2025

SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

DR NATALIA SZÓSTAK

ATTACHMENT 3

NAME

NATALIA SZÓSTAK

AWARDED DEGREES AND ACADEMIC TITLES

2018 **Doctor of Technical Sciences, discipline: Computer Science**

Poznań University of Technology

Faculty of Computing

Supervisor: Prof. hab. Eng. Jacek Błażewicz

Title: *"Bioinformatic Methods for Modeling and Verification of the RNA World Hypothesis"*

2010 **Master's Degree in Biology (specialization: Bioinformatics)**

Adam Mickiewicz University in Poznań

Faculty of Biology, Bioinformatics Laboratory

Supervisor: Prof. hab. Janusz Bujnicki

Title: *"Development of the RNAmap2D Program for Visualization and Analysis of Intra- and Intermolecular Contact and Distance Maps in RNA and Protein-RNA Complexes"*

INFORMATION ON PREVIOUS EMPLOYMENT IN RESEARCH INSTITUTIONS

02.2020 – present	Assistant Professor, Institute of Bioorganic Chemistry, Polish Academy of Sciences
08.2018 – 12.2018	Senior Scientific and Technical Specialist, Institute of Computing, Poznań University of Technology
03.2016 – 12. 2018	Assistant, Institute of Bioorganic Chemistry, Polish Academy of Sciences

DESCRIPTION OF THE ACHIEVEMENT

TITLE

The Role of the Human Gut Mycobiota in Organism Homeostasis and the Pathogenesis of Cancer Diseases

PUBLICATIONS INCLUDED IN THE ACHIEVEMENT

H1. **Szóstak N***, Szymanek A*, Havránek J, Tomela K, Rakoczy M, Samelak-Czajka A, Schmidt M, Figlerowicz M, Majta J, Milanowska-Zabel K, Handschuh L, Philips A. The standardisation of the approach to metagenomic human gut analysis: from sample collection to microbiome profiling. *Scientific Reports* 12 (1), 8470 (2022). doi: 10.1038/s41598-022-12037-3

IF_{2022} = 4,6; $5IF_{2022}$ = 4,9; MNiSW: 140

H2. **Szóstak N**, Handschuh L, Samelak-Czajka A, Tomela K, Schmidt M, Pruss Ł, Milanowska-Zabel K, Kozlowski P, Philips A. Host factors associated with gut mycobiome structure. *mSystems* 8 (2), e00986-22 (2023). doi: 10.1128/msystems.00986-22

IF_{2023} = 5; $5IF_{2023}$ = 6,1; MNiSW: 140

H3. **Szóstak N**, Handschuh L, Samelak-Czajka A, Tomela K, Pietrzak B, Schmidt M, Kaczmarek M, Galus Ł, Mackiewicz J, Mackiewicz A, Kozlowski P, Philips A. Gut Mycobiota Dysbiosis Is Associated with Melanoma and Response to Anti–PD-1 Therapy. *Cancer Immunology Research* 12 (4), 427-439 (2024). doi: 10.1158/2326-6066.CIR-23-0592

IF_{2023} = 8,1; $5IF_{2023}$ = 10,1; MNiSW: 200

H4. **Szóstak N**[#], Budnik M, Tomela K, Handschuh L, Samelak-Czajka A, Pietrzak B, Schmidt M, Kaczmarek M, Galus Ł, Mackiewicz J, Mackiewicz A, Kozlowski P, Philips A. Exploring correlations between gut mycobiome and lymphocytes in melanoma patients undergoing anti-PD-1 therapy. *Cancer Immunology, Immunotherapy* 74, 110 (2025). doi: 10.1007/s00262-024-03918-9

IF_{2023} = 4,6; $5IF_{2023}$ = 5,4; MNiSW: 140

H5. **Szóstak N**, Figlerowicz M, Philips A. The emerging role of the gut mycobiome in liver diseases. *Gut Microbes* 15 (1), 2211922 (2023). doi: 10.1080/19490976.2023.2211922

IF_{2023} = 12,2; $5IF_{2023}$ = 12,3; MNiSW: 100

*equal contribution
[#] corresponding author

ACHIEVEMENT SUMMARY

The research discussed below, comprising my scientific achievement entitled "*The role of the human gut mycobiota in organism homeostasis and the pathogenesis of cancer*", was conducted between 2020 and 2025 at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences (IBCH PAS) in Poznań. My scientific achievement consists of a collection of five multi-author publications, of which four present original research results, and one is a critical synthesis of the existing knowledge. The papers are published under an open access model. In all of these works, I am the first author, and in one, I am also the corresponding author. PDF files of these publications have been attached to the electronic version of the application.

INTRODUCTION

In recent years, microbiomes from various environments, including the diverse human body microbiome, have attracted significant attention from the scientific community. Of particular interest is the gut microbiota, which plays a crucial role in maintaining homeostasis and in the pathogenesis of numerous diseases. These dynamic and diverse ecosystems of microorganisms, encompassing representatives from different biological kingdoms, have become one of the key areas of biomedical research. Until now, researchers have primarily focused on bacteria, which constitute the most abundant component of the gut microbiota, while the role of other microorganisms, including gut fungi, referred to as the mycobiota, has long been marginalized. However, the development of modern next-generation sequencing (NGS) methods and advanced bioinformatics tools has enabled a deeper understanding of

the microbiome's composition, revealing that, alongside bacteria, other microorganisms, including fungi, play key roles in shaping human health (Zhang et al. 2021; Pérez 2021; Wu et al. 2021).

