JAGIELLONIAN UNIVERSITY IN KRAKÓW

Faculty of Biochemistry, Biophysics and Biotechnology


UNIWERSYTET JAGIELLOŃSKI

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Foreword

The Faculty of Biochemistry, Biophysics and Biotechnology (FBBB) is one of Poland's leading national research centres in the life sciences, as recognized by the last two evaluations conducted by the Polish Ministry of Education and Science.

We are dedicated to supporting research to the highest level, publishing in the best scientific journals and promoting national and international collaborations. We seek to translate our research to commercial applications. Of equal importance, is our commitment to delivering a high standard of academic teaching, sharing our knowledge and passion for science and discovery with our students.

The success of FBBB is a direct result of the joint efforts of the staff and students, past and present. The unique and exceptional atmosphere provides a solid foundation to guarantee our constant development. We encourage new ideas, creativity and vision through our thoughts and actions. I believe that with our new efforts and ambitious initiatives, the Faculty will enhance its scientific potential, providing an even better environment and infrastructure for its employees, whilst also enabling it to attract outstanding scientists from all over the world.

In a dynamically changing reality that is presenting us with challenging new trends and expectations in science and education, we all continue to be guided by the values of scientific integrity, reliability, friendship and trust, while undertaking research endeavors important for society, environment and economy.



Professor Jolanta Jura
Dean

About the FBBB

The Faculty of Biochemistry, Biophysics and Biotechnology (FBBB) is one of the leading scientific and educational institutions in Poland in the field of modern biology. It is also well-recognized internationally in several specific areas of research for its outstanding contributions and discoveries and pioneering methodological and conceptual approaches.

The FBBB was established in 2002, following the expansion of the Jan Zurzycki Institute of Molecular Biology which had been growing since the 1970s. The FBBB has 17 Departments/Laboratories and other Research Units conducting multidisciplinary research at the atomic and molecular level, as well as at the level of tissue and organisms.

Since 2013, the FBBB has been ranked in the highest category (A+) in the Polish national ranking of scientific and research institutions. In 2014, the FBBB was awarded the status of Leading National Research Centre (*KNOW*). In 2016, according to the Nature Index that tracks the affiliations of original research articles, the FBBB received the highest score among all life science research institutions in Poland. The FBBB contributed significantly to ranking of the JU in the list of Europe's Most Innovative Universities in 2018.



The FBBB employs about **150** scientists, including **21** full-time professors and **29** associate professors.



About the FBBB

The FBBB offers study programmes leading to Bachelor's, Master's and PhD degrees in the field of biotechnology, biophysics, biochemistry, bioinformatics, and molecular biology, as well as a two-year Master's programme in English for Polish and foreign students. In 2016, the programme in biotechnology came first in the ranking held by the “Perspektywy” Educational Fund. This success is has been repeated every year for the next 5 years.

The FBBB's building is located on the 3rd Campus of the JU, in close vicinity to other faculties and life sciences units of the JU, including the Faculty of Biology, the Jagiellonian Centre for Experimental Therapeutics, the Małopolska Centre of Biotechnology and the National Synchrotron Radiation Centre SOLARIS. The FBBB is linked with all four of these units under the Excellence Initiative – Research University, Structural and Translational Biology Priority Research Area (BiOS PRA) to support scientific collaboration and interdisciplinary projects and constantly expand methodological possibilities and areas of research activities.

The FBBB receives public funds from the Polish Ministry of Education and Science (*MEiN*, formerly the Ministry of Science and Higher Education) as it is a part of a public, state-funded university. In addition to this, research at the FBBB is financed by several national and international grant agencies including: the Polish National Science Centre (*NCN*), the Polish National Centre for Research and Development (*NCBiR*), and the Foundation for Polish Science (*FNP*), as well as by EU funds and private institutions providing support to science.

Research synopsis

Research at the FBBB covers broad areas of basic and applied science in:

- **biochemistry**
  – protein structure and dynamics, macromolecular interactions, lipids, peptides, proteomics, antibodies, metabolomics

- **molecular biology/biotechnology**
  – gene transcription regulation, post-transcriptional and post-translational modifications, cellular signalling pathways, genetic and cellular engineering, bioremediation

- **biophysics**
  – eye and skin photobiology, radiobiology, oxidative stress, bioenergetics of prokaryotic and eukaryotic cells, DNA damage and repair, quantum mechanics and modelling, advanced optical and magnetic spectroscopies and imaging of molecules, organelles, cells, tissues, and organs

- **immunology/microbiology**
  – regulation of immune response, host-pathogen interactions, bacterial infection, proteases and protein inhibitors, autoimmune diseases, periodontitis

- **cellular and tissue biology**
  – stem cell biology, vascular biology, cancer biology and therapy.

Specific research areas and key achievements are described in the sections on the individual Departments and Laboratories of the FBBB.

The FBBB organises Winter Schools, which are Faculty retreats to discuss science, exchange ideas and share achievements among Departments and Laboratories. The Winter Schools are organized every year since the time of creation of the Institute of Molecular Biology in the 1970s to gather employees and doctoral students from the FBBB, as well as invited guests from Polish and foreign institutions.

The FBBB hosts or co-hosts a number of conferences and specialist workshops (e.g. an international workshop on EPR spectroscopy), including large-scale events such as EUROBIOTECH (European Congress of Biotechnology).



Specialized laboratories and core facilities

In January 2020, the FBBB was put forward for inclusion in the Polish National Roadmap for Research Infrastructures with the proposal to establish the Biomedical Multiscale Imaging Centre (MultIMA). This long-term infrastructural project aims to develop a broad platform of core facilities that will enrich existing infrastructure with access to new, advanced and highly-specialized methodological approaches for conducting studies of biological systems at levels ranging from the sub-molecular to the organism level.

Cell Bank

The Cell Bank is a laboratory with a deployed quality assurance system (Good Manufacturing Practice) equipped with a set of equipment necessary to conduct cell cultures and with an HVAC ventilation system to ensure maintenance of aseptic conditions for the production of advanced therapy medicinal products for clinical use (Hospital Exemption Advanced Therapy Medicinal Products – HE-ATMPs).

The Cell Bank collaborates closely with hospitals by providing biological material for the treatment of burns and ulcerations.

Specialized laboratories and core facilities

Animal Facility
The Faculty's Animal Facility works to a specific pathogen-free (SPF) regime, certified by regular health screening performed according to Felasa recommendations. Rooms are fully equipped to perform all animal handling under sterile conditions. The facility is registered both for maintaining and breeding laboratory animals.
It is also certified to use genetically modified mice (GMO Bl1).

The Animal Facility provides access to the following labs, rooms and equipment:

- The Microsurgery Room equipped with anaesthesia stations, surgical microscopes, a micromanipulator for pre-zygotic injections, heating tables, and recovery chambers for animals.
- The *In vivo* Imaging Laboratory equipped with high-resolution ultrasonography equipment for small animals, an *in vivo* luminescence measurement system), and an blood flow measurement system.
- The Flow Cytometry Laboratory equipped with an flow cytometer, cell sorter, as well as an Image Stream System and a Multiplex assay system.
- The Histology Laboratory equipped with a tissue processor, a tissues stainer, a tissue embedding system, a microtome, a cryostat, and a laser capture microdissection system.



Cooperation with scientific institutions and industry

Much of the research at the FBBB has been conducted in collaboration with other research centres, including over 80 universities and research institutes in Poland, in other European countries, as well as in the USA, South America and Asia. Scientists from the FBBB are also involved in projects conducted within the framework of European Union consortia and programmes such as the EU Joint Programme – Neurodegenerative Disease Research, the European Research Area Network on Cardiovascular Diseases (ERA-CVD), UM Cure 2020, COST Actions and many others.

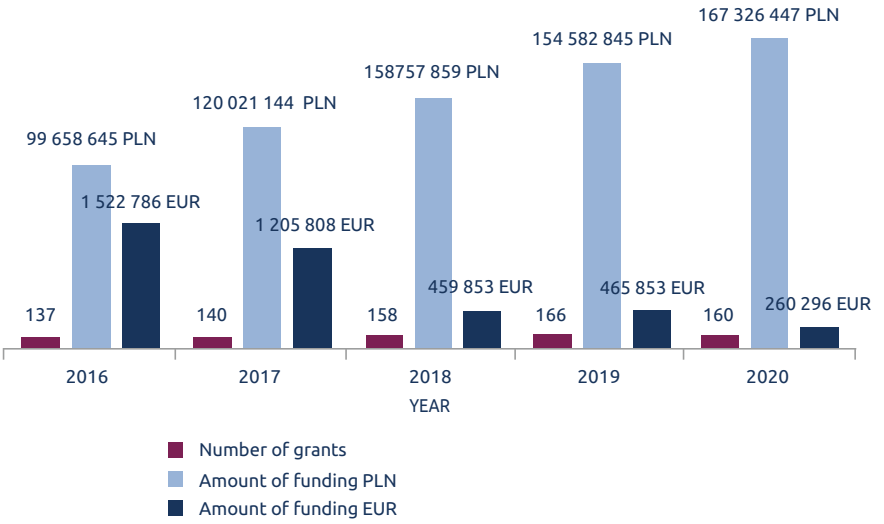
Work is conducted at the Faculty on a range of projects with a potential practical application and on "strict" research deployment projects carried out in cooperation with industrial partners. This cooperation includes commissioned studies – the preparation of expert opinions, conducting of analyses, consultancy in the field of research techniques, and as well as scientific projects. Our business partners include pharmaceutical and biotechnology companies, such as leading Polish firms like Adamed Pharma S.A., Selvita S.A. and Ryvu Therapeutics S.A.

Research studies conducted at the Faculty have resulted in numerous patent applications and patents. Just recently, a spin-off company, IntoDNA, commenced operations based on one of these and is now experiencing dynamic growth.

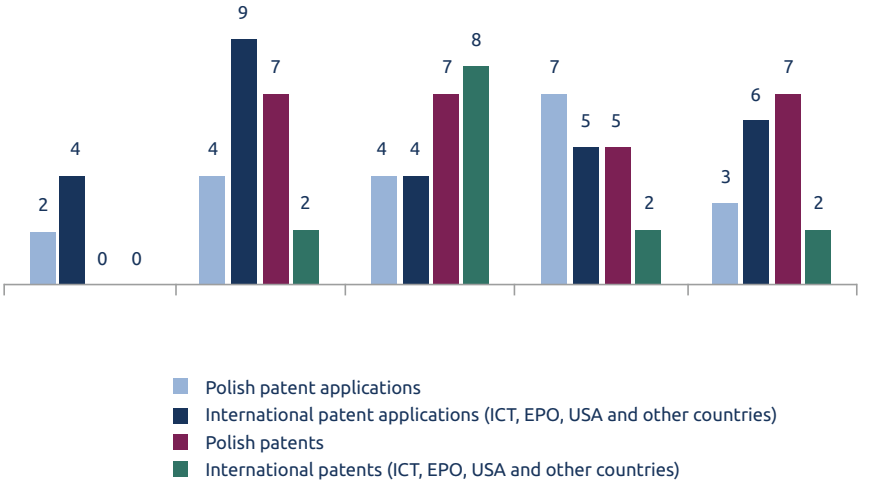


FBBB in numbers

Annual number of research grants carried out at the FBBB and the total amount of funding over the period from 2016 to 2020.



Number of patent applications and patents filed by scientists from the FBBB over the period from 2016 to 2020.

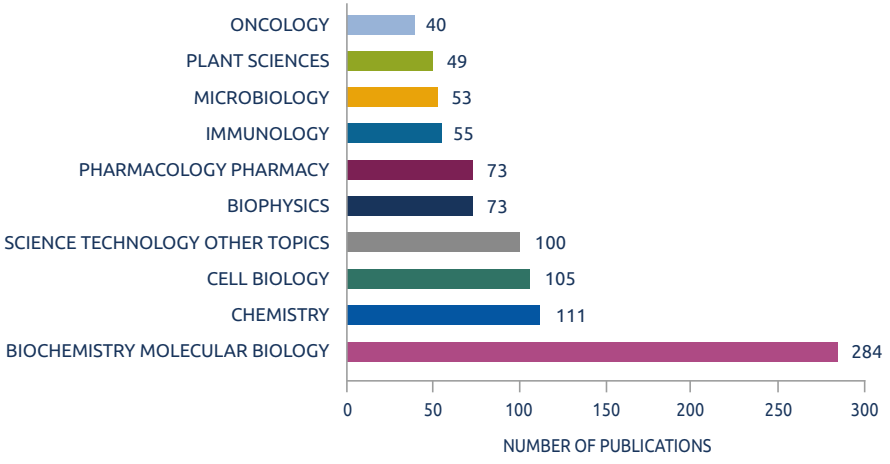




Number of scientific articles published by scientists from the FBBB over the period from 2016 to 2020.



Quality of scientific articles published by scientists from the FBBB over the period from 2016 to 2020 (based on a value of an average impact factor <IF> per work).



10 main areas of research in which scientists from the FBBB published scientific articles over the period from 2016 to 2020.

Education

The FBBB offers four Bachelor's degree programmes, four Master's degree programmes, as well as doctoral studies. Since 2007, foreign students have been welcome to enrol for a two-year Molecular Biotechnology Master's degree programme in English. All in all, around 700–750 students participate in our programmes every year.

Thanks to bilateral agreements signed with sixteen universities from nine European countries, the FBBB hosts foreign students – mainly from Spain, Germany, France, and Turkey – who come to Poland as participants in the Erasmus+ programme.

Several factors secure the high quality of our study programmes:

- Lectures, given by active researchers, include updates on the latest developments, ideas and breakthroughs in science as well as the discussions on the ongoing controversies and uncertainties.
- Curricula are flexible – numerous elective courses enhance individual development. At the same time, every study programme provides necessary backgrounds in biology, chemistry, physics, mathematics, and biochemistry.
- Classes are run in small groups.
- Students acquire practical skills in labs and they run their own projects and experiments.

Every year, undergraduate, graduate and postgraduate students from the FBBB receive awards and scholarships from many public and private institutions (such as the scholarships from the Polish Ministry of Education and Science *<Stypendium Ministra Edukacji i Nauki>*, the Polish Ministry of Education and Science Diamond Grant *<Diamentowy Grant>*, and the L'Oréal Scholarship for Women and Science). The study programmes are highly rated in numerous educational rankings.

Education

Biotechnology
3-year Bachelor's degree programme (in Polish)
Up to 80 students per year

Biochemistry
3-year Bachelor's degree programme (in Polish)
Up to 60 students per year

Molecular and Cellular Biophysics
3-year Bachelor's degree programme (in Polish)
Up to 30 students per year

Bioinformatics
3-year Bachelor's degree programme (in Polish)
Up to 25 students per year

Molecular Biotechnology
2-year Master's degree programme (in Polish)
Up to 60 students per year

Molecular Biotechnology
2-year Master's degree programme (in English)
Up to 30 students per year

Biochemistry
2-year Master's degree programme (in Polish)
Up to 40 students per year

Bioinformatics
2-year Master's degree programme (in Polish)
Up to 20 students per year

Molecular and Cellular Biophysics
2-year Master's degree programme (in Polish)
Up to 30 students per year

Doctoral programme in biochemistry, biophysics, and molecular biology
4-year Doctor's degree programme
Up to 25 students per year



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UHPLC

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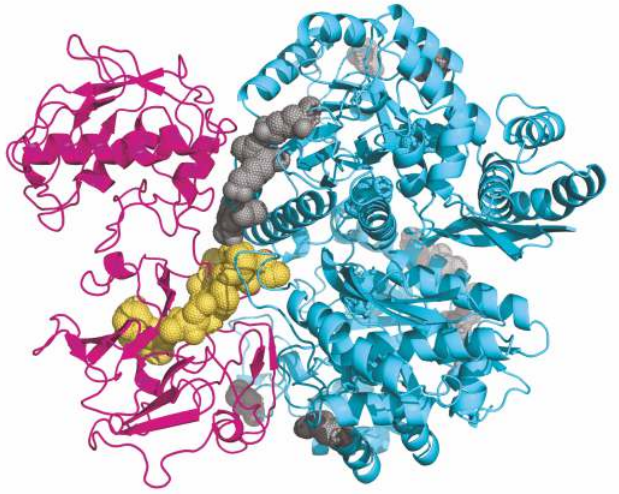
KEYWORDS
antibacterial peptides, bacteriocins, *Candida*, fungal adhesins, kinins, microbial infection, moonlighting proteins, staphylococci, toxin-antitoxin systems, virulence factors

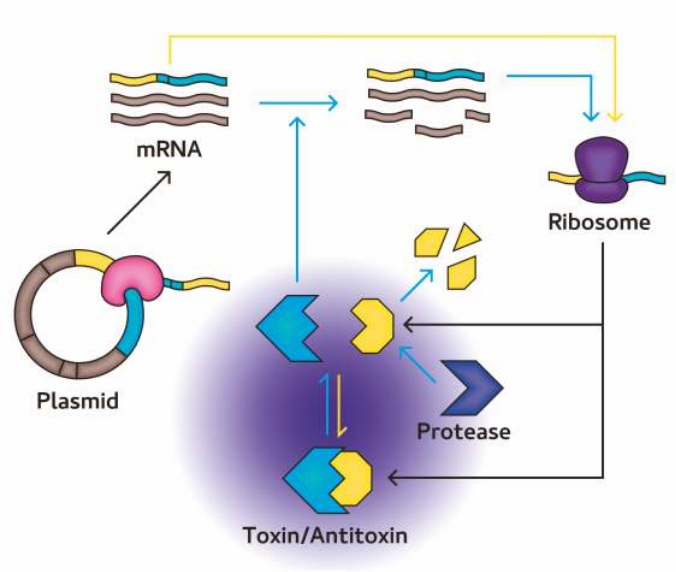
Department of Analytical Biochemistry

Scientists in the Department use methods of analytical biochemistry and peptide/protein chemistry (high-performance chromatography, mass spectrometry, automated amino acid analysis, surface plasmon resonance measurements) and molecular biology to study the structure, molecular interactions and physiological/pathophysiological functions of several groups of peptides and proteins. Currently, the main objects of studies include bacterial and fungal proteinaceous virulence factors, proteolytic enzymes involved in microbial infections, inflammatory states and haemostasis, protease inhibitors, antimicrobial and proinflammatory peptides, and bacterial toxin-antitoxin systems.



