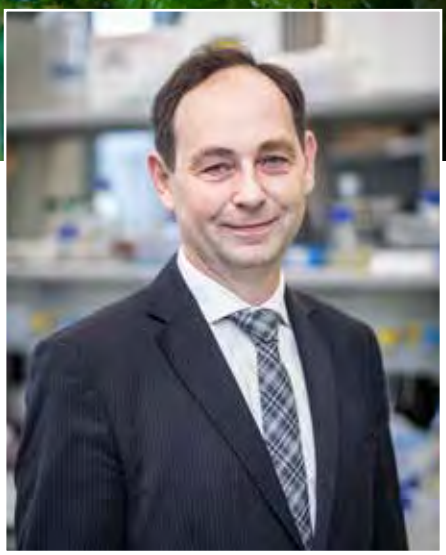
**Events for schools:** **Biologists' Night**, January 2018, 2019 and 2020 • **Academia Fair**, March 2018, 2019 and 2020 • **UG Open Days**, March 2018, 2019 • **The STARBIOS2 Vaccine Knowledge Quiz for secondary school pupils**, March, 2018 • **The STARBIOS2 GMO and GMM Knowledge Quiz for secondary school pupils**, March 2019 • **Speed-networking of journalists and scientists and a science communication workshop for scientists organised at IFB**, December 2018, (STARBIOS2) • **Medical University of Gdansk Open Day — Science for Health**, April 2018, 2019 • **Webinarium and Q&A „Coronaviruses: SARS-CoV-2",** April 2020

**Workshops for schools:** **Scientific picnics, "University - I like it"**, October 2018, Kwidzyń • **Kociewski Festival of Science**, Starogard Gdański, October 2018 • **Debate on GMO plants "Learning through fun as a tool to learn about myths and facts about GMOs!"**, May 2019 • **Laboratory workshops at IFB for the winners of STARIOS2 Knowledge Quiz**, September 2019 • **"The Small Big World of Microorganisms" organised by IFB Research and Development Laboratory**, January 2020 (STARBIOS2)



# Research Group Profiles

# Laboratory of Physical Biochemistry



## Prof. Bogdan Banecki

PhD, 1985, University of Gdańsk, PL; Postdoctoral training: ETH Zurich, University of Utah, USA, University of Ancona, Italy; Habilitation, 2003, University of Gdańsk, PL; Group leader since 2004; full-professor since 2020.

### Research Group:

Leszek Kadziński, PhD
Robert Łyżeń, PhD

PhD Students: Alicja Filipek, Grzegorz Gawron, Paulina Tomiczek

## Research

The Laboratory of Physical Biochemistry deals with functional analysis of the structure and function of proteins and other molecules. The team studies structural changes in proteins and the influence of such changes on the activity and stability of these compounds, using advanced spectroscopic, biophysical and biochemical techniques. The employed research techniques include stopped flow measurements, titration and scanning microcalorimetry, and biochemical and genetic fluorescent techniques. The team is interested in both basic and applied research. In addition to spectroscopic studies, members of the team specialize in chromatographic analyses using liquid (HPLC) and gas (GC) chromatography with absorption, fluorimetric, light scattering or mass spectrometry detection.

### Laboratory participates in five main projects:

Studies on the functions of the MTHFR and CBS genes during the formation of homocysteine in the context of therapeutic and preventive aspects in patients with ischemic brain stroke. The aim of the research, performed in collaboration with the Medical University of Gdańsk, is to describe the mechanism of homocysteine action in the organism as one of the risk factors in ischemic stroke. The initial research suggests that heat shock proteins significantly modify the activity of the MTHFR enzyme.

Development and studies of biocompatible and bioregenerative materials. We analysed the side effects of silicone implants by determining the influence on the stability and structure of proteins. The aim of the current research is to characterize the interactions of small-molecule polymethylsiloxanes with proteins. The conducted research indicates significant affinity of silicones to fibrinogen and collagen, which could be important in the development of clinical side effects of implants.

Isolation and implementation of new nutraceuticals containing natural extracts with the use of efficient, economical and environmentally friendly methods. The project involves the structural identification and determination of possible modifications for increasing the activity and bioavailability of anti-inflammatory and antimicrobial compounds. As a result, the project would yield a group of active compounds that will be implemented in industrial practice. With the collaboration between our team and the laboratory of Biovico sp. z o.o. Gdynia, Poland, we developed a new ointment preparation based on one of the nutraceuticals being examined in the project, which has already been released to the market.

Analysis of interactions between silica-based biomaterials and proteins. Silica-based biomaterials have attracted great interest among scientists due to their potential use in medicine as scaffolds and drug carriers. Their superiority over other biomaterials lies in the way of their synthesis as well as their nontoxicity and biocompatibility in vivo. Gained knowledge could benefit a better understanding of the wound healing process and bone regeneration.

Development and studies on an innovative dermo-protective gel based on active substances from snail mucus. The project involves three main parts. The first is the optimization of breeding conditions and the influence of physical and chemical factors on the properties of mucus. The second step is the development of a nondestructive mucus collection procedure and finally the formulation of a dermo-protective gel.

## Publications

- Janczak Monika, Hyz Karolina, Bukowski Michal, Łyżen Robert, Hydzik Marcin, Wegrzyn Grzegorz, Szalewska-Palasz Agnieszka, Grudnik Przemyslaw, Dubin Grzegorz, Wladyka Benedykt. Chromosomal localization of PemIK toxin-antitoxin system results in the loss of toxicity – Characterization of pemIKSa1-Sp from Staphylococcus pseudintermedius. Microbiological Research 2020, 240: 126529 (doi:10.1016/j.micres.2020.126529).
- Gawron Grzegorz, Krzyczkowski Wojciech, Lemke Krzysztof, Oldak Alicja, Kadzinski Leszek, Banecki Bogdan. Nigella sativa seed extract applicability in preparations against methicillin-resistant Staphylococcus aureus and effects on human dermal fibroblasts viability. Journal of Ethnopharmacology 2019, 244:UNSP 112135 (doi: 10.1016/j.jep.2019.112135).
- Kadziński Leszek, Banasiuk Rafał, Banecki Bogdan. Determination of ten sulfonamides in honey using tetrahydrofuran Salting Out Liquid Liquid Extraction and monolithic silica column. LWT – Food Science and Technology 2018, 96: 7-12 (doi: 10.1016/j.lwt.2018.05.007).
- Mozolewski Pawel, Jakobkiewicz-Banecka Joanna, Wegrzyn Grzegorz, Banecki Bogdan, Gabig-Ciminska Magdalena. Non-steroidal anti-inflammatory drugs are safe with respect to the transcriptome of human dermal fibroblasts. European Journal of Pharmacology 2018, 818: 206-210 (doi: 10.1016/j.ejphar.2017.10.040).
- Smolińska Elwira, Moskot Marta, Jakóbkiewicz-Banecka Joanna, Węgrzyn Grzegorz, Banecki Bogdan, Szczerkowska-Dobosz Aneta, Purzycka-Bohdan Dorota, Gabig-Cimińska Magdalena. Molecular action of isoflavone genistein in the human epithelial cell line HaCaT. PLoS ONE 2018, 13(2):e0192297 (doi: 10.1371/journal.pone.0192297).
- Moskot Marta, Bocheńska Katarzyna, Jakóbkiewicz-Banecka Joanna, Banecki Bogdan, Gabig-Ciminska Magdalena. Abnormal Sphingolipid World in Inflammation Specific for Lysosomal Storage Diseases and Skin Disorders. International Journal of Molecular Sciences 2018, 19: 247 (doi: 10.3390/ijms19010247).

## Research Grants

- Development of an innovative dermo-protective gel based on active substances from snail mucus. Smart Growth Operational Programme project POIR.02.03.02-28-0004/15.
- Development and commercialization of new immunomodulating and antibacterial nutraceutics. Innovative Economy Operational Programme project POIG.01.04.00-22-183/12.
- Development of a diagnostic test based on the determination of the concentration of metabolites of drugs in the patient's blood used in the treatment of autoimmune diseases and the technology of its production Polish Agency for Enterprise Development – 61/WDB/DPP/15.

## Patents

- The extraction method of sulfonamides, secondary metabolites or semi-synthetic compounds. The patent application (UP RP) No. 402299 dated 2012-12-28.

## Scientific collaboration

- Medical University of Gdańsk; Gdańsk, Poland.
- Biovico sp. z o.o. Gdynia, Poland.
- Polish Snail Holding, Olsztyn, Poland.

## Research Awards

- Team award of the 2nd degree of the Medical University of Gdańsk for Magdalena Prokopowicz, Chair and Department of Physical Chemistry.
- Bogdan Banecki, IFB UG and the MUG.
- Leszek Kadziński, IFB UG and MUG.
- Joanna Jakóbkiewicz-Banecka, Department of Molecular Biology, University of Gdańsk; Magdalena Gabig-Cimińska, Laboratory of Molecular Biology of the University of Gdańsk.
- Jerzy Łukasiak – for research on the preparation of a xerogel based on polydimethylsiloxane as a new, solid carrier for doxorubicin hydrochloride.

# Laboratory of Biophysics


## Assoc. Prof. Jacek Piosik

He graduated (1994), received PhD (1999) and habilitation (2011) at University of Gdansk in biological sciences. In 2012 he was appointed associate professor at the University of Gdansk

### Research Group:

Anna Woziwodzka, PhD
Grzegorz Gołuński, PhD

PhD student: Kamila Butowska

## Research

The Laboratory of Biophysics conducts research on biologically active low-molecular-weight compounds, such as anticancer drugs, antibiotics, environmental mutagens and carcinogens, toxins, substances with chemopreventive properties, and nanoparticles. The main objectives of our research are to describe the mechanisms of action of a wide variety of toxic substances, to search for protective compounds and to reveal their mechanisms of action, and to develop effective new methods to modulate the activity of drugs with particular emphasis on drugs used in anticancer therapy and antibiotics.

Our recent studies focused on examining possible direct interactions of nanoparticles and small biologically active compounds with commonly used antitumor drugs and antibiotics.

### Metallic (silver and platinum) nanoparticles can interact directly with the acridine mutagen ICR-191

Silver (AgNPs) and platinum (PtNPs) nanoparticles are among the most investigated metallic nanoparticles because of their stability, functionality, and documented antimicrobial properties.

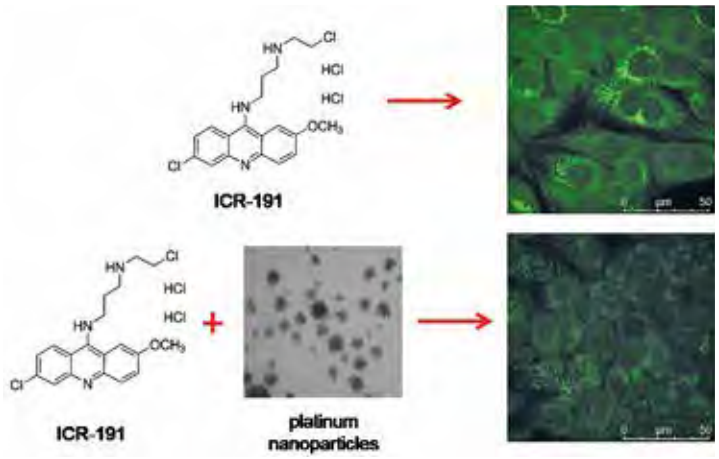



FIGURE 1. Platinum nanoparticles modulate the biological activity of the acridine mutagen ICR-191.

In our recently published work, we functionalized AgNPs with thiobarbituric acid (TBA) or 11-mercaptoundecanoic acid (MUA) residues to improve the nanoparticles' biological activities. We assessed the physicochemical properties of newly synthesized AgNPs using a wide range of biophysical methodologies and compared them to the properties of "naked" AgNPs. Then, we analysed direct interactions between all AgNPs and the model acridine mutagen ICR-191. We also examined the mutagenicity and toxicity of all AgNPs alone and in combination with ICR-191. We showed that AgNPs coated with MUA had the most interesting interactions with the tested ICR-191, slightly modulating its toxicity properties by decreasing the viability of treated cells. We also studied the direct interactions of newly synthesized PtNPs with ICR-191 and their possible influence on the biological activity of the tested substances. We used dynamic light scattering, fluorescence spectroscopy, and isothermal titration calorimetry to assess the thermodynamic parameters of the formation of ICR-191-PtNP non-covalent aggregates.

Moreover, to determine the biological activity of ICR-191-PtNP aggregates, we employed the Ames mutagenicity test, eukaryotic cell line analysis, and toxicity testing against the model organism *Caenorhabditis elegans*. We showed that PtNP lacked toxicity in a tested range of concentrations and had the ability to modulate ICR-191 biological activity, suggesting that these particles could be used successfully as potential delivery platforms for different biologically active substances (Fig. 1).

### Doxorubicin conjugates as potential drugs in targeted therapy

The main idea of this project was to conjugate the well-known anticancer drug doxorubicin (DOX) with transport molecules. We expected such conjugates to play an important role in the delivery of DOX molecules to cancer cells and possibly to modulate their activity.

First, we synthesized fullerene C60-doxorubicin conjugates (C60-DOX). The obtained C60-DOX molecule was characterized by a wide range of biophysical and biological methods. C60-DOX conjugates exhibited low cytotoxicity, probably due to their poor penetration through the nuclear membrane. Therefore, we decided to study the product of matrix metalloproteinase cleavage, a tetrapeptide-DOX conjugate (pep-DOX). We showed that pep-DOX can intercalate to dsDNA through the major groove, in contrast to DOX, which intercalates through the minor groove. Finally, we conclude that these conjugates may be an effective tool for the selective delivery of DOX molecules to cancer cells.

### Methylxanthines as modulators of antibiotics

Despite a broad spectrum of available antimicrobial drugs, the effective treatment of infectious diseases in the era of multidrug-resistant superbugs still appears to be a major challenge for modern medicine. One of the promising approaches to improve the efficacy of antibiotic treatment is the application of a combination therapy, in which, in addition to an antibiotic, an additional substance that improves the overall antimicrobial effects is used. There are some indications in the literature that caffeine – a purine alkaloid commonly consumed as a component of popular beverages – effectively reduces bacterial growth. On the other hand, caffeine and other xanthines are known to interact noncovalently with other aromatic compounds. As at least some commonly used antibiotics are aromatic, and xanthines may decrease their pharmacological activity. To date, knowledge about possible in vitro antagonistic or synergistic interactions between methylxanthines and antibiotics is scarce. Within the project, we are investigating whether caffeine and pentoxifylline are able to potentiate the effects of various antibiotics or do the opposite – reduce their antimicrobial activity – and assessing the possible mechanisms of their action as modulators of antibiotics.

### Research Publications


- Butowska Kamila, Żamojć Krzysztof, Kogut Mateusz, Kozak Witold, Wyrzykowski Dariusz, Wiczk Wiesław, Czub Jacek, Piosik Jacek, Rak Janusz. The Product of Matrix Metalloproteinase Cleavage of Doxorubicin Conjugate for Anticancer Drug Delivery: Calorimetric, Spectroscopic, and Molecular Dynamics Studies on Peptide–Doxorubicin Binding to DNA. International Journal o f Molecular Sciences 2020, 21, 6923 (doi:10.3390/ijms21186923).
- Maznychenko Andriy V., Bulgakova Nataliya V., Sokolowska Inna V., Butowska Kamila, Borowik Agnieszka, Mankivska Olena P., Piosik Jacek, Tomiak Tomasz, Gonchar Olga O., Maisky Volodymyr O., Kostyukov Alexander I. Fatigue-induced Fos immunoreactivity within the lumbar cord and amygdala decreases after C60 fullerene pretreatment. Scientific Reports 2020, 10: 9826 (doi: 10.1038/s41598-020-67034-1).
- Butowska Kamila, Kozak Witold, Zdrowowicz Magdalena, Makurat Samanta, Rychłowski Michał, Hać Aleksandra, Herman-Antosiewicz Anna, Piosik Jacek, Rak Janusz. Cytotoxicity of doxorubicin conjugated with C60 fullerene. Structural and in vitro studies. Structural Chemistry 2019, 30(6): 2327-2338 (doi: 10.1007/s11224-019-01428-4).
- Borowik Agnieszka, Butowska Kamila, Konkel Kinga, Banasiuk Rafał, Derewońko Natali, Wyrzykowski Dariusz, Davydenko Mykola, Cherepanov Vsevolod, Styopkin Viktor, Prylutskyy Yuriy, Pohl Paweł, Królicka Aleksandra, Piosik Jacek. The impact of surface functionalization on the biophysical properties of silver nanoparticles. Nanomaterials 2019, 9: 973 (doi: 10.3390/nano9070973).
- Borowik Agnieszka, Banasiuk Rafał, Derewonko Natalia, Rychłowski Michał, Krychowiak-Maśnicka Marta, Wyrzykowski Dariusz, Ziąbka Magdalena, Woziwodzka Anna, Królicka Aleksandra, Piosik Jacek. Interactions of newly synthesized platinum nanoparticles with ICR-191 and their potential application. Scientific Reports 2019, 9: 4987 (doi: 10.1038/s41598-019-41092-6).
- Gołuński Grzegorz, Woziwodzka Anna, Piosik Jacek. Potential use of pentoxifylline in cancer therapy. Current Pharmaceutical Biotechnology 2018, 19 (3): 206-216 (doi: 10.2174/1389201019666180528084641).
- Agnieszka Borowik, Prylutskyy Yuriy, Kawelski Łukasz, Kyzyma Olena, Bulavin Leonid, Ivankov Oleksandr, Cherepanov Vsevolod, Wyrzykowski Dariusz, Kaźmierkiewicz Rajmund, Gołuński Grzegorz, Woziwodzka Anna, Evstigneev Maxim, Ritter Uwe, Jacek Piosik. Does C60fullerene act as a transporter of small aromatic molecules? Colloids and Surfaces B: Biointerfaces 2018, 164: 134-143 (doi: 10.1016/j.colsurfb.2018.01.026).


### Research Grants


- National Science Centre SONATA 11, "Old drugs - new possibilities: modulation of antibiotic activity by caffeine and pentoxifylline", 464 600 PLN 2017-2020, PI Woziwodzka Anna.


### Research Awards

- Kamila Butowska, NAWA scholarship, 2019.

### Scientific collaboration

- Prof. Yuriy Prylutskyy (Department of Biophysics, Taras Shevchenko National University of Kyiv, Ukraine ).
- Prof. Maxim P. Evstigneev (Faculty of Physics, National Technical University of Sevastopol, Crimea ).
- Prof. Janusz Rak, Prof. Dariusz Wyrzykowski (Faculty of Chemistry, University of Gdańsk).
- Assoc. Prof. Aleksandra Królicka, Assoc. Prof. Rajmund Kaźmierkiewicz (IFB).

# Laboratory of Experimental and Translational Immunology

## Assoc. Prof. Danuta Gutowska-Owsiak

Danuta holds a MD degree from the Medical University in Gdańsk and a PhD in 'Infection and Immunity' from the University of Liverpool. She underwent 8 years of postdoctoral training at the MRC Human Immunology Unit (University of Oxford) in the group of Prof. Graham Ogg. This work earned her the *"BSID Young Investigator Award"* for her contribution to dermatology research. In 2017 she received a prestigious Marie Skłodowska-Curie Cofund POLONEZ Fellowship (NSC) and a startup First TEAM grant (FNP) to start her own research programme at IFB.

### Research Group:

Anna Biernacka, PhD
Ewa Czechowska, PhD
Amandine Hauer, PhD
Lilit Hovhannisyan, PhD
Joanna Łukomska, MSc

PhD students: Adrian Kobiela, Kinga Panek

Zhi Jane Chen, PhD, prof. UG
Agnieszka Jabłońska, PhD

PhD students: Dominika Miroszewska, Victor Urbiola Salvador

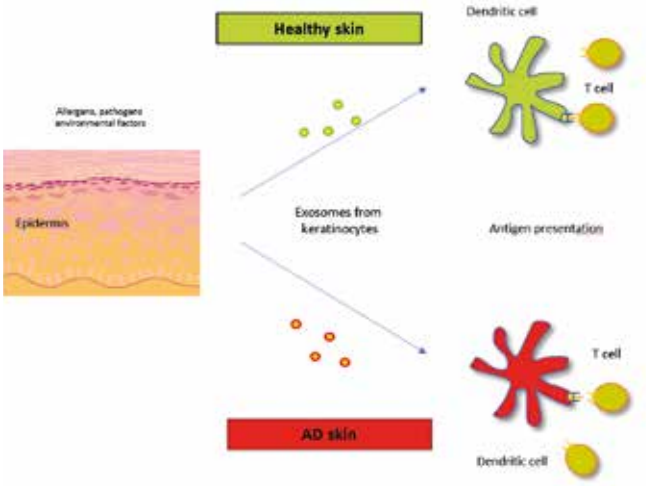
## Research

We aim to understand the principal immunological mechanisms underpinning physiology and disease status to inform novel diagnostic and therapeutic approaches for patients. Initially focused primarily on the skin and allergy, our interest and expertise have now expanded into the areas of tumour immunology and infectious diseases.

### Current projects

Keratinocyte-derived exosomes in the induction of allergy and tolerance to environmental allergens. We are investigating nanosized vesicles and exosomes, which may participate in communication between distant cells. In particular, we focus on exosomes released by keratinocytes in the epidermis during allergic inflammation in atopic dermatitis and their role in the skin and beyond. We are examining their involvement in the induction of sensitization to allergens, such as peanut. We are also interested in the interplay between the genetic susceptibility factor, a mutation in the gene encoding a critical epidermal barrier protein (filaggrin), and the exosomal compartment.

Targeted exosome-mediated crosstalk between epithelial and antigen-presenting cells for effective immunity at barrier sites.




The second project we are involved in focuses on skin infections, relevant to atopic dermatitis. Here, we are also interested in exosomes but are looking at the involvement of phospholipase A2 as a mediator of T cell reactivity in this model. In addition, we examine the differential adhesiveness and altered dendritic cell-targeted delivery of exosomal cargo upon keratinocyte exposure to infectious agents.

Artificial exosomes as a novel approach to immunotherapy Together with our research partner, prof. Jonathan Heddle (Małopolska Centre of Biotechnology, Cracow) we are investing artificial exosomes as potential novel approach to stimulating T cell-driven immune responses. Firstly, we are examining if this new methodology could be suitable for induction of protective responses against SARS-CoV2 virus (as a COVID-19 vaccine). The second arm of this project aims at proposing a broad immunotherapy approach for cancer patients.

Smart antigen provision for efficient induction of allergen tolerance Allergen-specific immunotherapy, the only curative for of treatment for allergic patients suffers from limitations that limit patient compliance and the efficacy of the treatment. Here, by taking advantage of the most recent nanotechnological advances we aim to improve allergen provision during desensitization process, with the hope of enhancing induction of tolerance to allergens.

Th17/Treg balance during immune response to cancerCancer is a leading cause of death worldwide. Even with recently developed immune checkpoint blockade therapy, durable responses are still observed in a fraction of cancer patients, suggesting better molecular understanding to develop new targeted therapies is urgently needed. In recent years, Th17 and Treg cells have been identified to play critical roles in inflammatory diseases and tumour. Manipulating Th17/Treg cell balance in the treatment of inflammation and tumour is the most active research area to develop tumour immunotherapy. We want to identify and characterize novel regulators of Th17/Treg cells for cancer immunotherapy by applying cutting-edge technologies in molecular and cell biology, proteomics and bioinformatics.