There is growing evidence linking alterations in the composition of the gut mycobiome to the development of various diseases, including autoimmune disorders (X. V. Li, Leonardi, and Iliev 2019; Underhill and Iliev 2014), metabolic conditions (X. Zhou, Zhang, and Yu 2024; Bao et al. 2023), and cancers (Narunsky-Haziza et al. 2022; Dohlman et al. 2022; Aykut et al. 2019). Some fungal species residing in the human gut are thought to exert beneficial effects, such as *Saccharomyces cerevisiae* var. *boulardii*, a component of commercially available probiotics (Ansari et al. 2021). Others, including certain species of *Candida* and *Aspergillus*, are considered harmful. The impact of gut fungi on the host is believed to involve the production of a wide array of metabolites that significantly influence host metabolic pathways (Kew 2013). Moreover, fungal cell wall components, such as chitin, β-glucan, and mannan, can interact with the host immune system, eliciting pro-inflammatory (Briard et al. 2021; Rizzetto et al. 2016).

Given that inflammation is a key contributor to cancer development (Multhoff, Molls, and Radons 2012; J. Yu et al. 2016), a dysbiotic mycobiota profile may promote systemic low-grade inflammation, thereby potentially fostering tumor development (Neagu et al. 2019; S. L. Schneider, Ross, and Grichnik 2015). In this context, emerging evidence suggests that the gut mycobiota may significantly influence patient responses to immunotherapy. However, the underlying mechanisms of these interactions remain poorly understood and warrant further investigation.

Unraveling the role of the gut mycobiota in disease processes and its impact on the success of immunotherapy is hindered by limited knowledge about its composition and variability. To date, no consensus has been reached regarding the existence or composition of a "core" human gut mycobiome, nor whether fungi form a stable, long-term community in the human intestine. Shifts in mycobiome composition are thought to be influenced by multiple factors, including host genotype, sex, age, comorbidities, medications, lifestyle, socioeconomic status, and diet. Nonetheless, studies examining the relationship between host-related factors and the human gut mycobiota have been mainly conducted in Chinese cohorts (Y. Sun et al. 2021), with the notable exception of research stemming from the Human Microbiome Project, which profiled fungal communities in 317 fecal samples from healthy donors (Nash et al. 2017). Other existing studies have been narrow in scope, focusing on specific aspects such as the influence of diet (Hoffmann et al. 2013), age and sex (Strati et al. 2016), body mass index (BMI) (Borges et al. 2018), or particular diseases, including liver (Gao et al. 2021; Zeng and Schnabl 2022), irritable bowel syndrome (Das et al. 2021; G. Hong et al. 2020), autoimmune diseases (Ben-Ami, Lewis, and Kontoyiannis 2010; Chiaro and Round 2021; Zhai et al. 2020), and the effects of antibiotics (Jiang et al. 2017; Krause and Reisinger 2005; Samonis et al. 1993; Seelbinder et al. 2020).

When analyzing the relationships between the mycobiota and host-related factors, it is important to remember that these factors can vary significantly depending on geographic region and culture, as well as environmental conditions and genotypes across different populations. Therefore, the results of gut mycobiota analyses and the conclusions drawn from them should be considered in the context of sociodemographic and geographic variables. Due to significant differences in diet, lifestyle, and environmental factors between China and Western countries, findings based on Chinese cohorts may not be fully applicable to other populations. Consequently, in order to better understand the relationships between gut mycobiota and various factors, it is crucial to conduct analyses across diverse cohorts, ideally with large sample sizes, to obtain a more comprehensive understanding of these interactions.

Furthermore, despite the growing number of metagenomic studies, the standard procedure for metagenomic data analysis remains a matter of debate. Due to the immense complexity and variability of microbial communities, studying the composition of gut microorganisms from stool samples is

subject to so-called batch effects, which may cause incompatibilities between different data sets, even those generated within the same study and/or laboratory (Schloss 2018). This effect stems from systematic differences caused by technical factors such as sample preparation methods, types of equipment used, or the analytical techniques applied, all of which can distort results and limit comparability. As a result, comparing findings obtained by different researchers, a standard practice in human genomics, becomes more difficult in metagenomic research.

Given the above, as well as the continuously growing volume of data generated in metagenomic experiments, it is essential to establish clear analytical standards. The lack of unified protocols and wide methodological diversity present a significant challenge, limiting comparability across studies and hindering the identification of universal prognostic markers that could be relevant to public health and therapeutic interventions. Although best practices in metagenomic research have been addressed in several publications (D'Amore et al. 2016; Quince et al. 2017; Wesolowska-Andersen et al. 2014) and are the primary objective of the International Human Microbiome Standards (IHMS) project, standardization of metagenomic data analysis procedures is still far from being achieved.

Finally, an open question remains as to how universal the influence of gut mycobiota might be across various diseases, including liver, autoimmune, and oncological conditions, and how these interactions may vary depending on specific pathological contexts. Although there are suggestions that mycobiota dysbiosis may have similar health effects across different diseases, further research is crucial to determine how gut mycobiota might affect various organs and the molecular mechanisms leading to disease development. Understanding these processes could be of great importance for the development of new therapeutic strategies that consider the role of the gut microbiota in disease treatment.