Interaction of candidal cell-surface adhesins with human host proteins
Numerous proteins exposed on the surface of *Candida* spp. yeast-like fungi contribute to adhesion of the pathogen to human host tissues. Some of these candidal cell surface factors are "moonlighting proteins", i.e. their adhesive functions add to the primary role in the intracellular metabolism. The pathogen-host protein-protein interactions, such as that in the depicted candidal enolase-human vitronectin complex, are studied by methods of protein chemistry, surface plasmon resonance measurements, mass spectrometry and molecular modelling.

In pursuit of new antibiotics: isolation and characterization of novel microbicidal peptides
Production of antimicrobial peptides (AMPs) is an ancient mechanism of immunity, encountered in most species of living organisms. They are also produced by bacteria, as so-called bacteriocins, acting as weapons against competing strains. The studies conducted by Dr. Paweł Mak and his co-workers are devoted to isolation and characterization of novel AMPs produced by different insect species, as well as those excreted by staphylococci. These compounds are then characterized in terms of their structure, mechanism of action and biological role. The results of this research are expected to help develop new antibacterial drugs.



Staphylococcal virulence factors and toxin-antitoxin systems

Staphylococci, as opportunistic pathogens in human and animals, produce an array of virulence factors (VFs), the expression of which undergoes orchestrated regulation. We focus on biochemical, structural and functional characterization of those molecules. Moreover, we conduct research into the distribution of VF genes and their expression using genomics, transcriptomics and proteomics. Toxin-antitoxin systems (TASs) are involved in the maintenance of genetic material and bacterial response to stress. We characterize TASs in staphylococci, elucidating their role in virulence, response to antibiotics and host-pathogen interactions.

Molecular interactions of cellular kinin receptors

Bradykinin-related peptides, collectively called kinins, are universal mediators of inflammation, involved in numerous human diseases such as neurodegeneration, vascular system dysfunctions and obesity. The biological effects of kinins are exerted via cell-membrane G protein-coupled B1 and B2 receptors. The interactions of these receptors with natural and pharmacological ligands, and with other cellular G protein-coupled receptors are studied in the Department using methods of molecular biology and physical biochemistry.

SELECTED PUBLICATIONS

1. Satala D, et al. Structural insights into the interactions of candidal enolase with human vitronectin, fibronectin and plasminogen. *Int J Mol Sci.* 2020;21:78438; doi:10.3390/ijms21217843
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3. Wladyka B, et al. A peptide factor secreted by Staphylococcus pseudintermedius exhibits properties of both bacteriocins and virulence factors. *Sci Rep.* 2015;5:14569; doi: 10.1038/srep14569
4. Niewiarowska-Sendo A, et al. Influence of bradykinin B2 receptor and dopamine D2 receptor on oxidative stress, inflammatory response, and apoptotic processes in human endothelial cells. *PLoS ONE.* 2018;13(11); doi:10.1371/journal.pone.0206443


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KEYWORDS
inflammation, regulation of gene expression, monoclonal antibodies, nanoparticle drug carriers, regulatory RNAs, RNA turnover, stress granules, lymphatic endothelium, gene silencing, genome editing

Department of Cell Biochemistry

The research conducted by the Department of Cell Biochemistry focusses on regulation of the expression of genes involved in inflammation and cancer; mechanisms involved in the regulation of transcript stability; the generation and characterization of monoclonal antibodies specific to selected proteins – their applications in research, diagnostics and therapy; and nanoparticle-based drug delivery systems.

Biological evaluation of nanomaterials and biomaterials for their use in medicine

- *In vitro* analyses of nanomaterial toxicity (haemolysis, oxidative stress and proinflammatory activity).
- Nanomaterials biodistribution, pharmacokinetic and toxicity studies *in vivo.*
- *In vitro* and *in vivo* evaluation of antitumor activity of encapsulated drugs.



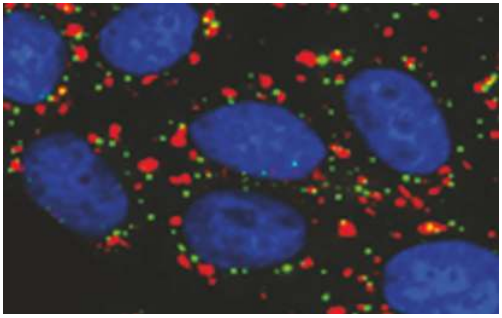
Production of monoclonal antibodies using two different technologies

- Hybridoma technology: generation of hybridoma cell lines producing monoclonal antibodies.
- Antibody phage display technology: production of soluble antibody fragments.
- Purification of monoclonal antibodies using affinity chromatography.
- Studies on unique properties of mouse monoclonal IgG3.

Mechanisms of degradation/inactivation of mRNAs and lncRNAs

The development and progression of inflammation partially depends on the rate of degradation of mRNAs and regulatory RNAs by specific RNases. We have been focussing on ZC3H12B-D RNases. To investigate their biological roles, we are employing new approaches such as:

- single-molecule RNA FISH (smRNA-FISH) combined with immunofluorescence (IF) to analyse RNA-protein interactions and subcellular localization in single cells
- the MS2 coat protein (MCP) system for immunoprecipitation and fluorescence imaging of particular mRNAs and lncRNA
- the *Sleeping Beauty* transposon system for inducible expression of the genes of interest
- next-generation sequencing (NGS) to reveal transcripts down-regulated by specific RNAses
- the CRISPR and CRISPRi systems for knock-out and knock- down of mRNA or lncRNA.



Immunofluorescence images of HeLa cells exposed to oxidative stress with visualized G3BP1, a key marker of stress granules (red) and EDC4, a marker of processing bodies (green). Nuclei are shown in blue (DAPI).

Novel therapeutic targets for the treatment of lymphangioleiomyomatosis

Lymphangioleiomyomatosis (LAM) is a rare, progressive lung disease characterized by uncontrolled proliferation of smooth muscle-like cells within the lungs of young women, leading to cyst formation and gradual decline in lung function.

We have been analysing the role of TGFβ and two sheddases: ADAM17 and ADAM10 in processes that are crucial for LAM progression. Using different *in vitro* assays, we evaluate the role of these molecules in:

- the migration, transmigration and proliferation of LAM cells
- crosstalk of LAM cells with cells probably involved in LAM development such as LAM-associated fibroblasts
- lymphatic endothelial cells and immune cells
- the expression of genes crucial for LAM progression.

SELECTED PUBLICATIONS

1. Czarnek M, Bereta J. Proteolytic processing of neuregulin 2. *Mol Neurobiol.* 2020 Apr;57(4):1799-1813
2. Karabasz A, et al. In vivo studies on pharmacokinetics, toxicity and immunogenicity of polyelectrolyte nanocapsules functionalized with two different polymers: poly-L-glutamic acid or PEG. *Int J Nanomedicine.* 2019; 14: 9587-9602
3. Czarnek M, Bereta J. SmartFlares fail to reflect their target transcripts levels. *J.Sci Rep.* 2017 Sep 15;7(1):11682
4. Wawro M, et al. ZC3H12B/MCPIP2, a new active member of the ZC3H12 family. *RNA.* 2019 Jul; 25(7):840-856
5. Kochan J, Wawro M, Kasza A. Simultaneous detection of mRNA and protein in single cells using immunofluorescence-combined single-molecule RNA FISH. *Biotechniques.* 2015 Oct 1;59(4):209-12
6. Kochan J, et al. Ultra-soft X-ray system for imaging the early cellular responses to X-ray induced DNA damage. *Nucleic Acids Res.* 2019 Sep 26;47(17):e100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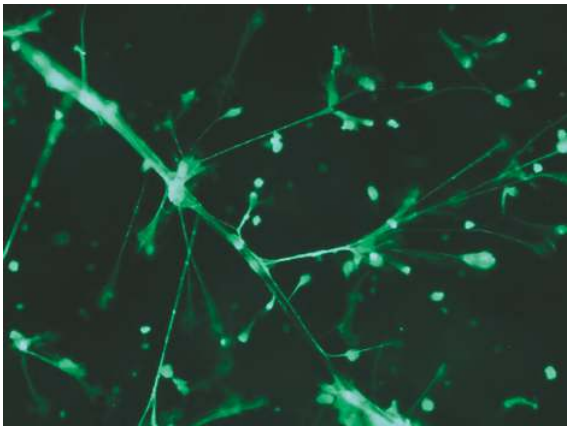
KEYWORDS
microbial infection, human pathogenic fungi, *Candida albicans,* host-pathogen-interactions, mixed species biofilm, fungal adhesins, fungal moonlighting proteins, extracellular vesicles, neutrophil extracellular trap, periodontitis, aspiration pneumonia

Department of Comparative Biochemistry and Bioanalytics

The polymorphic yeasts of the *Candida* genus are by far the most important causes of invasive mycoses in Europe. The Department's research focuses on the identification of mechanisms used by these opportunistic microbes in direct human-host colonization or the modulation of host immune responses. The possible cooperation collaboration of fungal cells with bacteria during the formation of mixed-species biofilm offers the microbes an increasing resistance to antimicrobial treatment and host defence. The overcoming of such limitations in order to expand options for antifungal therapy is therefore another challenge which is being addressed by our group.



Neutrophil extracellular traps (NETs) produced during contact with *Candida albicans* cells.

Host responses to fungal infections triggered by opportunistic yeast species of *Candida*

The host's defence cells use many defence mechanisms to fight infection caused by pathogens. One of them is the formation of extracellular traps by neutrophils in which human cells use their DNA and a series of proteins to recognize and incapacitate the enemy. Our research has identified several mechanisms in which fungi, engaging mannans and glucans present on the cell surface, as well as aspartyl proteases secreted into the environment, quorum-sensing molecules (farnesol), and yeast RNA fragments exposed by fungal biofilm, trigger the process of NET release [1,2]. However, analysis of neutrophils and yeasts indicates that both types of cells can inactivate enemy-activated mechanisms and coexist without mutual destruction.
We are currently conducting genetic, proteomic and interactomic research aimed at finding an answer to the question of what processes are used by yeast cells and human cells to regulate the type of response to mutual contact, which could be put to further use in the search of modern and effective methods of antifungal treatment.

Cross-talk between fungal and bacterial species forming biofilms

The possibility of common colonization of some human body niches by bacteria and yeasts creates favourable conditions for microbial coexistence. We considered such cooperation between *C. albicans* cells forming a biofilm under aerobic conditions, in contact with anaerobic bacteria – *Porphyromonas gingivalis* [3].
The cooperation and protection provided by the fungal biofilm matrix composed of glucans, mannans, proteins, and nucleic acids, not only allows the bacterial cells to survive in an unfavourable environment, but also makes

it difficult for them to be detected by the host's defence system, something which was confirmed in mouse models of mixed infection [4]. Currently, the mechanisms of fungal protection towards bacterial pathogen are being considered in the context of aspiration pneumonia, where popularly used antibiotic therapies are ineffective. The microbial extracellular vesicles supplying the biofilm matrix structure by their cargo [5] are the subject of special focus. We used 3D models of host cell infection to determine the changes in host responses to mixed-species infections and to demonstrate the effectiveness of mixed antimycotic and antibiotic treatment.

KEY ACHIEVEMENTS

- Assignment of destructive activity towards human defence factors such as elastase inhibitor (α1-proteinase inhibitor), antimicrobial peptide LL-37 and histones to *C. albicans* aspartyl proteases, and determination of their participation in the activation of NETs at the initial stages of fungal infection.
- Proving the protective role of microbial biofilm formed by yeasts in co-infections with anaerobic bacteria *Porphyromonas gingivalis*, concerning both increased survival under unfavourable conditions for bacteria and increased adhesive properties of *P. gingivalis* to the filamentous morphological form of *C. albicans* cells.
- Indication of the mechanism involved in microbe interaction within the mixed biofilm, engaging the fungal surface adhesins Als3 or moonlighting proteins and bacterial agglutinin – like proteinase – gingipain RgpA, as well as fungal surface proteins modification performed by bacterial protein peptidylarginine deiminase (PPAD).
- Indication of a reduced level of identification of both pathogens forming biofilm by host cells: monocytes, keratinocytes, and fibroblasts and confirmation of the chronic nature of the mixed infection in a mouse model.

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1. Zawrotniak M, et al. Aspartic proteases and major cell wall components in Candida albicans trigger the release of neutrophil extracellular traps. *Front Cell Infect Microbiol.* 2017, 7:414
2. Zawrotniak M, et al. Farnesol, a quorum-sensing molecule of Candida albicans triggers the release of neutrophil extracellular traps. *Cells* 2019, 8(12), 1611
3. Bartnicka D, et al. Adhesive protein-mediated cross-talk between *C*andida albicans and Porphyromonas gingivalis in dual species biofilm protects the anaerobic bacterium in unfavorable oxic environment. *Sci. Rep.* 2019, 9(1), 4376
4. Bartnicka D, et al. *Candida albicans* shields the periodontal killer Porphyromonas gingivalis from recognition by the host immune system and supports the bacterial infection of gingival tissue. *Int J Mol Sci.* 2020 Mar; 21(6): 1984
5. Karkowska-Kuleta J, et. al. Characteristics of extracellular vesicles released by the pathogenic yeast-like fungi Candida glabrata, Candida parapsilosis and Candida tropicalis. *Cells.* 2020 Jul; 9(7): 1722


epi
der
K10/K14
WT - MCPIP1
eKO – K14
epi – epidermis
der – dermis

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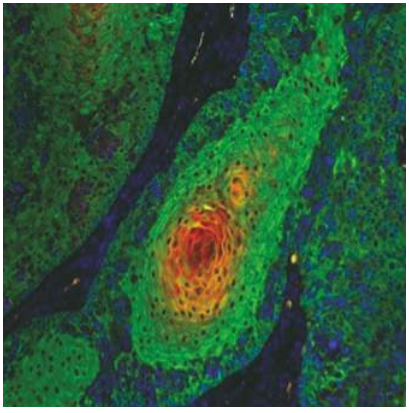
KEYWORDS
proteins with PIN-like domains, inflammation, metabolic diseases, skin disorders, tumorigenesis, RNA degradation

Department of General Biochemistry

Inflammation is a physiological response triggered by infection or tissue damage. Persistent or misdirected inflammation may become detrimental and is associated with many diseases. Our research employs a wide range of molecular techniques to investigate how inflammation is controlled in health and disease. We focus on the role of proteins with PIN-like domains that act as RNases regulating the half-life of transcripts (e.g. MCPIP family). To answer the more specific questions outlined below, we utilize a range of *in vitro*- and *in vivo*- models, including tissue-specific knockout mice, unique zebrafish models and xenotransplants.