## Publications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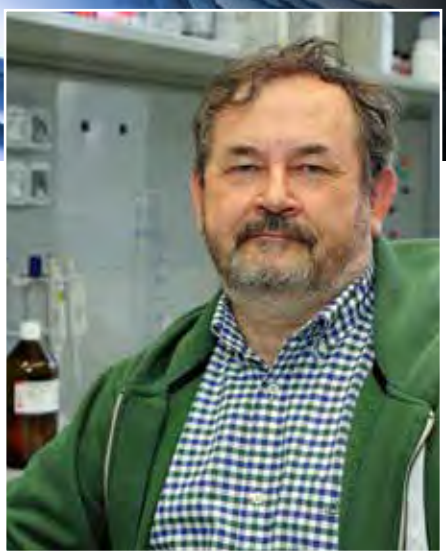
## Research Grants

- Foundation for Polish Science (FNP), First TEAM 2, Keratinocyte-derived exosomes in the induction of allergy and tolerance to environmental allergens, to DGO; 5,331,550 PLN (ca. € 1,200,000), 2017-2022; PI Danuta Gutowska-Owsiak.
  Including:
  - → COVID-19 research grant Artificial exosomes, a new approach towards the COVID-19 vaccine; 1,227,500 PLN (ca. €276,000); 2020.
  - → Collaborative research grant ArtExo: Artificial Exosomes For T-cell activation, 1,000,000 PLN (ca. €231,000); 2019.
  - → Research equipment grant; 904,050 PLN (ca. € 212,000); 2018.
- National Science Centre (NCN), Sonata BIS 9; Smart antigen provision for efficient induction of allergen tolerance; 3 824 800 PLN (ca. € 900,000); 2020-2025; PI Danuta Gutowska-Owsiak.
- National Science Centre (NCN) POLONEZ 3 Fellowship Marie Skłodowska-Curie Cofund; Targeted exosome-mediated crosstalk between epithelial and antigen presenting cells for effective immunity at barrier sites; 946,464 PLN (ca. € 224 000); 2017-2019; PI Danuta Gutowska-Owsiak.
- National Science Centre (NCN) SHENG 1; Application of new experimental approaches to characterize the balance between helper T lymphocytes (Th17) and regulatory T lymphocytes (Treg), necessary for antitumor immune response; 2 743 764 PLN (ca. € 600,000); 2018-2023; PI Zhi Jane Chen.

## Scientific Collaborations

- University of Oxford, UK.
- Jagiellonian University, Poland.
- Medical University of Gdansk, Poland.
- Karolinska Institutet, Sweden.
- Imperial College London, UK.
- Queen Mary, London, UK.
- University of Hong Kong.
- University of Oulu, Finland.
- Shanghai Jiao Tong University School of Medicine, China.

# Laboratory of Biopolymer Structure



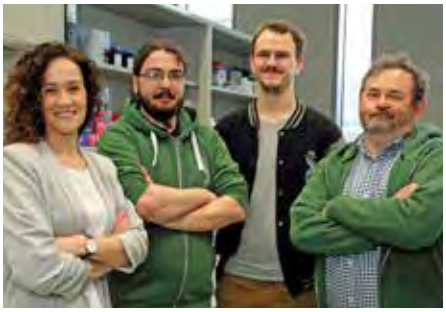
## Assoc. Prof. Stanisław Ołdziej

Received his PhD in Chemistry at the University of Gdańsk (1995). He carried out his postdoctoral training at the University of Montreal, Canada (1996-1998) working with prof. F. Major on modeling structure of small RNA molecules. In the years 2001-2004 he carried out his second post-doctoral training at the Cornell University, USA working with prof H.A. Scheraga on predicting three-dimensional structure of proteins. He became a habilitation doctor at 2005. Author of more than 130 per-reviewed publications.

### Research Group:

Wioletta Żmudzińska, PhD

PhD students: Aleksandra Lewandowska, Anna Fel, Marcel Thiel, Michał Puchalski



## Research

Our research is focused mainly on understanding the mechanisms of action of small proteins and peptides in biological systems. As a part of a large international (Poland, South Korea, USA, Argentina, France, Sweden) informal research group, we participate in a project to develop a coarse-grained (simplified) model of the polypeptide chain called UNRES (UNited RESidues). UNRES (www.unres.pl) can be used in computer simulations of the protein folding process to simulate conformational changes in proteins over a long period of time. Recently, the model has been extended (NARES) to treat DNA and RNA molecules alone or in complex with proteins. Our role in UNRES development is to provide experimental information (based on NMR measurements) on the influence of temperature on the three-dimensional structure of proteins (see Fig. 1). The temperature-induced folding/unfolding of a protein is a well-known phenomenon studied extensively because complete or partial unfolding very often leads to protein aggregation and/or fibril formation and consequently to cell/tissue malfunction.

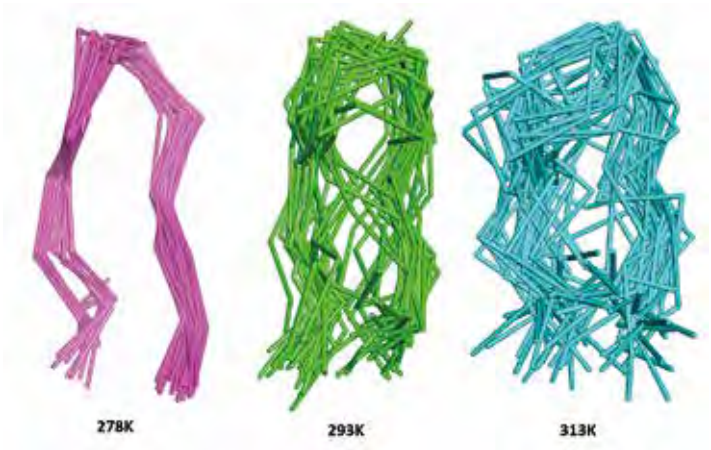
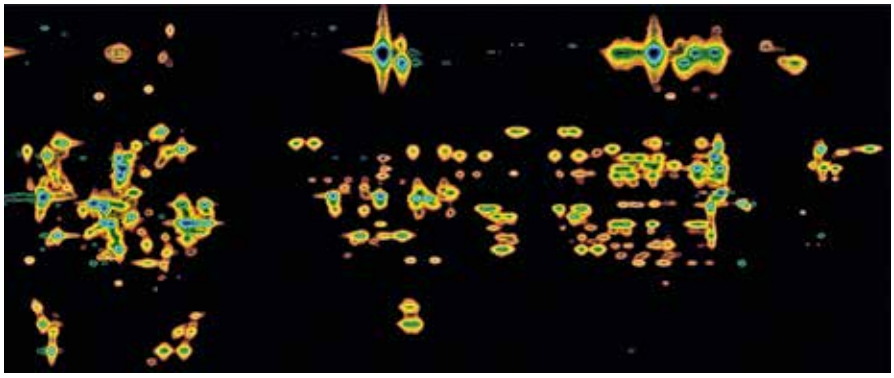



FIGURE 1. Ensembles of conformations (only backbone is shown) obtained from DYANA simulations based on restraints derived from the NMR spectra of the TRPZIP6 peptide registered at 278, 293, and 313 K.

During our studies related to the effect of temperature on the protein structure, we observed that some short sections of the polypeptide chain (6-15 amino acid residues long) occur in very well-defined conformations, often over a wide temperature range. It is believed that such fragments of amino acid sequences with a well-defined structure can play a key role in the processes of spatial self-organization of proteins (nucleation sites). In our research, we identified more than a dozen such sequences.

Since a well-defined spatial structure can often be associated with some biological function, we have undertaken studies to determine the possible biological function of such peptides. We were able to identify several peptides (the structure of one of them is shown in Fig. 1.) that show the ability to hydrolyse polyphosphate bonds in ATP, GTP, or UTP molecules. Some of these peptides can also catalyse phosphodiester bonds in single-stranded DNA or RNA chains. The identification of short peptides with a function (e.g., catalytic) can be relevant for two reasons. Peptides with simple but well-defined chemical/physical functions may have been an important part of the prebiotic chemistry system that paved the way for the first life forms on Earth. On the other hand, peptides with a well-defined three-dimensional structure can be the basis for creating simple tools that can be used in biotechnology. For example, we tried to modify the selected peptide sequence so that it could be used as a fluorescent marker in biological systems as the Green Fluorescence Protein (GFP) substitute.



In addition to research related to the structure and function of small proteins and peptides, the Laboratory of Biopolymer Structure is engaged in proteome and peptidome research. In cooperation with Prof. Lukaszuk from the Medical University of Gdańsk/Invicta Clinic, we are carrying out a large research project funded by NCN aimed at the identification of molecular markers that may be of diagnostic importance in assessing the development potential of human ova. Our research is focused on the proteomic and peptidomic composition of a medium, which is the environment of oocyte growth and development in the ovarian follicle follicular fluid (hFF). We developed a procedure for the qualitative and quantitative assessment of peptides and proteins in hFF from separate follicles of a single patient. We determined a preliminary list of protein/peptide markers, which we tested (in progress) in clinical studies to identify possible protein/peptide markers connected with oocyte development potential. A preliminary list of potential peptide biomarkers also includes small open reading frame-encoded peptides.

## Publications


- Urban Aleksandra, Volokhina Elena, Felberg Anna, Stasiłojć Grzegorz, Blom Anna M., Jongerius Ilse, van den Heuvel Lambertus, Thiel Marcel, Ołdziej Stanisław, Arjona Emilia, Rodriguez de Córdoba Santiago, Okrój Marcin. Gain-of-function mutation in complement C2 protein identified in patient with Ahus. Journal of Allergy and Clinical Immunology 2020, 146(4): 916-919 (doi: 10.1016/j.jaci.2020.02.014).
- Lewandowska Aleksandra Ewa, Macur Katarzyna, Czaplewska Paulina, Liss Joanna, Łukaszuk Krzysztof, Ołdziej Stanisław. Human follicular fluid proteomic and peptidomic composition quantitative studies by SWATH-MS methodology. Applicability of high pH RP-HPLC fractionation. Journal of Proteomics 2019, 191: 131-142 (doi: 10.1016/j.jprot.2018.03.010).
- Keasar Chen, McGuffin Liam J, Wallner Bjorn, Chopra Gaurav, Adhikari Badri, Bhattacharya Debswapna, Blake Lauren, Bortot Leandro Oliveira, Cao Renzhi Dhanasekaran, Dimas, Itzhel, Faccioli Rodrigo Antonio, Faraggi Eshel, Ganzynkowicz Robert, Ghosh Sambit, Ghosh Soma, Gieldon Artur, Golon Lukasz, He Yi, Heo Lim, Hou Jie, Khan Main, Khatib Firas, Khoury George A, Kieslich Chris, Kim David E, Krupa Pawel, Lee Gyu Rie, Li Hongbo, Li Jilong, Lipska Agnieszka, Liwo Adam, Maghrabi Ali Hassan A., Mirdita Milot, Mirzaei Shokoufeh, Mozolewska Magdalena A., Onel Melis, Ovchinnikov Sergey, Shah Anand, Shah Utkarsh, Sidi Tomer, Sieradzan Adam K., Slusarz Magdalena, Slusarz Rafał, Smadbeck James, Tamamis Phanourios, Trieber Nicholas, Wirecki Tomasz, Yin Yanping, Zhang Yang, Bacardit Jaume, Baranowski Maciej, Chapman Nicholas, Cooper Seth, Defelicibus Alexandre, Flatten Jeff, Koepnick Brian, Popovic Zoran, Zaborowski Bartlomiej, Baker David, Cheng Jianlin, Czaplewski Cezary, Botazzo Delbem Alexandre Claudio, Floudas Christodoulos, Kloczkowski Andrzej, Ołdziej Stanislaw, Levitt Michael, Scheraga Harold, Seok Chaok, Soeding Johannes, Vishveshwara Saraswathi, Xu Dong, Crivelli Silvia N. An analysis and evaluation of the WeFold collaborative for protein structure prediction and its pipelines in CASP11 and CASP12. Scientific Reports 2018, 8: 9939 (doi: 10.1038/s41598-018-26812-8).
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- Fel Anna, Lewandowska Aleksandra E., Petrides Petro E, Wiśniewski Jacek R.. Comparison of Proteome Composition of Serum Enriched in Extracellular Vesicles Isolated from Polycythemia Vera Patients and Healthy Controls. Proteomes 2019, 7(2): 20 (doi: 10.3390/proteomes7020020).
- Liwo Adam, Sierdzan Adam K., Lipska Agnieszka G., Czaplewski Cezary, Joung InSuk, Żmudzińska Wioletta, Hałabis Anna, Ołdziej Stanisław. A general method for the derivation of the functional forms of the effective energy terms in coarse-grained energy functions of polymers. III. Determination of scale-consistent backbone-local and correlation potentials in the UNRES force field and force-field calibration and validation. The Journal of Chemical Physics 2019, 150: 155104 (doi: 10.1063/1.5093015).


## Research Grants


- National Science Center 2017/01/X/ST5/00178, "Mini fluorescent protein (MFP)", 25 740,00 PLN, 2017-2018, PI: Wioletta Żmudzińska


## Collaboration

- Prof. K. Łukaszuk, Invicta Clinic/Medical University of Gdańsk, Poland.
- Prof A. Liwo, University of Gdańsk, Poland.
- Dr Jacek Wiśniewski, Max-Planck-Institute of Biochemistry, Germany.

# Laboratory of Biomolecular System Simulations


## Assoc. Prof. Rajmund Kaźmierkiewicz

Born in 1970. He obtained his master degree in chemistry in 1992 at the Chemistry Department of Gdańsk University, where he was employed until 2007. He defended his PhD thesis in 1997. After postdoctoral fellowship (1997-1998) at the Department of Chemistry, University of Arizona, Tucson, USA he visited several times (overall 3 years) the Department of Chemistry and Chemical Biology, Cornell University, USA. Since 2007 is a team leader of Biomolecular Systems Simulations Research Group.


### Research group

PhD students: Inga Jamrożek, Paweł Przygocki, Monika Romanik-Błońska

## Research

The tools used in our research activities are all contemporary molecular modelling techniques, including but not limited to quantum ab initio methods, semiempirical methods, empirical molecular mechanics (classical force field methods), molecular dynamics simulations, Monte Carlo methods, and calculations taking into consideration the free energy changes and the influence of solvent. The computers used in this research include five supercomputers placed near the top of the top 500 list, including the second fastest computer in the EU, which is the IBM Blue Gene located in Forschungszentrum Juelich, Germany.

Each of our computer modelling projects takes into consideration the complete environment with the presence of all molecules, as they appear in living organisms. We are engaged in several state-of-the-art research activities:

Investigation of the folding mechanism, i.e., the path molecules follow to accommodate a tertiary structure, for several selected proteins. We use the clustering analysis of molecular structures obtained from simulations, build a Markov state model using obtained clusters and subsequently prepare the transition graphs between obtained groups of structures.

Computer-aided molecular design of potential inhibitors of the NDM-1 enzyme, selection of the best candidates to be effective drugs, and assessment of their toxicity. Each year, the threat of bacterial resistance to antibiotics increases. One of the enzymes responsible for this state of affairs is New Delhi Metal-Beta-Lactamase 1 (NDM-1). NDM-1 belongs to a group of enzymes called carbapenemases and is capable of hydrolysing the amide bond in the beta-lactam ring.

FIGURE 1. Potential ligand interaction sites on the NDM-1 protein surface as detected by the Cavity module of the Ligbuilder program

## Research 2018-2020 continued

Molecular protein-protein docking studies. This project is in accord with current trends, as "blind" protein-protein docking studies are the subject of another series of experiments termed the "communitywide experiment on the comparative evaluation of protein-protein docking for structure prediction", in short, CAPRI. This project involves molecular dynamics simulation of the catalytic domain of the serine-threonine PrkC kinase and its complexes with ATP derivatives. PrkC is able to trans-autophosphorylate the second molecule of this enzyme; therefore, we also investigate the possible structures of the PrkC dimers.

To obtain a dynamic molecular model from a static model, one needs to perform the molecular dynamics (MD) simulations must be performed using tools such as GROMACS. We created a plugin for PyMOL (a popular and easy-to-use programme for displaying and manipulating molecular models). This plugin enables the easy use of molecular dynamics simulations using GROMACS through a graphic interface. It transfers the results of those calculations and displays them back in PyMOL.

Computer simulations of possible ligand escape pathways. There are quite a few known protein-ligand complexes. The still unsolved problem is how they are formed. Currently, it is possible only to simulate realistic physics-based processes of dissociating ligands from complexes or simulations of so-called "escape pathways". For that purpose, we use the computer modelling technique called Random Acceleration Molecular Dynamics.

Molecular modelling of the TraR protein and autoinducer molecules involved in the quorum sensing mechanism. Dominika modelled TraR/DNA complexes with N-acyl-L homoserine lactones (OOHL, OHL, HHL, OHHL) and inhibitors (patulin, droseron, plumbagin and 3-chloroplumbagin) for the first time, using AutoDock and molecular dynamics in the AMBER empirical force field.

QM/MM studies on the possible mechanism of action of the AiiO enzyme interacting with N-acyl-L homoserine lactones. There are contradictory literature reports regarding the substrate degradation pathways of AiiO. They can be verified using the QM/MM simulations.

## Future plans

Prof Kaźmierkiewicz's team plans to predict all stages of motion while kinesin transports its "cargo". Another subject of future research is to implement a molecular dynamics algorithm in torsional space into the ECEPP empirical force field. One of subjects involves computer simulations of the self-assembly process of protein capsids of viruses. It is currently possible with the exception that one needs to design coarse-grained protein models and evaluate protein-protein motions using the simplified empirical force field.

## Publications


- Borowik A, Prylutskyy Y, Kawelski Ł, Kyzyma O, Bulavin L, Ivankov O, Cherepanov V, Wyrzykowski D, Kaźmierkiewicz R, Gołuński G, Woziwodzka A, Evstigneev M, Ritter U, Piosik J. „Does C60 fullerene act as a transporter of small aromatic molecules?" Colloids Surf B Biointerfaces. 2018 Apr 1; 164:134-143.
- Grela A, Jamrożek I, Hubisz M, Iwanicki A, Hinc K, Kaźmierkiewicz R, Obuchowski M. (2018) Positions 299 and 302 of the GerAA subunit are important for function of the GerA spore germination receptor in Bacillus. PLoS ONE 13(6): e0198561.


## Research Awards

- 2019 Award from Rector of the Medical University of Gdańsk.

## Scientific Collaboration

- Prof. Harold A. Scheraga, Department of Chemistry and Chemical Biology, Cornell University, USA.
- Prof. Baldomero M. Olivera, Department of Biology, University of Utah, USA.
- Prof. Grzegorz Bulaj, Department of Medicinal Chemistry University of Utah, USA.
- Prof. Ulrich H. E. Hansmann, John von Neumann Institute for Computing, Germany.
- The past collaboration with prof. Victor Hruby from Department of Chemistry and Biochemistry University of Arizona, Tucson, USA.

# Laboratory of Biologically Active Compounds

## Assoc.Prof. Aleksandra Królicka

She graduated from the Academy of Agriculture and Forestry in Olsztyn in 1994, received a PhD in biology (1999) at the University of Gdansk and habilitation in biotechnology sciences (2011) at Wrocław University of Environmental and Life Sciences. Group leader since 2012. Author of 75 peer-reviewed publications and 3 textbooks, supervisor of 1 finished and 3 ongoing PhD's.

### Research Group:

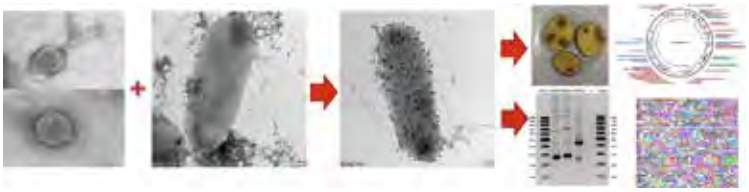
Assoc. Prof. Robert Czajkowski, Marta Krychowiak-Maśnicka, PhD

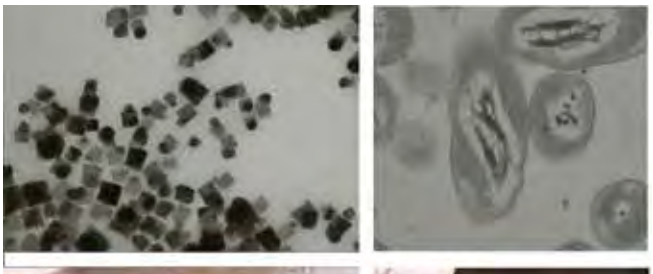
PhD Students: Angelika Michalak, Weronika Wojciechowska, Wojciech Makowski

## Research

Bacterial diseases in plants, as well as in animals and humans, comprise a serious economic problem on a global scale. The use of antibiotics is restricted in agriculture worldwide, and in medicine and veterinary medicine, microbial drug resistance, i.e., the ability of microorganisms to survive in the presence of antibiotics or chemotherapeutics, limits possible therapies and significantly prolongs hospital stays. Despite multiple preventive efforts, as well as a better understanding of resistance mechanisms and many new drug discoveries, we are facing a postantibiotic era. Although the rate of acquired resistance may continue to decrease for a period of time, due to a sustainable approach to drug administration, it is not possible to completely eliminate this serious threat. In other words, it is necessary to engage in the effort to extend the list of possible treatments to be one step ahead of pathogenic microorganisms.

Our research is mostly focused on the development of new antimicrobial approaches to address the issue of bacterial infections in agriculture, as well as the problem of multidrug resistance in human and veterinary medicine. Currently, we concentrate on the three major research areas:





### 1) Nanomaterials for the treatment and prevention of infectious diseases

Nanoparticles possess unique properties due to their ultrafine size (up to 100 nm), which determines their interaction with cells and molecules. Silver nanoparticles

(AgNPs) are broadly studied for their antimicrobial potential and the significance of silver in wound treatment. According to their antibacterial properties, AgNPs were applied successfully as middle ear prosthesis additives that prevent postoperative infections after implantation. In our laboratory, we also study the interactions of AgNPs and drug molecules, as well as the pharmacological relevance of nanoparticle-drug combinations. Moreover, our research relates to a deeper understanding of cell-nanoparticle interactions and seeks to improve AgNP antimicrobial properties and reduce their toxicity.

### 2) Bioactive compound from plant origin

An estimated 50 000 biologically active compounds are derived from plants. They are termed secondary metabolites, as they are not involved in the basic metabolism of plants but are developed as a result of specialized metabolic pathways. Plants have been a source of pharmaceutical compounds since the 7$^{th}$ century B.C. In the second half of the 19th century, interest in phytotherapy decreased, and herbs were replaced with synthetic pharmaceuticals. Currently, according to the development of plant tissue cultures and phytochemistry, active compounds of plant origin can be intensively explored. Our research focuses on antimicrobial compound isolation and identification, as well as enhancement of their production in in vitro cultured plants.