MOTIVATION AND RESEARCH OBJECTIVE

The motivation for my research stemmed from the growing awareness of the crucial role of the gut microbiome in human health and the pathogenesis of various diseases. The gut microbiome, including the mycobiome, plays an essential role in maintaining homeostasis and regulating immune responses, which has direct implications for treatment efficacy, particularly in the context of immunotherapy. Since previous studies have primarily focused on bacteria, the literature lacks sufficient data on the role of the gut mycobiome. Therefore, I decided to focus my research on this aspect.

The main objective of my work was to explore the role of the human gut mycobiome in disease pathogenesis, with particular emphasis on cancer, and to investigate its impact on treatment effectiveness, especially immunotherapy. To achieve this goal, it was necessary to address the challenges associated with metagenomic studies, such as the influence of methodological approaches on the outcomes of gut microbiota profiling based on stool samples, and the limited knowledge regarding the human gut mycobiome and its natural variability.

The first step was *(i)* the development and standardization of protocols for collecting and analyzing metagenomic data, particularly for profiling the composition of the gut microbiota from stool samples (**H1**). This was crucial to ensure reliable and reproducible results in the subsequent phases of the research. Then *(ii)* I focused on characterizing the gut mycobiome in healthy volunteers from Poland, examining the influence of demographic factors, diet, and lifestyle on mycobiome composition (**H2**). The results of this study provided insights into the natural variability of the mycobiome and its associations with general health status.

The next aim was *(iii)* to investigate differences in gut mycobiome composition between healthy individuals and cancer patients and assess the impact of mycobiome composition on the efficacy of immunotherapy (**H3**). For this purpose, I analyzed stool samples collected from melanoma patients

treated with anti-PD-1 therapy or receiving a vaccine. The research hypothesis assumed that the mycobiome of cancer patients differs from that of healthy individuals and that its composition may influence the response to immunotherapy. The obtained results confirmed this hypothesis, prompting further investigation into potential links between specific fungal species and the immune response in these patients.

To explore this question, *(iv)* I analyzed correlations between individual B and T lymphocyte subtypes and fungal species identified in melanoma patients (**H4**). The results indicated that the gut mycobiome composition may have the potential to modulate lymphocyte activity, which could affect the efficacy of anti-PD-1 therapy and the course of treatment. Finally, *(v)* I also undertook a critical review and synthesis of the existing knowledge on the role of the gut mycobiome in other diseases, including liver diseases, to determine whether similar mechanisms might be present in other conditions (**H5**). This part of the research provided new insights into potential universal mechanisms by which the mycobiome may influence human health.

STANDARDIZATION OF THE PROTOCOL FOR THE COLLECTION AND ANALYSIS OF METAGENOMIC FECAL DATA

The first objective of my work was to standardize the protocol for obtaining and analyzing metagenomic data from stool samples, which is a key step in ensuring the reliability of gut microbiome research results (**H1**). The growing number of metagenomic studies in recent years has highlighted the need to unify procedures in order to reduce confounding factors arising from DNA isolation, sequencing, and bioinformatic analyses. Only in this way can we ensure that the observed differences in microbiome composition are actual biological effects rather than artifacts of differing methodologies. To address the question of how individual steps and the methods used within them affect the results of gut microbiome profiling based on stool samples, I analyzed the impact of several key stages in the preparation and analysis of metagenomic data.

The first step that was directly analyzed was DNA isolation from stool samples. A critical factor that appears to play a role at this stage is the homogenization time of the samples before isolation. To investigate the impact of homogenization time on the resulting taxonomic profiles, a commercially available bacterial mix, ATCC Bacterial Mix MSA-2006 (referred to as BL), composed of 12 known bacterial strains, was used, along with three stool samples obtained from healthy volunteers (S1, S2, S3). The ATCC samples used in this study originate from authenticated microbial communities selected based on relevant phenotypic and genotypic attributes such as Gram staining, GC content, genome size, and sporulation, thereby mimicking mixed metagenomic samples. Moreover, since host cells are also abundant in stool (Tortora and Anagnostakos 1987), approximately 10,000 human peripheral blood mononuclear cells (PBMCs) were added to the ATCC Bacterial Mix to examine whether the DNA isolation process shows any preference for human versus bacterial DNA. Meanwhile, the real stool samples were enriched with so-called Zymo-spikes – two bacterial strains (Gram-positive *Allobacillus halotolerans* and Gram-negative *Imtechella halotolerans*) that are not naturally present in the human gut. These Zymo-spikes were added to evaluate potential differences in the extraction efficiency of Gram-negative and Gram-positive bacteria, as structural differences in their cell walls may lead to significant deviations in DNA isolation and subsequent results (Ketchum et al. 2018). Both the BL and real samples were then subjected to homogenization for three different durations: 10, 15, and 20 minutes.

In the second stage, I tested the impact of different reagent kits used for NGS library preparation on the results of gut microbiota profiling. For this analysis, I selected three reagent kits from leading manufacturers – KAPA, Illumina (Nextera), and Qiagen, based on their popularity and differences in DNA fragmentation protocols. At this stage, a new sample was introduced: a ready-to-use isolate, the

ATCC Genomic Mix DNA (ATCC® MSA-1003™, hereafter referred to as GD), which served as a reference sample that did not require DNA isolation. This resulted in three additional libraries (hereafter referred to as KAPA, Nextera, and Qiagen). In total, thirty-nine libraries were prepared – 13 with each kit. All libraries were then subjected to NGS sequencing.