Role of inflammation in the systemic physiology

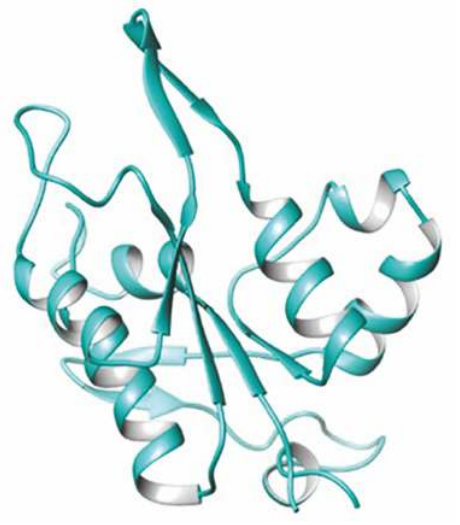
The primary objective of our group is to understand the physiological consequences of prolonged low-grade as well as acute inflammation on the homeostatic balance of an organism as a whole. Based on this holistic approach, we conduct a wide range of experimental research at multiple organizational levels, such as basic molecular and cellular biology, investigation of inflamed tissue dysfunction and implementation of a battery of behavioural tests in different animal models of systemic inflammation. On our multidisciplinary team, we are particularly focused on: i) adipose tissue and liver function in metabolic diseases (obesity, non-alcoholic fatty liver disease); ii) skin integrity, keratinocyte differentiation and inflammation associated with skin disorders (psoriasis); iii) regulations of neuroinflammation and a biological dialogue between the immunological system and brain function.



Immunofluorescence staining of skin squamous cell carcinoma tissue with keratin pearl.

Role of inflammation in tumorigenesis

Our research includes the analysis of the molecular basis of tumour development and metastasis formation. We are interested in particular in the influence of inflammatory processes and its regulatory molecules on tumour initiation, growth, vascularization and progression. Our studies concern the role of the MCPIP family in activation of growth factors that sustain proliferative signalling, survival factors that limit cell death, proangiogenic factors and extracellular matrix-modifying enzymes that facilitate angiogenesis. We study the influence of inflammation and its regulatory proteins in the activation of EMT and the resulting invasion, metastasis and resistance to therapy. Our research covers the development of kidney, skin and liver cancers. We work with cell lines isolated from primary tumours and metastases, animal models, and patient tissues.



Crystal structure of the MCPIP1 PIN domain, determined at the JU and the Argonne National Laboratory.

Mechanism of mRNA turnover catalysed by MCPIP1

In our research, we focus on the mechanism of RNA molecules degradation (mRNA, miRNA, lncRNA and circRNA) by RNases containing a PIN (PilT N-terminus) domain. RNA turnover is analysed during inflammatory response and tumour development. We have shown that MCPIP1 RNase recognizes and cleaves single-stranded stem-loop structures by binding to RNA with a nanomolar dissociation constant.

In order to learn the principles of directing RNA molecules for degradation, as well as the exact mechanism of RNA cleavage, it is necessary to understand the structure of RNases. Our goal is to characterize the structure of selected RNases, their interaction with RNA molecules, as well as to analyse the protein complexes with which RNases interact. For this purpose, we use advanced methods, such as X-ray crystallography, CryoEM and mass spectrometry. Using X-ray crystallography, we determined the structure of the MCPIP1 PIN domain. We also study post-translational modifications of RNases and their influence on nucleolytic activity.

KEY ACHIEVEMENTS

- Identification of MCPIP1 as an RNase.
- Assessment of PIN domain in the regulation of RNA half-life.
- Role of PIN domain containing proteins in biological processes and cell pathology.

SELECTED PUBLICATIONS

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KEYWORDS
protein-ligand interactions, intrinsically disordered proteins, molecular recognition, G protein-coupled receptor heteromers, signalling platforms, nanomedicine, antipsychotics, proteomics

Department of Physical Biochemistry

At the Department of Physical Biochemistry, we seek to understand the structural, thermodynamic, and kinetic aspects of macromolecules in living organisms. We focus on proteins, often in the context of their interactions with other partners such as nucleic acids, biological membranes, or low molecular weight ligands. We are interested in the functioning of these systems in living organisms and in understanding the general principles governing their function. The purpose of our early research was to gain a detailed understanding of the function of prokaryotic and eukaryotic transcription factors. Over time, our research interests have evolved and we are currently conducting studies which touch upon a range of very distinct scientific issues. What they all have in common is our extensive expertise, commitment and constant desire to broaden our research base.

Structure-function relationships in intrinsically disordered human transcription factors (A. Górecki)
We focus on the human transcription factor Yin Yang 1 (YY1), which is crucial in organism development from its early stages but also plays a role in oncogenesis and neurological disorders. We have shown that YY1 is an intrinsically disordered protein – it has extensive regions that do not form any stable spatial structures. These regions can adopt different folds, thus determining different functions upon binding to a diverse range of molecular partners. We use a variety of biochemical methods such as CD, fluorescence, SEC, ITC and NMR to characterize the structural features of YY1 and its binding interactions.

Thermodynamics of molecular recognition in the β-lactoglobulin-ligand model system (P. Bonarek)
Determining the individual energy contribution of all processes occurring during molecular recognition in the interaction of two biological molecules is key to its understanding. We conduct such studies for the model lipocalin, β-lactoglobulin, using ITC as the primary technique. We achieve modifications in the model system by selecting ligands and introducing point mutations into the recombinant protein. Our goal is to understand the ligand binding mechanism sufficiently to rationally design the protein to achieve the intended properties, such as the ability to bind a selected molecular target with a specific affinity.

Effects of cell membrane organization on G protein-dependent signal transduction (A. Polit)
We study the mechanisms and structural aspects of signal transduction involving G protein-coupled receptors and G proteins. The cell membrane contains specialized signalling platforms composed of lipids and proteins that enhance signal transduction efficiency and enable functional selectivity of receptor molecules to G proteins. We focus on the initial steps of signal transduction and activation of heterotrimeric G proteins. Our research is concerned with protein-lipid interactions, which are key modulators of the spatial and temporal distribution of signalling proteins in the cell membrane.

Exploring heteromerization of GPCRs in the context of finding novel sites of therapeutic action and controlled drug delivery to determined brain areas (S. Łukasiewicz)
G protein-coupled receptor heterodimers are formed when interacting receptors coexist in cells. Targeting drugs to heterodimers increases their selectivity. Our studies include: i) assessing dimerization of selected receptor pairs and the effect of ligands/drugs on it; ii) developing ligands for heterodimers (Phage Display generated antibodies) and using them to functionalize nanocarriers that transport therapeutic substances, such as clozapine, across the blood-brain barrier to areas of the brain expressing heterodimers; iii) studying nanocarrier-target cell interactions that will allow efficient and safe nanocarriers for drugs to be developed.

Investigating the molecular mechanism of action of antipsychotic drugs (S. Kędracka-Krok)
We use rat brain tissue, human neurons and astrocytes to elucidate the molecular mechanisms underlying the effects of the antipsychotic drugs – clozapine and risperidone. We verify the results obtained by mass-spectrometry-based proteomics with targeted proteomics methods, antibody-based and functional studies.
To understand the effects of antipsychotic drugs on transcription and epigenetic processes, we study the nuclear subproteome. We use the CRISPR/Cas9 system to investigate the effect of silencing selected genes, encoding chromatin remodelling proteins or transcription factors, on neuronal cell function and their response to antipsychotics.

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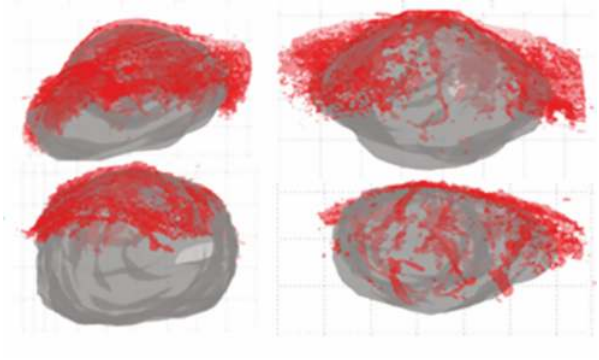
KEYWORDS
oxidative stress, reactive oxygen species, antioxidants, lipids, peroxides, photosensitizers, melanin pigments, nitric oxide, imaging, cancer, melanoma, tumour microenvironment, hair cycle, biomechanics

Department of Biophysics

The Department of Biophysics consists of two large laboratories: the Laboratory of Photobiophysics and the Laboratory of Radiospectroscopy of Cancer and Radiobiology. The focus of teaching is biophysics, photobiology, cancer biology, imaging and bioethics. Bachelor, Master and PhD students take part in our research projects. The Department is a partner in multiple long-term international collaborations and organizes a Workshop on Biology and Medicine in Electron Paramagnetic Resonance every 3 years.



Murine breast cancer E0771 vasculature.

Tumours and their microenvironment (M. Elas)
The main focus of the team is cancer biology, especially in the context of the tumour microenvironment, development of metastases and therapy. Various cellular and animal models, including spheroids, CAMs and orthotopic tumours, as well as PDXs, are used in preclinical testing of anti-cancer therapies, including radio-, photo- and thermotherapy and their combinations with chemotherapy. Of particular interest are melanoma, breast, lung and pancreas cancer. Tissue hypoxia is one of the main microenvironmental factors of interest to us. A variety of imaging techniques are extensively used, especially oxygen and redox state mapping of tumours using electron paramagnetic resonance, vasculature imaging using ultrasound, computer tomography, MRI, as well as microscopy. Biomechanical aspects of tumours are studied using atomic force microscopy. We are involved in several international collaborations of vital importance, including with the University of Chicago, the University of West Virginia, the University of Orléans, the Marie Curie Institute, and Geneva University. Our research is funded by grants from Polish and European sources.

Oxidative Processes (W. Korytowski)
Our research is conducted within two major aspects of Oxidative Stress: i) Investigation of mechanisms of tumour cell responses to photodynamic therapy (PDT), and ii) elucidation of the role and metabolic fate of lipid hydroperoxides (LOOHs) in cells under normoxic vs. oxidative stress conditions. An example of the research in the first category are studies of the bystander effect (responses of cells located in the vicinity of cells undergoing photodynamic treatment) and the role of nitric oxide (NO) in this process. Research into the second aspect concentrates on the postulated role of lipid hydroperoxides mobility and transfer in the development

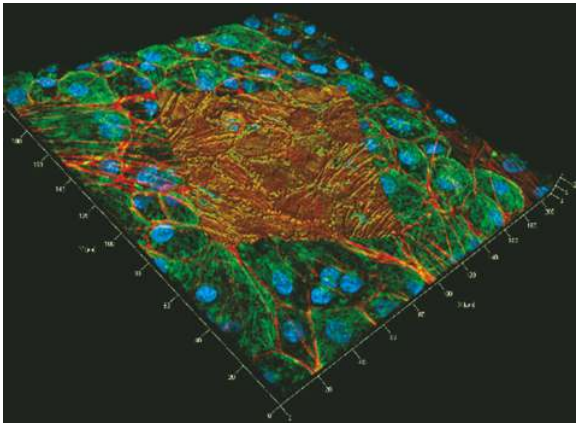
of several pathologies, including infertility and atherosclerosis. Research conducted in cooperation with the Medical College of Wisconsin has been continuously supported for over 20 years by grants from the National Institute of Health, the Fogarty Institute, and the Polish National Science Centre (*NCN*).

Oxidative stress in the retina (A. Pawlak)
Our research focusses on oxidative stress in the human retina and mechanisms involved in protection of the retina against photodamage. The role of lipids and their oxidation products in the ageing and photodamage to the retina, as well as the onset and development of retinal degenerative diseases is of particular interest. Antioxidant properties of plasmalogens, a specific subclass of phospholipids characterized by a vinyl-ether bond in sn-1 position of the glycerol backbone, and their impact on photoreactivity of other lipids present in the retina constitutes a significant part of the scientific work being conducted in this area. The second important area of research interest is the mechanism by which tauroursodeoxycholic acid (TUDCA) protects photoreceptors against photodamage. Biophysical and molecular biology methods are widely used. In our latest work, we analysed the antioxidant properties of TUDCA and its impact on biophysical properties of membranes containing high and low concentrations of cholesterol.



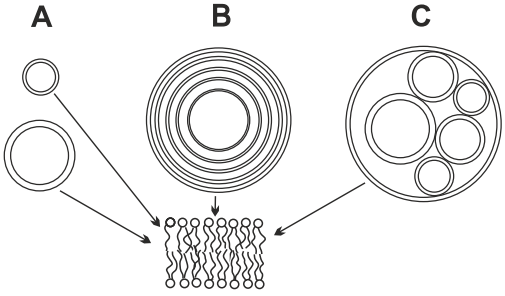
A fractal-like *in vitro* culture of *Fuligo septica* L. on the bottom surface of a Petri dish cover.

Biophysics as metabiology (P. M. Płonka)
The research subject of P. M. Płonka has undergone a long path of transformation, from the EPR measurement of complexes of nitric oxide *in vivo* and *ex vivo*, and analysis of the melanization of tumours and other pigmented tissues, through more general aspects of melanization, free radicals, and EPR, up to the general problems of history and philosophy of science. At present, Dr. Płonka interests are focused on *in cerebro* analysis and modelling of biological systems, including tumours *in silico*, semiotics, and definition of genetic information. Dr. Płonka attempts to adapt true slime moulds (Mycetozoa) to biomedical research as interesting alternate model organisms.



Combined AFM and SLCM image of ARPE-19 cells.

Biophysics of pigmentary systems (T. Sarna)
The research concerns two main pigmentary systems: epidermal melanocytes and keratinocytes, and retinal pigment epithelium (RPE).
Key physicochemical properties of melanin pigments and so-called age pigments accumulating in the RPE, are studied by advanced spectroscopic and imaging methods. The main goal of the research is to determine the mechanisms of photoprotective and antioxidant action of melanin in cells and to identify biologically relevant experimental conditions that may stimulate photosensitizing and pro-oxidant properties of melanin and age pigments.
The results we have obtained to date indicate that the predominant mode of the protective action of melanin is sequestration of redox active metal ions, scavenging of reactive radicals and quenching of electronically excited states. At the same time, the prooxidant action of melanin and age pigments involves enhanced photogeneration of reactive oxygen species and reduced efficiency to neutralize them. Our research into the nanomechanical properties of RPE and melanoma cells have demonstrated that melanin granules determine the cell elastic properties critical for the proposed mechanical barrier function of RPE and of key importance for cancer cell metastasis.



Different types of liposomes.

Structure and dynamics of lipid membranes
(A. Wiśniewska-Becker)
Our research is focused on changes in physical and chemical properties of lipid membranes caused by different chemical agents such as drugs, natural compounds (carotenoids, curcumin), photosensitizers, as well as physical factors (light, oxygen, etc.).
In this context, the domain structure of membranes including lipid rafts is of special interest.

As membrane models mostly various kinds of liposomes are used. However, alternative models such as nanodiscs have also been introduced. In the latter models, the effect of membrane scaffold protein on the lipid bilayer is being investigated. The main method used in our research is electron paramagnetic resonance (EPR) – both continuous wave (CW) and saturation recovery (SR), and nitroxide spin labels. International collaborations include partnerships with the Biophysics Department of the Medical College of Wisconsin, USA, and the University of Helsinki, Finland.

Photobiology of the skin (A. Wolnicka-Głubisz)
Our team studies a role of newly recognized proteins in response of skin cells to UVB or UVA radiation, in a context of photooxidative stress, signalling, type of cell death (apoptosis/necrosis), inflammatory response, and photocarcinogenesis. We study mechanisms of phototoxicity of new-generation photosensitizers or drugs in normal and tumour skin cells *in vitro* at the cellular and biochemical levels. Recently, our studies have focussed on the role of receptor-interacting protein kinase 4 (RIPK4) in melanoma biology, especially in the context of signalling pathways related to tumour growth, invasiveness and resistance to therapy using western blots and qRT-PCR, as well as animal models.

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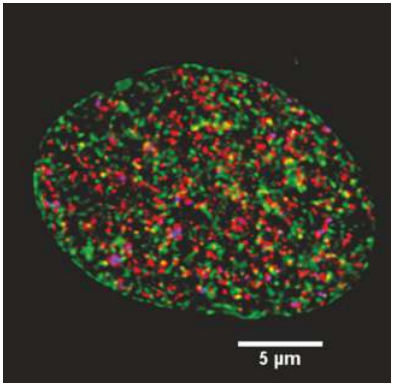
KEYWORDS
DNA damage, DNA repair, DNA-drug interactions, fluorescence live cell imaging, fluorescence confocal microscopy, FRAP, FCS, super-resolution imaging, dSTORM

Department of Cell Biophysics

Research conducted at the Department of Cell Biophysics is focused on understanding the mechanisms of induction and repair of DNA damage, and on developing new analytical techniques facilitating investigations into these problems. Current projects aim to gain an understanding of the architecture of DNA repair foci and the dynamics of repair proteins (HP1, 53BP1, XRCC1), and the influence of hypoxia on mechanisms of DNA repair. New methods include a method of detecting individual single- and double-strand DNA breaks (STRIDE).