### 3) Phage therapy for the control of agriculturally relevant bacterial pathogens

From their discovery, it was hoped that bacteriophages could cure bacterial infections in humans and animals. Over time, this idea was abandoned entirely in Western Europe and the USA due to the discovery and use of the first antibiotics and new developments in that field. Recently, however, in Western Europe, bacteriophages have been more widely used in the control of bacterial infections transmitted through water, in the food industry, and in the management of nosocomial drug-resistant bacteria. The use of bacteriophages in the biological control of bacterial infections in plants has never been so routine as their use in the prevention and treatment of diseases in humans and animals. Likewise, no large-scale research has been done thus far on the ecological interactions between bacteriophages and plant-associated beneficial and pathogenic bacteria. Such studies are expected to broaden our knowledge of global plant-bacteria-bacteriophage environmental systems.

## Publications


- Ziąbka M, Kiszka J, Trenczek-Zając A, Radecka M, Cholewa-Kowalska K, Bissenik I, Kyzioł A, Dziadek M, Niemiec W, Królicka A (2020). Antibacterial composite hybrid coatings of veterinary medical implants. Materials Science&Engineering C 112: 110968.
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- Krzyzanowska D, Maciąg T, Siwinska J, Krychowiak M, Jafra S, Czajkowski R (2019). Compatible mixture of bacterial antagonists developed to protect potato tubers from soft rot caused by Pectobacterium spp. and Dickeya spp. Plant Disease 103: 1374-1382.
- Czajkowski R (2019). May the Phage be With You? Prophage-Like Elements in the Genomes of Soft Rot Pectobacteriaceae: Pectobacterium spp. and Dickeya spp. Frontiers in Microbiology 10:138.


## Research Grants

- Innovation Incubator 4.0., "Synergistic combinations of silver and naphthoquinones: formulation optimization and its antibacterial potential in infected wound model", 100 000 PLN. 2020-2021. PI: Marta Krychowiak-Maśnicka.
- Innovation Incubator 2.0., "Global metabolic analysis of the plant microconsortium of five agriculturally beneficial bacterial strains with the use of an innovative technique of the phenotypic microarrays BIOLOG EcoPlates and GEN III", 30 750 PLN, 2019-2020, PI: Robert Czajkowski.
- National Science Centre OPUS 13, "Host receptors involved in bacteriophage adsorption to Dickeya solani and Pectobacterium parmentieri cells and the ecological costs of phage resistance in vitro and in planta", 1 301 600 PLN, 2018-2022, PI Robert Czajkowski.

## Patents

- Patent nr P.405473: Sposób oczyszczania z chlorofili ekstraktów roślinnych zawierających metabolity wtórne. Banasiuk R, Michalak A, Krychowiak M, Królicka A, 30.09.2013.
- Patent nr P. 236445: Mieszanina szczepów bakterii antagonistycznych Serratia plymuthica szczep A294, Enterobacter amnigenus szczep A167, Rahnella aquatilis szczep H145, Serratia rubidaea szczepy H440, Serratia rubidaea szczep H469 i zastosowanie do ochrony lub leczenia zakażeń roślin powodowanych przez bakterie Pectobacterium i Dickeya. Czajkowski R, Maciag T, Jafra S, Krzyzanowska D, Siwinska J, 08.12.2017.
- Patent nr P. 229466: Sposób zagęszczania preparatów bakteriofagowych oraz jego zastosowania. Czajkowski R, Ozymko Z, Lojkowska E, 13.11.2014.

## Scientific Collaboration

- Université de Lorraine France Laboratoire Agronomie et Environnement Nancy-Colmar, France (Prof. Frederic Bourgaud).
- Medical University of Gdansk, Department of Pharmacognosy, Poland (Prof. Mirosława Krauze-Baranowska and Prof. Maria Łuczkiewicz).
- Medical University of Lodz, Department of Biology and Pharmaceutical Botany, Poland (prof. Halina Wysokińska).
- AGH-University of Science and Technology, Poland (Magdalena Ziabka, PhD);
- University of California-Berkeley, Berkeley, USA (Prof. Steven Lindow).
- Wageningen University and Research Center, Wageningen, Holandia (Dr. Jan M. van der Wolf).
- Université Claude Bernard Lyon 1, Villeurbanne Cedex, France (Dr. Erwan Gueguen).

# Laboratory of Protein Biochemistry

## Prof. Krzysztof Liberek

He graduated from physics at the Technical University of Gdansk (1982), received his PhD in molecular biology at the University of Gdansk (1990) and received habilitation in biological sciences at the University of Gdansk (1996). He carried out postdoctoral work at University of Utah, USA (1990-91) and University of Geneve, Switzerland (1992-93) working with prof. Costa Georgopoulos. He is a full-professor since 2002. In 2006 he was elected to EMBO.

### Research group:

Agnieszka Kłosowska, PhD
Igor Obuchowski, PhD
Szymon Ziętkiewicz, PhD

PhD students: Piotr Karaś, Dagmara Mróz, Wiktoria Sztangierska, Hubert Wyszkowski

## Research

We are interested in the role and mechanisms of chaperone proteins action in different cellular processes. The chaperone protein network controls both initial protein folding and the maintenance of proteins in the cell. The linear polypeptide chains newly synthesized on ribosomes have to fold into native three-dimensional structures. Although the native structure of a protein is principally encoded in its amino acid sequence, the process of folding in vivo very often requires the assistance of molecular chaperones. Chaperones also play a role downstream of protein synthesis in a quality-control system and thus are required to maintain the proper conformation of proteins in changing environmental conditions. Many factors leading to the unfolding and misfolding of proteins result eventually in protein aggregation. High temperature was one of the first aggregation-inducing factors studied and remains one of the main model approaches. With massive protein aggregation occurring in response to heat exposure, the cell needs chaperones to control and counteract the aggregation process. Aggregates can be eliminated by two alternative pathways, the solubilization of aggregates and either the refolding of liberated polypeptides or their proteolysis. In our research, we focus on the mechanisms by which Hsp70, Hsp100 and small heat shock proteins (sHsps) liberate and refold polypeptides trapped in protein aggregates.


FIGURE 1. Model presenting the mechanism of Hsp70-Hsp100 dependent refolding of proteins from aggregates

**Cooperation between Hsp100 and Hsp70 in protein disaggregation.** The Hsp100 family of chaperone proteins contributes to a wide variety of important cellular functions, including survival under environmental stress, the regulation of genetic composition, transposition, proteolysis, and the control of protein-based genetic elements (prions). These different roles are unified by a common biochemical

mechanism, the ability of Hsp100 proteins to promote the disassembly of aggregated proteins and high-order protein complexes. During disaggregation, a polypeptide is disentangled from the aggregate, translocated through the central channel, and enabled to fold into the native structure. However, Hsp100 chaperones are not able to act alone. Functional cooperation between Hsp100 and Hsp70 and their cochaperones is required at several stages of this process. In our research, we seek to determine the molecular mechanism of cooperation between chaperones from the Hsp70 and Hsp100 families in the disaggregation and refolding of aggregated proteins.

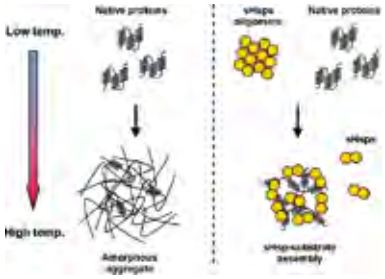


FIGURE 2. Small Hsps influence the substrate aggregation process. Temperature increase causes deoligomerization and activation of sHsps, which start binding to partially unfolded polypeptide substrates. This modifies the aggregation process and leads to the formation of sHsp-substrate complexes

**Role of sHsps in the control of protein aggregation.** Small Hsps are an evolutionary conserved class of ATP-independent molecular chaperones. Members of this family are characterized by a low molecular mass (15-43 kDa) and the presence of the conserved α-crystallin domain. Under physiological conditions, sHsps form oligomeric structures. Under heat stress conditions, the deoligomerization process takes place, and sHsps form complexes with misfolded proteins, preventing them from further aggregation and keeping them in a refoldable state that facilitates subsequent solubilization and refolding by ATP-dependent Hsp70 and Hsp100 chaperones. Using different biochemical approaches, we are investigating the molecular events leading to the deoligomerization of sHsps and the subsequent interaction between these chaperones and their substrates, leading to the formation of sHsp-substrate complexes. We also analysed the mechanism of protein refolding from such complexes. While the initial binding of sHsps to substrates is beneficial because it prevents the formation of large aggregates and increases the accessible surface for Hsp70-Hsp100 action, this interaction also hampers Hsp70-Hsp100 association and has to be broken to allow substrate refolding. Recently, we identified novel Hsp70 activity, displacing sHsp molecules from the surface of the complex at the very initial phase of the reactivation process. Currently, we are trying to understand the mechanistic principles of functioning of a two-component sHsp system and compare this system with single-protein sHsp.

## Publications


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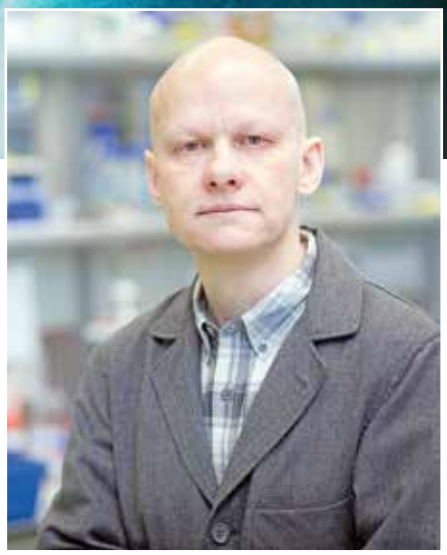
## Research Grants


- National Science Centre OPUS, "Emergence of the two-component small heat shock protein system – a fortified first line of defense against stress in Enterobacterales"; 2 131 200 PLN, 2020-2023, PI Krzysztof Liberek.
- National Science Centre OPUS, "Hsp70 and its co-chaperones in protein recovery from aggregates": 2 450 400 PLN, 2020-2024, PI Krzysztof Liberek.
- National Science Centre SONATA BIS, "Determination of biochemical properties and interaction partners of human CLPB protein - its physiological significance and role in MEGCANN syndrome pathogenesis" 659 960 PLN, 2016-2021, PI Szymon Ziętkiewicz.
- National Science Centre MAESTRO, "Molecular mechanisms of chaperones in protein disaggregation" 2 635 900 PLN, 2013-2019, PI Krzysztof Liberek.



# Laboratory of Evolutionary Biochemistry

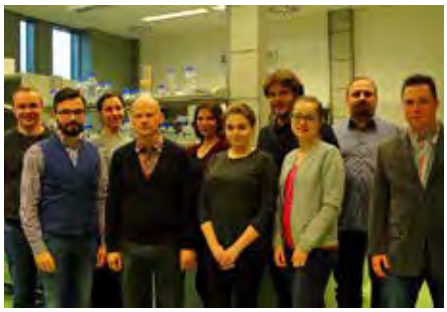
## Prof. Jarosław Marszałek

He graduated from the University of Gdansk (1982), received his PhD in biochemistry at the Medical University of Gdansk (1988) and received his habilitation in biological sciences at the University of Gdansk (1997). He carried out postdoctoral work at the Michigan State University, USA (1989-1993) and Universitat Muenchen (1995). He has been a Professor in biological sciences since 2004. Beginning in 1999, he has been a Visiting Associate Professor at the University of Wisconsin-Madison, USA.

### Research Group:

Rafał Dutkiewicz, Assoc Prof.
Bartłomiej Tomiczek, PhD
Marta Uzarska, PhD

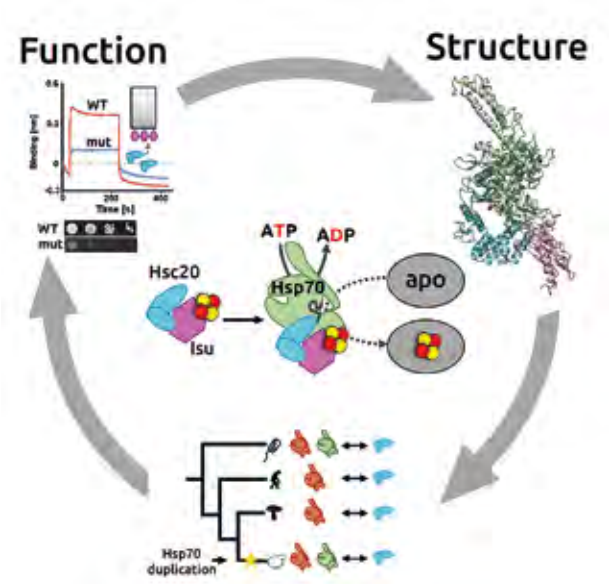
PhD students: Małgorzata Kleczewska, Marcin Jeleń, Aneta Grabińska, Milena Stolarska, Igor Grochowina



## Research

Molecular chaperone systems comprised of an Hsp70 chaperone and its J-domain protein (JDP) cochaperone are well known for their function in protein folding, preventing protein aggregation and intracellular protein trafficking. However, JDP/Hsp70 systems are also involved in more specialized essential functions. For example, JDP, termed Hsc20, and its Hsp70 partner play a critical role in the biogenesis of iron-sulfur clusters (FeS), which are cofactors required for the activity of many cellular proteins. Hsc20/Hsp70 interact with the scaffold protein (Isu), on which FeS clusters are first assembled, and facilitate cluster transfer to recipient proteins.

Our goal is to unravel the molecular mechanisms behind Hsc20/Hsp70 function in the process of FeS biogenesis. In our research, we combine biochemical experiments and evolutionary analyses because only such an approach can comprehensively explain the molecular and functional properties of the proteins involved in this complex process. As model systems, we study yeast (Saccharomyces cerevisiae) and bacteria (Escherichia coli); the differences between these systems have allowed us to investigate the molecular mechanisms behind changes in the JDP/Hsp70 partnership that took place during evolution.




Because the biochemical mechanisms underlying JDP/Hsp70 function and their interaction with protein substrate(s) are highly conserved, our research has an impact beyond FeS biogenesis. We use this Hsc20/Hsp70 system to ask questions relevant to all JDP/Hsp70 systems: (i) how the specificity of the JDP/Hsp70 interaction is determined and (ii) how JDP/Hsp70 systems interact with native protein substrates, i.e., FeS scaffolds promote (iii) what evolutionary mechanisms are responsible for changes in the JDP/Hsp70 partnerships.

## Publications

- Tomiczek Bartlomiej, Delewski Wojciech, Nierzwicki Łukasz, Stolarska Milena, Grochowina Igor, Schilke Brenda, Dutkiewicz Rafał, Uzarska Marta A., Ciesielski Szymon J., Czub Jacek, Craig Elizabeth A., Marszałek Jarosław. Two-step mechanism of J-domain action in driving Hsp70 function. PLOS Computational Biology 2020, 16(6): e1007913 (doi: 10.1371/journal.pcbi.1007913)
- Kleczewska Małgorzata, Grabińska Aneta, Jeleń Marcin, Stolarska Milena, Schilke Brenda, Marszałek Jarosław, Craig Elizabeth A., Dutkiewicz Rafał. Biochemical Convergence of Mitochondrial Hsp70 System Specialized in Iron–Sulfur Cluster Biogenesis. International Journal of Molecular Sciences 2020, 21(9): 3326 (doi: 10.3390/ijms21093326)
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- Craig Elizabeth A., Marszalek Jaroslaw. How do J-Proteins get Hsp70 to do So many different things? Trends in Biochemical Sciences 2017, 42(5): 355-368 (doi: 10.1016/j.tibs.2017.02.007)
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## Research Grants

- Foundation for Polish Science, TEAM, "Hsp70/J-protein chaperones substrate binding cycle: molecular mechanisms and functional consequences", 3 456 130 PLN, 2018-2021, PI Jarosław Marszałek.
- National Science Centre, OPUS, "Biochemical reconstitution of key steps of mitochondrial iron-sulfur cluster (FeS) biogenesis", 1 083 200 PLN, 2016-2020, PI Rafał Dutkiewicz.
- National Science Centre, MAESTRO, "Molecular mechanisms behind functional diversification of mitochondrial Hsp70 chaperones", 2 653 260, 2013-2019, PI Jarosław Marszałek.

## Scientific Collaboration

- Prof. Elizabeth A. Craig, Department of Biochemistry, University of Wisconsin - Madison, Madison, WI 53706 USA.
- Prof. Jason E. Gestwicki, Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA 94158, USA.
- Prof. Benoit D'Autreaux, Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198 Gif-sur-Yvette cedex, France.

# Laboratory of Plant Biochemistry

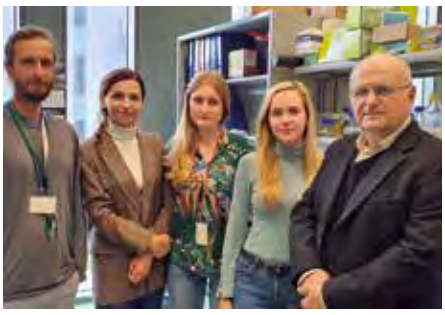
## Prof. Antoni Banaś

Graduated from the University of Gdańsk in 1978, where he received PhD in biology (1985). In 1994 received habilitation in biological sciences at University of Warsaw. He carried out post-doctoral work at Swedish University of Agricultural Sciences and at Scandinavian Biotechnology Research AB (totally around 15 years). He is a full-professor in biological sciences since 2009. He is co-author of 97 articles (some of them published in very high ranking journals e.g. Sciences – IF 41,058, PNAS – IF 9.504, Plant Physiology – IF – 5,949, Journal of Experimental Botany – IF 5,354) citied over 1600 time. Hi is also co-author of around 165 conference communications and co-inventor of 59 patents. He supervised 6 finished PhD and is a supervisor of 3 ongoing ones. In 2011 he organised 5th European Symposium on Plant Lipids in Gdańsk. He was also a member of Scientific Committees/ chairman of the Scientific Sessions of several European and International Conferences connected to plant lipids. He also popularised science by interviews in local and national journals/TV/radio.

### Research Group:

Bartosz Głąb, PhD
Katarzyna Jasieniecka-Gazarkiewicz, PhD

PhD students: Ada Połońska,
Sylwia Klińska



## Research

The research interests of this laboratory mainly concern the biochemistry and biotechnology of plant lipids.

During the last few years, the laboratory participated in the worldwide integrated project "Industrial Crops producing added-value Oils for Novel chemicals – ICON" financed by the Seventh EU Framework Programme. The key goal of this endeavour was to produce different kinds of wax esters in industrial crops, such as *Crambe abbysinica*, *Camelina sativa* or Brassica carinata. Our laboratory has specialized in the characterization of substrate species of various fatty acid reductases (FARs) and wax synthases (WSs) – the key enzymes in wax ester synthesis. Additionally, the biochemistry of wax ester mobilization in germinating is as follows: (a) jojoba seeds (plants that naturally accumulate wax esters) and (b) seeds of transgenic industrial crops producing wax esters have been investigated. Several of these research strands are continued now outside the ICON project

Beside the abovementioned research we are involved in:

- biochemical characterization and determination of substrate specificity and physiological functions of selected acyl-CoA:lysophospholipid acyltransferases,
- biochemical characterization, determination of substrate specificity and physiological function of enzyme of DGAT type (acyl-CoA:diacylglycerol acyltransferase) and PDAT-type (phospholipid:diacylglycerol acyltransferase) – key enzymes involved in triacylglycerols biosynthesis,
- potential use of biotechnology to create commercially viable plants accumulating modified storage lipids.

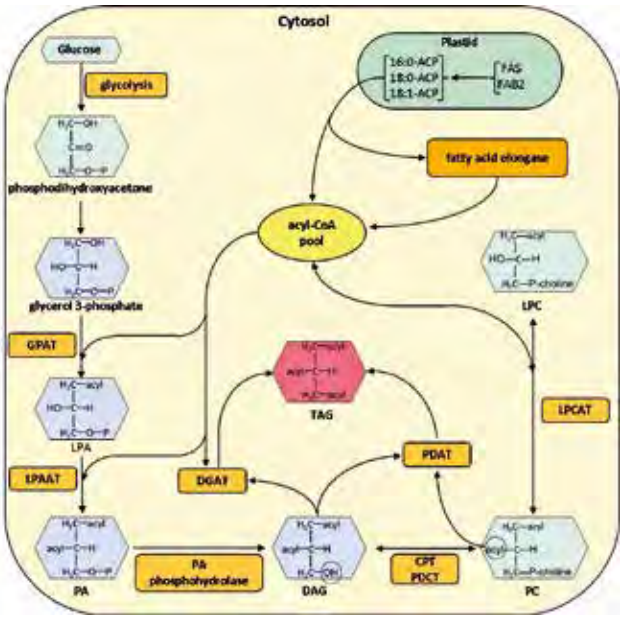



FIGURE 1.
The general scheme of the biosynthesis of triacylglycerols in seeds of oilseed plants

**GPAT** – acyltransferase acyl-CoA:3-phosphoglycerol | **LPAAT** – acyl-transferase acyl-CoA:lysophosphatidic acid |**DGAT** – acyltransferase acylo-CoA:diacylglycerol | **PDAT** – acyltransferase phospholipid:diacylg-lycerol | **CPT** – cholinephosphotransferase CDP-choline:diacylglycerol |**PDCT** – cholinephosphotransferase phosphatidylcholine: diacylglycerol | **LPCAT** – acyltransferase acyl-CoA: lysophosphatidylcholine | **FAS** – fattyacid synthase |**FAB2** – stearyl-ACP desaturase

Generally, the group aims to characterize different enzymes connected with lipid metabolism (Fig. 1), evaluate their potential application in the production of different kinds of oil in transgenic industrial oil crops and provide a biochemical basis for genetic engineering aimed at increasing the oil production capacity of oilseed crops.

### Applied research methods/laboratory techniques:

- We work with a wide variety of model microorganisms (*Escherichia coli, Saccharomyces cerevisiae, Agrobacterium tumefaciens*) and plants (Arabidopsis thaliana, Camelina sativa, rapeseed, soybean).
- In our research, we incorporate "the best of both worlds"; we utilize the classic methods of biochemistry (thin-layer and gas chromatography, in vitro assays, autoradiography, microsomal isolation) with modern approaches of molecular biology (q-RT-PCR, gatewaying, microorganism and plant transformation, plant in vitro cultivation, generating knock-outlines using CRISPR, site-directed mutagenesis).