Simultaneously, two *in silico* samples were generated, containing a mixture of reads derived from the genomes of the bacterial strains in the ATCC bacterial mix (ISE and ISS). One simulated sample reflected the actual composition of the ATCC mix, while the other was constructed using a log-normal distribution. These two simulated samples served as controls for evaluating the performance of bioinformatic tools used for community composition analysis. At this stage, two leading taxonomic profiling tools were employed: MetaPhlAn2 (Truong et al. 2015) and a combination of Kraken2/Bracken (Lu et al. 2017; Wood, Lu, and Langmead 2019). MetaPhlAn2 relies on mapping sequencing reads to predefined clade-specific microbial marker sequences, while Kraken uses k-mer matching between sequencing reads and a database of k-mers derived from microbial genomes.

In addition, to assess the content of human DNA in the sequenced samples, reads obtained from both the BL and real stool samples were mapped against the human genome (V. A. Schneider et al. 2017) using the BWA MEM algorithm (H. Li 2013). Similarly, reads from the real stool samples were mapped to the reference genomes of the bacterial species used as Zymo-spikes, allowing for comparison of read coverage and analysis of potential bias in the isolation efficiency of Gram-negative versus Gram-positive species. Furthermore, the ratio of Gram-positive to Gram-negative bacterial species in the GD and BL samples was evaluated against the expected known proportions in the ATCC mixes used in the experiment.

Studies have confirmed the impact of bacterial cell structure (expressed by Gram staining status) on the abundance of individual bacterial species. Results obtained from human samples with added Zymo-spike and BL samples indicate that several factors may contribute to the bias in the isolation of Gram-positive and Gram-negative bacteria when considering the effect of homogenization. While the lysis of Gram-positive bacterial cell walls may hinder DNA isolation, the fragility of Gram-negative bacteria may lead to mechanical fragmentation of their DNA. Depending on which of these phenomena predominates, we may observe an underrepresentation of either Gram-positive or Gram-negative bacteria. The results suggest that 10 minutes of homogenization best balances these opposing factors, enabling a more accurate reflection of Gram-positive and Gram-negative bacterial abundances. Moreover, the resulting microbiome profiles show the lowest degree of heterogeneity in sample diversity (*beta-diversity*).

At the same time, a comparison of the obtained results demonstrated that the chosen NGS library preparation kit significantly impacts gut microbiome profiling. The tagmentation-based Nextera kit yielded the most homogeneous and reproducible results, which is a key element of any experiment, including metagenomic studies. Additionally, bioinformatics analysis revealed that the choice of computational tool for determining gut microbiota composition significantly affects the results. In the conducted *in silico* experiments, the analytical pipeline based on Kraken2/Bracken tools outperformed MetaPhlAn2 in terms of effectiveness. In particular, the studies showed that MetaPhlAn2 failed to detect all expected species, demonstrating especially poor performance for the ISS sample, which better reflects the abundance distribution expected in real samples. The Kraken2/Bracken tandem was also able to identify all expected species for GD and BL samples while maintaining a low level of false positives. However, in both cases, individual abundances deviated from actual values. Analysis of the deviations from true values indicated that the differences in abundances were likely due to errors introduced during the DNA isolation step for the BL samples.

The study of the influence of human DNA content in the isolated material on profiling results showed that in nearly all tested samples, the number of reads mapping to the human genome was less than 3%. Furthermore, statistical testing showed that the library preparation kits played a significant role in the fraction of reads mapping to the human genome ($F=74.012$, $R^2=0.866$, $p=0.001$), unlike the homogenization time or the combination of homogenization time and library preparation kit type. Nevertheless, further bioinformatics analysis demonstrated that the presence of human DNA in samples does not significantly impact the results of metagenomic composition profiling. The resulting metagenomic profiles did not differ significantly, regardless of whether human reads were filtered out or not. This allowed us to conclude that the commonly used step of host read filtering in various metagenomic analysis protocols is unnecessary to obtain reliable metagenomic profiles.

The conducted studies confirmed that experimental design and protocol can have a crucial impact on determining the taxonomic profile of the gut microbiome. In response to the need for standardization in metagenomic analysis methodology, the results of my research enabled the development of recommendations for metagenomic studies regarding homogenization time (suggested 10 minutes), reagents used for NGS library preparation (tagmentation-based methods, Nextera, Illumina), tools for taxonomic composition profiling (advantage of Kraken2/Bracken tools), and the lack of necessity to filter out host-derived reads. Such studies and findings are important for a wide range of research aiming to better understand the gut microbiome's role in health and disease.

FACTORS SHAPING THE STRUCTURE OF THE GUT MYCOBIOTA

By applying the optimized strategy described above, I characterized the gut mycobiota of healthy volunteers, investigating the influence of demographic factors, diet, and lifestyle on its composition (**H2**). I focused on the gut mycobiota because its role in health and disease remains insufficiently understood, and most available studies concentrate on the bacterial microbiota. These studies were conducted within the framework of the Polish Microbiome Map project (NCT04169867), which enabled the collection and analysis of stool samples from 923 healthy volunteers in Poland – currently the largest cohort analyzed in the context of the gut mycobiota. The large sample size allowed for more precise identification of potential associations between host factors and the composition of gut fungi, as well as capturing inter-individual variability typical of the Eastern European population.