Architecture of DNA repair foci and dynamics of repair proteins
When a DNA double-strand break (DSB) is detected by cellular surveying machinery, phosphorylation of histone H2AX in a large region of chromatin on both sides of the lesion occurs, protein repair factors are recruited to the site of damage and form a repair focus, and chromatin higher order structures undergo reorganization (the image shows DNA repair foci (γH2A.X, red; 53BP1, blue) and replication foci (green) in the cell nucleus (image by Dr P. Rybak).

Some repair foci formed in response to DSB are relatively large and appear to have a well-defined internal architecture (super-foci), with 53BP1 localized in the outermost region, and Rad51 in the centre.



The existing knowledge about DNA repair super-foci is scarce, primarily due to a lack of suitable analytical methods. The toolbox which is available to the researchers interested in chromatin structure and DNA repair contains several new methods developed in the Department of Cell Biophysics:

1) super-resolution imaging of DNA in the cell nuclei (Żurek-Biesiada et al., 2016; Szczurek et al., 2017),
2) STRIDE – a method capable of detecting individual DNA breaks (Kordon et al., 2020),
3) inducing individual DSB in a predetermined region of the cell nucleus or in a region of a known sequence in the genome (Solarczyk et al., 2016, Kordon et al., 2020).

Current work is focussed on determining the spatial positions of several repair proteins within repair foci and their dynamic behaviour (exchange with the surrounding nucleoplasm), using FLIP and FCS, in order to gain further insight into the mechanism of formation of super-foci and their role in promoting repair of DNA by homologous recombination or non-homologous end-joining pathway.

The Department's recent and ongoing work is being carried out in collaboration with researchers from the University of Massachusetts Medical School, the Brander Cancer Center in New York, USA, the Institute of Molecular Biology in Mainz, Germany, the Czech Academy of Sciences in Prague, and the Technical University in Wrocław, Poland.

KEY ACHIEVEMENTS

The Cell Biophysics Group was the first to demonstrate the involvement of Heterochromatin Protein 1 (HP1) in DNA repair, and also to demonstrate that visible light can induce DNA damage and that this damage can be controlled in order to study repair of single- and double-strand breaks. The group has developed a technique of super-resolution imaging of DNA in fixed cells, and a technique capable of detecting individual single- or double-strand DNA breaks (STRIDE).

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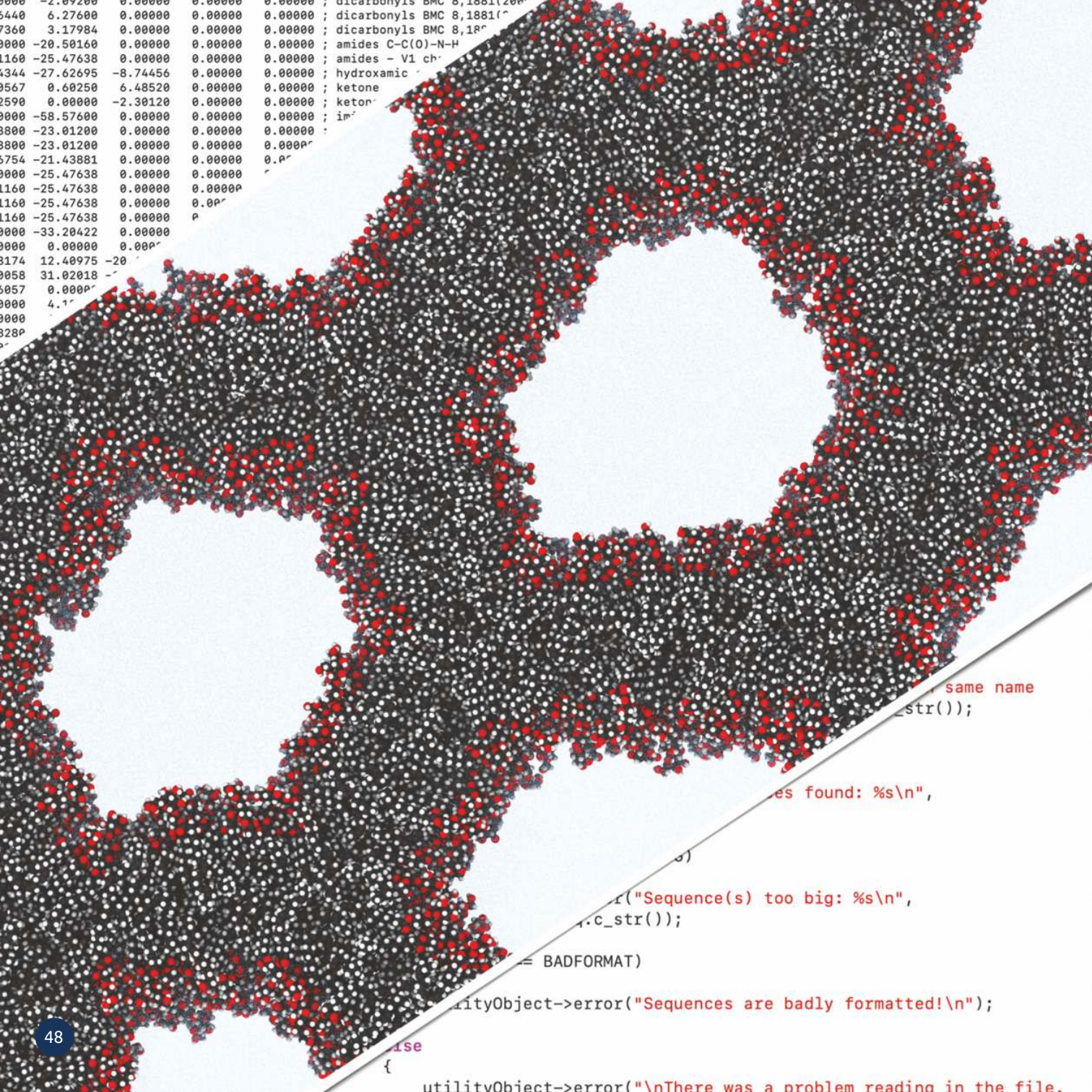
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KEYWORDS
molecular modelling, phospholipids, cholesterol, force field development, lamellar and nonlamellar lipid phases, computational biophysics, sequence bioinformatics, free-alignment methods, chaos game representation, machine learning

Department of Computational Biophysics and Bioinformatics

We study the dynamic structure of both lamellar and non-lamellar lipid phases with different molecular compositions, develop and refine empirical force fields parameters used in biomolecular modelling, and expand applications of various bioinformatics sequence analysis methods employing machine learning, protein and RNA structure prediction, biomedical text mining and a chaos game representation. We also participate actively in expanding the teaching offer of the Faculty and teach numerous courses offered mostly for students of Bioinformatics and Molecular and Cellular Biophysics programmes.

The dynamic structure of lipid lamellar and non-lamellar phases
We construct and validate computer models of fully hydrated bilayers and inverse hexagonal phases made of various phospholipids, cholesterol, and glycolipids. In our studies, we also include biologically active compounds which are known to influence the overall structure of lipid membranes and lipid-lipid interactions. In one of our recent papers published in the *Computational and Structural Biotechnology Journal*, we studied how lutein, a hydroxylated carotenoid, inserts into phospholipid membranes. Our studies allowed us to elucidate details of the dynamics and energetics of lutein intercalation from the water to the lipid phase. We also compared the kinetics of lutein intercalation to appropriate studies focussing on interactions of helical peptides with membrane phosholipids. In another of our studies published in the *Journal of Physical Chemistry B*, we investigated the properties of a hexacyl lipid A bilayer as the simplest model of interactions between lipid parts of lipopolysaccharides in the external layer of the outer membrane of Gram-negative bacteria. This study will be a reference for an asymmetric bacterial outer membrane made of phosphatidylethanolamine and sample lipopolysaccharides.

Development and validation of empirical force fields parameters
We critically compare alternative sets of force field parameters used in molecular modelling of lipids. In our recent papers published in the *Journal of Physical Chemistry B*, we provided two refined sets of OPLS-AA parameters, which address unsatisfactory reproduction of the dynamical structure of the hydrophobic core of phospholipid bilayers. We carefully validated the new parameters using both the thermodynamic and kinetic properties of liquids made of appropriate model compounds.

Development of free-alignment methods
Such methods are used for comparing sequences without an alignment step. The whole process is faster and requires only a few parameters. In our recent paper published in *Genomics*, we extended the chaos game representation and showed the benefits of such an approach to a nucleotide sequence analysis. The new chaos game representation is more robust to mutations events and provides more reliable phylogenetic trees.

Department of Computational Biophysics and Bioinformatics

KEY ACHIEVEMENTS

- The molecular basis of ordering effect of cholesterol and its analogues (380+ citations).
- A computer model of the inner bacterial membrane (190+ citations).
- The role of water at the lipid membrane interface (180+ citations).

SELECTED PUBLICATIONS

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5. Kania A, Sarapata K. The robustness of the chaos game representation to mutations and its application in free-alignment methods. *Genomics.* 2021 113:1428-1437



A section of the home-designed and assembled system for high-resolution UV/VIS spectrophotometry at liquid-helium temperatures.

Department of Molecular Biophysics

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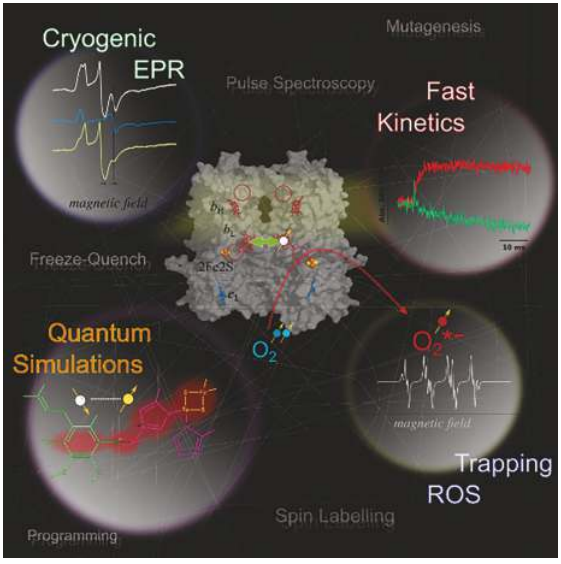
KEYWORDS
molecular bioenergetics, respiration, photosynthesis, redox enzymes, quinones, reactive oxygen species, mitochondrial diseases, electron paramagnetic resonance (EPR), spin labelling, electron and proton transport, quantum mechanical simulations

Department of Molecular Biophysics

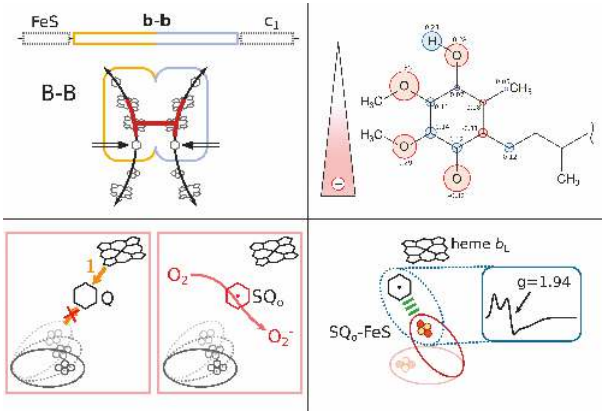
The Department gathers scientists with an interest in investigating molecular mechanisms of operation of biological complex systems using advanced spectroscopic approaches – mainly time-resolved optical spectroscopy and electron paramagnetic resonance (EPR), as well as theoretical approaches – quantum mechanical calculations and mathematical modelling. Our main research themes aim to develop an understanding of the function and regulation of key redox enzymes engaged in biological energy conversion. These include membrane complexes of mitochondrial respiration, plant photosynthesis and various prokaryotic electron transfer systems. We ask fundamental questions related to electron and proton transfer reactions, protein dynamics and protein-protein interactions, as well as the chemistry of catalytic redox reactions. We also develop new techniques, hardware and software to provide support in solving particular scientific issues.



Research synopsis

We focus on the ubiquitous group of metalloproteins consisting of multi-subunit complexes responsible for oxidation/reduction of quinol/quinone, the evolutionary-conserved reactions that contribute to generation of the proton-motive force necessary for production of ATP. Specific enzymes which are of interest to us include mitochondrial complex III, its bacterial homologue – cytochrome (cyt) bc_1, plant cyt b_6f and alternative complex III fulfilling the function of cyt bc_1 in some bacteria. We comprehensively investigate these proteins at molecular and sub-molecular levels to understand the mechanism of operation of the catalytic sites, the thermodynamic basis of the involvement of the metal centres (haems, iron-sulphur clusters), engineering of electron and proton transfers within and between the catalytic sites, the dynamics of macromolecular interactions with substrates and redox-protein partners (such as cyt *c*, plastocyanin, ferredoxin, FNR), the physiological aspects of regulation of respiratory and photosynthetic electron flow and engagement in redox signalling. Our conceptual framework considers both the energy-conserving catalytic reactions, as well as the energy-

wasting short-circuits or leaks of electrons and, associated with them, generation of reactive oxygen species. Bacterial models are exploited for genetic manipulations to target specific structural elements for functional studies. This includes mimicking mitochondrial disease-related or adaptive mutations to unravel their molecular effects and modifying proteins for site-directed spin-labelling used to probe various molecular interactions and conformational dynamics.



KEY ACHIEVEMENTS

- We have defined short (submillisecond) lifetime of the cyt *c* – cyt bc_1 complex implicating diffusion-coupled mechanism of inter-protein electron transfer [1].
- We broke the inherent symmetry of cyt bc_1 and revealed that the enzyme is a functional homodimer [2]; *left top.*
- We have described new molecular mechanism (*"Semireverse – Rieske off"*) of superoxide production by cyt bc_1 [3] and identified its relevance to the effects of mitochondrial mutations [4]; *left bottom.*
- We discovered new spin-coupled radical intermediate in cyt bc_1 and cyt b_6f [5]; *right bottom.*
- We have described charge/spin polarization of semiquinone as key catalytic element of enzymatic redox reactions of quinone [6].

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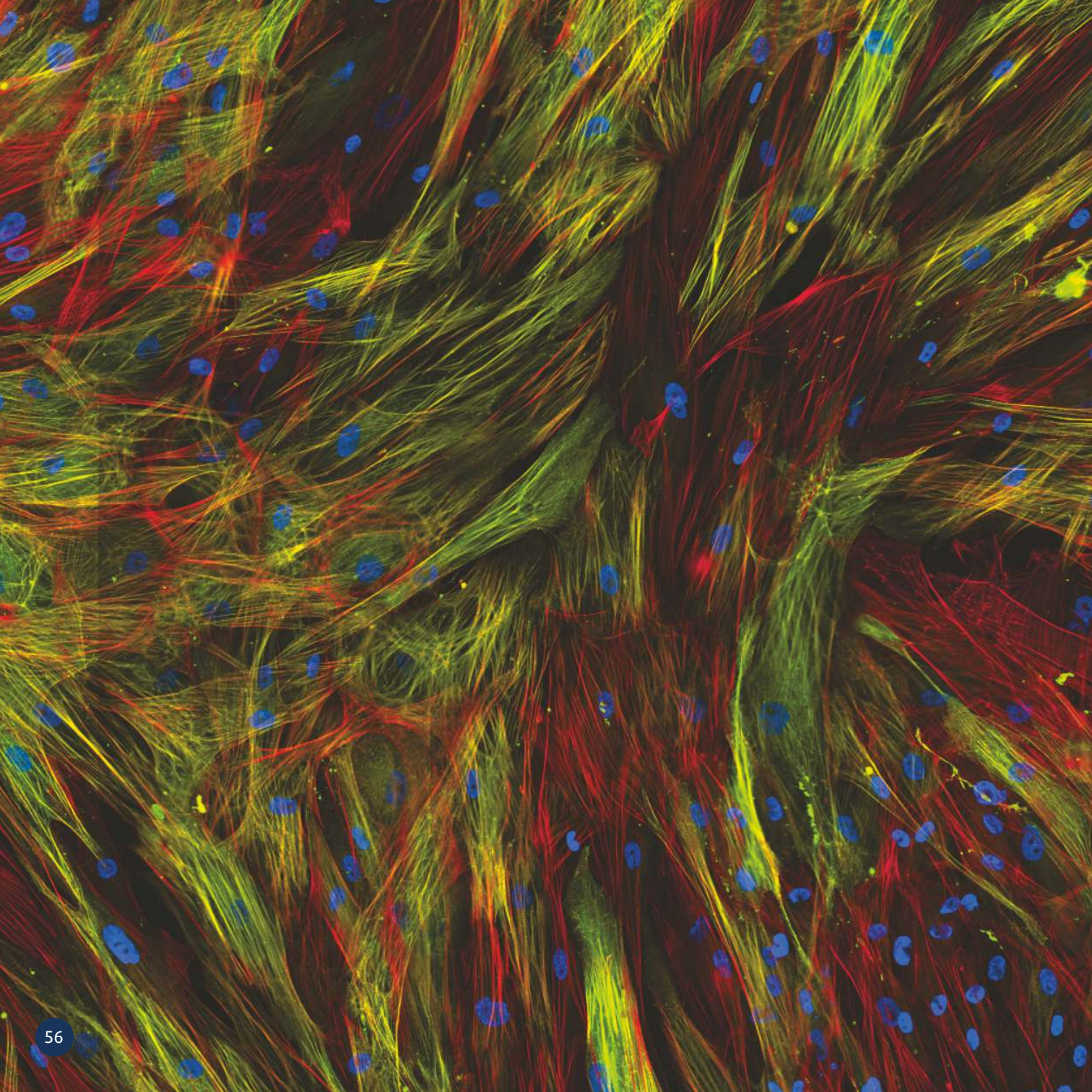

Department of Cell Biology

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KEYWORDS
electrotaxis, lipofection, gap junctions, tumour cells, tumour microevolution, myofibroblasts, asthma, wound healing, pancreas, calcium signalling, stem cells, regenerative medicine, extracellular vesicles, tissue engineering

Department of Cell Biology

The Department of Cell Biology conducts research in the following areas: direct cell-cell interactions in regulating cancer cell migration activity; involvement of connexins, gap junctions and cell adhesion molecules in tumour progression; mechanisms of electrotaxis; mechanisms of lipofection; mechanisms of epithelial–mesenchymal transition (EMT); cancer stem cells, metabolic reprogramming and multi-drug resistance; fibroblast-myofibroblast transition (FMT) and its role in respiratory tract remodelling during bronchial asthma; *in vitro* skin cell cultures in regenerative medicine: wound treatment and plastic surgery; and the application of stem cells and their derivatives (extracellular vesicles, EVs) in tissue regeneration.