## Publications


- Jasieniecka-Gazarkiewicz, K., Lager, I., Carlsson, AS., Gutbrod, K., Peisker, H., Dörman, P., Stymne, S., Banaś, A. 2017 Acyl-CoA:lysophosphatidylethanolamine acyltransferase activity regulates growth of Arabidopsis. Plant Physiol 174: 986-998.
- Kawiński, A., Miklaszewska, M., Stelter, Sz., Głąb, B., Banaś, A. 2021. Lipases of germinating jojoba seeds efficiently hydrolyze triacylglycerols and wax esters and display wax ester-synthesizing activity. BMC Plant Biology, 21:50; doi.org/10.1186/s12870-020-02823-4.
- Jasieniecka-Gazarkiewicz, K., Demski, K., Gidda, S., Klińska,i S., Niedojadło J., Lager, I., Carlsson, A., Minina, E., Mullen, R., Bozhkov, P., Stymne, S., Banaś, A. Subcellular localization of acyl-CoA:lysophosphatidylethanolamine acyltransferases (LPEATs) and the effects of knocking-out and overexpression of their genes on authophagy markers level and life span of A. thaliana. Int. J. Mol. Sci. 2021, 22, 3006. https://doi.org/10.3390/ijms.22063006.
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- Demski, K., Jeppson, S., Lager, I., Misztal, A., Jasieniecka-Gazarkiewicz, K., Waleron, M., Stymne, S., Banaś, A. 2019. Isoform of acyl-CoA:diacyloglicerol acyltransferase2 differ substantially in their specificities toward erucic acid. Plant Physiol 181: 1468-1479.
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- Klińska, S., Jasieniecka-Gazarkiewicz, K., Demski K., Banaś, A. 2020. Editing of phosphatidic acid and phosphatidylethanolamine by acyl-CoA:lysophospholipid acyltransferases in developing Camelina sativa seeds. Planta, 252: 4 (1-17).
- Demski, K., Łosiewska A., Jasieniecka-Gazarkiewicz, K., Klińska S., Banaś A. 2020. Phospholipid:diacylglycerol acyltransferase1 overexpression delays senescence and enhances post-heat and cold exposure fitness. Frontiers in Plant Siences, 11: 611897; doi: 10.3389/fpls.2020.611897.


## Research Grants

- National Science Centre, "The role of acyl-CoA:lysophosphatidylethanolamine acyltransferases (LPEATs) in plants ", 1 187 000 PLN, 2018-2022, PI Antoni Banaś
- National Science Centre, "Substrate specificity of LPCATs from photosynthetic oleaginous microalgae in forward and reverse reactions and characterization of their functions in acyl editing of phosphatidylcholine", 1 347 200 PLN, 2019-2023, PI Antoni Banaś.

## Scientific Collaboration

- Department of Plant Breeding and Biotechnology of the Swedish University of Agricultural Science.
- Several leading laboratories from Europe, USA, Canada and China.

# Laboratory of Molecular Biology

## Prof. Igor Konieczny

Received his PhD at the University of Gdansk in 1994 and habilitation in 2000. He is a full-professor since 2004. He carried out postdoctoral training at University of California San Diego, USA. From 1998 a member of International Society for Plasmid Biology. A member of COST Biomedical Domain (2008-2015). He was awarded by EMBO, HHMI YIP and Foundation for Polish Science. Author of 45 peer-reviewed publications, supervisor of 16 defended PhD thesis. Dean of IFB UG & MUG (2012-2020).

### Research Group:

Katarzyna Bury, PhD
Marta Gross, PhD
Dorota Pomorska, PhD
Małgorzata Ropelewska, PhD
Katarzyna Węgrzyn, PhD

PhD students: Monika Oliwa, Magdalena Sroka, Ewelina Wysocka

## Research

Our research is focused on the analysis of the molecular mechanisms responsible for plasmid and chromosomal DNA replication in bacterial cells. One model used in our investigations is the RK2 plasmid, which can be stably maintained and transferred between cells of various bacterial species, including plant, animal and human pathogens. Research utilizing such a unique system makes a significant contribution to the general understanding of fundamental cellular processes. In our investigations, achieving the controlled proteolysis of essential replication factors is a long-term task as a potential tool in developing new strategies for antimicrobial therapies. We currently concentrate on the regulation of DNA replication in bacterial cells during stress conditions. We analyse the multimolecular complexes formed on DNA by proteases, replication initiation proteins (Rep) and components of plasmid-encoded toxin/antitoxin (TA) systems.

Our major recent achievement is the discovery of PolyP-induced DnaA proteolysis (PDAP) (Gross MH. and Konieczny I., NAR. 2020; 48(10):5457-5466). It was known for years that when a bacteria encounters stress, e.g., starvation, it launches a stringent response to arrest cell proliferation. A vast amount of polymer composed of phosphate residues, i.e., inorganic polyphosphate (PolyP), is synthesized from ATP, but the role of PolyP is still ambiguous. We found that during the stringent response in Escherichia coli cells, both PolyP and Lon protease accumulate, and Lon is stimulated by PolyP proteolysis DnaA-ADP but not DnaA-ATP to arrest replication initiation. Unlike DnaA-ADP, DnaA-ATP does not interact with PolyP but binds to the dnaA promoter to block dnaA transcription. The uncovered regulatory mechanism interlocks PolyP-dependent protease activation with the ATP/ADP cycle of DnaA. These data are a continuation of our previously published work on Lon protease, where we demonstrated the engagement of positively charged amino acid residues located on the ATPase domain surface in interaction with DNA (Karłowicz A., Węgrzyn K. et al., JBC 2017). We also found that E. coli Lon and ClpAP interact and disrupt the handcuff complex by degrading Rep proteins interacting with DNA, thus affecting plasmid stability (Bur K. et al., NAR 2017). Additionally, our investigations of the plasmid-encoded TA system (Dubiel A., et al., Sci Rep. 2018) showed that the ParD antitoxin is degraded by ClpAP homologous proteases, and dsDNA stimulates this process. We challenge the question about the molecular mechanism(s) of protease activity modulation by interaction with nucleic acids and PolyP.

POLYP-DEPENDENT DNAA PROTEOLYSIS (PDAP)

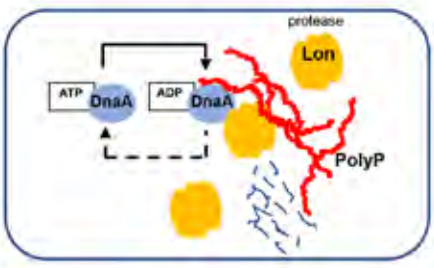


FIGURE 1.
Polyphosphate induces the proteolysis of ADP-bound fraction of initiator to inhibit DNA replication initiation upon stress in Escherichia coli (Gross and Konieczny NAR 2020).

Our second major recent achievement is the progress we made in the analysis of intrinsically disordered Rep proteins and their complexes with double-stranded and single-stranded DNA. After discovery that Rep proteins bound both double-stranded DNA (dsDNA) and similar structures as chromosomal DnaA, they also interact with

ssDNA (Wegrzyn K. et al., NAR 2014) we structurally characterize full length 33 kDa TrfA protein, an RK2 plasmid replication initiator, complex on dsDNA and defined a novel domain that provides an essential contribution to site-specific interaction of Rep protein with DNA (Wegrzyn K, Zabrocka E, et al., NAR 2021). In our studies, we applied a vast number of experimental approaches, including mutant construction and phenotypic analysis, reconstituted DNA replication assays, real-time kinetics of protein-DNA complexes, crosslinking studies combined with mass spectrometry (MS), and advanced bioinformatic approaches. It is a multidisciplinary study involving broad collaboration with many research groups. We are currently conducting crystallographic analysis to solve the structure of the Rep complex with ssDNA.

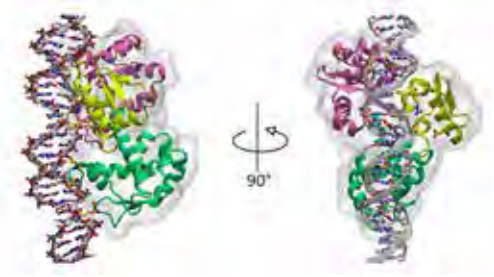


FIGURE 2.
Structural model of 33kDa TrfA protein in a complex with double-stranded DNA. A novel domain that provides an essential contribution to site-specific interaction of Rep protein with DNA is marked in magenta (Wegrzyn and Zabrocka et. al. NAR 2021).

We also recently set up the new high-speed atomic force microscope MS-NEX (RIBM Co., Ltd.), which would allow us to implement this technique in our research.

## Publications


- Romanowska A, Węgrzyn K, Bury K, Sikorska E, Gnatek A, Piwkowska A, Konieczny I, Lesner A, Wysocka M. (2021) Novel Cell Permeable Polymers of N-Substituted L-2, 3-Diaminopropionic Acid (DAPEGs) and Cellular Consequences of Their Interactions with Nucleic Acids. Int J Mol Sci. 22(5):2571. doi: 10.3390/ijms22052571.
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## Research Grants

- Fundation for Polish Science, TEAM (POIR.04.04.00-00-5C75/17-00) "Condition-dependent protease activation for targeted proteolysis in the regulation of DNA replication", 3 470 070 PLN, 2018-2022, Igor Konieczny.
- National Science Center SONATA (2017/26/D/NZ1/00239) "Structural-functional analysis of nucleoprotein complexes of plasmid Rep proteins and ssDNA DUE origin region", 833 000 PLN, 2018-2022, Katarzyna Węgrzyn.
- National Science Center MINIATURA 1 (2017/01/X/NZ1/00079) "Analysis of ORC proteins interactions with ssDNA of origin region – preliminary research", 2017-2018, Katarzyna Węgrzyn.

## Scientific Collaboration

- Rafael Giraldo, Fernando Moreno-Herrero, CNB-CSIC (Madrid, Spain).
- Jan Lowe, MRC Laboratory of Molecular Biology (Cambridge UK).
- Dmitri Svergun, EMBL (Hamburg, Germany).
- Martin Zacharias, Technische Universität München (Germany).
- Eva Top, University of Idaho (USA).
- Chris Thomas, University of Birmingham (UK).
- Jolanta Zakrzewska-Czerwińska University of Wrocław (Poland).
- Janusz Bujnicki, Marcin Nowotny IIMCB (Warsaw, Poland).
- Jacek Czub, Technical University of Gdansk (Poland).

## Research Awards

- 2019 COST Action grant for Training School "Workshop for the hydrodynamic and thermodynamic analysis of biological macromolecules and their interactions: multi-method approaches and global data analysis", KW.
- 2018 Distinction from Committee of the Cell Molecular Biology of Polish Academy of Science (PAN) for the publication in Nucleic Acid Research DOI: 10.1093/NAR/GKX166.

# Laboratory of Virus Molecular Biology

## Prof. Krystyna Bieńkowska-Szewczyk

Graduated in 1976 at University of Gdansk, obtained PhD in biochemistry (1982) at the Faculty of Biology, Geography and Oceanology of University of Gdansk, habilitation awarded at the same Faculty in 2002. Full professorship since 2008. Postdoctoral research: Max Planck Institute, Tubingen (Germany), University of California San Francisco (USA), University of Utah, (USA), The Institute for Animal Science and Health (The Netherlands). Since 1998 a partner in five EU international projects founded by European Framework Programs. Vice-dean of IFB for 6 years. The host of European Congress of Virology in 2022.

### Research Group:

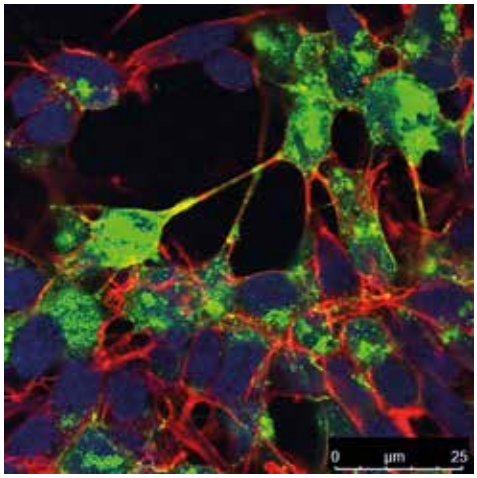
Andrea Lipińska, PhD,
Alicja Chmielewska PhD,
Michał Rychłowski PhD,
Katarzyna Grzyb PhD,
Malgorzata Graul PhD

PhD students: Natalia Derewońko, Kinga Grabowska, Magda Wąchalska, Patryk Zalewski, Marcin Lubocki

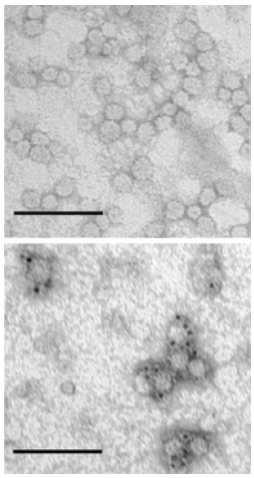
## Research

Our research is focused on investigating various aspects of virus-host interactions, including virus entry, spread, and the modulation of the immune response. We are also interested in the construction of viral vectors for vaccine and therapeutic purposes. Until 2020, our main models were animal and human herpesviruses and human hepatitis C virus (HCV). Since the beginning of the SARS-CoV 2 pandemic, our research interest has also turned to coronaviruses. To study our model viruses, we use other viruses as tools: adenoviruses to create vaccine vectors, retroviruses to introduce genes into mammalian cells and baculoviruses to produce proteins in insect or mammalian cells. Our experimental expertise covers a wide range of cell biology and virology methods, such as the construction of live viral mutants with deleted or modified genes and the construction of viral pseudoparticles and stable cell lines expressing the viral genes of interest. Laser confocal microscopy is widely used in our laboratory for studies of viral proteins in infected cells and analysis of fluorescent viral mutants spread in live cells. The examples of recent research projects include:

Investigation of virus direct transmission, "cell-to-cell" spread mode. Some viruses use this mode of transmission, which is faster than entry from outside and allows them to move between adjacent cells without being exposed to the immune response of the host. Using a panel of recombinant fluorescent animal herpesvirus (BHV-1) mutants constructed in our laboratory, we demonstrated that herpesviruses can be transmitted between distant cells via specialized long-range intercellular connections, tunnelling nanotubes (TNTs). We explore the molecular mechanism of cell-to-cell spread using new methods developed in our laboratory.




Human neuroblastoma cells infected by BHV-1-GFP



Virus-like particles formed by HBV surface antigen exposing hepatitis C virus epitopes

**The mechanism of immune evasion:** We study various strategies used by viruses to prevent recognition by the immune system of the host. Structural studies and protein modelling followed by side-directed mutagenesis are employed to elucidate the mechanism of activity of the UL49.5 protein, the main immunomodulatory protein in some herpesviruses, which downregulates major histocompatibility class I (MHC I). Using fluorescently labelled cellular target protein-TAP transporter and genome-wide siRNA screens, we search for cellular proteins involved in the mechanism of inhibition.

The methodology developed for our research on herpesviruses will be used to study the properties of SARS-CoV-2 and other coronaviruses with the aim of contributing to the understanding of the pathogenesis of these viruses and identifying targets for future therapies and vaccines. We are currently adapting our core BSL3 virus culture laboratories to the highest safety level required for work with human respiratory pathogens.

The development of a new oncolytic vector, BHV-1, the model alpha-herpesvirus used in many studies in our laboratory, is a species-specific virus that replicates only in cells of bovine origin. However, we have shown that BHV-1 can also infect and destroy human cancer cells while leaving healthy cells intact, which makes BHV-1 a promising candidate for cancer therapies. At present, the project is focused on thorough characterization of the oncolytic potential of this virus and understanding its interaction with cancer cells.

Viral infection in the context of the regulation of interferon (IFN) signalling: antiviral properties of interferon-induced transmembrane proteins (IFITMs) are investigated during infection and spread of hepatitis C virus (HCV), tick-borne encephalitis virus (TBEV) and coronaviruses. SARS-CoV-2 entry studies are based on pseudoparticles, a model system based on pseudotyped lentiviruses, which we previously used during our work on HCV vector vaccines.

Construction of an experimental bivalent prophylactic vaccine against HBV and HCV: hepatitis B (HBV) surface antigen (sHBsAg) forms subviral particles that are widely used in the control of HBV infection. We analyze the immunogenic properties of conserved epitopes of HCV E2 glycoprotein and nonstructural protein NS3 exposed to sHBsAg particles to trigger both humoral and cellular responses against HCV, aiming at the development of a cost-effective bivalent prophylactic vaccine against the two main hepatotropic human pathogens.

Apart from research activity, our group has been involved since 2020 in many SARS-CoV-2 pandemic-related activities: diagnostics, media activity, and the popularization of virology and vaccine science in the forms of lectures, webinars, and articles for the medical community.

## Publications


- Brzozowska A, Lipińska AD, Derewońko N, Lesiak D, Rychłowski M, Rąbalski Ł, Bieńkowska-Szewczyk K.(2018) Inhibition of apoptosis in BHV-1-infected cells depends on Us3 serine/threonine kinase and its enzymatic activity. Virology,513:136-145.
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## Scientific Grants


- National Science Centre (NCN) - MAESTRO 2: Mechanism of virus transmission between cells: strategies to survive, 2011-2018, 2 300 000, PI Krystyna Bienkowska-Szewczyk.
- National Science Centre (NCN) -SONATA-BIS: The molecular mechanism of activity of alphaherpesvirus-encoded key immune evasion proteins. 2015-2021, 1 870 760 PLN, PI: Andrea Lipinska.
- National Science Centre (NCN) — OPUS 14 Immunogenic properties of hepatitis C virus NS3 protein epitopes fused to hepatitis B small surface antigen ,1 410 400 PLN, 2018-2021, PI: Katarzyna Grzyb.
- Foundation for Polish Science (FNP) — Powroty : Antiviral activity of interferon induced transmembrane proteins (IFITM) as a novel therapeutic strategy to control viral infections — evaluation in vitro and in vivo, 2018-2021, 999.713 PLN, PI: Alicja Chmielewska.
- Ministry of Science and Higher Education (MNISW) infrastructure grant for adaptation of virology laboratory to BSL3+ safety level 2020-2021 , 4 000 000 PLN, PI: Krystyna Bienkowska-Szewczyk.


## Patents


- An immunogenic vaccine against the HCV and/or HBV, 2019-08-28, nr: EP3244921, European Patent Office, Anna Czarnota, Katarzyna Grzyb.


## Scientific Collaboration

- Institute of Virology, MRC Glasgow (Prof. Arvind Patel).
- Reithera company, Italy (Prof. Alfredo Nicosia).
- Gent University, Belgium (Prof.Philip Meuleman).
- University of Edinburgh, UK (Prof. Kathryn Ball).
- University of Copenhagen, Denmark (Dr. Judith Gottwein).
- University of Utrecht, Netherlands (Prof. Emmanuel Wiertz).

# Structural Biology Laboratory

## Assoc. Prof. Michał R. Szymański

Completed his studies in Biochemistry and Biophysics at the University of Houston, USA (2007) and earned his PhD in Biochemistry and Molecular Biology from the University of Texas, USA (2011). Carried out Postdoctoral Fellowship at the Department of Biochemistry and Molecular Biology (2011-2013) and Department of Pharmacology (2013-2016), UTMB, USA where he later was a Research Scientist (2016-2017). After receiving POLONEZ (NCN) and FIRST TEAM (FNP) grants, he joined the IFB in 2017. In 2019 he won the prestigious ERC Starting Grant, EMBO Installation Grant and received his Habilitation. He is a Head of the Structural Biology Laboratory since 2019.

### Research Group:

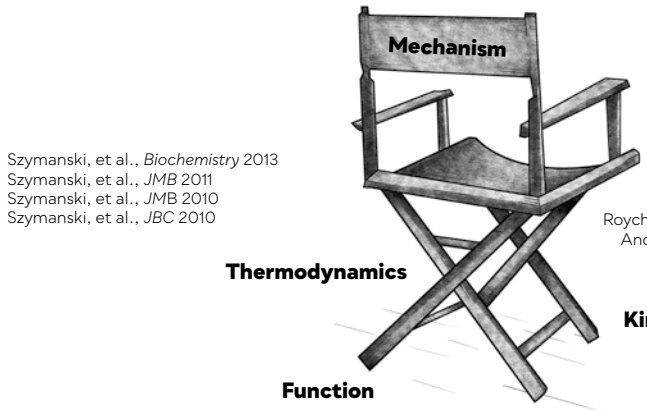
Anna Karłowicz, PhD
Ewa Dzik, PhD
Karolina Kwiatkowska-Semrau, PhD
Andrzej Dubiel, PhD

PhD Students: Adela Bledea, Marta Grzelewska, Piotr Purzycki, Michał Majewski
Olga Jóźwiak - undergraduate student

## Research

We aim to understand the basic principles underlying the assembly of the multiprotein macromolecular machines involved in nucleic acid metabolism and to characterize their structure and function to gain insight into their mechanism of action and regulation.

FIGURE 1. Research philosophy in the Szymanski lab




Szymanski, et al., *Biochemistry* 2013
Szymanski, et al., *JMB* 2011
Szymanski, et al., *JMB* 2010
Szymanski, et al., *JBC* 2010

Roychowdhury, et al., *Biochemistry* 2009
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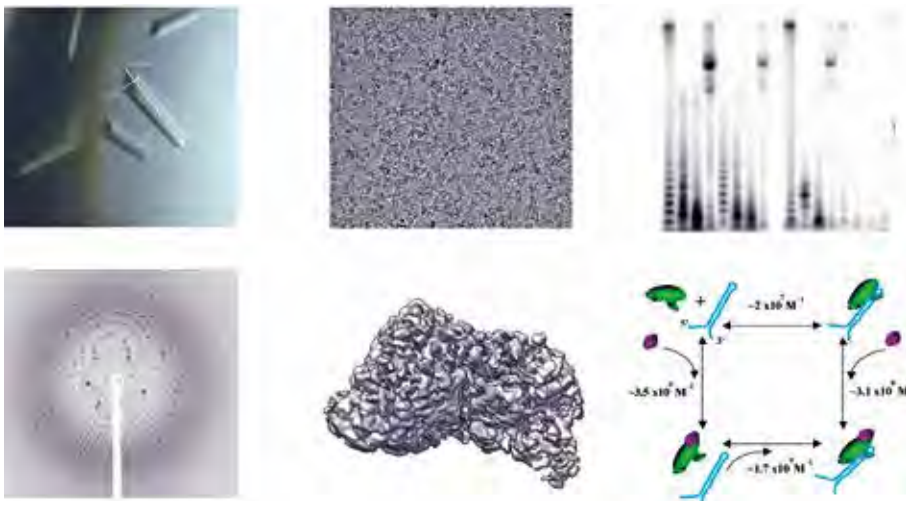
Jain, et al., *Science* 2020
Szymanskix, et al., *JMB* 2009
Duret, Szymanski, et al., *JBC* 2008
Szymanski, et al., *Biochemistry* 2011

Szymanski, et al., *EMBO J* 2015
Reyes-Aldrete, et al., *Viruses* 2020
Sohl*, Szymanski*, et al., *PNAS* 2015
Szymanski, et al., *Nature Comm* 2017

Research philosophy in the Szymanski laboratory: kinetics and thermodynamics are, along with structure and function, the legs of a chair on which we set the understanding of the macromolecular mechanism (Fig. 1). Thus, we combine structural biology (X-ray crystallography and cryo-electron microscopy), molecular biology, protein biochemistry and thermodynamic and kinetic approaches to understand the function of large macromolecular machines at the molecular level (Fig. 2).

FIGURE 2. The latest results form the Szymanski lab



We are currently investigating several large macromolecular machines. For example, we aim to understand the assembly, structure and function of MitoRepairosome, a multiprotein DNA repair toolkit from human mitochondria. In another project, we aim to target enzymes responsible for mitochondrial DNA repair with small molecule inhibitors to modulate their activity. We are also interested in the mitochondrial replisome, a multiprotein macromolecular machine responsible for human mitochondrial DNA replication, and the primosome and DNA replication restart machinery from E. coli. We purify these complexes, reconstitute their biochemical activities, and determine their structures to gain mechanistic insights.