In 88.1% of samples (813 samples), I detected the presence of fungi, identifying a total of fifty-five species belonging to thirty-seven genera. The presence of fungi in nearly 90% of the samples supported the hypothesis that the mycobiota is a common component of the gut microbiome. At the same time, the studies confirmed the low abundance of fungi within the overall gut microbiome – my analysis showed that the mycobiota constituted about 1% of the entire microbiota (Nash et al. 2017). The study also demonstrated that despite high inter-individual variability, the presence of several species was consistent and observed in the majority of samples, supporting the concept of a core gut mycobiota. In this context, the most abundantly represented phylum in the analyzed samples was *Ascomycota* (94.0% of samples), while *Basidiomycota* was present in 40.0% of samples. This finding is consistent with earlier reports indicating the stability of *Ascomycetes* in the human gastrointestinal tract (Richard and Sokol 2019; Shuai et al. 2022). The most frequently detected genera were *Saccharomyces* (59.0% of samples), *Candida* (38.7% of samples), and *Sporisorium* (23.4% of samples), which may indicate their widespread presence in the diet or environment, or specific conditions favoring their colonization. The prevalence of *Saccharomyces* and *Candida* species in the human gut is consistent with previous reports (Hoffmann et al. 2013), while the genus *Sporisorium* has not been previously described as a component of the human gut microbiota.

S. cerevisiae, *Candida albicans*, and *Sporisorium* graminicola were the species found in the highest number of samples. Among them, *S. cerevisiae* is commonly found in the environment and food, which points to potential sources of fungi in the human gut and reflects the notion that environment and diet may have an inherent impact on human health. On the other hand, *C. albicans*, frequently detected in healthy individuals' stools, appears to lack a primary environmental reservoir, suggesting that it has co-evolved with its host and co-existing microorganisms (Pérez 2021). It is worth noting that *S. graminicola*, a plant pathogen, had not previously been identified as a component of the human gut. This may result from specific dietary habits of the studied cohort or the geographical presence of plants that serve as reservoirs for *S. graminicola*.

The analysis of host factors influencing gut mycobiota composition included fifty-three variables, such as diet, age, sex, health status, lifestyle, and socioeconomic factors, allowing for a comprehensive exploration of potential associations. I found significant correlations between the mycobiota composition and ten factors (Fig. 3A from publication H2), most of which were related to diet, including consumption of chips, meat, soft drinks, sweeteners, processed food, and alcohol, including both frequency and type (e.g., beer). In addition, marital status and age group were also significant factors shaping the mycobiota composition. In contrast, health status and the use of medications and supplements had no significant impact on the mycobiota composition, suggesting that environmental and dietary factors play a key role in this context.

I also examined correlations between the presence of specific fungal genera and the observed traits. The analysis revealed that many fungal genera were associated with various factors, predominantly disease- and diet-related, and that the patterns of association varied considerably depending on the specific factor (Fig. 3C from publication H2). For example, the genera *Nakaseomyces*, *Neurospora*, and *Sporisorium* were associated with depression, which may indicate their involvement in processes related to this condition. Meanwhile, the genera *Aspergillus*, *Candida*, and *Yarrowia* correlated with autoimmune disorders, suggesting their potential role in immune responses. In the context of diet, the genus *Candida* was linked to the consumption of sweets, salt, chips, coffee, fish, and vegetables, indicating that its presence may be strongly modulated by dietary composition. The genus *Debaryomyces* showed similar associations, particularly with salt, chips, coffee, and tea. Another potentially relevant genus, *Malassezia*, correlated with the consumption of fish and tea, while *Yarrowia* was associated with chip consumption. These observations suggest that different fungal genera may respond to or originate from specific dietary products.

Concerning diet, the studies also showed that vegetarians had significantly higher gut fungal diversity, both in terms of species richness (*alpha-diversity*) and Shannon diversity, which may suggest that this diet promotes a broader range of fungi, including those of plant origin. Additionally, differential abundance analysis revealed numerous fungal species that varied in abundance depending on the type of diet. Frequent alcohol consumption, especially wine, was associated with greater fungal diversity, and alcohol intake in general was linked to higher abundance of *S. cerevisiae*, likely due to alcohol being a potential source of exogenous yeasts. The results confirmed that diet significantly impacts gut mycobiota variability, particularly in terms of species diversity and the abundance of specific fungal genera. This phenomenon appears to have a dual nature. On one hand, consuming food products that contain fungi, such as yeasts, can directly affect mycobiota composition by introducing yeasts into the gastrointestinal tract (as in the case of wine). On the other hand, dietary components such as sugars and alcohol may promote the growth of certain fungal species in the gut.

In particular, the conducted studies confirm earlier findings suggesting that a diet rich in carbohydrates promotes the growth of *Candida*, whereas a high-protein and high-fat diet may limit its presence (Hoffmann et al. 2013). These findings are especially relevant in the context of gut health and diseases

associated with mycobiota dysbiosis, such as candidiasis or autoimmune disorders. Excessive proliferation of *Candida* species in the gut can lead to disturbances in gut microbiota balance, which has numerous health consequences, including impacts on immune system function and the development of inflammatory conditions. My research suggests that dietary manipulation, especially reducing simple carbohydrates and increasing protein and fat intake, may be one way to control the excessive growth of *Candida* species.