Unravelling the mechanism of electrotaxis
– directional cell migration in response to electric fields (S. Lasota, Z. Madeja)
Electric fields (EFs) are present in all developing and regenerating animal tissues and play a key role in major biological processes such as embryogenesis, wound healing and tissue regeneration by induction of electrotaxis. Although a variety of cultured cells respond to application of the EFs *in vitro*, still very little is known regarding the general mechanisms of the process. Two main hypotheses in that field are based either on the activation of specific ion channels or on the redistribution of cell membrane receptors responsible for the sensing of directional signals from chemokines/growth factors. Currently, we are running a project in which a hypothesis regarding the biphasic mechanism of electrotaxis that involves a complementary role of both the components mentioned above is being verified. Another part of our research is concerned with electrotaxis of cells presenting varied strategies of migration (mesenchymal and amoeboid/blebbing).

Polyprenyl-based lipofection – applications and mechanism (M. Rak, Z. Madeja)
Lipofection (cationic lipids-based DNA/RNA delivery) is a widely used technique, as well as one of the most promising strategies for non-viral gene therapy and DNA vaccine delivery. However, its main drawback is the lack of efficient and safe lipofecting formulations. Recently, we have developed an innovative lipofection system utilizing cationic polyprenyl derivatives – trimethylpolyprenylammonium iodides (PTAIs). Since some basic mechanisms underlying efficient lipofection remain unknown, we are also investigating cellular uptake mechanisms of polyprenyl-based vehicles. We conduct our research in cooperation with the Institute of Biochemistry and Biophysics, Warsaw, Poland and the Institute of Organic Chemistry of the Polish Academy of Sciences in Warsaw.

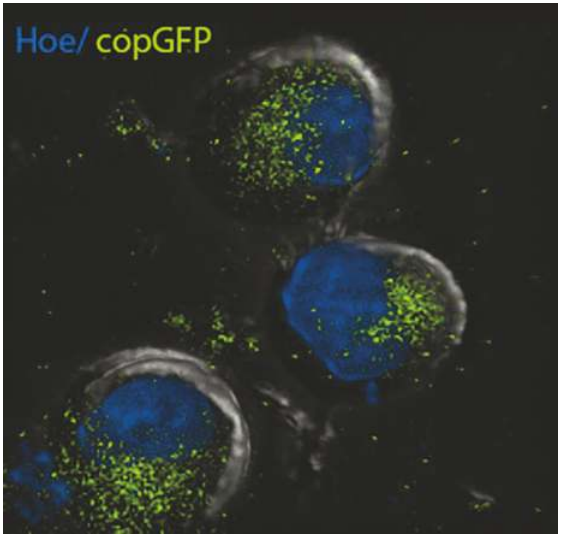
Impact of the environment on pluripotent stem cells (S. Bobis-Wozowicz, Z. Madeja)
Pluripotent stem cells (PSCs), which are characterized by an unlimited ability to self-renew and extensive differentiation potential, constitute an excellent tool in both, basic science and regenerative medicine.

These cells rapidly respond to exogenous factors present in their environment, either by differentiation, or apoptosis. Through our studies, we try to understand the mechanisms that regulate PSCs fate decisions. In particular, we are investigating the impact of oxygen concentration and temperature, on genetic stability and biological properties of PSCs and their derivatives. We utilize fluorescence-based reporters and the CRISPR/Cas9 system, as well as *in vivo* models, to design novel therapies for monogenic diseases (cystic fibrosis) and to alleviate tissue fibrosis, which affects millions of people globally.

Metabolic microevolution of tumour cells (K. Piwowarczyk, M. Paw, J. Czyż)
Tumour microevolution implies processes that facilitate cells' adaptation to chemotherapeutic stress and their further expansion. In particular, it increases metabolic flexibility of tumour cells and enhances their drug resistance. In our hands, tumours cells adapt to chemotherapeutic stress-induced energy consumption with the reprogramming towards energetically effective aerobic respiration. This increases their vulnerability to metabolic blockers, such as fenofibrate and metformin. Along with the enhancing effects of fenofibrate on the endothelial barrier function during diapedesis, our data suggest their application in the treatment of drug-resistant tumours. However, tumour cells can adapt to the chemotherapeutic/metabolic stress by generating CD44+ stem cells. The ongoing research on this topic is focused on i) the mechanisms underlying metabolic adaptation of tumour cells to chemotherapy and ii) to metabolic blockers; iii) consequences of metabolic reprogramming for the invasion of tumour cells and diapedesis; iv) the role of connexin(Cx)43, stromal and stem cells in these processes and on v) dietary compounds interfering with the metabolic adaptation and drug resistance of tumour cells.

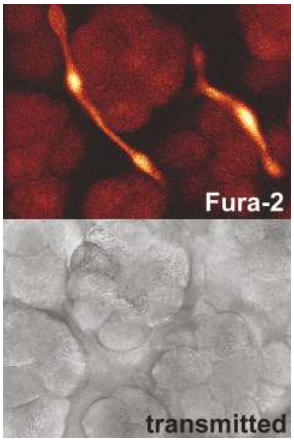
Advanced Therapy Medicinal Products manufacturing for clinical trials (M. Piejko, D. Wnuk, J. Drukała)
Our GMP laboratory specializes in the manufacture of skin tissue-engineered products used for extensive burn treatment in the clinic. Additionally, we prepare mesenchymal cells products in combination with extracellular equivalents for connective tissue regeneration and chondrocytes for chondromalacia treatment.
We are constantly working on the improvement of wound healing strategies using skin cells and acellular matrices, conducting basic research in this area. The long-term experience of the Cell Bank in development of ATMPs allow us to offer a service to implement the new results of research in the field of ATMPs for clinical application. In addition to clinical research, we are working on a new source of epidermal stem cells to obtain a complete regeneration of the skin together with its appendages. Furthermore, we use *in vitro*-cultured epidermal cells as a model to study mechanisms related to the pathology of psoriasis and atopic dermatitis.

Myofibroblasts in bronchial remodelling in asthma (M. Paw, D. Wnuk, M. Michalik)
The research focusses on the role of myofibroblasts in the fibrotic lung tissue. Studies primarily discuss: i) the nature of phenotypic fibroblast-to-myofibroblast transition (FMT) and its underlying mechanisms during bronchial airway wall remodelling in asthma; ii) differences between asthmatic and non-asthmatic bronchial fibroblasts (HBFs) in response to the transforming growth factor β (the main profibrotic cytokine); iii) mechanisms of the enhanced asthma-related epithelial–mesenchymal transition; iv) functional interactions between human bronchial epithelial cells (HBECs) and HBFs within the epithelial-mesenchymal trophic unit, and v) the effects of some selected compounds (e.g. flavonoids, statins, chalcones, PPAR agonists) on FMT efficiency in populations of HBFs.



Role of stem cells and their bioactive derivatives in tissue regeneration- focus on cardiovascular and bone repair (E. Karnas, A. Łabędź-Masłowska, E. Zuba-Surma)
The research of our group is focused on studying a role and mechanisms of action of selected adult and pluripotent stem cells (SCs) in tissue repair, including cardiovascular and bone regeneration. Our recent projects conducted in international cooperation are also focused on exploring bioactive properties of SC-derived extracellular vesicles (EVs), examined in several cellular *in vitro*- and animal *in vivo*-models. Another interdisciplinary branch of the research is dedicated to combining SCs with biomaterial scaffolds for effective tissue repair and translational aspects to develop novel therapeutic approaches in humans, an area in which we are very actively engaged in collaborating with clinical centres and conducting clinical trials.

Cell signalling in physiology and pathophysiology of the pancreas (P. Ferdek)
Pancreatitis is a serious disease in which the tissues becomes destroyed by prematurely activated digestive enzymes stored in acinar cells, often leading to permanent tissue scarring – fibrosis. Pancreatic fibrosis remains very poorly studied, possibly because the main cellular mediators – pancreatic stellate cells – have long been overlooked. In our research, we apply real-time imaging techniques to investigate signalling events (Ca^{2+} or ROS signals) induced by pathophysiological stimuli in pancreatic acinar or stellate cells; and we aim to understand the implications these signals have for the development of pancreatitis and pancreatic cancer. We also use animal models of pancreatic diseases to test new therapeutic strategies based on selective regulation of cell death in pancreatic cells.



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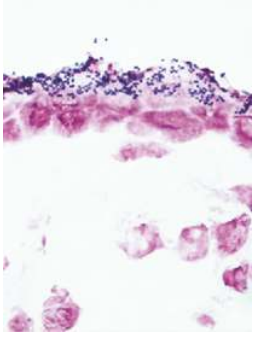
KEYWORDS
skin, neutrophils, eosinophils, macrophages, antimicrobial peptides, chemerin, SLPI, Regnase, chaperone, PAMPs

Department of Immunology

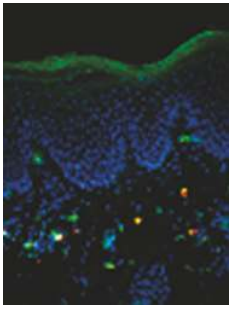
At the Department of Immunology, we are interested in investigating the mechanisms that underlie the protective function of environmentally exposed organs, with the skin as a model. We explore how major anatomical layers of the skin react to and interpret microbial stimuli, and study various functional links that engage the immune and nervous system for skin defence. At the cellular level, we focus on keratinocytes and leukocytes, such as neutrophils and eosinophils. At the molecular level, one of our major interests is in multifunctional proteins such as regulatory and antibiotic-like molecules, including chemerin, secretory leukocyte protease inhibitor (SLPI) and Regnase. Modern approaches in immunology, biochemistry and genetics are applied to the analysis of skin defence under homeostatic and pathologic conditions associated with chronic inflammation, with the goal of gaining a better understanding of fundamental aspects of body barrier defence. A further, complementary area of interest is in molecular pattern recognition by macrophages.



Histology of skin of chemerin KO mice topically infected with *S. aureus* (blue).

Antimicrobial peptides in maintaining skin homeostasis
Natural antibiotic-like molecules (antimicrobial peptides – AMPs) are produced at body barriers and have the potential to regulate skin immunity and bolster skin defence against microbial assaults. These include chemerin and SLPI. The microbial communities inhabiting barrier organs contribute to the health of the organism but also to skin disorders, such as psoriasis and atopic dermatitis. The burden and composition of cutaneous bacteria is likely influenced by SLPI and chemerin, that are differentially regulated in the skin under homeostatic and disease conditions. We investigate the impact of these agents on skin barrier protection.

Neutrophil function in psoriasis and regulatory role of SLPI in neutrophil biology
Skin-infiltrating neutrophils are the distinguishing hallmark of psoriasis, yet a role of neutrophils in pathophysiology of this condition remains elusive. Neutrophils are broadly classified into conventional neutrophils (PMNs) and low density neutrophils (LDNs) based on their cellular density. Neutrophil heterogeneity is also revealed by differential immunostaining with neutrophil elastase (NE) and its controlling inhibitor SLPI. The NE/SLPI staining pattern identifies common attributes of both circulating



Immunofluorescence image of psoriasis skin showing SLPI+ (green) and NE+ (red) neutrophils, cell nuclei (blue).

and skin-infiltrating neutrophils, and may determine the location of LDG-mediated cutaneous pathology. We are studying the impact of both NE and SLPI on the biology of neutrophils and the functional significance of neutrophils in the pathophysiology of psoriasis.

Recognition of molecular patterns by monocytes and macrophages

Our conventional perception of innate immunity does not fully rationalize how an adequate response is readily initiated and coordinated by a broad array of diverse particles, ranging from lipoproteins to the organism's own cells. Recent conceptual advances have highlighted synapse-like, dynamic associations of membrane receptors and other proteins, which enable response to a variety of disparate stimuli. Our group is focused on pattern recognition where we have found that subtle modifications in the target surface dictated the manner of its recognition by macrophages, and identified HSP90 as an essential component of signalling complexes. In recent years, we have extended our interest to tumour-associated macrophages.



Confocal microscopy 3D images of hMDMs labelled with anty-CD11b mAbs (white) and specific HSP90 inhibitor – geldanamycin (red).

KEY ACHIEVEMENTS

Important discoveries made at the Department of Immunology include making the finding that chemoattractant protein chemerin is the skin natural antibiotic molecule. Inspired by chemerin antimicrobial function, we have also designed a chemerin-based antimicrobial peptide that effectively controls various skin-associated microbes, including bacteria resistant to commonly-used antibiotics. We also discovered the important regulatory role of SLPI that, as a component of neutrophil extracellular traps (NETs) and an inhibitor of NET deposition, may be involved in chronic inflammatory skin diseases.

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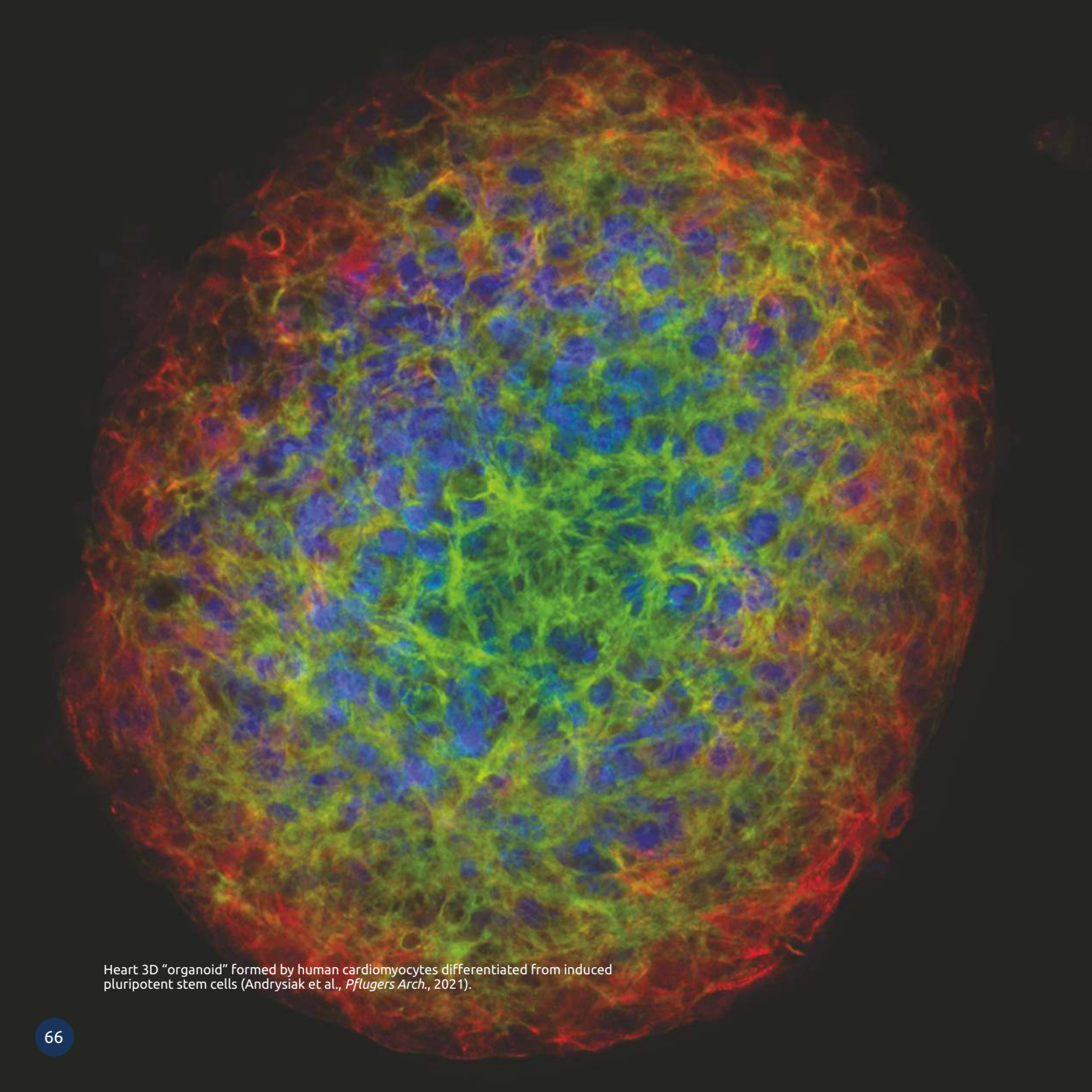

Heart 3D “organoid” formed by human cardiomyocytes differentiated from induced pluripotent stem cells (Andrysiak et al., *Pflugers Arch.*, 2021).