## Publications


- Jain N., Blauch, L.R., Szymanski, M.R., Das, R., Tang, S.K.Y., Yin, Y.W., Fire, A.Z. Transcription polymerase-catalyzed emergence of novel RNA replicons. Science. 2020 Mar 26. pii: eaay0688. doi: 10.1126/science.aay0688. (doi: 10.1126/science.aay0688).
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- Szymanski, M.R., Jezewska, M.J., and Bujalowski, W. The Escherichia coli PriA helicase specifically recognizes gapped DNA substrates: effect of the two nucleotide-binding sites of the enzyme on the recognition process. Journal of Biological Chemistry. 2010 Mar 26;285(13):9683-96.
- Szymanski, M.R., Fiebach, A.R., Tratschin, J.D., Gut, M., Ramanujam, V.M., Gottipati, K., Patel, P., Ye, M., Ruggli, N., and Choi, K.H. Zinc binding in pestivirus N(pro) is required for interferon regulatory factor 3 interaction and degradation. Journal of Molecular Biology. 2009 Aug 14;391(2):438-49.


## Research Grants

- European Research Council (ERC) Starting Grant; "Dissecting the mechanism of DNA repair in human mitochondria (MitoRepairosome)"; 1.5 million €; 2020-2025; Michal R. Szymanski.
- European Molecular Biology Organization (EMBO), Installation Grant; "Structural basis for DNA repair in human mitochondria"; 150k €; 2019-2022; Michal R. Szymanski.
- The Foundation for Polish Science (FNP), First Team 3 Grant; "Targeting mitochondrial DNA repair for novel anti-cancer therapies"; 855k €; 2018-2023; Michal R. Szymanski.
- National Science Centre of Poland (NCN), POLONEZ 2 Grant; Unraveling the molecular basis of DNA damage recognition and processing in human mitochondria; 240k €; 2017-2019; Michal R. Szymanski.

## Patents

- Patent Application, U.S. Application Serial No. 62/872,540, RNA Replication Using Transcription Polymerases, July 10, 2019.

## Scientific Collaboration

- Dr. Andrew Fire, Stanford University, CA, USA.
- Dr. Bartosz Szczesny, University of Texas Medical Branch at Galveston, Galveston, TX, USA.
- Dr. Whitney Yin, University of Texas Medical Branch at Galveston, Galveston, TX, USA.
- Dr. Ted Hupp, International Centre for Cancer Vaccine Science, UG, Gdansk, Poland.
- Dr. Roman Szczęsny, IBB PAN, Poland.

## Research Awards

- University of Gdansk Rectors Award for Research Achievements (2019).
- EMBO YIP Member.
- EMBO Best Poster Award, Svartsjö, Sweden (2018).

# Laboratory of Molecular Diagnostics

## Prof. Krzysztof P. Bielawski

He graduated from University of Gdansk, received PhD in medical biology at Medical University of Gdańsk. He carried out postdoctoral work in Germany, Sweden and France. He is a full-professor in biological sciences. Author of > 100 peer-reviewed publications, supervisor of 11 finished and 3 ongoing PhD's. He is a Director of the Technology Transfer Office at UG and CEO of TechTransBalt Ltd. at UG. Currently, he is a Vice-Rector for Innovation and Liaison with Business and the Community of University of Gdansk.

### Research group

Agnieszka Bernat-Wójtowska, PhD
Mariusz Grinholc, PhD
Joanna Nakonieczna PhD
Magda Rybicka, PhD
Aleksandra Rapacka-Zdończyk, PhD

PhD students: Jan Barczyński, Beata Kruszewska, Klaudia Michalska, Michał Mikitiuk, Patrycja Ogonowska, Paulina Pełka, Michał Pierański, Agata Woźniak

## Research

Our research includes two fields of interest: research related to the photodynamic activation (PDI) of bacterial infections and the application of modern tools of molecular biology to the diagnosis of cancer and the treatment of metabolic and infectious diseases.

PDI is based on the concept that metabolically active cells, such as bacteria and fungi, accumulate a photosensitizer (PS), a small-molecule compound, that is excited by visible light. In an oxygen-rich environment, the surplus of energy or an electron is passed to molecular oxygen, giving rise to singlet oxygen and other reactive oxygen species with a cytotoxic effect on living cells. PDI is a method in which the generation of resistance has never been shown so far. Moreover, PDI acts equally effectively on microbial strains that are either susceptible or resistant to classical antimicrobial drugs. Therefore, it seems to be a promising therapeutic option for the treatment of multidrug-resistant local infections.

The research carried out in our laboratory focuses on both basic and applied research. In our group, we are studying the mechanisms that govern the different responses of microorganisms to photoinactivation, including biofilm formation, antioxidant enzyme activity, DNA damage, and bacterial transporters (Fig. 1). As one of the main limitations of the method is the low selectivity of the PS used in PDI, the idea is to design a PS molecule that is efficient against gram(+) and/or gram(-) pathogens, specifically multiresistant strains, and functionalization of the PS molecule with cell delivery systems to improve the action of PDI. PS must accumulate inside the bacterial cell or in proximity to its envelope to efficiently kill bacteria while remaining nontoxic to human tissue.

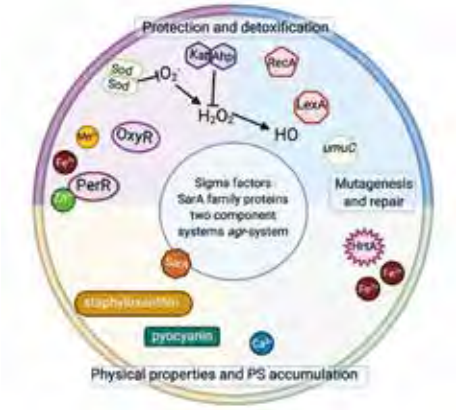



FIGURE 1. Bacterial molecular targets involved in antimicrobial photoinactivation (PDI).

Despite the establishment of monitoring of resistant strains and the level and structure of antibiotic consumption, the problem of bacterial infections continues to grow, especially in the case of multidrug-resistant strains belonging to the ESKAPE pathogens (*Enterococcus spp., Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp.). Our research projects focus on the development and creation of therapeutic options to reduce the use of antibiotics and the rate of emerging drug resistance mechanisms. For the main human pathogens that are resistant to multiple drugs, such as extensively drug-resistant (XDR) microorganisms, we analysed the effect of photodynamic inactivation (PDI), both using blue light (405 nm) and in combination with photosensitizing compounds to help combat microorganisms in vitro and in vivo.

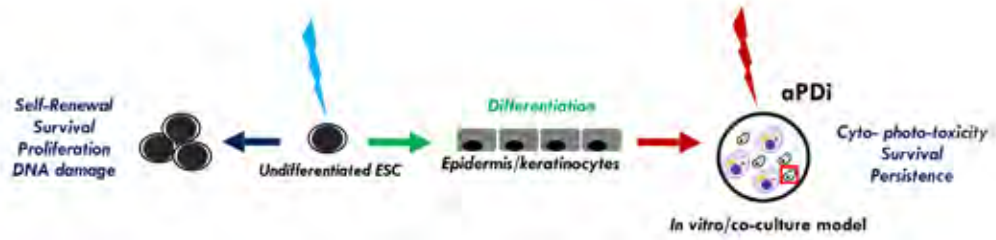



FIGURE 2 Photoinactivation (PDI) studied at eucaryotic models.

We also focus on the eradication of skin-colonizing S. aureus (including MRSA) in atopic dermatitis with PDI, and more recently, our interest has been directed toward studying the response of eukaryotic cells to light treatment. We implemented the coculture method of immortalized keratinocytes infected with S. aureus for the analysis of bacterial persistence in cells on one side, and the influence of bacterial infection on the proliferation of keratinocytes in real time (Fig. 2). Additionally, we aim to study the effect of light on self-renewal, proliferation, DNA damage and cell differentiation toward epidermal linage in embryonic stem cells.

In our research, we apply a mouse model of a wound infected with bioluminescent MRSA or skin colonized with S. aureus to mimic the same disease in humans. The use of bioluminescent strains allows us to monitor infection in mouse wounds in real time. We believe that the PDI approach would be an alternative therapeutic option for treating infected wounds or decolonizing skin.

## Recent Publications


- Genetic variation in IL-10 influences the progression of hepatitis B infection. Rybicka M, Woziwodzka A, Sznarkowska A, Romanowski T, Stalke P, Dręczewski M, Verrier ER, Baumert TF, Bielawski KP.
- Ogonowska P, Nakonieczna J. Validation of stable reference genes in Staphylococcus aureus to study gene expression under photodynamic treatment: a case study of SEB virulence factor analysis. Sci Rep. 2020 Oct;10(1):16354.
- M. Pieranski, I. Sitkiewicz, M. Grinholc. Increased photoinactivation stress tolerance of Streptococcus agalactiae upon consecutive sublethal phototreatments. Free Radicals Biology and Medicine, Volume 160, 20 November 2020, Pages 657-669.
- Rybicka M, Woziwodzka A, Sznarkowska A, Romanowski T, Stalke P, Dręczewski M, Verrier ER, Baumert TF, Bielawski KP. Liver Cirrhosis in Chronic Hepatitis B Patients Is Associated with Genetic Variations in DNA Repair Pathway Genes. Cancers (Basel). 2020 Nov 7;12(11):3295. doi: 10.3390/cancers12113295.
- Kossakowska-Zwierucho M, Szewczyk G, Sarna T, Nakonieczna J. Farnesol potentiates photodynamic inactivation of Staphylococcus aureus with the use of red light-activated porphyrin TMPyP. J Photochem Photobiol B. 2020 Mar 21;206:111863.
- Rapacka-Zdonczyk A, Wozniak A, Pieranski M, Woziwodzka A, Bielawski KP, Grinholc M. Development of Staphylococcus aureus tolerance to antimicrobial photodynamic inactivation and antimicrobial blue light upon sub-lethal treatment. Scientific Reports, 2019, 1;9(1):9423. doi: 10.1038/s41598-019-45962-x.
- Fila G., Krychowiak M., Rychlowski M., Bielawski K.P., Grinholc M. Antimicrobial Blue Light Photoinactivation of Pseudomonas aeruginosa: Quorum Sensing Signaling Molecules, Biofilm Formation and Pathogenicity. Journal of Biophotonics, 2018, 11:e201800079, DOI: 10.1002/jbio.20180 0079.
- M. Brasel, M. Pieranski, M. Grinholc. An extended Logistic model of photodynamic inactivation for various levels of irradiance using the example of Streptococcus agalactiae. Scientific Reports, 10, 14168 (2020). https://doi.org/10.1038/s41598-020-71033-7.
- Nakonieczna J, Wolnikowska K, Ogonowska P, Neubauer D, Bernat A, Kamysz W. Rose Bengal-Mediated Photoinactivation of Multidrug Resistant Pseudomonas aeruginosa Is Enhanced in the Presence of Antimicrobial Peptides.
- M. Brasel, M. Pieranski, M. Grinholc. An extended Logistic model of photodynamic inactivation for various levels of irradiance using the example of Streptococcus agalactiae. Scientific Reports, 10, 14168 (2020). https://doi.org/10.1038/s41598-020-71033-7.


## Research Grants


- National Science Centre (NCN) SHENG, Development and study of photodynamic and Ga loaded highly efficient and dual-functional antimicrobial agents; 1 752 800 zł; 2019-2022; PI Mariusz Grinholc.
- National Science Centre (NCN) OPUS; The influence of antimicrobial photoinactivation on virulence of Staphylococcus aureus strains colonizing atopic dermatitis patients: in vitro and in vivo studies; 949 000 zł; 2018-2022; PI Joanna Nakonieczna).
- National Science Centre (NCN) OPUS; Derivation and indentification of pluripotency traits of induced pluripotent stem cells from Bison bonasus bonasus; 829 920 zł; 2018-2021; PI Agnieszka Bernat-Wójtowska.
- National Science Centre (NCN) OPUS; Evaluation of photoinactivation potential in the eradication of Streptococcus agalactiae carrier-state in the urogenital system: in vitro and in vivo studies; 671 600 zł; 2017-2021; PI Mariusz Grinholc).
- National Science Centre (NCN) OPUS; Antimicrobial photoinactivation as an effective tool for sensitization of multidrug resistant ESKAPE pathogenes to antimicrobials; 662 400 zł; 2016-2020, PI Mariusz Grinholc.


## Scientific Collaboration

- University Hospital Miguel Servet, Zaragoza, Spain (Prof. Yolanda Gilaberte).
- Medical University of Gdansk, Poland (Prof. Wioletta Barańska-Rybak).
- University of Toronto, Canada (Prof. Lothar Lilge).
- Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University (Prof. Tadeusz Sarna).
- INSERM U1208 Stem-cell and Brain Research Institute, France (Prof. Pierre Savatier).
- Experimental Embriology Department, Institute of Genetics and Animal Biotechnology Polish Academy of Sciences (Prof. Anna Piliszek, dr Katarzyna Filimonow).
- School of Chemical Engineering and Technology, Tianjin University, China (Prof. Lei Zhang).

# Laboratory of Biological Plant Protection

## Assoc. Prof. Sylwia Jafra

Received her PhD in biological sciences (1999) at the University of Gdansk. In 1996-1998, she carried out several lab trainings in INSA de Lyon, France. She accomplished her postdoctoral training at PRI (WUR), Wageningen, The Netherlands (2001-2003). She got habilitation in biochemistry in 2011. She held the post of a Vice-Dean for students' and educational affairs for two terms of the office (2012-2020). In 2013 and 2020, she was awarded the Mrongovius Teacher of the Year awards to the best academic teachers at the University of Gdansk.

### Research Group:

Dorota Krzyżanowska, PhD
Magdalena Rajewska, PhD

PhD students: Tomasz Maciąg, Marta Matuszewska, Magdalena Jabłońska,

## Research

Our research focuses on understanding the plant-bacteria interaction occurring in the plant rhizosphere, emphasizing plant-beneficial bacteria with application potential in biological control. Our research goal is to understand the molecular mechanisms

i) involved in plant-bacteria interactions, including adhesion and biofilm formation; and

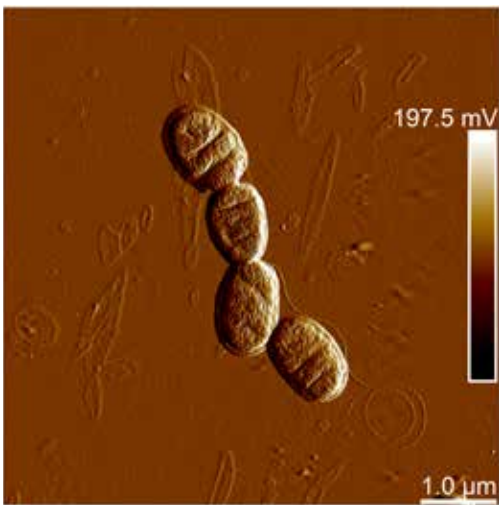
ii) Determining the antimicrobial activity of the plant-associated bacteria from the *Pseudomonas*, *Bacillus* and *Ochrobactrum genera*.

We are also interested in bacterial cell-to-cell communication (quorum sensing, QS) and gene expression regulation mechanisms in response to environmental cues mediated via signalling molecules. Our key focus here is the silencing of QS (quorum quenching, QQ) via enzymatic disruption of the signalling molecules. We explore this topic both in terms of the ecological role of QQ in bacteria that produce QS-interfering enzymes and its potential to attenuate the QS-dependent virulence of plant pathogens.

Knowledge concerning successful competitive strategies of microbes could be applied to improve the efficiency of ecological, microbial-based plant protection products. The competitive mechanisms employed by the plant-beneficial bacteria confer a better attachment to the root surface, effective catabolism of the available nutrients, iron sequestration and successful competition with microbial cohabitants.

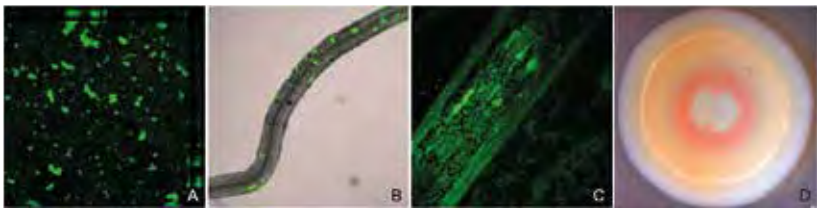
In the reporting period, our primary research focused on the characterization of *Ochrobactrum* sp. A44 strain isolated from the potato rhizosphere. *Ochrobactrum* sp. A44 interferes with the QS mechanism of the plant pathogenic bacterium Pectobacterium carotovorum and attenuates plant tissue maceration caused by this pathogen. *Ochrobatrum* sp. A44 degrades N-acyl homoserine lactones (AHLs), the signalling molecules of gram-negative bacteria, due to AiiO hydrolase activity. Our goals were to classify the A44 strain taxonomically and determine the endogenous role of AiiO in this strain's metabolism and ecological fitness.

The taxonomic classification is based on genetic and phenotypic traits of the strain. We determined the phylogenetic relationship of the A44 strain based on 16S rRNA and MLST analyses, followed by genome-based phylogeny and subsequent phenotypic characterization. All these factors allowed us to classify A44 as a novel species, *Ochrobactrum quorumnocens*, and establish this QS-interfering strain, A44T, as the type strain.




Micrograph of Ochrobactrum quorumnocens A44T cells taken with atomic force microscope.

We are continuing our research on the role of AiiO hydrolase in the metabolism and fitness of A44. For this purpose, we developed RT-qPCR to quantify gene expression changes in A44. First, the reference genes for the normalization of the data obtained for *O. quorumnocens* A44 were established. Subsequently, aiiO gene expression upon exposure to two AHLs was studied. We found that the synthesis of AiiO is not activated via the tested AHLs, as the expression of the aiiO gene did not increase above the 1-fold significance threshold.




Biofilm formed by wild-type *Pseudomonas donghuensis* P482 on the abiotic surface glass (A) under minimal M9 medium conditions and on host plant root tissues tomato (B) and maize (C), showing efficient plant colonization by P482 (CLSM images). (D) P482 wt colony morphology on Congo Red/CBB medium plates (stereomicroscopy).

We also participated in the project Lider VI led by R. Czajkowski, focusing on selecting and applying the artificial bacterial consortium to protect potato tubers against soft rot disease caused by bacteria from the Pectobacteriaceae family. A successful consortium was formulated for tuber application, and its efficacy was verified in potato tubers under storage conditions.

Another project carried out within the group concerns biofilm formation by *Pseudomonas donghuensis* P482. We aim to identify and analyse genetic factors associated with biofilm formation by P482 on artificial surfaces and plant tissues in relation to environmental factors. For this purpose, we constructed mutants of genes involved in bacterial motility, the synthesis of exopolysaccharides and other biofilm matrix components, proteins determining cells' adhesion to various surfaces. We have studied how the carbon source, iron availability, temperature, and plant host presence influence biofilm-related processes in P482.

## Publications


- Krzyżanowska DM, Supernat A, Maciąg T, Matuszewska M, Jafra S. 2019. Selection of reference genes for measuring the expression of aiiO in Ochrobactrum quorumnocens A44 using RT-qPCR. Sci. Rep. 9, 13129.
- Krzyżanowska DM, Maciąg T, Ossowicki A, Rajewska M, Kaczyński Z, Czerwicka M, Rąbalski Ł, Czaplewska P, Jafra S. 2019. Ochrobactrum quorumnocens sp. nov., a quorum quenching bacterium from the potato rhizosphere. PLoS ONE 14(1): e0210874.
- van der Wolf J, De Boer S, Czajkowski R, Cahill G, van Gijsegem F, Davey T, Dupuis B, Ellicott J, Jafra S, Kooman M, Tsror L, Yedidia I, van der Waals J. 2020. Disease Management (Chapter 7) - in "Plant Diseases Caused by Dickeya and Pectobacterium Species". Ed. 1. Eds.: F. Van Gijsegem, J. M. van der Wolf, I. Toth. Springer International Publishing, Springer Nature Switzerland AG – doi:10.1007/978-3-030-61459-1
- Krzyżanowska DM, Maciąg T, Siwińska J, Krychowiak M, Jafra S, Czajkowski R. 2019. Compatible mixture of antagonistic bacterial strains developed to effectively control soft rot caused by Pectobacterium spp. and Dickeya spp. on potato tubers. Plant Disease 2019 103:6, 1374-1382.
- Czajkowski R, Fikowicz-Krosko J, Maciąg TM, Rabalski L, Czaplewska P, Jafra S, Richert M, Krychowiak-Masnicka M, Cotte-Pattat N. 2020. Genome-wide identification of Dickeya solani transcriptional units upregulated in response to plant tissues from a crop-host Solanum tuberosum and a weed-host Solanum dulcamara. Front. Plant Sci. 11:580330.
- Szpakowska N, Kowalczyk A, Jafra S, Kaczynski Z. 2020. The chemical structure of polysaccharides isolated from the Ochrobactrum rhizosphaerae PR17T. Carbohydr. Res. 497:108136.
- Maciag T, Krzyzanowska DM, Jafra S, Siwińska J, Czajkowski R. (2020) The Great Five - an artificial bacterial consortium with antagonistic activity towards Pectobacterium spp. and Dickeya spp.: formulation, shelf life, and the ability to prevent soft rot of potato in storage. Appl. Microbiol. Biotechnol. 104: 4547-4561.
- Lisicka W, Fikowicz-Krosko J, Jafra S, Narajczyk M, Czaplewska P, Czajkowski R. 2018. Oxygen availability influences expression of Dickeya solani genes associated with virulence in potato (Solanum tuberosum L.) and chicory (Cichorium intybus L.). Front. Plant Sci. 21:374.


## Research Grants


- National Science Centre (NCN), OPUS 13 (2017/25/B/NZ9/00513) "Comprehensive analysis of the interactions between plant-beneficial strain P482 of Pseudomonas donghuensis and the mono- (maise) and dicot plants", 1 251 000 PLN, 2018-2022, PI Sylwia Jafra.
- National Science Centre (NCN), OPUS 7 (2014/13/B/NZ9/02136) "The role of the aiiO gene in the metabolism of Ochrobactrum sp. A44 and the ability of this bacterium to efficiently colonise the potato rhizosphere", 551 478 PLN, 2015-2019, PI Sylwia Jafra.
- National Science Centre (NCN), SONATA 8 (2015/19/D/NZ9/03588) "Genetic background and impact of environmental factors on biofilm formation by the beneficial strain of bacteria, Pseudomonas donghuensis P482, in biotic and abiotic conditions", 677 560 PLN, 2016-2021, PI Magdalena Rajewska.


## Scientific Collaboration

- The University of Nottingham, Centre for Biomolecular Science, Nottingham, UK (Prof. Paul Williams).
- Julius-Kuhn Institute, Braunschweig, Germany (Dr Adam Schikora).
- Nederlands Instituut voor Ecologie (NIOO-KNAW). Wageningen, The Netherlands (Dr Paolina Garbeva)
- Wagenignen UR, Plant Research International, Wageningen, The Netherlands (Dr Jan van der Wolf).
- ARO, The Volcani Center, Institute of Plant Sciences, Ornamental Plants and Agricultural Biotechnology Dep., Bet-Dagan, Israel (Dr Iris Yedidia).