With regard to sex, differences in microbiota composition between men and women are well documented; however, research has mainly focused on bacteria (Kim et al. 2020; Valeri and Endres 2021), with relatively little attention given to differences in fungal composition between sexes. Similar to bacteria, fungal composition differences between sexes could be attributed to the role of sex hormones in modulating microbiota composition (Markle et al. 2013) and indirectly to diet, which may vary by gender (Bolnick et al. 2014). Despite these assumptions, in my study, the only observed sex-based difference was in the level of gut mycobiota evenness (Pilou's index), with women showing more even mycobiota. Thus, sex does not appear to influence gut fungal composition significantly.

In contrast to sex, my study showed that age is associated with gut mycobiota composition. Previous studies on human mycobiota and age have found that the gut mycobiota richness of infants (0–2 years) and children (3–10 years) is higher than that of adults (18 years) (Strati et al. 2016). My analysis also demonstrated that changes in gut fungal composition can still be observed during adulthood: individuals aged 46–65 had higher mycobiota diversity than participants in younger age groups (18–25 and 26–45). The conducted analyses also allowed for the identification of many fungal species that differentiate age groups, with the most significant differences observed in comparison with older adults (65+ age group).

In the context of BMI, the analyses showed that overweight individuals had a less even gut mycobiota compared to those with normal or low BMI. Additionally, obese individuals exhibited an increased abundance of *D. hansenii* and *Saccharomyces paradoxus*, yeasts from the *Saccharomycetaceae* family. The high abundance of *D. hansenii* may be related to the dietary habits of overweight and obese individuals, as *D. hansenii* is a common species found in all types of cheese (Fleet 1990) and sausages (Dalton, Board, and Davenport 1984). On the other hand, studies conducted on lean and obese mice suggest that the gut microbiota affects energy balance by influencing the efficiency of calorie extraction as well as how that energy is used and stored (Bäckhed et al. 2004; Turnbaugh et al. 2008; 2006). The gut fungus *Candida parapsilosis* has also recently been identified as a critical commensal fungus associated with diet-induced obesity in mice (S. Sun et al. 2021). The results I obtained, together with references to other studies on this topic, indicate that the gut mycobiota may play a key role in the development of obesity and related metabolic disorders.

In the cases I analyzed, probiotics had a significant impact on the gut mycobiota – daily use was associated with lower fungal diversity and varied changes in the composition of specific species compared to individuals who took probiotics sporadically or not at all. In contrast to probiotics, dietary supplements did not have an apparent effect on the gut mycobiota. Antibiotics also did not significantly affect overall fungal diversity; however, species composition analysis revealed some differences in the abundance of particular species depending on the antibiotic regimen. Although antibiotics do not directly affect fungal communities, commensal bacteria can theoretically limit fungal colonization and invasion through several mechanisms, such as the production of antifungal compounds (Cabral et al. 2018), competition for available nutrients, chemotaxis, and physicochemical changes in the local environment (Frey-Klett et al. 2011; Peleg, Hogan, and Mylonakis 2010). Therefore, antibacterial drugs may be an important risk factor for fungal infections. For this reason, studies on gut mycobiota in the context of antibiotics are critical, as they may contribute to a better understanding of microbiome balance mechanisms and ways to minimize the risk of fungal infections resulting from antibacterial therapy.

Additionally, these studies may help develop more balanced treatment strategies that not only effectively eliminate bacterial pathogens but also minimize negative effects on other gut microbiota components, including fungi.

The obtained results constitute a significant contribution to understanding the factors shaping the composition of gut mycobiota in the European population. Previous studies on gut mycobiota were mainly conducted in Asian populations, while European data remained limited, preventing a complete understanding of the impact of environmental and demographic factors characteristic of this part of the world. The studied cohort's size and the large number of analyzed factors allowed for capturing subtle relationships between demographic and environmental factors and mycobiota composition. These results provide valuable insights into the role of gut mycobiota in human health, indicating the need for further research that takes into account regional and cultural specificity, especially related to regional diets. They may also serve as a reference point for mycobiota analysis in future studies, enabling more precise identification of factors influencing its composition, e.g., in specific disease states or studied conditions. The work based on the analyses was nominated for the Director's Award of ICHB PAN for the best experimental article published in 2023.

DYSBIOSIS OF THE GUT MYCOBIOTA IN MELANOMA PATIENTS AND ITS IMPACT ON THE EFFECTIVENESS OF ANTI-PD-1 IMMUNOTHERAPY

Knowing the relationships between a wide range of environmental, dietary, and demographic factors in a healthy cohort and the composition of the gut mycobiota, I decided to analyze the role of gut mycobiota in melanoma progression and response to anti-PD-1 therapy (**H3**). Immunotherapy, particularly immune checkpoint inhibitors, has brought a breakthrough in cancer treatment; however, its effectiveness varies significantly among patients – only 20–40% achieve a durable response (Zhao, Zhao, and Zhao 2020). My interest in this topic arose from the growing body of evidence highlighting the importance of the microbiome in modulating immune responses, while detailed data on the specific role of gut fungi in the context of immunogenic cancers such as melanoma were lacking. My goal was to investigate how changes in the composition and diversity of the gut mycobiota may influence the development of this disease and the effectiveness of immunotherapy, an area that has so far remained poorly understood in the scientific literature on melanoma. By undertaking this task, I aimed to fill a significant knowledge gap, combining microbiological analysis with clinical oncology aspects to better understand how gut fungi may shape disease progression and response to treatment.