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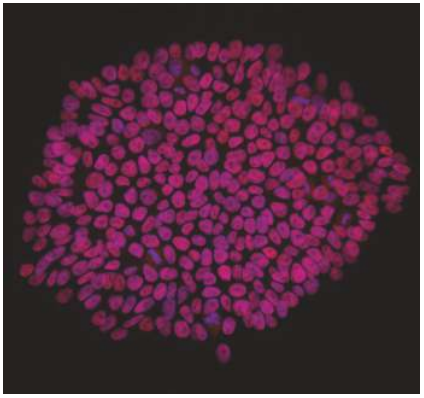
KEYWORDS
stem cells, induced pluripotent stem cells, vascular biology, Duchenne muscular dystrophy, cardiovascular biology and diseases, regenerative medicine, inflammation, hematopoiesis and leukemogenesis, heme metabolism, microRNA

Department of Medical Biotechnology

The main areas of our interest are the molecular mechanisms of stem cell differentiation in the context of vascular biology and mechanisms of inflammation, with particular emphasis on the importance of these processes in cardiovascular diseases, Duchenne muscular dystrophy, neoplastic diseases – including leukemia, psoriasis, and rheumatoid inflammation. In our research, we use numerous animal models, gene and cell therapy methods, and the CRISPR/Cas9 gene-editing technique. We work with clinicians and biotech companies in translational research and in developing new drugs and treatments.

Stem cells in the study of disease mechanisms and regenerative medicine

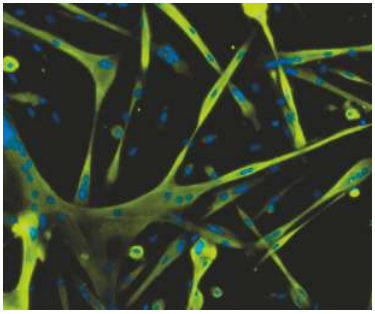
Induced pluripotent stem cells (iPSCs), obtained by reprogramming of somatic cells, e.g. patients' blood leukocytes, are an excellent tool in medical biotechnology. They can be differentiated into difficult-to-reach human cells, e.g. cardiomyocytes, neurons, or endothelial cells, and thus, as a so-called "patient in a dish" model, are used to study disease mechanisms and to test drugs. We use this strategy to study the mechanisms of cardiomyopathy in Duchenne and Becker muscular dystrophy (DMD/BMD), endothelial dysfunction in HNF1A-MODY diabetes, neuromuscular junction disorders in spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). We also utilize this model to examine the relationship between rheumatoid arthritis and psoriasis and heart disease. We apply cardiomyocytes obtained from iPSCs in experimental therapies for the treatment of heart failure in mice, and their implantation improves heart functions after a heart attack. In research into the mechanisms of diseases, we also investigate human and animal tissue-specific stem cells, in particular muscle satellite cells, hematopoietic cells, and skeletal stem cells.



Colony of human iPSCs expressing Oct4.

Molecular mechanisms of cardiovascular disease, muscle disease, and cancer

We revealed the existence of new mechanisms influencing the muscle damage in Duchenne muscular dystrophy (DMD). Recently, using unique animal models – *mdx* dystrophic mice, additionally devoid of heme oxygenase (HO-1) or miR-378 genes, we have shown that the lack of HO-1 intensifies the processes of muscle damage, while the absence of miR-378 has the opposite effect. These effects are related to the modulation of inflammation, as well as the differentiation of satellite cells. Understanding the role of HO-1 and miR-378, as well as hydrogen sulphide,

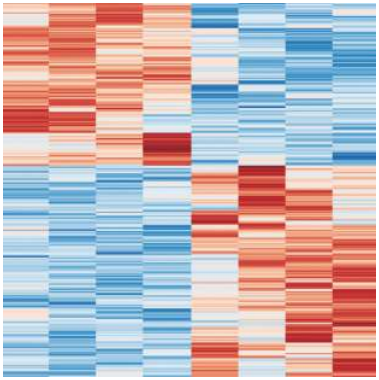


Skeletal muscle myoblasts differentiated from satellite cells of dystrophic mice.

as factors affecting the course of heart and skeletal muscle damage is the goal of the current projects on which we are working, as part of which we are also studying the importance of angiogenesis in DMD, and investigating the effects of drugs – statins and other means to limit the inflammation. We aim to understand the impact and function of mesenchymal and endothelial cells and their crosstalk during atherogenesis, and to identify potential therapeutic targets for the treatment of atherosclerosis. Our research has also demonstrated the protective effects of HO-1 in ischemia of skeletal muscles and the heart. In turn, excessive expression of HO-1 contributes to the development of cancers, enhancing the proliferation of tumour cells and angiogenesis, and also increases the resistance of neoplastic cells to therapies.
We focussed on developing new small-molecule HO inhibitors and we have demonstrated their anti-cancer potential. We have shown that increased activity of HO-1 by disturbing the expression of microRNAs inhibits the differentiation of muscle cell precursors, being one of the mechanisms in the development of rhabdomyosarcoma.

Molecular mechanisms of regulation of gene expression in protective and pathological inflammatory processes

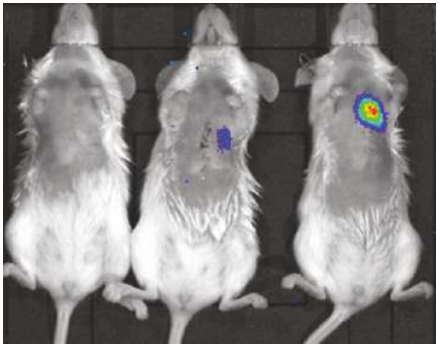
The induction of antioxidant and anti-inflammatory protective genes plays a key role in the regulation of inflammation following sterile tissue damage (myocardial infarction, limb ischemia, wound healing), as well as pathological phenomena in developing neoplasms. The most important of them include the transcription factor Nrf2, inducing the expression of heme oxygenase-1 (HO-1), and transcription factors HIF-1 and HIF-2, activated by hypoxia and non-coding microRNAs. By applying various methods, including RNA sequencing and cell/animal models of gene silencing/deletion (knockout), we are investigating the mechanisms by which Nrf2, HO-1, and various microRNAs influence the expression of other genes. We are looking into the effect of HO-1 and its activity products on the expression of microRNAs that regulate key differentiation processes of stem cells, e.g. satellite cells.
We also determine the role of microRNAs in the regulation of cell differentiation, inflammation, angiogenesis, and tissue repair.



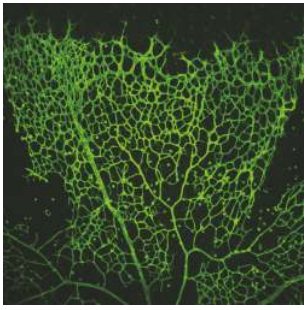
Heat map of RNA seq analysis of gene expression In iPSC-derived cardiomyocytes.

Gene transfer and gene editing in the study of disease mechanisms and experimental therapies

We have developed numerous gene transfer and gene editing methods based on plasmids and viral vectors. Lentiviral vectors allow cells, e.g. stem cells (iPSCs), to be permanently modified and thus make it possible to study the effect of overexpression of protective genes on the course of healing processes. In *in vivo* studies, we use adeno-associated virus (AAV) vectors, different serotypes of which allow for specific transduction of e.g. cardiomyocytes after systemic administration. We investigate the factors influencing AAV transduction efficiency in various types of cells and the therapeutic potential of AAV vectors regulated by endogenous microRNAs. Knowledge of molecular mechanisms prompts us to use regulated gene therapy in which the expression of therapeutic genes is induced, for example, by reduced oxygen concentration in cells (hypoxia). The CRISPR/Cas9 gene-editing technique allows the repair of mutations in iPSCs obtained from the cells of patients with DMD, SMA, ALS, or HNF1A-MODY diabetes to be repaired, thus making it possible to study the mechanisms of these diseases using the "patient in a dish" strategy.



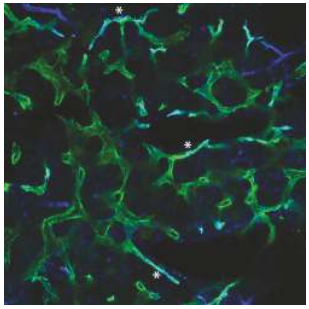
Bioluminescent signal from iPSC-derived cardiomyocytes injected into murine heart after myocardial infarction.



Blood vessels of murine retinas isolated from pups (5 days after birth), stained with isolectin B4.

Aging and biology of endothelial cells

Endothelial cells play a major role in the maintenance of vascular integrity. Our goal is to identify the mechanisms, which drive the premature senescence of endothelial cells and to characterize the switch between the senescent and apoptotic fate. We focus on Nrf2/Keap1 system, protein S-nitrosation and miRNA-34a. Our recent findings are that: i) endothelial cell fate is determined by Keap1-dependent protein S-nitrosation; ii) Keap1/NOS/GAPDH is an enzymatic complex for S-nitrosation in mammals; iii) Nrf2 regulates angiogenesis independent of its transcriptional activity. We are also studying the role of Nrf2-induced gene, heme oxygenase-1 (HO-1), and heme metabolism in vascular pathophysiology. Our focus is on the involvement of the nuclear form of HO-1 in preventing replication stress and resolving G-quadruplexes and non-B DNA structures stabilized by heme.



Endothelial cells in the mouse bone marrow, stained for Endomucin (green) and Sca1 (blue).

Hematopoiesis and leukemogenesis: the role of the vascular niche

All blood cells derive from hematopoietic stem cells (HSCs). However, during aging, the blood-forming potential of this dynamic system declines. Additionally, HSCs accumulate mutations, which may lead to leukemia. For proper functioning, HSCs require a specialized microenvironment – the niche. Using multiparameter flow cytometry, single-cell transcriptomic, genomic and functional analyses, as well as new transgenic and humanized mice models, we study how HSCs contribute to blood production and how their functions change with age. Together with clinicians, we trace the roots of leukemia within the pool of human HSCs. Our research focusses on HSCs' heterogeneity and mechanisms that regulate the specific HSC-niche interactions. We also want to understand the importance of clonogenicity and plasticity of endothelial cells in maintaining the hematopoietic niche. Our recent findings are that: i) a lack of extrinsic heme oxygenase-1 triggers premature exhaustion of hematopoietic stem cells in mice; ii) cobalt protoporphyrin IX (CoPP) induces endogenous G-CSF and has mobilizing properties superior to recombinant G-CSF. In cooperation with the Irving Weissman Laboratory, we demonstrated that iii) Neogenin-1 marks the myeloid-biased fraction of strictly defined Hoxb5^{+} HSC in mice. Now, we are looking for a similar subpopulation in humans.

KEY ACHIEVEMENTS

- Demonstration of the importance of heme oxygenase-1 (HO-1) in the processes of blood vessel formation (angiogenesis) and reduction of inflammation in skeletal muscle ischemia, myocardial infarction, wound healing, and toxic kidney damage.
- Discovery of novel regulators of skeletal muscle damage in Duchenne muscular dystrophy, in particular, the role of mir-378 and HO-1.
- Demonstration of the role of HO-1 and some microRNAs in tumour development, and the importance of HO-1 as a target for cancer therapy.
- Development of unique animal models of diseases, gene transfer and editing techniques, and stem cell research tools.

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KEYWORDS
periodontitis, *Staphylococcus aureus*, proteolytic enzymes, *Porphyromonas gingivalis*, inflammation, advanced diagnostics, genetics

Department of Microbiology

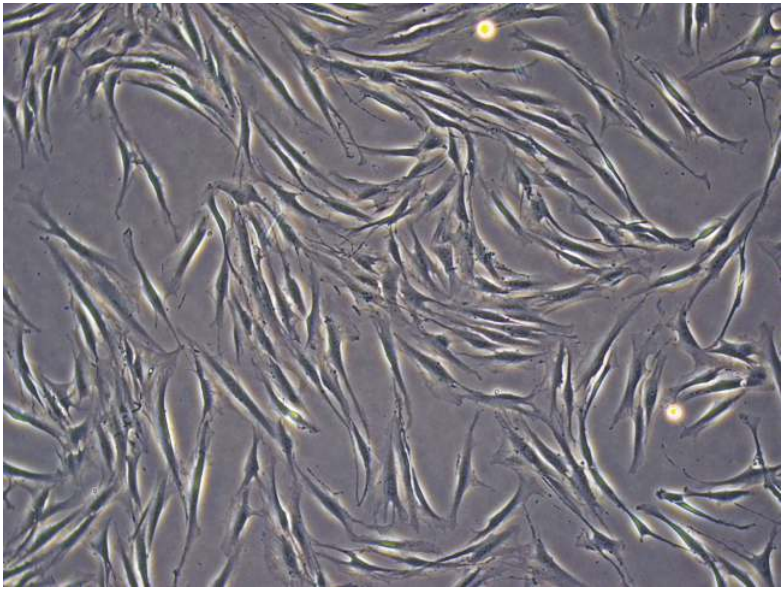
Research at the Department of Microbiology is focused on studies of the structure and function of bacterial virulence factors, including proteolytic enzymes of bacterial pathogens that play pivotal role in the deregulation of a number of physiological pathways and evasion of host immunity. Specifically, investigations of proteolytic systems of staphylococci and periodontal pathogens have led to the discovery of new families of proteases (e.g. gingipains, interpains) and protease inhibitors (e.g. staphostatins). The results of these studies cast a new light on host-pathogen interaction with the innate immunity system, a mechanistic link between periodontitis and various chronic inflammatory disease (rheumatoid arthritis, COPD, Alzheimer disease) and led to the discovery and characterization of a novel protein secretion system (PerioGate/T9SS) used by periodontopathogens to secrete their virulence factors. These discoveries open pathways for the development of novel and effective prevention and treatment therapies.

***Staphylococcus aureus* and coagulase-negative staphylococci infections** (J. Międzobrodzki)
Research is focused on molecular mechanisms of opportunistic bacterial infections caused by the genera *Staphylococcus*, *Streptococcus* and *Enterococcus*. We identify and type bacterial strains with genetic and phenotypic methods, which reveal the genetic profiles and clonal structures of strains responsible for hospital infections, as well as making it possible to assess animal-to-man and man-to-animal transmissions of bacterial strains. Based on next-generation sequencing (NGS) methods, we perform in-depth characterization of the species content of clinical samples, as well as the antibiotic resistome and transcriptome (RNASeq) of bacterial infections for both clinical and diagnostic needs.

Host-microbe Interactions (J. Kozieł)
Research is focused on innate immune responses to bacterial infection on mucosal surfaces with an emphasis on leukocytes (macrophages, neutrophils, lymphocytes), antibacterial peptides, and toll-like receptor signalling. Specifically, studies of bacterial infections caused by *Staphylococcus aureus, Streptococcus anginosus*, and periodontal pathogens aim to identify and characterize virulence factors and strategies that impair host innate immune defence. To this end, the group is applying a broad array of modern cell biology approaches, as well as transgenic/knockout mice and disease models, to delineate molecular mechanisms underlying the role of innate immunity in the inflammatory reaction triggered by infection.