# Laboratory of Plant Protection and Biotechnology

## Prof. Ewa Łojkowska

Graduated in 1977, PhD in 1984, habilitation in 1991. Postdoc at University of Madison-Wisconsin, USA (1986-1988) and INSA Lyon, France (1992-1993). Full professor since 2001. Dean of IFB UG & MUG (2005-2012). Author of more than 120 peer-reviewed publications, supervisor of 20 finished PhDs. Head of the Committee for Biotechnology PAS, President of the Jury L'Oreal Poland for Women in Science and member of the L'Oreal International Rising Talents Selection Committee. President of the Board of Waclaw Szybalski Foundation.

### Research Group:

Małgorzata Waleron, Assoc Prof.
Anna Ihnatowicz, PhD
Joanna Jońca, PhD
Natalia Kaczyńska, PhD
Anna Kawiak, PhD
Agata Motyka-Pomagruk, PhD
Wojciech Śledź, PhD

PhD students: Weronika Babińska, Izabela Perkowska

## Research

Studies conducted in the Laboratory of Plant Protection and Biotechnology can be subdivided into the following research topics:

**1) Genomic and phenomic-oriented studies on soft rot Pectobacteriaceae (SRP) aiming to reveal molecular mechanisms responsible for their virulence or reevaluation of their taxonomy.**

We developed a genome assembly pipeline for handling large amounts of raw sequencing Dickeya solani and Pectobacterium parmentieri genomic reads. Implementation of comparative genomics tools showed that highly homogenous D. solani possesses a closed pangenome in contrast to the open pangenome of heterogeneous species

P. parmentieri. In both D. solani and P. parmentieri, COGs associated with phages and mobile genetic elements in addition to the regulation of transcription were overrepresented in the dispensable pangenome parts. (Zoledowska et al., BMC Genomics 2018; Motyka-Pomagruk et al., BMC Genomics 2020).

As a result of comprehensive genomic and phenomic analyses, four novel Pectobacterium species, i.e., *P. peruviense*, *P. polonicum*, *P. zantedeschiae* and *P. parvum* have been successfully differentiated, described and published (Waleron et al., 2018, Waleron et al., 2019a, 2019b, Waleron et al., 2020).


FIGURE 1.
Whole genome comparison of 22 strains of D. solani

**2) Monitoring of the occurrence of SRP and development of novel diagnostic and eradication methods against phytopathogens Zoledowska et al, 2018 Plant Dis.; Motyka-Pomagruk et a., 2021.**

We have shown that Pectobacterium spp. are widespread in the territory of Poland and outnumber the isolates belonging to Dickeya spp. P. parmentieri forms a notable fraction of all SRPs in our country, contrary to, for instance, Norway. We investigated various ways of implementing cold atmospheric pressure plasma technology to the plant protection sector – more details on this topic and derived publications and patents can be found on the page of the Research and Development Laboratory of IFB UG & MUG.

**3) Biosynthesis of plant secondary metabolites and the role of these compounds in plant resistance to abiotic and biotic stresses.**

We aimed to gain insight into plant responses to iron deficiency by characterizing an enzyme of unknown function encoded by the At3g12900 gene, which is highlighted in the literature as one of the most strongly induced Fe-responsive genes. We elucidated its biological role as a scopoletin 8-hydroxylase (S8H) involved in the last step of fraxetin biosynthesis. We proved that S8H is involved in the biosynthesis of coumarins, which play a crucial role in Fe acquisition in plants (Siwinska et al., J. Exper. Bot. 2018).

**4) Genetics, molecular taxonomy and stress adaptation of Cyanobacteria.**

A new Siberian strain of edible cyanobacteria of the genus Arthrospira that can be used for mass breeding in a temperate climate, e.g., Poland, was isolated and fully characterized. Moreover, we isolated, identified and characterised several new bacteria naturally associated with Arthrospira, and a new genus of the heterotrophic bacterium *Arthrospiribacter ruber* was differentiated (Waleron et al., Sys. App. Microb. 2020).

**5) Role of plant secondary metabolites in the modulation of chemoresistance in breast cancer cells.**

Research has included the analysis of signalling pathways involved in cancer cell chemosensitization and cell death induction in drug-resistant cancer cells (Kawiak et al., 2019 Front. Pharm.; Kawiak et al., 2019 J. Nat. Prod.).

## Research Publications


- Golanowska M, Potrykus M, Motyka-Pomagruk A, Kabza M, Bacci G, Galardini M, Bazzicalupo M, Makalowska I, Smalla K, Mengoni A, Hugouvieux-Cotte-Pattat N, Lojkowska E. Comparison of highly and weakly virulent Dickeya solani strains, with a view on the pangenome and panregulon of this species. Front. Microbiol. 2018, 9:1940.32.
- Siwińska J, Siatkowska K, Alexandre A, Grosjean J, Hehn A, Bourgaud F, Meharg A A., Manus C, Łojkowska E, Ihnatowicz A. Scopoletin 8-hydroxylase: anovel enzyme involved in coumarin biosynthesis and iron-deficiency responses in Arabidopsis. Journal Exper. Bot. 2018, 69: 1735-1748.
- Żołędowska S, Motyka-Pomagruk A, Śledź W, Mengoni A, Łojkowska E. High genomic variability in the plant pathogenic bacterium Pectobacterium parmentieri deciphered from de novo assembled complete genomes. BMC Genomics 2018, 19:751.
- Kawiak A, Domachowska A, Łojkowska E. Plumbagin Increases Paclitaxel-Induced Cell Death and Overcomes Paclitaxel Resistance in Breast Cancer Cells through ERK-Mediated Apoptosis Induction. J. Nat. Prod. 2019, 82: 878-885.
- Waleron M, Misztak A, Waleron M, M, Franczuk M, Jońca J, Wielgomas B, Mikiciński A, Popović T, Waleron K. Pectobacterium zantedeschiae sp. nov. a new species of a soft rot pathogen isolated from Calla lily (Zantedeschia spp.). System. and Appl. Microb. 2019, 42: 275-283.
- Waleron M, Misztak A,Waleron M, M, Furmaniak Magda, Mrozik A, Waleron K. Arthrospiribacter ruber gen. nov., sp. nov., a novel bacterium isolated from Arthrospira cultures. Systematic and Applied Microbiology 2020, 43: 126072.
- Motyka-Pomagruk A, Żołędowska S, Misztak A, E., Śledź W, Mengoni A, Łojkowka E. Comparative genomics and pangenome-oriented studies reveal high homogeneity of the agronomically relevant enterobacterial plant pathogen Dickeya solani. BMC Genomics 2020.
- Kaczyńska Natalia, Łojkowska Ewa, Narajczyk Magdalena, Czajkowski Robert. Genome-Wide Analyses of the Temperature-Responsive Genetic Loci of the Pectinolytic Plant Pathogenic Pectobacterium atrosepticum. International Journal of Molecular Sciences 2021, 22: 4839 (doi: 10.3390/ijms22094839).


## Research Grants


- National Science Centre HARMONIA 6, "Study of the pangenome of plant pathogenic bacteria from species Dickeya solani i Pectobacterium wasabiae causative agent of losses in potato crops in Europe", 1 134 900,00 PLN, 2015-2019, PI: E. Łojkowska.
- National Science Centre PRELUDIUM BIS , "Elucidating the role and mechanism of regulatory network of genes encoding dioxygenases in terms of plant adaptation to land conditions", 532 800 PLN, 2020-2024, PI: E. Łojkowska.
- National Science Centre OPUS 17, "Assessment of the antibacterial properties of post-plasma solutions generated with the use of cold atmospheric pressure plasmas against economically important phytopathogens and impact of the obtained liquids on the growth of crops and vegetables", 2 131 200 PLN, 2020-2022, PI: W. Śledź.
- National Science Centre SONATA 15, "Application of cold atmospheric pressure plasmas generated in contact with a flowing liquid for direct degradation of antibiotics and limitation of multi-drug resistance in the natural environment", 796 404 PLN, 2020-2023, Co-PI: W. Śledź.
- National Science Centre PRELUDIUM 1, "Evaluation of long-term survival of phytopathogenic bacteria from the genera Dickeya and Pectobacterium in natural waterways of diverse pollution status", 144 800 PLN, 2016-2019, PI: A. Motyka-Pomagruk.
- National Science Centre PRELUDIUM 7, "Estimating the influence of distinct metabolic profiles on virulence of bacterial plant pathogens from Dickeya solani species", 149 500 PLN, 2015-2018, PI: M. Potrykus.
- National Science Centre OPUS 8, "The role of iron- and oxoglutarate-dependent dioxygenases in Arabidopsis thaliana responses to environmental stresses and regulation of iron homeostasis", 851 900 PLN, 2015-2019, PI: A. Ihnatowicz.
- Characteristics of ubiquitous bacteria of the genus Pectobacterium (plant pathogens)-identification of genes involved in the adaptation to the environment, the rapid spread of and changes in host plant. 2015/17/B/NZ9/01730.
- National Science Centre OPUS 18, "Ecological biochemistry of Pectobacterium - decoding of molecular interactions between bacteria and plants", 1 945 200 PLN, 2020-2024, PI: M. Waleron.


## Patents

- Pat.233502; 31.10.2019.
- Pat.236055; 30.11.2020.
- Pat.236377; 11.01.2021.
- Pat.236665; 08.02.2021.

## Research Awards

- A. Motyka-Pomagruk — Prime Minister Award for outstanding PhD thesis.

## Scientific Collaboration

- EUPHRESCO – Phytosanitary ERA NET.
- Global Initiative of Crop Microbiome and Sustainable Agriculture.
- Prof. A. Mengoni, University of Florence, Italy.
- Dr. N. Hugouvieux-Cotet-Pattat, INSA, Lyon, France.
- Prof. A. Hehn ENSAIA, Nancy, France.
- Prof. A. Wilmotte, University of Liege, Belgium.
- Prof. P. Pohl Wroclaw University of Science and Technology, Poland.
- Prof. Z. Kaczyński, University of Gdańsk, Poland.

# Laboratory of Recombinant Vaccines

## Prof. Boguslaw Szewczyk

Graduated in 1974 at the Faculty of Chemistry, Technical University of Gdansk. Obtained PhD degree at the Faculty of Chemistry, University of Gdansk in 1984. Habilitation was awarded in 1996 at the Faculty of Biology, Geography and Oceanology, University of Gdansk. Full professorship since 2001. Group leader at IFB since 1996. Postdoctoral research was done at: University of California San Francisco, USA; University of Utah, Salt Lake City, USA; University of London, United Kingdom.

### Research Group:

Ewelina Król, Assoc. Prof.
Łukasz Rąbalski, PhD
Martyna Krejmer, PhD
Krzysztof Łepek, PhD

PhD students: Gabriela Brzuska, Marta Zimna, Karolina Gackowska, Maciej Kosiński, Klaudia Barańska, Aurelia Schweda

## Research

The main field of interest of the Laboratory is the application of different expression systems for the production of viral proteins that can be used as potential vaccines or constituents of modern diagnostic tests. Our research interests are also focused on identifying antiviral compounds that are potent against many dangerous viral human and animal pathogens.

The Laboratory has also actively joined the world efforts to combat the SARS-CoV-2 pandemic. Our scientific efforts to contribute to fighting this disease are listed in the later part of this description. Some of the research projects carried out in the Laboratory are also listed below:

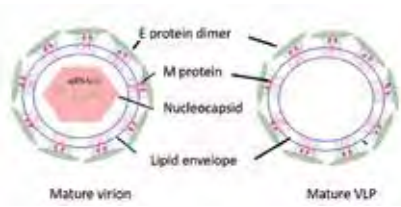
Construction of recombinant vaccines against human pandemic and seasonal influenza viruses, against Newcastle virus (NDV) and against Zika and tick-borne encephalitis (TBEV) viruses. We are trying to develop a universal vaccine against influenza virus that may protect against many strains of the virus. We plan to achieve this goal with a combined mRNA/virus-like particle (VLP) vaccine. The project is financed by the Polish National Science Agency and performed in collaboration with the Centre of New Technologies University of Warsaw. Another devastating disease is Newcastle disease. The very effective vaccine that was constructed and tested in collaboration with the Polish National Institute of Veterinary Sciences is a recombinant construct based on live attenuated turkey herpesvirus with two inserted genes of Newcastle virus. Moreover, we are trying to develop a safe and cost-effective recombinant vaccine against Zika virus. The approach to potential vaccines is based on recombinant VLPs composed of prM and E proteins produced in insect and mammalian cells. The experience gained from this project is also used to produce a potential VLP-based vaccine against tick-borne encephalitis virus (TBEV).

Combatting SARS-CoV-2 – our contribution. We submitted the first full virus genome isolated directly from patients in Poland. Using Nanopore (internally) and Illumina (in collaboration with Gdansk Medical University) next-generation sequencing (NGS) technologies, we have been performing SARS-CoV-2 mutation surveillance on isolates from northern Poland on a daily basis. In collaboration with the University Clinical Centre in Gdansk and the International Centre for Cancer Vaccine Science, our laboratory is executing a study on respiratory viral coinfections in SARS-CoV-2-positive patients. In addition, under the Polish National Science Centre COVID-19 programme, we are characterizing the role of glycans and their modifications on the production and maturation of SARS-CoV-2 spike protein. Various modifications of glycoprotein S are used to produce a potential VLP-based vaccine. We plan to explore the possibilities for the improvement of a potential anti-SARS-CoV-2 vaccine leading to the elimination or at least reduction of antibody-dependent enhancement of the infection effect.

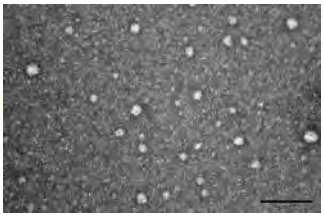
Effect of synthetic glycosylation inhibitors on viral entrance and propagation in host cells. The lack of effective drugs against most viral diseases calls for intensive research in this field. We have concentrated our efforts on the development of structural analogues and mimetics of tunicamycin, an effective inhibitor of N-glycosylation of glycoproteins. A large number of synthetic compounds were obtained from Silesian Technical University in Gliwice. In the past, we tested these compounds using a few model viruses, including hepatitis C virus and influenza A virus. At present, their action is tested for Zika, TBEV and SARS-CoV-2 viruses.

Future plans. As the case of SARS-CoV-2 shows, the threat of viral diseases forces us to constantly pursue new pathogenic viruses. The present situation also shows that

modern vaccinations are the most efficient tools to combat viral diseases. Hence, the construction of vaccine prototypes is of paramount importance for future therapies. We shall pursue this line of research with respect to the pathogens that we are already studying but shall also be prepared to contribute to the fight against new and re-emerging viral diseases.




A.



B.

FIGURE 1.
Schematic representation of mature virion and mature virus-like particles (**A**). Electron micrograph of exemplary flavivirus-like particles (**B**).

## Research Publications


- Krol, E., Pastuch-Gawolek, G., Chaubey, B., Brzuska, G., Erfurt, K., Szewczyk, B. (2018) Novel uridine conjugates, derivatives of 4-aminophenyl 1-thioglycosides, as potential antiviral compounds. Molecules 23, 1435; doi:10.3390/molecules23061435.
- Krol, E., Wandzik, I., Pastuch-Gawolek, G., Szewczyk, B. (2018) Anti-hepatitis C virus activity of uridine derivatives of 2-deoxy sugars. Molecules 23, 1547; doi:10.3390/molecules23071547.
- Marsberg, T., Jukes, M.D., Krejmer-Rabalska, M., Rabalski, L., Knox, C.M., Moore, S.D., Hill, M.P., Szewczyk, B. (2018) Morphological, genetic and biological characterization of a novel alphabaculovirus isolated from Cryptophlebia peltastica (Lepidoptera: Tortricidae). Journal of Invertebrate Pathology 157, 90-99.
- Krejmer-Rabalska, M., Rabalski, L., Jukes, M.D., Lobo de Souza, M., Moore, S.D., Szewczyk, B. (2019) New method for differentiation of granuloviruses (betabaculoviruses) based on real-time polymerase chain reaction (real-time PCR). Viruses, 11, 115; doi:10.3390/v1102115.
- Kopera, E., Zdanowski, K., Uranowska, K., Kosson, P., Sączyńska, V., Florys, K., Szewczyk, B. (2019) High-titre neutralizing antibodies to H1N1 influenza virus after mouse immunization with yeast expressed H1 antigen: A promising influenza vaccine candidate. Journal of Immunology Research, vol. 2019, Article ID 2463731, doi.org/ 2019/2463731.
- Krol, E., Wandzik,I., Brzuska,G., Eyer,L., Růžek, D., Szewczyk, B. (2019) Antiviral activity of uridine derivatives of 2-deoxy sugars against Tick-borne Encephalitis Virus. Molecules, 24, 1129, doi:10.3390/molecules24061129.
- Smietanka, K., Tyborowska, J., Olszewka-Tomczyk, M., Domanska-Blicharz, K., Minta, Z., Rabalski, L., Czarnota, A., Kucharczyk, K., Szewczyk, B. (2019) A recombinant turkey herpesvirus expressing F and NH genes of avian avulavirus (AAvV-1) genotype VI confers cross-protection against challenge with virulent AAvV-genotypes IV and VII in chickens. Viruses, 11, 784; doi:10.3390/v11090784.
- Krol, E., Brzuska, G., Szewczyk, B. (2019) Production and biomedical application of flavivirus-like particles. Trends in Biotechnology, 37 (11), 1202-1216, doi.org/10.1016/j.tibtech.2019.03.013.
- Ndolo, D., Njuguna, E., Adetunji, C.O., Harbor, C., Rowe, A., Den Breeyen, A., Sangeetha, J., Singh, G., Szewczyk, B., Anjorin, T., Thangadurai, D., Hospet, R. (2019) Research and development of biopesticides: challenges and prospects. Outlooks on Pest Management 30, 267-276, doi: 10.1564/v30_dec_08.
- Brzuska G., Pastuch-Gawołek G., Krawczyk M., Szewczyk B., Krol E. (2020) Anti-tick-borne encephalitis virus activity of novel uridine glycoconjugates containing amide or/and 1,2,3-triazole moiety in the linker structure. Pharmaceuticals, 13, 460; doi:3390/ph13120460.


## Research Grants

- National Science Centre OPUS, "Mammalian cell delivery of stable therapeutic mRNA packaged into virus-like particles", 2 300 000 PLN, 2020-2023, PI Bogusław Szewczyk.
- National Science Centre EXPRESS CALL TO FUND RESEARCH ON COVID-19 grant, "Towards safe anti-SARS-CoV-2 coronavirus vaccine - modifications of the spike protein leading to the elimination of ADE effect", 999 600 PLN, 2020-2021, PI Ewelina Król.
- National Science Centre SONATA, "Tick-borne encephalitis virus - search for mechanisms useful in treatment and prophylaxis", 563 650 PLN, 2016-2021, PI Ewelina Król.
- National Science Centre SONATA, "Public health risk assessment, associated with potentially dangerous zoonotic influenza A virus strains, circulating among livestock in Poland", 747 040 PLN, 2017-2021, PI Krzysztof Łepek.
- National Centre for Research and Development LIDER, " Anti-Zika vaccine - innovative methods for antigen construction", 1 195 500 PLN, 2017-2020, PI Ewelina Król.
- National Centre for Research and Development LIDER, "Virus-Like particles produced in live bioreactors as a vaccine against the most dangerous disease of poultry", 1 200 000 PLN, 2017-2020, PI Łukasz Rąbalski.
- Ministry of Science and Higher Education, Poland, "Co-infections with SARS-CoV-2, Database of COVID-19 co-infections", 965 000 PLN, 2020-2022, PI Łukasz Rąbalski.

## Patents

- Patent No.: US 10,398,770 B2 – Influenza virus hemagglutinin protein as a vaccine antigen.
- Patent: EP3083660A1 – Antigen, influenza vaccine, system for vaccine manufacturing, method of antigen production, and use of antigen to produce an influenza vaccine.
- PL 228449 B1: Selected strain of LdMNPV-PL for protection of plants.
- PL 227745 B1: Collection of oligonucleotide starters and application of these starters for detection and differentiation of NDV strain.
- PL 236026: Application of antibody for detection of influenza virus, immunosensor and method of its construction.

## Research Awards

- Rector's First-Degree Team Award for Scientific Achievements.
- "L'Oréal-UNESCO For Women in Science" award in the category of habilitation theses for Ewelina Król.
- Rector's Second-Degree Individual Award for Ewelina Król.

## Scientific Collaboration

- Rhodes University, Grahamstown, South Africa.
- Biological Control Unit EMBRAPA, Brasilia, Brazil.
- Friedrich Loeffler Institute (FLI), Riems, Germany.
- Veterinary Research Institute, Brno, Czech Republic.
- Laboratoire de Chimie Biologique, University of Cergy-Pontoise, France.
- Centre of New Technologies University of Warsaw, Poland.
- Silesian University of Technology, Gliwice, Poland.

# Laboratory of Molecular Bacteriology

## Prof. Michał Obuchowski

Graduated from University of Gdansk in 1993, received PhD in biology (1997) University of Gdansk and habilitation in biological sciences (2006) and was awarded the full professorship in 2014. Since 2004 he works at Medical University of Gdańsk. He carried out postdoctoral work at University of Paris XI, France. Author of more than 50 peer-reviewed publications.

### Research Group:

Adam Iwanicki, PhD
Krzysztof Hinc, PhD
Alessandro Negri, PhD

PhD student: Roberta Gatta, Katarzyna Pruska

## Research

Our research is conducted on two gram-positive microorganisms: *Bacillus subtilis* and *Clostridium difficile*. To date, our investigation on *B. subtilis* concern spore formation and its possible application in biotechnology. We pay attention to the germination process when dormant spores are converted into vegetative cells. Accordingly, we investigate the mechanism of action of GerA receptors, which trigger germination in response to the presence of L-alanine or L-valine in the environment. As part of this pathway, we are


FIGURE 1.
Swarming motility *B. subtilis* (blue spot) and *D. solani* (red spot). The dots marked point of inoculation.