In the study, I analyzed stool samples from eighty-six melanoma patients and 134 healthy participants recruited as part of the Polish Microbiome Map project. The healthy participants were a subset of the cohort analyzed in my previous work on factors shaping the gut mycobiota in general (**H2**). Among the melanoma patients, two groups could be distinguished: sixty-two individuals with advanced stage III or IV melanoma treated with anti-PD-1 antibodies (nivolumab or pembrolizumab), and 24 patients with clinically stable long-term melanoma receiving the therapeutic vaccine AGI-101H.

For the anti-PD-1 group (n=62), samples were collected at three time points: before treatment initiation (BT), after 3 months of therapy (T3), and after 12 months of treatment (T12), which allowed assessment of the dynamics of gut mycobiota changes during immunotherapy. In the AGI-101H vaccine group (n=24), samples were collected at two points: before and 6–10 days after vaccination, to evaluate the stability of the mycobiota in this group.

In patients treated with anti-PD-1, treatment response was additionally assessed according to RECIST criteria (Response Evaluation Criteria In Solid Tumors), classifying their responses as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). Responders (R)

were defined as patients with complete (CR) or partial (PR) responses, whereas non-responders (NR) were those with stable disease (SD) or progression (PD). The study included 27 responders and 34 non-responders. An independent classification divided patients into those showing clinical benefit (CB) from immune checkpoint inhibitor (ICI) therapy (CR, PR, and SD) and those without such benefit (NB, including PD). The study included 37 CB and 24 NB patients. This classification enabled me to analyze the relationship between mycobiota composition and clinical treatment outcomes.

Using NGS sequencing techniques and bioinformatic analyses, I characterized the gut mycobiome structure in melanoma patients compared to the control group. My research revealed dysbiosis in the gut mycobiome of melanoma patients relative to healthy individuals. I found a higher abundance of opportunistic pathogenic species such as *C. albicans*, *Candida dubliniensis*, and *Neurospora crassa*, while potentially beneficial species like *S. cerevisiae* and *D. hansenii* were less abundant compared to healthy controls. This novel finding indicates a specific mycobiome profile associated with melanoma that had not been previously described in the literature.

Moreover, analyzing samples collected during anti-PD-1 therapy, I observed dynamic changes in the gut mycobiota composition. Notably, the relative abundance of *Malassezia restricta* and *C. albicans* increased during immunotherapy, suggesting that immune treatment may modulate the intestinal fungal microbiota. Although no statistically significant effect was observed for *S. cerevisiae* in this context, its abundance gradually decreased over the course of immunotherapy.

A key result of my study was demonstrating an association between mycobiome composition and response to anti-PD-1 therapy. Comparing patients who derived clinical benefit from anti-PD-1 therapy (CB) with those who did not (NB), I found that CB patients had lower fungal richness and Shannon diversity than NB patients, which may indicate a less diverse mycobiota environment favoring treatment efficacy. Additionally, the fungus *Tetrapisispora blattae* was less abundant in patients who benefited from anti-PD-1 therapy, suggesting that its presence may be linked to resistance to immunotherapy. Comparison of responders (R) versus non-responders (NR) showed similar results regarding overall gut mycobiota structure as in the CB vs. NB comparison (R patients had lower fungal richness and Shannon diversity than NR). Taxonomic analysis distinguishing R from NR revealed a higher abundance of *S. paradoxus* in patients responding positively to treatment (R), providing the first indication that this species may play a protective role in the context of immunotherapy, potentially by supporting a beneficial immune response.

Next, I investigated whether and how specific fungal species identified in the guts of melanoma patients correlate with disease progression. For this purpose, I performed Kaplan–Meier survival analysis and used the Cox proportional hazards model (CPH), including LDH concentration as a prognostic biomarker in the model. The analyses showed that patients with high levels of *M. restricta* had a 2.2-fold higher risk of melanoma progression, while those with elevated levels of *C. albicans* had twice the risk compared to patients with low levels of these species (Fig. 6A in publication H3). Survival analysis indicated that higher abundance of *C. albicans* and *M. restricta* was associated with a tendency toward shorter progression-free survival ($p = 0.09$ and $p = 0.07$), although the results did not reach full statistical significance (Fig. 6B in publication H3) – the lack of significance may be due to limited sample size. Moreover, median progression time was significantly shorter in patients with elevated *M. restricta* (5 vs. 13 months) and *C. albicans* (3 vs. 14.5 months), highlighting the potential importance of these fungal species as predictors of poor prognosis in patients treated with anti-PD-1 therapy.

My research contributed new knowledge to oncology, immunology, and microbiology by indicating that the gut mycobiota plays an important, previously underestimated role in melanoma progression and immunotherapy efficacy. The results suggest that not only bacteria but also gut fungi may actively influence the body's immune response to cancer, an aspect previously overlooked in microbiome studies

on melanoma. These findings expand the understanding of mechanisms underlying this disease and open new clinical opportunities. They suggest potential biomarkers such as *M. restricta*, *C. albicans*, and *S. paradoxus* that may help predict treatment response and therapeutic strategies based on mycobiome modulation, for example, using probiotics containing *Saccharomyces* species. These results may serve as a starting point for further studies on interactions between the mycobiome and the immune system in the context of cancer immunotherapy.