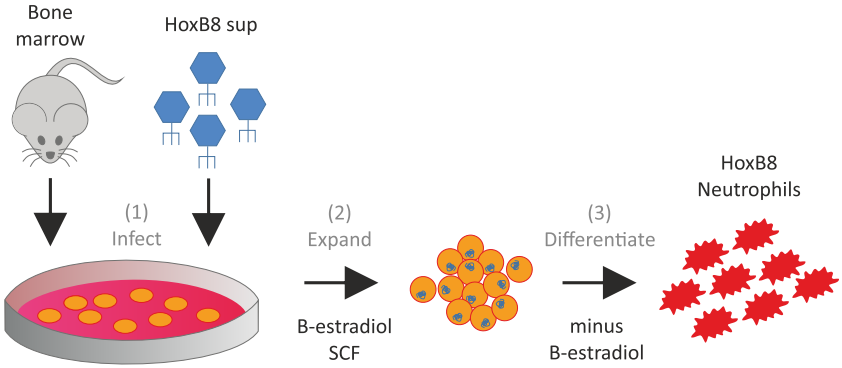
Gingival fibroblasts as key effector cells in the pathogenesis of periodontitis (A. Grabiec)
Research is focused on understanding contributions of gingival fibroblasts (GFs) to the pathogenesis of periodontitis. It is now recognized that fibroblast are important sentinel cells that can respond to pathogenic microorganisms and danger signals. In periodontitis, GFs exposed to oral pathogens and their products respond

with inflammatory mediators. The main goal is to understand how oral pathogens manipulate gene expression in GFs, in particular through epigenetic regulatory mechanisms, to drive pathological activation of GFs, and to identify new anti-inflammatory therapeutic strategies targeting fibroblast activation.



Primary human gingival fibroblasts isolated from a healthy gingival tissue specimen (magnification 100x).

Neutrophils in periodontitis (M. Sochalska)
The response of neutrophils (PMNs) to bacterial infection plays a pivotal role in the pathobiology of periodontitis. PMNs produce many bactericidal compounds, like enzymes and free radicals able to kill pathogens. However, these substances may damage tooth-surrounding tissues if PMN life and death in the inflamed tissue is not tightly regulated. We aim to study the molecular mechanisms of PMNs deregulation during interaction with the periopathogen, *P. gingivalis* using a novel *in vitro* approach, which significantly reduces the use of animals, while allowing functions of PMNs to be studied in a precise, controlled way and making it possible to develop new diagnostic markers and therapies for periodontitis.



A novel approach to analyse neutrophil survival and function. (1) Bone marrow cells can be isolated from mice of different genotypes and infected with retrovirus encoding oestrogen-dependant HoxB8 transcription factor. (2) HoxB8 progenitor cells are cultured with β-estradiol and SCF. (3) β-estradiol withdrawal leads to full PMNs differentiation after 4 days in culture.

Role of post-translational modifications in immune response (P. Mydel)
Post-translational modifications (PTMs) are pivotal steps in protein maturation. PTMs increase the functional diversity of the proteome and play a key role in multiple cellular processes. Non-enzymatic conversion of lysine residues and the N-terminal amino group to homocitrulline (carbamylation) is a common post-translational modification of proteins and peptides, which occurs in inflammatory foci and in a uremic environment.
In our research, we seek to determine the effects of carbamylation on the immunomodulatory functions of peptides/proteins and the impact this modification has on immune response, the resolution of inflammation and coagulation cascade.

Studies of the structure and function of periodontal pathogens virulence factors, protein secretion and nutrient acquisition (M. Książek and M. Madej)
The main aetiological factors of periodontitis, *P. gingivalis* and *T. forsythia* secrete many proteins, including proteases. Except gingipains, their function, structure, secretion pathways are not very well understood. Also, the mechanism of nutrients acquisition is unknown. Therefore, the goal of this group is: i) identification of the protein complexes located in the cell envelope engaged in the secretion of proteins and acquisition of nutrients; ii) detailed characterization of secretion of proteases of these bacteria, including determination of their biological functions and activation mechanisms; and iii) finding low-molecular-weight specific inhibitors of these proteases.

Molecular biology of periodontal pathogens (D. Mizgalska and Z. Nowakowska)
The study aims to understand the molecular mechanisms underpinning the functioning of bacterial systems regulating expression, the secretion and processing of virulence factors, reactions to environmental cues, interspecies communication, the acquisition of nutrients and responses to challenges from the host immune system. To this end, the group has designed and constructed a large number of mutant strains of *P. gingivalis* and *T. forsythia*.

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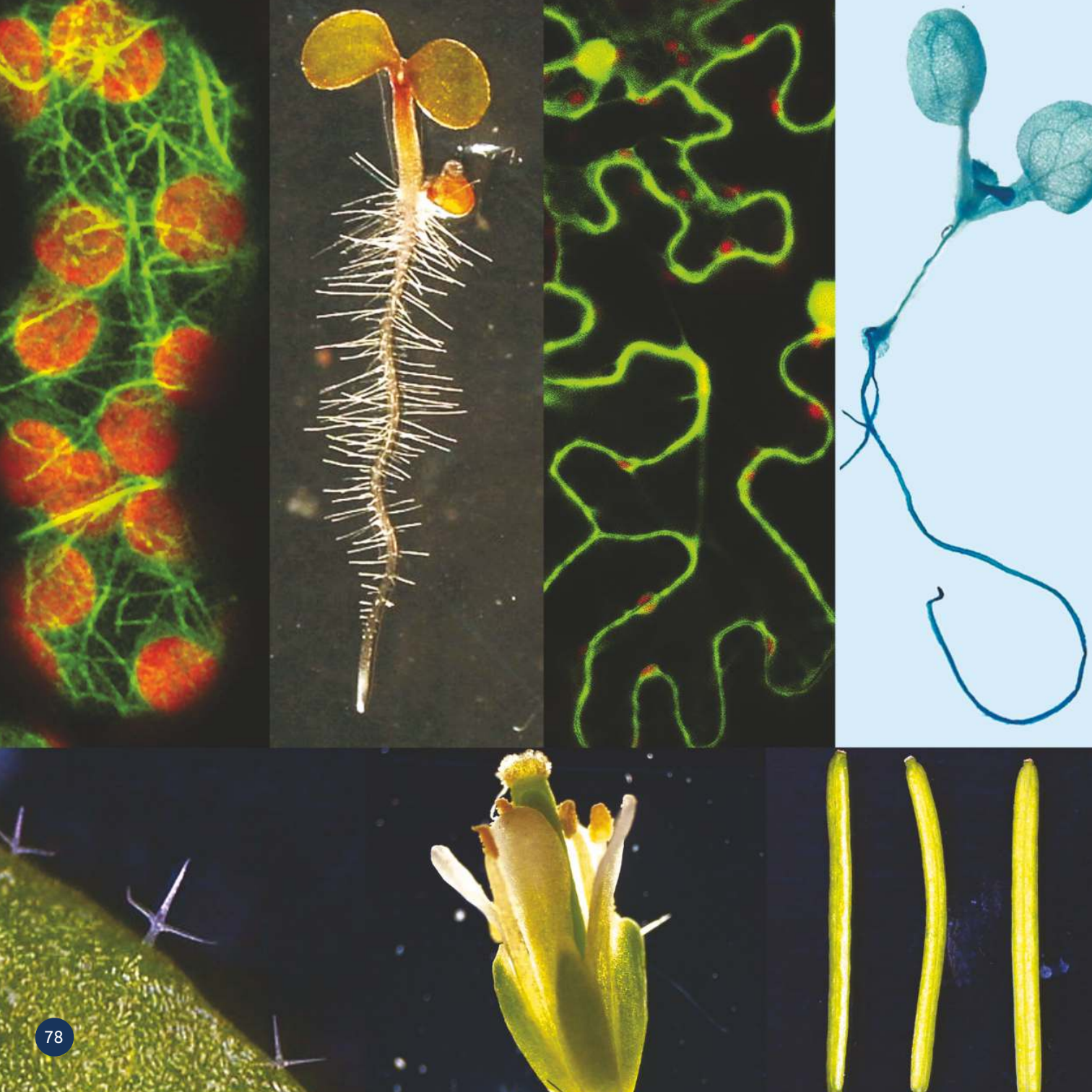

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KEYWORDS
blue light, chloroplasts, DNA aptamer, DNA metabolism, glutamate receptors, signalling, transgenic plants, plant polysaccharides, wood development, trees

Department of Plant Biotechnology

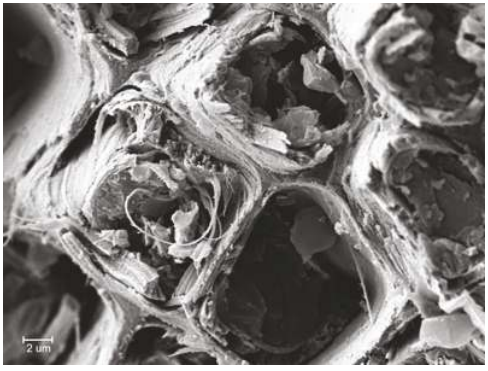
Research at the Department of Plant Biotechnology is conducted by scientists from various areas of specialization and covers a number of topics of molecular plant science. This includes the study of DNA replication and repair, chloroplast movements and characterization of light signalling pathways in plant cells. In addition to that, we are actively involved in developing biotechnological applications of DNA aptamers.

Plant DNA: replication, damage and repair

We study Arabidopsis proteins involved in DNA replication and repair – a proliferating cell nuclear antigen (PCNA), FEN1 endonuclease and putative photolyases which mediate UVA/blue light-dependent repair of pyrimidine dimers and can also function as blue light photoreceptors. In our experiments, we are investigating the role of these proteins in plant cell response to abiotic stress, for example by evaluating DNA repair after UV or chemical damage. We are also interested in understanding the biochemical and structural features contributing to the activity of the above-mentioned proteins. In particular, we are investigating their protein-protein interactions and post-translational modifications, such as phosphorylation and SUMOylation. In our work, we use T-DNA insertional mutants and complemented lines of *Arabidopsis thaliana*. To study sub-cellular localization of the proteins that are of interest to us, we employ transient expression system in *Nicothiana benthamiana* and confocal microscopy. We use RT-PCR to understand the influence of light, chemical mutagens and abiotic stress on the expression of the studied genes. In our research, we are particularly interested in uncharacterized Arabidopsis genes and proteins. As such our work is novel and has the potential to contribute to the identification of new functions of these genes and proteins.

Signalling pathways leading to or modulating chloroplast responses to blue light

Current projects are focussing on signalling pathways leading to, or modulating, chloroplast responses to blue light. While chloroplast accumulation in response to weak light helps plants to optimize photosynthesis, their avoidance of strong light protects the photosynthetic apparatus from damage. As Ca^{2+} ions are assumed to play a role as secondary messengers in the control of chloroplast movements, we try to find out which calcium channels are involved in the signal transduction. In particular, we are testing for the potential involvement of GLR channels, plant homologues of animal ionotropic glutamate receptors active in the nervous system. We are also studying plasma membrane proton pumps since they may also be involved in primary light signalling. Another line of investigation aims to identify potential signals coming from chloroplasts. Here, we are testing the role of the malate valve, a transport system present in the organelle membrane. We also try to explain the physiological significance of sugar signalling in chloroplast redistribution and find out whether it is associated with stress signals produced by fungal pathogens in the apoplast. Separate areas being examined more closely as a result of our interest in GLR channels are light regulation of genes encoding their subunits and the role of these channels in scoto- and photomorphogenesis.



Spruce wood visualized with low temperature scanning electron microscopy.

Environment-driven variation in the molecular architecture of softwood

Ever-increasing environmental pollution and the process of climate change mean it is necessary to develop a novel, sustainable, bio-based economy. As the majority of carbon in terrestrial ecosystems is stored in forests as wood, it is a material that offers an unprecedented renewable resource of energy and biodegradable materials.

Variation in the properties of wood and a lack of understanding of their origin is one of the main challenges in the development of novel applications of wood. On this project, we are investigating the molecular structure of the building blocks of wood and the impact of environmental signals on the process of wood formation. In particular, we are trying to understand how light influences wood deposited by coniferous trees such as pine and spruce.

We hope that our results will inform future forestry practices looking to generate desired types of timber and will contribute to the advancement of knowledge in plant developmental biology, providing particularly important information connecting environmental signals with the molecular structure of timber produced by trees.

KEY ACHIEVEMENTS

- Identification of a blue light receptor phototropin 2 and its role in chloroplast responses to light.
- Development of a DNA aptamer capable of binding a human PCNA and inhibiting DNA replication through a unique mechanism. This breakthrough discovery has numerous potential applications, for example in the development of novel pharmaceuticals.

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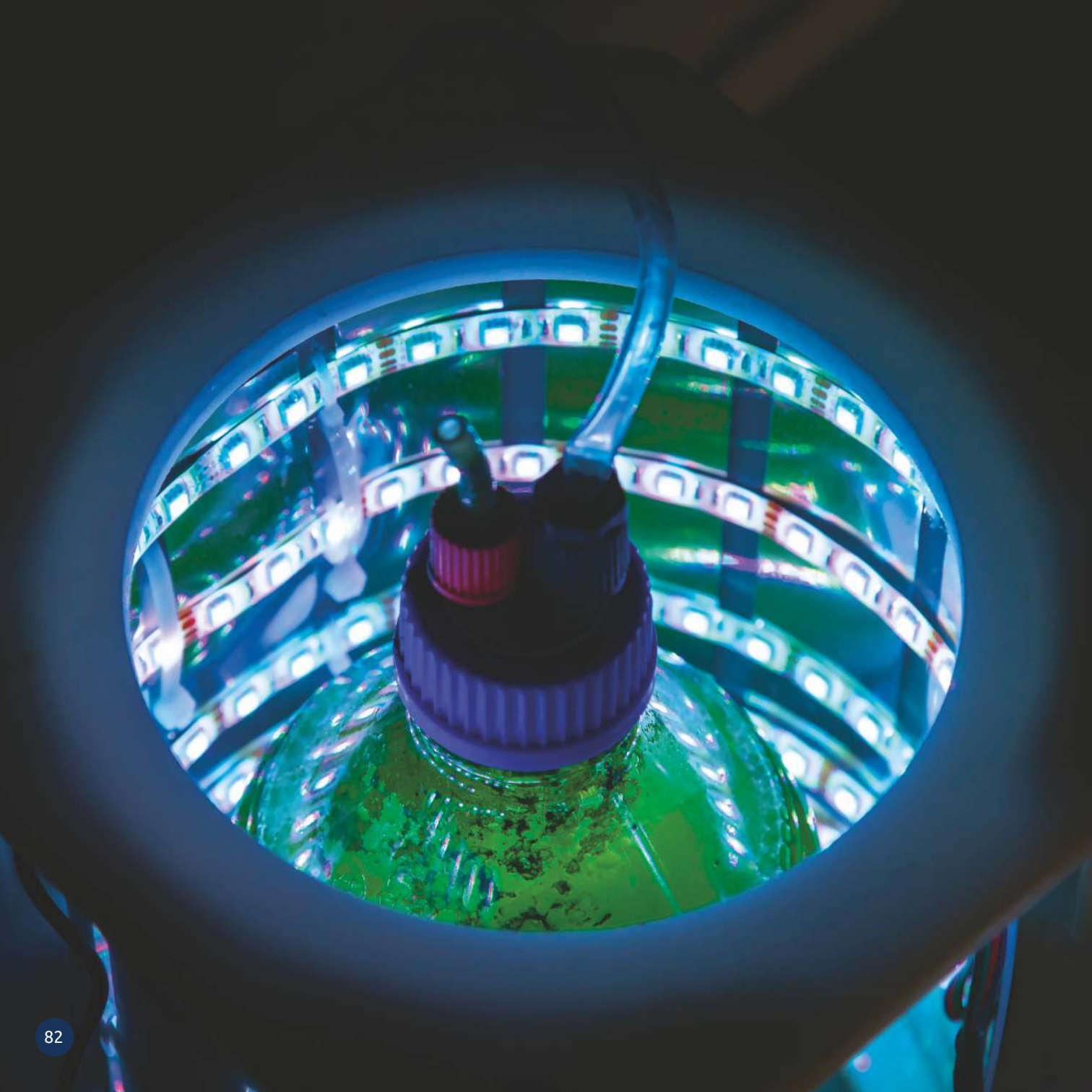

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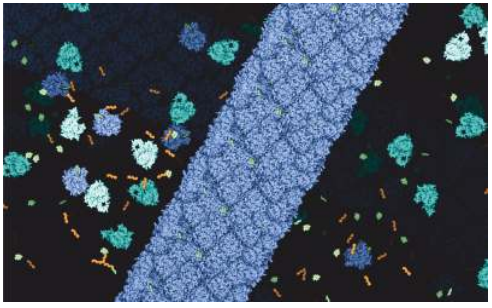
KEYWORDS
antioxidants, heavy metals, oxidative stress, chlorophyll biosynthesis, xanthophyll cycle, phytobioremediation, plant-microbe interactions, diatoms, photosynthetic pigments

Department of Plant Physiology and Biochemistry

The research being conducted at the Department covers broad aspects of biochemistry and molecular biology of plants and photosynthetic prokaryotes, such as biosynthesis and the function of chlorophylls and carotenoids, biogenesis and the structure of the photosynthetic apparatus, photomorphogenesis, occurrence, signalling and antioxidant functions of prenyllipids, mechanisms of heavy-metal toxicity, interaction of biological compounds with plant lipids, molecular mechanisms of the abiotic stress response of photosynthetic organisms, the biotechnological application of autotrophs in phytoremediation and waste biotransformation. Research methods include: molecular biology techniques, chromatography, molecular spectroscopy, computer modelling, application of model systems, and chemical synthesis.