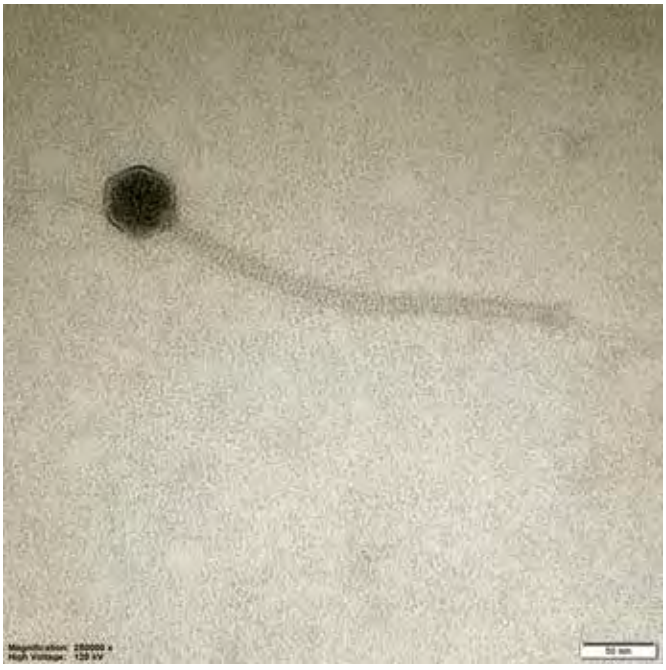


FIGURE 2.
The bacteriophage isolated in our lab from clinical strain of *C. difficile*.

trying to build a model of the GerA receptor using a bioinformatics approach supported by biochemical experiments. In our second research focus, we are investigating the interaction between *B. subtilis* and *Dickeya solani*. Using the MALDI imaging technique, we seek to identify factors secreted by both bacterial species that are responsible for their interaction. As a complementary part, we also look for mutants in both species that failed to show cross-species interactions. Applied research conducted in our laboratory also concerns spores but from different angles. The spores, which should survive different harsh conditions, are extremely resistant to temperature, changes in humidity, low pH, UV irradiation and proteolytic enzymes. All these properties make spores a very good choice as carrier particles. This is also supported by the fact that the external shield of the spore consists of proteins. This creates an opportunity to place peptides or proteins of choice on the surface of spores by the simple creation of fusion genes between the genetic material coding coat proteins and that coding for passenger parts. Using this approach, we created a set of spores presenting antigens of two pathogenic bacteria: *Helicobacter pylori* and *C. difficile*. Such recombinant spores are used for the oral or intranasal immunization of laboratory animals to protect them from pathogen infection. As a new possible application of spores, we have begun constructing spore-producing strains that present human inhibitor of tissue metalloproteases. These spores will be used to reduce symptoms of periodontitis.

The research path concerning C. difficile is focused on finding and characterizing bacteriophages that can infect such microorganisms. The major goal of this approach is to find a bacteriophage suitable for phage therapy against these bacteria. To achieve this, we would like to arm selected phages with some *B. subtilis*-origin genes that can cause growth arrest of *C. difficile* in the intestine.

## Research Publications


- Piekarska A., Pełka P., Peszyńska-Sularz G., Negri A., Hinc K., Obuchowski M., Iwanicki A., 2017, The choice of the anchoring protein influences the interaction of recombinant Bacillus spores with the immune system. Acta Biocim. Pol. 64: 239-244.
- Potocki W., Negri A., Peszyńska-Sularz G., Hinc K., Obuchowski M., Iwanicki A., 2017, The combination of recombinant and non-recombinant Bacillus subtilis spore display technology for presentation of antigen and adjuvant on single spore. Microb Cell Fact. 16:151. doi: 10.1186/s12934-017-0765-y.
- Grela A., Jamrożek I., Hubisz M., Iwanicki A., Hinc K., Kaźmierkiewicz R., Obuchowski M., 2018, Positions 299 and 302 of the GerAA subunit are important for function of the GerA spore germination receptor in Bacillus subtilis. Plos ONE 13 (6): e0198561.
- Potocki W., Negri A., Peszyńska-Sularz G., Hinc K., Obuchowski M., Iwanicki A., 2018, IL-1 fragment modulates immune response elicited by recombinant Bacillus subtilis spores presenting an antigen/adjuvant chimeric protein. Mol Biotechnol. 60:810-819. doi: 10.1007/s12033-018-0117-0.
- Zyśk M., Gapys B., Ronowska A., Gul-Hinc S., Erlandsson A., Iwanicki A., Sakowicz-Burkiewicz M., Szutowicz A., Bielarczyk H., 2018, Protective effects of voltage-gated calcium channel antagonists against zinc toxicity in SN56 neuroblastoma cholinergic cells. PLOS One 13(12):e0209363. doi: 10.1371/journal.pone.0209363
- Gonciarz W., Walencka M., Moran A.P., Hinc K., Obuchowski M., Chmiela M., 2019, Upregulation of MUC5AC production and deposition of LEWIS determinants by Helicobacter pylori facilitate gastric tissue colonization and the maintenance of infection. J Biomed Sci. 26:23. doi: 10.1186/s12929-019-0515-z.
- Gonciarz W., Krupa A., Hinc K., Obuchowski M., Moran A.P., Gajewski A., Chmiela M., 2019, The effect of Helicobacter pylori infection and different H. pylori components on the proliferation and apoptosis of gastric epithelial cells and fibroblasts. PLoS One. 2019 14:e0220636. doi: 10.1371/journal.pone.0220636.


## Research Grants


- National Science Center project no. 2018/29/B/NZ9/02339 "Spcoal interaction between B. subtilis and D. solani. 1 100 000pln, 2019-2021. PI: Michał Obuchowski.
- National Science Center project no 2018/02/X/NZ6/01360 Isolation of lytic bacteriophages infecting clinical strains of Clostridium difficile. 36 300 PLN 2018-2019 PI: Krzysztof Hinc.


## Patents

- Pentapeptydy zawierające 3-[2-(2-chinolino) benzokazaol-5-ylo]alaninę, kompozycja farmaceutyczna oraz ich zastosowanie; number: 407032; granted date: 2017-01-25.
- Oral vaccinec containing the Bacillus subtilis spores and its application to immunise against Helicobacter pylori; Granted date: 10-09-2018; application number: 13 723 573.5.

## Scientific Collaboration

- Laboratory of microbiology, University of Frideric II (Naples, Italy), professor Ezio Ricca.
- Department of Periodontology and Oral Mucosa Diseases, Medical University of Gdańsk, prof. Aida Kusiak.
- Department of Medical Microbiology, Medical University of Silesia, prof. Gajane Martirosian.
- Department of Medical Microbiology Medical University of Warsaw, prof. Hanna Pituch.

# Laboratory of Cell Biology and Immunology

## Prof. Jacek Bigda

Received his PhD in Medicine at the Medical University of Gdansk (1990). He carried out postdoctoral training at the Weizmann Institute of Science (1991-1993), working with Prof. David Wallach. He underwent also shorter trainings in the Central Laboratory of Dutch Red Cross (1991) and Medical University of Hannover, Germany (1995, 1996). He became a habilitated doctor at 1996. In 2001 he was appointed associate professor at the Medical University of Gdansk and was awarded the full professorship at 2005.

### Research Group:

Marcin Okrój, Assoc. Prof.
Patrycja Koszałka, Assoc. Prof.
Grzegorz Stasiłojć, PhD

PhD student: Anna Felberg, Aleksandra Urban, Paweł Serafin

## Research

Our research is focused on cancer cell biology, with special emphasis on invasion and metastasis. We are interested in mutual interactions of cancer cells with their microenvironment, and their implications for prognosis and therapy of cancer patients. Experiments are carried in vitro, in vivo in animal models and in the clinical setting using broad spectrum of methods. There are three main lines of research related to: I) interaction of cancer cells with endothelium, II) the role of complement system in tumor evasion. Our research should lead to novel biomarkers of cancer progression.

First line of research involves the complement system, which is one of the defense lines in the framework of innate immunity. On one hand, complement is an effector mechanism of numerous immunotherapeutics and its enhancement may ensure a more efficient clinical response. On the other hand, dysregulation of complement pathways often leads to autoimmune and inflammatory diseases. We are interested in a screening of patients suffering from rare kidney diseases for unique variants of complement genes. Functional analyses brought us to the identification of several gain-of-function mutations as previously unknown, potential etiologic factors. Concurrently, we design complement C2 variants that support the formation of hyperactive convertases. Convertases are the nodal points in the complement cascade that cleave the major complement protein C3 to its active fragment C3b and anaphylatoxin C3a. Such gain-of-function (GoF) C2 proteins form convertases, which are insensitive to complement inhibitors produced by tumor cells. Supplementation of patients' sera with GoF C2 variants in combination with antitumor monoclonal antibodies (mAbs) enables the killing of malignant cells normally resistant to mAbs alone. We are also working on antibodies that recognize tumor cells resistant to first-line immunotherapeutics. Contact of the cell surface with mAb leaves a footprint, namely C4d fragment of complement C4 protein. The formation of C4d is fueled by complement inhibitors that render tumor cells resistant to complement attack. Targeting C4d would preferably select resistant tumor cells and engineering anti-C4d antibodies for enhanced effector functions would offer a novel and universal therapeutic. Other scientific research aims to elucidate the role of complement proteins produced by tumor cells in cancer progression. Apart from their obvious role in the modulation of the immune response, they are suspected to steer the intracellular signaling that influences cell motility, adhesion, and differentiation.

Second line of our research involves the influence of nucleotide metabolism in tumor microenvironment on cancer development and progression. Many tumors overexpress enzymes involved in nucleotide metabolism, such as ecto-5'-nucleotidase (CD73). CD73 catalyzes the conversion of nucleoside 5'-monophosphates to nucleosides, preferentially 5'-AMP to adenosine, and functions also as a cell adhesion molecule for T lymphocytes and cancer cells. In cancer, CD73-adenosine axis is associated with tumor invasiveness and an impaired anti-tumor immune response mainly through regulatory T (Treg) cells induced inhibition of CTL response. Using CD73 knockout mice we, and others, have demonstrated in syngeneic cancer models that CD73 depletion decreases cancer growth and metastasis. It pointed out CD73 as an attractive target for novel anti-cancer therapy. However, when clinical data is analyzed the role of CD73 is controversial for some cancer types, such as breast cancer. Therefore, to properly assess the role of CD73 in breast cancer progression and its effect on a major players in cancer immunity we have decided to apply a cancer model of chemical mammary tumorigenesis, a model able to reflect developmental pathway of human disease. Using mice with knockout of *cd73* gene we analyze changes at both initiation and progression stage of breast cancer. Furthermore, we also analyze an accumulation of metabolic intermediates in tumor microenviron-


ment. Such intermediates were shown to function as an "oncometabolites" and modulate invasive potential of tumour cells, their survival during dissemination stage, and regulate inflammatory state of cancer microenvironment. We also analyze if we can modulate cancer progression with an application of phytochemicals. As the amount of cancer-related deaths is still increasing, and metastasis formation is their leading cause, determination of the main regulatory mechanism and the pathways to modulate them is a primary concern in oncology research.

## Research Publications


- Felberg A, Taszner M, Urban A, Majeranowski A, Jaskuła K, Jurkiewicz A, Stasiłojć G, Blom AM, Zaucha JM, Okrój M. Monitoring of the Complement System Status in Patients With B-Cell Malignancies Treated With Rituximab. Front Immunol. 2020; 11: 584509. doi: 10.3389/fimmu.2020.584509.
- Urban A, Volokhina E, Felberg A, Stasiłojć G, Blom AM, Jongerius I, van den Heuvel L, Thiel M, Ołdziej S, Arjona E, de Córdoba SR, Okrój M. Gain-of-function mutation in complement C2 protein identified in a patient with aHUS. J Allergy Clin Immunol. 2020; 146, 4: 916-919.e11. doi: 10.1016/j.jaci.2020.02.014.
- Stasiłojć G., Felberg A., Urban A., Kowalska D. Ma S., Blom A.M., Lundin J., Österborg A., Okrój M. Calcein release assay as a method for monitoring serum complement activity during monoclonal antibody therapy in patients with B-cell malignancies. J. Immunol. Methods 2019; 112675. doi: 10.1016/j.jim.2019.112675.
- Felberg A., Urban A., Borowska A., Stasiłojć G., Taszner M., Hellman A., Blom A.M., Okrój M. Mutations resulting in the formation of hyperactive complement convertases support cytocidal effect of anti-CD20 immunotherapeutics. Cancer Immunol. Immunother. 2019; 68, 4: 587-598.
- Okrój M., Potempa J. Complement activation as a helping hand for inflammophilic pathogens and cancer. Front. Immunol. 2019; 9, 3125: 1-11.
- Stasiłojć G., Felberg A., Okrój M. Parameters critical for the effector mechanism of anti-CD20 antibodies revisited. Br. J. Haematol. 2018; 180, 6: 777-779.
- Urban A., Borowska A., Felberg A., van den Heuvel L., Stasiłojć G., Volokhina E., Okrój M. Gain of function mutant of complement factor B K323E mimics pathogenic C3NeF autoantibodies in convertase assays. Autoimmunity 2018; 51, 1: 18-24.
- Kutryb-Zajac B, Koszalka P, Mierzejewska P, Bulinska A, Zabielska MA, Brodzik K, Skrzypkowska A, Zelazek L, Pelikant-Malecka I, Slominska EM, Smolenski RT. Adenosine deaminase inhibition suppresses progression of 4T1 murine breast cancer by adenosine receptor-dependent mechanisms. J Cell Mol Med. 22(12):5939-5954, 2018.
- Stasiłojć G, Nagel A, Koszałka P, Bigda JJ., Defective apoptosis of U937 cells induced by benzyl isothiocyanate (BITC). Acta Biochim Pol. 24;66(4):401-407, 2019.
- Mierzejewska P, Zabielska MA, Kutryb-Zajac B, Tomczyk M, Koszalka P, Smolenski RT, Slominska EM. Impaired L-arginine metabolism marks endothelial dysfunction in CD73-deficient mice, Mol Cell Biochem. 458(1-2):133-142, 2019.


## Research Grants


- National Science Centre (NCN), OPUS 18, No 2019/35/B/NZ6/02450, "Concept of the universal antibody targeting tumor cells resistant to first-line immunotherapeutics", 1.704.000 PLN, 2020-2024, PI: Marcin Okrój
- National Science Centre (NCN), HARMONIA 7, No 2015/18/M/NZ6/00334, "Functional characteristics and therapeutic potential of gain-of-function mutations in complement C2 protein", 1.291.200 PLN, 2016-2021, PI: Marcin Okrój
- National Science Centre (NCN), SONATA Bis 4, No 2014/14/E/NZ6/00182 „Role of the complement system in cancer and therapeutic approaches", 1.945.000 PLN, 2015-2021, PI: Marcin Okrój
- National Science Centre (NCN), OPUS 14, No 2017/27/B/NZ5/02192 "CD73 (ecto-5'-nucleotidase) function in breast cancer progression and promotion, and its assessment as a therapeutic target", 1.286.240 PLN, 2018-2021, PI: Patrycja Koszałka
- The National for Research and Development (NCBiR), No 256 09-0835/18, „Prostacyclin, nitric oxide and carbon monoxide-based pharmacotherapy of endothelial disfunction and platelet activation-a novel strategy to inhibit cancer metastasis" (METENDOPHA), STRATEGMED1/233226/11/NCBR/2015. budget 24 834 510 PLN; 2015-2019, PI: Prof. Stefan Chłopicki, JCET (consortium), Prof. Ryszard T. Smoleński, MUG (MUG).


## Patents

- Patent no. P.425133 „Point mutations of complement factor B and C2 protein applied to enhance the cytotoxic activity of antibodies in the treatment of cancer", February 2021, Patent Office of Republic of Poland

## Scientific collaboration

- Prof. Anna Blom, Department of Translational Medicine, Lund University, Sweden
- Prof. Santiago Rodriguez de Cordoba, Centre of Biological Research, Madrid, Spain
- Prof. Ruben Pio, Department of Biochemistry and Genetics, School of Sciences, University of Navarra, Pamplona, Spain
- Prof. Anders Österborg, Department of Hematology-Oncology, Karolinska Institut, Stockholm, Sweden
- Prof. Robbert Spaapen, Sanquin Research, Amsterdam, The Netherlands
- Prof. Lambertus van den Heuvel, Department of Department of Laboratory Medicine, Radboud University Medical Center, Nijmegen, The Netherlands.

## Research Awards

- 2020: Medical University of Gdańsk Rector's prize for scientific achievements (individually to Marcin Okrój).

# Laboratory of Molecular Enzymology and Oncology

## Prof. Andrzej C. Składanowski

Received PhD in Biochemistry at the Medical University of Gdansk (1980); postdoctoral positions have been held at the Bern University, Switzerland and the Wales University College of Medicine in Cardiff, UK. Studied metabolism of ischemic heart at the Erasmus University Rotterdam, The Netherlands and structure of enzyme binding sites at the Bremen University, Germany. Became a habilitated doctor in 1997. In 2001 was appointed Associate Professor at the Medical University of Gdansk and awarded the full professorship at 2007. Is a member of the Editorial Board of Elsevier's Toxicology in Vitro and is chairing the Gdansk Section of the Polish Biochemical Society.

### Research Group:

Rafał Sądej Assoc. Prof.
Kamila Kitowska PhD
Dominika Piasecka PhD
Kamil Mieczkowski PhD

PhD Students: Monika Górska, Barbara Galikowska, Izabela Żarczyńska, Dima Antoun

## Research

Our research focuses on breast cancer cell biology, with emphasis on the interactions between cancer cells and the tumour microenvironment (TME). The coevolution of a tumour stroma with neoplastic cells is recognized as one of the key factors of disease progression and patient response to treatment. The search for new biomarkers and insight into the mechanism of developing resistance to therapies are the top challenges in contemporary oncology. Our research focuses on analysis of the heterogeneity of the tumour cell population induced by the TME. The projects carried out in the laboratory include three fields of interest:

1) Evolution of noninvasive ductal carcinoma in situ into invasive breast cancers upon inflammatory stimulation,
2) Involvement of the FGFR family in cancer cell progression and metastasis, and
3) Interaction of FGFRs with HER2 in acquisition of resistance to anti-HER2 therapies in breast cancer. The highest quality of research is ensured by the provided analyses at three complementary levels: in vitro, in vivo and in clinical analyses.

One main aim is to identify new markers for the invasive progression of ductal carcinoma in situ (DCIS). These may accelerate diagnosis and assist in the appropriate selection of patients for the best therapy. The last results suggest that the inflammatory microenvironment, as a possible bottleneck in the promotion of proliferative dominance and invasion of more responsive tumour subclones, might contribute to DCIS evolution toward HER2-negative (more aggressive) IDC and highlight the clinical value of the heterogeneity of DCIS lesions.

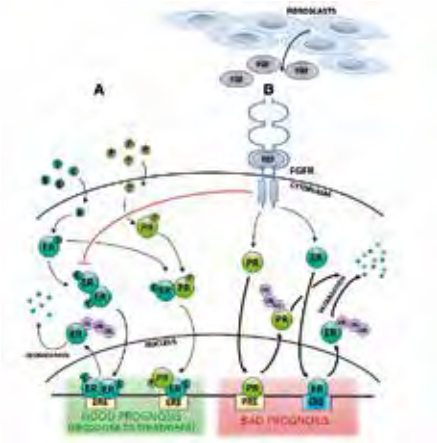



FIGURE 1. Ligand-dependent and –independent activation of ER/PR – an impact on patient prognosis in luminal IDC. (A) ER is activated in response to estrogen. In addition, progesterone induces PR/ER dimerization and recruits ER away from the classical ER-binding sites to the new PR-directed sites, promoting expression of a gene set associated with GOOD PROGNOSIS. (B) There are two major mechanisms of FGFRs-induced steroid hormone-independent ER/PR regulation, both associated with POOR PROGNOSIS: a FGFRs-triggered shift in ER binding to DNA (ERE, in blue), and FGFRs-dependent rapid activation of ER and PR leading to their subsequent degradation. E – estrogen; ER – estrogen receptor; ERE – estrogen responsive element; P – progesterone; Ub – ubiquitin (**from Piasecka D et al., J Exp Clin Cancer Res 2019: 29; 38:230**)

Another main field of interest is the fibroblast growth factor receptor (FGFR1-4) family, consisting of four transmembrane receptors responding to auto and paracrine TME signals. FGF-triggered signal transduction via Ras-dependent mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/AKT or STAT-dependent pathways was found to be involved in the regulation of numerous fundamental physiological processes. However, aberrant FGF/FGFR signalling has been associated with several developmental abnormalities and malignancies, including breast, lung, stomach and bladder cancer.

Over the last few years, the team has aimed to decipher a variety of FGFR-dependent signalling pathways in human malignancies. We focused on understanding how communication between cancer cells and the surrounding stroma controls tumour initiation, progression and response to therapies. A wide range of strategies were employed to investigate a set of FGFR actions, including comprehensive cancer cell line-based techniques (3D cultures, migration/invasion assays, fluorescence microscopy, clonogenic assays, etc.), biochemical and molecular biology methods (co-immunoprecipitation, Western blotting, proximity ligation assay, immunocytochemistry, RT-qPCR, etc.) complemented by analyses of patient samples (immunohistochemistry, quantitative gene expression analysis using Nanostring technology) and in vivo mouse xenograft studies (NSG mouse model). Currently, numerous projects are carried out with the goal of translating laboratory results into clinical care to find putative prognostic factors as well as new targets for more precise therapies.

## Research Publications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- Sadej R, Lu X, Turczyk L, Novitskaya V, Lopez-Clavijo AF, Kordek R, Potemski P, Wakelam MJO, Romanska H, Fedor Berditchevski. CD151 regulates expression of FGFR2 in breast cancer cells via PKC-dependent pathways. J Cell Sci 2018; 131: pii: jcs220640.
- Piasecka D, Braun M, Kordek R, Sadej R, Romanska-Knight H. MicroRNAs in regulation of triple-negative breast cancer progression. J Cancer Res Clin Oncol 2018; 8: 1401-1411.
- Kitowska K, Kowalska A, Mieszkowska M, Piasecka D, Skladanowski AC, Romanska HM, Sadej R. Progesterone impairs Herceptin effect on breast cancer cells. Oncology Lett 2018; 15: 1817-1822.


## Research Grants


- National Science Centre SONATA BIS,"FGFR2 role in ER/PR relationship — molecular mechanism and predicative significance in patients with luminal A breast cancer", 2 499 000 PLN, 2019-2024, PI Rafał Sądej.
- National Science Centre OPUS, "FGFR2 role in autophagy regulation in breast cancer — predicative significance", 1 499 200 PLN, 2018-2021, PI Rafał Sądej.
- National Science Centre PRELUDIUM, "FGFR2 role in initiation of cancer changes in mammary gland epithelial cells – molecular and clinical analyses", 150 000 PLN, 2016-2020, PI Kamil Mieczkowski.
- National Centre for Research and Development, STRATEGMED, "CELONKO – Development of novel biomarkers and innovatiove FGFR kinases inhibitor as an anti-cancer therapy", 1 901 250 PLN, 2015-2019, PI Andrzej C. Składanowski.


## Scientific Collaboration

- Prof. Hanna M. Romanska, Prof. Radzisław Kordek, Department of Pathology, Chair of Oncology, Medical University of Lodz.
- Prof. Fedor Berditchevski, Institute of Cancer and Genomic Sciences, The University of Birmingham, UK.

## Research Awards

- 2020 Scientific Award of Rector of Medical University of Gdańsk (for the team).
- 2019 Scientific Award of Rector of Medical University of Gdańsk (for the team).
- 2018 Scientific Award of Rector of Medical University of Gdańsk (for the team).
- European Network of Breast Development and Cancer (ENDBC) — Rafal Sadej, Steering Committee Member (from 2019).

# Laboratory of Translational Oncology

## Assoc. Prof. Anna J. Żaczek

Received her Ph.D in biochemistry (2004) at University of Gdansk and D.Sc. (habilitation) in medicine, specialization: tumor biology (2015) at Medical University of Gdansk. Scholar at Stanford University within TOP500 Innovators Program and Starship Innovation Fellow (EIT Health) with 1-year training in Bioinnovation at University of Coimbra and IESE Business School. Principal investigator and investigator in 17 externally funded projects. Author of >50 peer reviewed publications.

### Research Group:

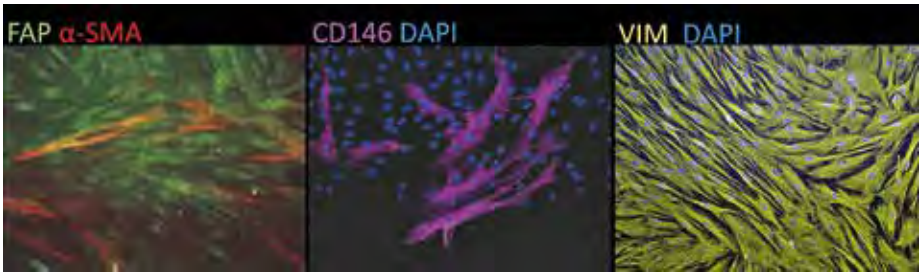
Natalia Bednarz-Knoll, PhD
Aleksandra Markiewicz, PhD
Paulina Nastały, PhD
Anna Supernat, PhD
Barbara Bollin-Matysiak, research assistant
Beata Pieczyńska-Uziębło, PhD, research assistant

PhD student: Marta Łukasiewicz, Anna Nagel, Anna Muchlińska, Krzysztof Pastuszak, Marta Popęda, Julia Smentoch, Justyna Topa, Magdalena Warnik, Michał Żuk

## Research

Our research team has existed for over a decade as a part of the Laboratory of Cell Biology but was constituted as a separate research unit in 2019. The projects are embedded in translational oncology, combining various methodologies to study multilayered and multifactor aspects of tumour development and progression. Our studies are based on both clinical material (primary tumours, blood, and metastases) and in vitro models.

We work on various tumour entities with a main focus on breast, lung, ovarian and prostate cancer. We search for prognostic markers and investigate the biology of cancer progression by studying specific molecular markers/signatures (e.g., epithelial-mesenchymal transition, BRCA1, EGFR, pictures of platelet transcriptome) and underlying mechanisms in both cancer cells and the tumor microenvironment (incl. studies on cancer-associated fibroblasts, platelets and neutrophils).




CAFs from breast cancer patients

Our team is experienced in carrying out projects related to the development and validation of biomarkers as well as in designing diagnostic methods for reliable investigation with routine approaches.

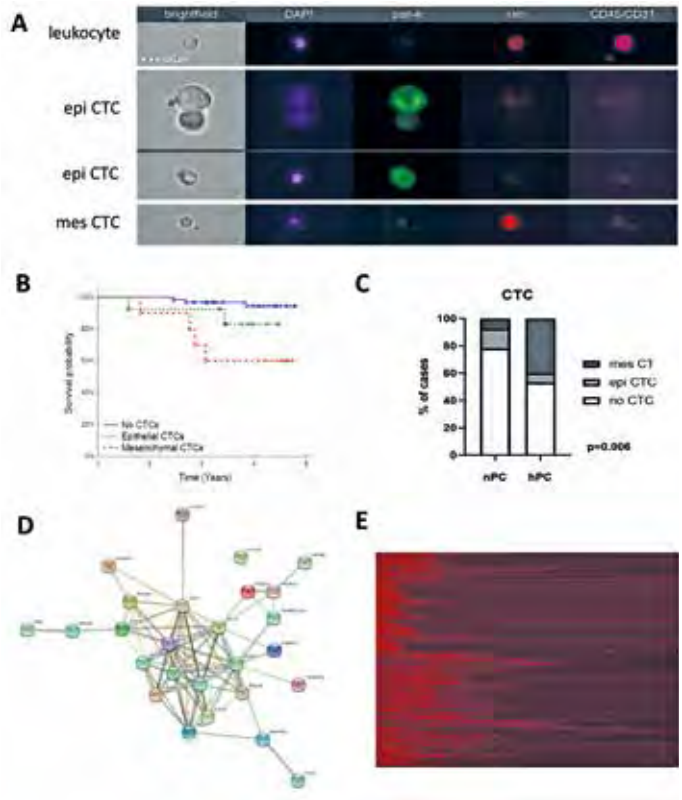
The research of the team is situated at the edge of molecular biology and clinical oncology with the mission of translating the results obtained in the laboratory into clinical practice, thus contributing to the more effective and individualized management of cancer patients.


For several years now, we have also been interested in so-called liquid biopsy, i.e., diagnostics based on blood samples collected from a patient in a non-invasive way. We are focused in particular on circulating tumour cells (CTCs), their phenotyping and transcriptional analysis, circulating tumour DNA (ctDNA) and tumour-educated platelets

(TEPs). In various projects (7 ongoing projects), we explore their potential to identify patients characterized by more aggressive disease and/or to monitor progression and response to therapy.

Among other techniques, we perform RNA sequencing, qPCR, immune-cytohistochemistry and histochemistry, single-cell isolation and micropopulation, primary cell and cell line culture and various in vitro molecular assays. We developed an advanced platform to study single cells. micromanipulation system (Transfer-Man, Eppendorf), isolation system Parsortix (Angle), imaging flow cytometry (Amnis® ImageStream® XMArk II, Luminex) and system for single-cell RNA sequencing (Chromium Controller, 10x Genomics).

→ *One of our projects at a glance: Investigation of potential interactions between disseminating tumor cells and platelets in breast cancer. We detect both epithelial and mesenchymal circulating tumor cells (epi and mes CTCs, respectively) by qPCR, immunoflorescent stainings and/or imaging flow cytometry (*panel A*). We established that mesCTCs have adverse impact on overall survival (*panel B *ref: Markiewicz et al, Transl Oncol, 2018), correlate with higher platelets counts (hPC,* panel C, *ref: Bednarz-Knoll et al, under review in Br J Cancer) and specific phenotype of primary tumors related to NF-kappa B signaling (*panel D, *ref: Popeda et al, Cancers, 2019). In addition, we are able to profile tumor-educated platelets (*panel E*) in order to explore their diagnostic potential.*




## Publications


- Nastały, Stoupiec, Popeda, Smentoch, Schlomm, Morrissey, Żaczek, Beyer, Tennstedt, Graefen, Eltze, Maiuri, Semjonow, Pantel, Brandt, Bednarz-Knoll. EGFR as a stable marker of prostate cancer dissemination to bones. British Journal of Cancer 2020, 123(12): 1767-1774.
- Popęda, Stokowy, Bednarz-Knoll, Jurek, Niemira, Bielska, Kretowski, Kalinowski, Szade, Markiewicz, Żaczek. NF-kappa B Signaling-Related Signatures Are Connected with the Mesenchymal Phenotype of Circulating Tumor Cells in Non-Metastatic Breast Cancer. Cancers 2019, 11(12): 1961.
- Markiewicz, Nagel, Szade, Majewska, Skokowski, Seroczynska, Stokowy, Welnicka-Jaskiewicz, Żaczek. Aggressive Phenotype of Cells Disseminated via Hematogenous and Lymphatic Route in Breast Cancer Patients. Translational Oncology, 2018, 11(3): 722-731.
- Markiewicz, Topa, Nagel, Skokowski, Seroczynska, Stokowy, Welnicka-Jaskiewicz, Żaczek. Spectrum of Epithelial-Mesenchymal Transition Phenotypes in Circulating Tumour Cells from Early Breast Cancer Patients. Cancers 2019, 11(1): 59.
- Supernat, Vidarsson, Steen, Stokowy. Comparison of three variant callers for human whole genome sequencing. Sci Rep. 2018 Dec 14;8(1):17851.
- Brandt Burkhard H., Bednarz-Knoll Natalia, Kleinheinz Johannes, Franke Andre, Fillies Thomas. RE: Oral Leukoplakia and Risk of Progression to Oral Cancer: A Population-Based Cohort Study. JNCI-Journal of the National Cancer Institute 2020, 112(9): 968-969.
- Shen Rui, Liu Peipei, Zhang Yiqiu, Yu Zhao, Chen Xuyue, Zhou Lu, Nie Baoqing, Żaczek Anna J., Chen Jian, Liu Jian. Sensitive detection of single-cell secreted $H_2O_2$ by integrating a microfluidic droplet sensor and Au nanoclusters. Analytical Chemistry 2018, 90(7): 4478-4484.
- Macintyre G, Goranova TE, De Silva D, Ennis D, Piskorz AM, Eldridge M, Sie D, Lewsley LA, Hanif A, Wilson C, Dowson S, Glasspool RM, Lockley M, Brockbank E, Montes A, Walther A, Sundar S, Edmondson R, Hall GD, Clamp A, Gourley C, Hall M, Fotopoulou C, Gabra H, Paul J, Supernat A, Millan D, Hoyle A, Bryson G, Nourse C, Mincarelli L, Sanchez LN, Ylstra B, Jimenez-Linan M, Moore L, Hofmann O, Markowetz F, McNeish IA, Brenton JD. Copy number signatures and mutational processes in ovarian carcinoma. Nat Genet. 2018 Sep;50(9):1262-1270.


## Research Grants

- National Science Centre OPUS, "Deciphering progression of triple negative breast cancer – analysis of tumor-associated cells in the bloodstream using single cell sequencing and methods of artificial intelligence", 2 074 080 PLN, 2021-2025, PI Anna Żaczek.
- National Science Centre SONATA, "Analysis of interaction between Tumor Educated Platelets and ovarian cancer cells", 1 044 088 PLN, 2019-2022, PI Anna Supernat.
- National Centre for Research and Development — Polish — Chinese Programme, "Highly Efficient Enrichment, Single-cell Analysis, and Drug Screening of Circulating Tumor Cells for Personalized Medicine (HESCAP)", 2 000 000 PLN, 2019-2022, PI Anna Żaczek.

## Patents

- Polish Patent Office, Patent number 227588, Markiewicz A, Żaczek A, Method of detection of aggressive cancer cells of stem cell-like and/or mesenchymal features, a set for detection and its application for in vitro detection of aggressive cancer cells of stem cell-like and/or mesenchymal features in order to predict breast cancer outcome.

## Scientific Collaboration

- Institute of Functional Nano and Soft Materials (FUNSOM), Soochow University, Suzhou, Jiangsu, China.
- Department of Pathology, Chair of Experimental Medicine, University of Regensburg, Germany.
- Department of Neurosurgery, Cancer Center Amsterdam, VU University Medical Center, Amsterdam, the Netherlands.

## Research Awards

- Biotechnology Committee of the Polish Academy of Science, Prof. Wacław Szybalski Foundation Award, 2020 – Aleksandra Markiewicz.
- FNP START, 2019 – Anna Supernat.
- Franco-Polish Pharmaceutical Prize: Polonium 2018 – Natalia Bednarz-Knol.

# Important Dates and Facts

**2020** The Polish Accreditation Committee (PKA) awarded the Biotechnology study program at IFB with the Certificate of Educational Excellence in the category **"Excellent programme - excellence in education"**

- PLN 4 million Ministry of Education and Science subsidy granted to Prof. Krystyna Bieńkowska-Szewczyk for reconstruction and adaptation of the virological laboratory at IFB to the high safety standard (BSL3+) required for working with respiratory viruses.
- The Research and Development Laboratory at IFB received the Accreditation Certificate No. AB1760 from the Polish Centre for Accreditation
- The biotechnology programme offered at IFB received the **highest rating from the Polish Accreditation Committee.** Accreditation was granted for the longest possible period of five years

**2019** IFB awarded with the Symbol of the **Synergy of Science and Business 2019**

- Medical University of Gdańsk (MUG) with the **status of a research university.** MUG has been selected to the elite group of the top 10 Polish universities awarded in the prestigious competition **"Initiative of Excellence - Research University".**
- IFB coorganizes *Molecular Biology for Polish Young Researchers and Students Promoting Embc/Embo And Embl Activities* together with the Ministry of Science and Higher Education of Poland
- IFB coorganizes *ScanBalt Forum 2019* on "Molecular Biology and Immunology of Cancer – R&D perspectives:"
- XV edition of the Biotechnology Summer School organized by IFB

**2018** IFB coorganizes 3rd Congress of Polish Biosciences BIO2018 *"Through interdisciplinary approach into new solutions"*

- Alumni Reunion at IFB
- 25th Anniversary of IFB
- IFB Research and Development Laboratory granted **ISO certificate** in accordance with PN-EN ISO/IEC 17015:2005+Ap1:2007 norm as the first at the University of Gdańsk
- XIV edition of the Biotechnology Summer School dedicated to Responsible Research and Innovation (RRI) in the framework of STARBIOS2 Horizon 2020 EU Project organized by IFB

**2017** Committee for Evaluation for Scientific Units completed omprehensive evaluation of the Polish scientific units granting IFB the highest possible mark A+ as a leading unit with the highest standard of scientific activities and research potential

- XXIII edition of the Biotechnology Summer School organized by IFB

**2016** The first Academic Year inauguration in the new building of IFB

- XXII edition of the Biotechnology Summer School organized by IFB
- Project STARBIOS 2 (Structural Transformation to Attain Responsible BIOSciences) funded for the EU Horizon 2020 research and innovation has started in May 2016
- Official opening of the new IFB building at the campus of University of Gdańsk – one of the most modern research and educational academic units in Poland

**2015** The last Academic Year inauguration in the old IFB premises at ul. Kladki 24 in Gdańsk

- The completion of the construction of the new building of the Institute of Biotechnology
- Prof. Krzysztof Bielawski re-elected Vice – President of ScanBalt
- XXI edition of the Biotechnology Summer School organized by IFB

**2014** 25 April – laying of the cornerstone for the new biotechnology building at University of Gdansk Campus. of the construction of the new building of the Institute of Biotechnology at University of Gdańsk Campus

- XX edition of the Biotechnology Summer School organized by IFB
- Establishing the units of Core Facility Laboratories and Teaching Laboratories at IFB

**2013** Establishing of a new unit at IFB dedicated to facilitating research management & administration: Dean's Office for Research and Project Management

- Start of the FP7 project MOBI4Health - Centre Of Molecular Biotechnology for Healthy Life Biotech solutions bringing health to living organisms and environment supported by mass spec-focused research platform" (2013-2016, 7. Framework Programme of the European Union), coordination by Prof. Krzysztof Bielawski
- XX Anniversary of IFB

**2012** Funding from the National Centre for R&D for the construction of a new building of the Institute of Biotechnology obtained (EU Structural Funds)

- Consortium agreement between IIMCB (Warsaw) and IFB signed
- IFB again receives funding for the so-called commissioned study programmes and for improving the alumnis' opportunities on the labour market
- Biotechnology at IFB as one of the best study courses receives additional funding from the Mini- stry for Science and Higher Education for further improving the study programme
- Distinction for the Best Major for the study course Biotechnology at IFB granted by the Ministry for Science and Higher Education

**2011** Distinction for the study course Biotechnology (B.Sc. and M.Sc.) at IFB granted by the Polish Accre- ditation Committee

- Establishing of IFB Advisory Council to intensify cooperation with institutions and companies from the biotech sector
- International Institute of Molecular and Cell Biology in Warsaw (IIMCB) joins the Life Sciences and Mathematics Interdisciplinary Doctoral Studies (LiSMIDoS)

**2010** IFB obtains the first category in the National Evaluation of Scientific Institution (tor the 3rd time) and is ranked in the 1st place within the group of biology at Polish universities

- Prof. Ewa Łojkowska is elected vice-chairwoman of the ScanBalt Association
- The Central Commission for Scientific Titles and Degrees licenses the Faculty to confer habilitation in biological sciences in the discipline of biochemistry
- Establishing of Life Sciences and Mathematics Interdisciplinary Doctoral Studies (LiSMIDoS)
- Opening of the International Doctoral Project, financed by the Polish Science Foundation from the Innovative Economy Programme, EU Structural Funds, coordination by Prof. Jarosław Marszałek
- IFB obtains the premium category in the National Evaluation of Scientific Organizations

**2009** IFB is funded by Ministry of Science and Higher Education within the frame of Commissioned fields of studies in Polish universities under the Human Capital Program, EU Structural Funds, co- ordination by Prof. Igor Konieczny.

**2008** Start of collaboration and exchange activities for Master degree students with the University of Chicago, USA and University of Virginia, USA

**2007** IFB is granted with accreditation for teaching biotechnology by the Polish University Accreditation Commission (for the 2nd time)

**2006** IFB becomes partner in the INTERREG III project ScanBalt Campus (2006-2008). The Centre of Knowledge on Molecular Diagnostics for Medicine, Plant and Animal Diseases in Gdańsk, coordina- tion by Prof. Ewa Łojkowska and Prof. Jacek Bigda

- Prof. Krzysztof Liberek elected as member of the European Molecular Biology Organization

**2005** IFB is granted the accreditation for teaching biotechnology granted by the State Accreditation Commission

- Opening of new laboratories belonging to the Faculty at the Medical University of Gdańsk (Tricity Academic Experimental Animal House – Research Service Centre of the Medical University of Gdańsk)
- IFB obtains the first category in the National Evaluation of Scientific Institution (for the 2nd time)

**2003** IFB establishes the Centre of Excellence BioMoBiL – Centre of Bio-safety Research and Molecu- lar Biomedicine – Integration in Education and Research Towards the Knowledge and Technology Transfer Level (2003-2006, 5. Framework Programme of the European Union); coordination by Prof. Jacek Bigda and Prof. Ewa Łojkowska.

**2002** IFB is granted with accreditation for teaching biotechnology by the Polish University Accreditation Commission

**2001** IFB becomes member of the ScanBalt association. Prof. Anna Podhajska elected as vice-chairwo-man of ScanBalt (2001-2005)

- IFB obtains first category in the National Evaluation of Scientific Institutions
- The Faculty becomes a partner in the consortium of 10 European Universities and participates in the establishment of a new teaching program: Job Creation Biotechnology Diploma – International First Level Degree (3 years), coordinated by prof. Mariapia Viola Magni from Universita degli Studi di Perugia, Italy

**2000** Prof. Wacław Szybalski is granted the title of Doctor Honoris Causa of the Medical University of Gdańsk. The initiative comes from and is supervised by the IFB. Prof. Wacław Szybalski is also ho- noured at the Marie Curie-Skłodowska University of Lublin (1980) University of Gdańsk (1989) and Technical University of Gdańsk (2002)

**1999** Opening of new laboratories in the Institute of Biotechnology, UG. Main lecture hall named in the memory of late prof. Karol Taylor, the founder of molecular biology in Gdańsk

- Central Commission for Scientific Titles and Degrees licenses IFB to confer PhD degrees in biologi- cal sciences in the discipline of biochemistry

**1994** Successful application for a first EU project: Creation and development of a novel Faculty of Bio- technology (TEMPUS, 1994-1997), coordination by Prof. Wiesław Makarewicz, Dean of IFB

- 1st Biotechnology Summer School organized at Wilga, near Warsaw, by Prof. Anna Podhajska. Prof. Wadaw Szybalski is the honorary guest at the event

**1993** Senate of the Medical University of Gdańsk and Senate of the University of Gdańsk decide to es tablish the Intercollegiate Faculty of Biotechnology UG & MUG

**1992** Crystallization of the idea to create a joint unit for teaching biochemistry among the universities in Gdansk. Rectors of the University of Gdańsk, Medical University of Gdansk and Technical Uni versity of Gdansk appoint Prof. Anna J. Podhajska as the person responsible for organizing and establishing the structure of this faculty

# Administration Team

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**Aleksandra Krejmer**
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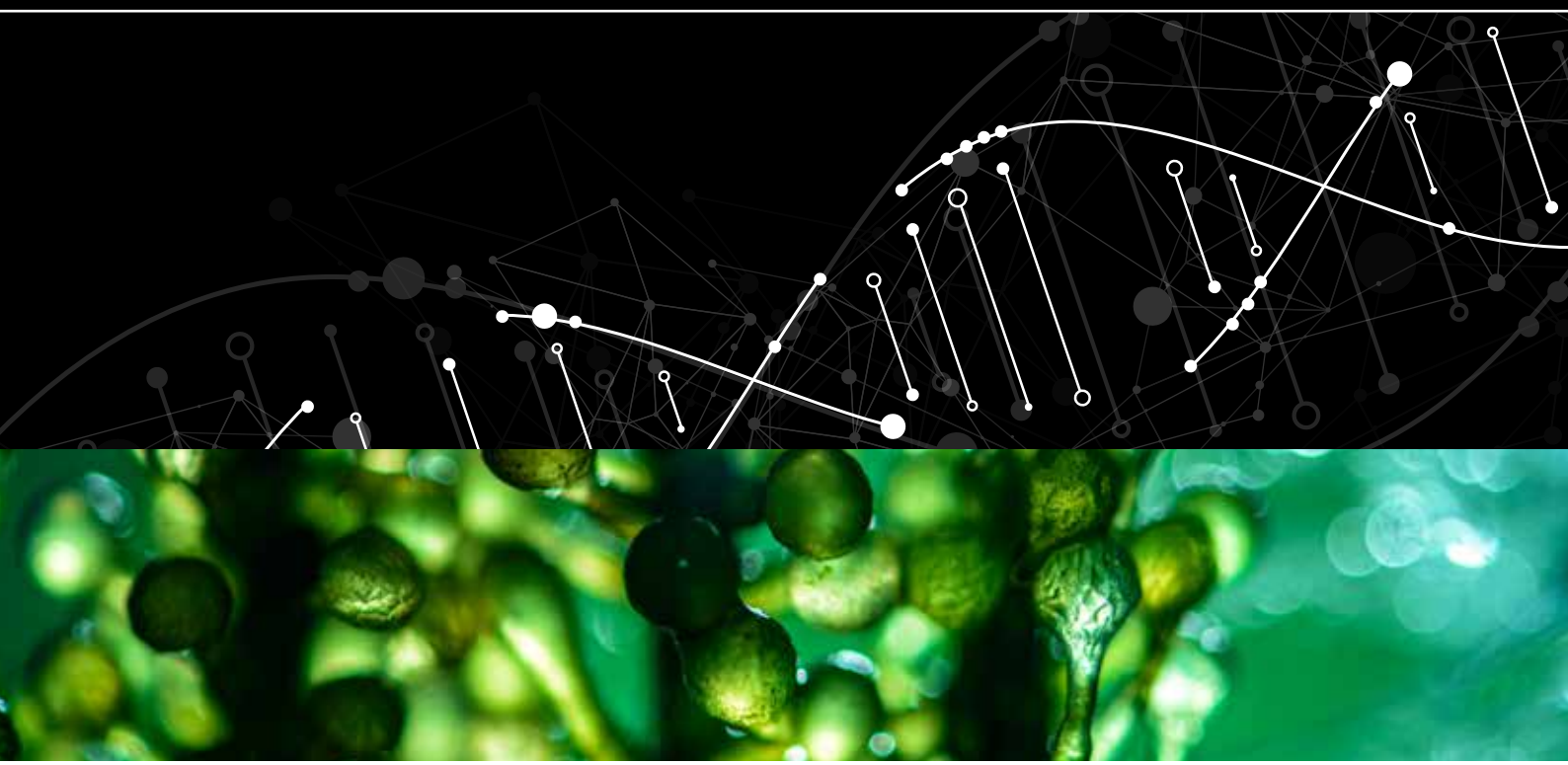

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