Antioxidant and signalling functions of prenyllipids (J. Kruk, B. Nowicka)
The research topics include: antioxidant and signalling functions of plastoquinones, plastochromanols, tocopherols, occurrence and function of rare and novel prenyllipids, generation and scavenging of reactive oxygen species in photosynthesis, and measurements of the redox state of the plastoquinone pool in response to different stress factors.

Participation of antioxidant response in heavy metal-induced stress in algae (J. Kruk, B. Nowicka)
One of the most important mechanisms of heavy-metal toxicity is the induction of oxidative stress. Therefore, antioxidant response is very important to provide the tolerance to the enhanced concentrations of heavy metal ions in the environment. The role of hydrophilic (ascorbate, glutathione, and proline) and hydrophobic (carotenoids, isoprenoid quinones and chromanols) low-molecular-weight antioxidants, as well as antioxidant enzymes (such as superoxide dismutase, catalase, and ascorbate peroxidase) in response to chronic and acute heavy metal-induced stress is examined in a model organisms *Chlamydomonas reinhardtii*.



Self-organizing light-dependent complexes for efficient plant growth (J. Kruk, M. Gabruk)
Our research is focused on light-dependent protochlorophyllide oxidoreductase (LPOR), an enzyme that forms large filamentous complexes with plant lipids and a chlorophyll intermediate. Recently, we determined the structure of the complex with the use of cryogenic electron microscopy. Now we are investigating the mechanism of the light-dependent reaction and how to alter it. Additionally, we are exploring the natural diversity

of LPOR variants which will help us to understand how the oligomeric properties of the enzyme evolved and how they can be modified. Moreover, we are taking advantage of directed evolution and looking for novel, interesting, non-natural LPOR variants. Our approach will allow us to determine how the properties of LPOR variants contribute to efficient chlorophyll synthesis and plant growth.

Structure-function relationships in photosynthesis (L. Fiedor, M. Zbyradowski, W. Rajwa)
The research topics include: structure-function relationships in photosynthetic pigments and complexes, the photochemistry and photophysics of chlorophylls and carotenoids, the anti- and pro-oxidant properties of carotenoids, the mechanisms of assembly of photosynthetic pigment-protein complexes, regulation of biosynthesis and biodegradation of photosynthetic pigments, the application of chlorophyll derivatives in photodynamic therapy, chlorophylls as xenobiotics – and interactions of chlorophylls with animal and human organism. Research methods include: chemical modifications of chlorophylls and carotenoids, synthesis of model pigments and photosensitizers, reconstitution of native and model pigment-protein complexes, electronic absorption and emission spectroscopy, time-resolved emission spectroscopy, spectroscopy under high pressure, chiralo-optical techniques, chromatographic methods, and *ab initio* quantum chemical computations on chlorophylls and carotenoids.

Molecular mechanisms of de-etiolation (B. Myśliwa-Kurdziel, P. Jedynak, K. Trzebuniak, W. Tokarek)
The main scientific interest of our group is the etioplast-to-chloroplast transformation occurring during the de-etiolation of angiosperm seedlings. Our research is focused on the light-dependent protochlorophyllide oxidoreductase (LPOR), which is the key regulatory enzyme of chlorophyll biosynthesis. It is also crucial for angiosperm de-etiolation. We aim to explain whether LPOR plays a regulatory role in the biosynthesis and assembly of photosynthetic pigment-protein-lipid complexes in developing seedlings. We employ multiple plant models including *Arabidopsis thaliana*, wheat, and other important crops. Using them we monitor plastid development and photosynthetic activity starting from the light triggered protochlorophyllide reduction in etioplasts through to chloroplast maturity and fully-developed photosynthetic complexes. We investigate these processes in plants cultivated under physiological conditions and undergoing stress e.g. drought, salt, and excessive light. Using *A. thaliana* mutants, we are also looking for other regulators of the de-etiolation process. Furthermore, we are investigating the photophysical properties of tetrapyrroles and other plant pigments in model systems and native membranes. We are also involved in studies of the photosynthetic activity of lichens collected from various natural habitats. We want to explain the mechanisms of adaptation of these organisms to various environmental conditions, including those polluted by heavy metals.

Molecular mechanism and functions of the xanthophyll cycle (D. Latowski, M. Bojko, E. Capała)
Violaxanthin de-epoxidases from higher plants and diatoms are studied in cells and model systems. We have shown the role of diatom de-epoxidation in induction of the "dynamic effect" and "stable effect" as two components in the molecular mechanism in short-term regulation of thylakoid membrane fluidity during changing temperatures. We explained why the main thylakoid lipid is required for the activity of de-epoxidases.

***Phaeodactylum tricornutum* as a model organism in diatom biology** (D. Latowski, M. Bojko, S. Listwan)
P. tricornutum is used to investigate molecular mechanisms of resistance to the abiotic stress, such as stress toxic metals, arsenic, and high temperature. *P. tricornutum* shows the ability to not only withstand very harsh conditions, but also to remove some stress factors from their environment. Upregulating of the secondary metabolites production in diatoms is also being investigated.

Role of the heavy metal resistance bacteria in phytobioremediation (D. Latowski, A. Kowalczyk, M. Rogala)
Our researchers are screening bacterial strains with optimal plant growth promoting properties (PGP) and heavy metal resistance for phytobioremediation purposes – culturing accumulative holobiont (plant/algae plus microorganism) to decontaminate soils and water polluted with As, Hg and Cr.

Fungal endophytes and saprophytes as biocontrol agents in combating ash (*Fraxinus excelsior* L.) dieback (N. Marcol, J. Pukalski, D. Latowski)
The main aim of our research is checking if endophytic and saprophytic fungi isolated from European ash can act against ash pathogenic fungus *Hymenoscyphus fraxineus.* We analyse inhibition zones and characterize metabolites secreted in dual cultures. Our second field of interest is studying the role of melanins in interfungal interactions.

Abiotic stresses in plants and lichens (K. Strzałka)
The research topics include: abiotic stresses in plants and lichens (excess of light, heavy metals, dehydration, freezing), extremophiles, physiological, biochemical and biophysical mechanisms of tolerance and adaptation to stresses.

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KEYWORDS
toxic cyanobacteria, lichens, cyanophages, bioremediation, phytoremediation, secondary metabolites

Laboratory of Metabolomics

Bioremediation of freshwaters from cyanobacterial pollutants
Bioremediation of toxic secondary metabolites produced by cyanobacteria is an alternative/complementary method in cyanobacterial management. At our Laboratory, we are developing two directions of research: microorganism based- and plant based-remediation.

A collaboration with McCormick Lab has been established to extend the MlrA (microcystinase that detoxifies microcystins, common cyanotoxins) expression in autotrophic hosts to gain a better understanding of heterologous protein production in cyanobacterial chassis. The final aim is to enable the heterologous production of an industrially relevant enzyme via photoautotrophic biomass from wastewaters.

Using plants to improve water quality and restrict or remove cyanobacterial blooms and cyanotoxins is one of the most environmentally-friendly methods of achieving these goals. Some of the cyanotoxins might be phytodegraded directly, through metabolic activities within plant tissues or through released enzymes. Others cyanotoxins may bind to the plant and be removed from the water with the plant biomass (phytoextraction), or the plants can stabilize the concentration of toxins in the water. The main purpose of these studies is to introduce suitably resistant plants to contaminated water bodies to eliminate pollution and threats.

Cyanobacteria and cyanophages
The invasive character of toxic and potentially toxic species of cyanobacteria in aquatic environments poses important problems for public health. Our research aims to determine how the *Raphidiopsis raciborskii* strains and other bloom-forming freshwater cyanobacteria adapt to crucial environmental conditions and how it can influence their invasive character. We are thus comprehensively investigating adaptation mechanisms, including genetic, biochemical, physiological and environmental backgrounds. The project (conducted in cooperation with *UAM* Poznań, Poland and CSIRO, Australia) combines a set of laboratory-based experiments and a field sampling campaign, as well as chemical, morphological and next-generation RNA sequencing supplemented by sophisticated physiological analyses.

There is also a growing interest in investigating how cyanophages impact the dynamic of cyanobacterial population. Our goal is to investigate the cyanophage infection process. In cooperation with NRC, Vilnius and the University of Haifa, we are attempting to investigate the physiology of invasive cyanobacteria during the viral infection to describe the overall strategies of freshwater cyanophages. Such research on interactions between ecologically important species and the cyanophages will give an insight into the mechanisms by means of which viruses influence cyanobacterial bloom dynamics.

Secondary metabolites of cyanobacteria and lichens

Cyanobacteria and lichens occurring worldwide are well known to produce a wide range of secondary metabolites with high structural diversity. In our laboratory, we conduct the isolation, identification and quantification of cyanobacterial toxins and oligopeptides, as well as lichen unique polyketides by high-performance liquid chromatography (HPLC) and mass spectrometry (MS) methods. We also investigate the biological activity/toxicity as well as the impact of various abiotic factors on the stability of these secondary metabolites.

Some lichen species are able to grow in extremely polluted post-industrial areas. One of the aims of our research is to determine the mechanisms of myco- and photobiont resistance to heavy metals. We also investigate the translocation of secondary metabolites of epiphytic lichens into tree tissues and their effect on the biochemistry of host trees.

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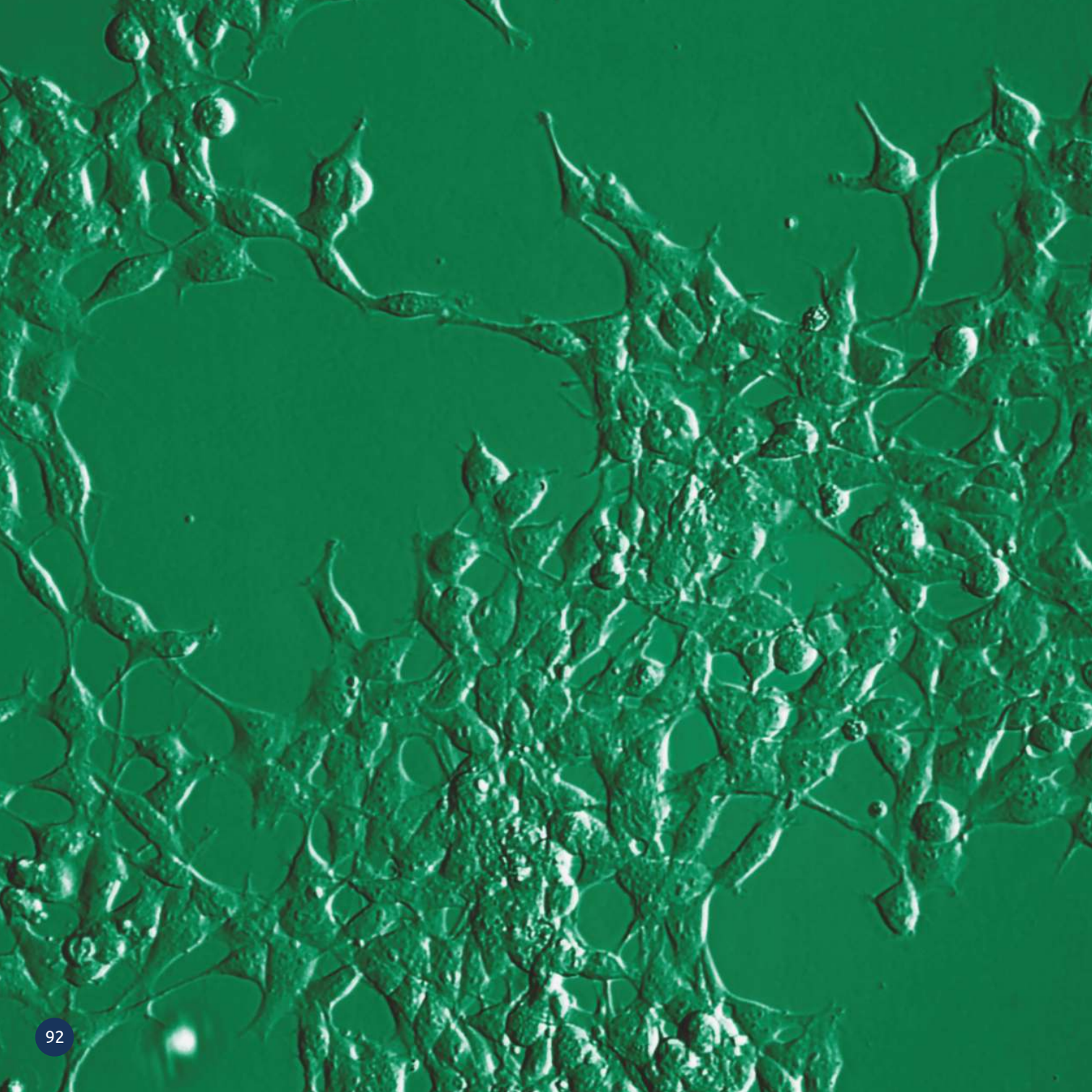

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KEYWORDS
cancer, neuroblastoma, melanoma, chemoimmunotherapy, GD2 ganglioside, peptide mimics, MCPIP1, PHLDA1, microRNA

Our recent *in vitro* studies are devoted to elucidation of molecular mechanisms of neuroblastoma cell death induced by GD2 ganglioside-recognizing therapeutic antibodies used in clinical practice to treat the residual, drug-resistant form of high-risk neuroblastoma. The structural basis of recognition of GD2 ganglioside and its peptide mimics by monoclonal antibody 14G2a was also studied. Additionally, a role of the monocyte chemoattractant protein-induced protein 1 (MCPIP1) as a potential tumour suppressor was described in human neuroblastoma and melanoma.

Molecular mechanisms of neuroblastoma cell death induced by GD2 ganglioside-recognizing monoclonal antibodies. Contribution of PHLDA1 to response of neuroblastoma cells to clinical and experimental treatment of high-risk neuroblastoma

Targeting GD2 ganglioside (a glycolipid expressed on neuroblastoma cells) with therapeutic antibodies including their chimeric form – ch14.18 now constitutes one of the major and most important ways of treating the residual, drug resistant form of high-risk neuroblastoma. Our recent *in vitro* studies have shown that treating neuroblastoma cells with the therapeutic antibody causes a significant decrease in cell viability and proliferation and increases apoptotic cell death. Through gene expression profiling, we have identified the *PHLDA1* gene as the most upregulated gene in the human neuroblastoma cells studied. Induction of PHLDA1 expression in our neuroblastoma cell model correlated with apoptosis induction, a decrease in aurora A kinase protein level and activity-related phosphorylation status, a decrease in the cytoplasmic level of MYCN, and an increased level of P53. At the same time, effective knockdown of PHLDA1 at the protein level (with a shRNA interference) caused enhanced cellular ATP levels and an increase in mitochondrial membrane potential, as compared to control and non-transduced cells. We found that downregulation of PHLDA1 leads to a significant increase in expression of aurora A kinase and TRKB that are markers of poor prognosis in neuroblastoma. Additionally, PHLDA1-silenced cells were less susceptible to apoptosis than the control cells. The current project is devoted to finding molecular networks of PHLDA1 and identifying its partners in neuroblastoma cells.

Elucidation of the mechanisms underlying the suppression of human neuroblastoma and melanoma cells overexpressing MCPIP1 protein

Neuroblastoma and melanoma cells overexpressing MCPIP1 exhibited a broad range of alterations in the transcriptome and proteome, as well as in the phosphorylation status of a number of proteins, strongly indicating MCPIP1-dependent cell cycle arrest and inhibition of Akt/mTOR signalling in these cells. Moreover, it was shown that MCPIP1 ribonuclease can bind and cleave the transcript of Aurora A kinase in *MYCN*-amplified neuroblastoma cells. It was found that the ribonuclease overexpression can affect microRNA expression profile in the cells. Evaluation of the MCPIP1 expression in melanoma tissues by immunohistochemical staining of primary and metastatic melanomas implicated decreased MCPIP1 expression in melanoma tumours compared

to normal tissues and positive correlation between high ribonuclease expression and melanoma-specific survival of patients. The data strongly suggests a potential tumour suppressor role and possible future application as a positive prognostic marker of MCPIP1 protein in melanoma.

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