ANALYSIS OF CORRELATIONS BETWEEN THE MYCOBIOME AND LYMPHOCYTES IN MELANOMA PATIENTS UNDERGOING ANTI-PD-1 THERAPY

The next stage of my work involved investigating the relationships between the gut mycobiome composition and various populations of lymphocytes circulating in the blood of melanoma patients undergoing anti-PD-1 therapy (**H4**). For this purpose, I used flow cytometry data obtained for a cohort of patients enrolled in the Polish Microbiome Map project, integrating them with taxonomic data derived from NGS sequencing of stool samples from these patients. Once again, data collected at three time points were analyzed: before the start of therapy (BT), after 3 months of treatment (T3), and after 12 months of treatment (T12), which allowed the assessment of changes in the patients' immune system throughout immunotherapy in the context of gut mycobiome composition. I also divided the patients into groups based on their response to therapy, which made it possible to track changes in the composition of the gut mycobiota and lymphocyte profiles depending on treatment effectiveness.

Before analyzing the relationships between the gut mycobiome and lymphocytes, I examined the correlations between lymphocyte populations and cohort characteristics such as age, serum LDH levels, overall survival (OS), and progression-free survival (PFS). I selected age due to its association with immune system aging, LDH level as a prognostic factor linked to response to anti-PD-1 therapy, and OS and PFS because of their direct relevance to immunotherapy effectiveness. Pre-treatment data (n=61) were used to analyze the correlations between selected factors and lymphocyte levels. The correlation analysis revealed numerous associations (Table 2 in publication H4), with OS and LDH level showing correlations with the highest number of lymphocyte types. Most correlations were positive, except for inverse relationships for LDH, PFS, and OS, which is expected, as a high LDH level is associated with a poorer response to immunotherapy and, consequently, shorter OS and PFS. For example, an increase in LDH was associated with a decrease in the percentage of B cells, while longer OS and PFS correlated with an increase in this percentage. Similarly, inverse relationships were observed for late memory B cells: a positive correlation with LDH and a negative correlation with OS and PFS. Additionally, I divided patients into groups based on thresholds for selected parameters (Table 3 in publication H4). The most significant changes in lymphocyte levels were associated with OS and PFS. Elevated LDH was associated with a higher percentage of late memory B cells and CD273+/CD274+ T cells, and a lower percentage of T cells overall. The opposite patterns were observed in patients with longer OS.

Next, I analyzed changes in lymphocyte levels in patients undergoing anti-PD-1 therapy, comparing the levels before and after three months of therapy. I observed that after three months of treatment, the levels of transitional regulatory B cells, naïve non-memory B cells, and PD-1-/CD152- T cells were significantly higher compared to pre-treatment values (Table 4 in publication H4). Conversely, the level of PD-1+ T cells was considerably higher before the start of therapy than after three months. Further analyses showed that greater differences in lymphocyte levels before treatment were observed between patients deriving clinical benefit (CB) and those not deriving benefit (NB) than between responders (R) and non-responders (NR). Particularly interesting was the reversal of the pattern in the case of late memory B cells. Before therapy, their level was lower in the R group compared to the NR group, whereas after three months of treatment, the level of these cells was higher in the R group. These results indicate

dynamic changes in lymphocyte populations during anti-PD-1 therapy, which may be important for assessing treatment response and predicting long-term therapy outcomes.

Having identified patterns of changes in the analyzed lymphocyte populations and their dependence on factors related to treatment efficacy, age, and LDH levels, I proceeded to characterize the patterns of associations between the gut mycobiota and lymphocytes in melanoma patients undergoing anti-PD-1 therapy. One of my key achievements was identifying specific correlation patterns between the presence of gut fungi and the levels of various lymphocyte types, which changed depending on treatment time and clinical response. I paid particular attention to two fungal species – *M. restricta* and *C. albicans* – which, in my previous studies [**H3**], showed correlations with increased risk of melanoma progression and poorer response to anti-PD-1 therapy.

In this study, I demonstrated that in the CB group, *M. restricta* exhibited positive correlations with numerous lymphocyte types, including B cells, regulatory T cells, and CD152+ T cells, as well as negative correlations with CD273-/274- T cells. Analysis of paired samples (BT vs. T3) revealed that in the NB group, changes in the abundance of *M. restricta* were associated, among other things, with an increase in PD-1+/CD152+ T cells, PD-1+ T cells, and total T cells. These findings may suggest a differential, treatment-response-related, strong immune reaction against antigens or metabolites associated with *M. restricta* in melanoma patients treated with anti-PD-1, or even an immune response induced by *M. restricta*.

On the other hand, in the CB group, *C. albicans* positively correlated with lymphocytes associated with long-term immunity, such as CD27+ memory B cells, EM Th among regulatory T cells, and uncommitted memory T cells. Notably, the Tp55 protein encoded by the CD27 gene is a member of the TNF receptor superfamily required for the generation and long-term maintenance of T cell immunity. TNF, which can exhibit both pro- and antitumor effects depending on the context (Gutierrez et al. 2022), is involved not only in carcinogenesis but also in nonspecific immunity to *C. albicans* ((Richardson and Moyes 2015; Wang and Lin 2008). This diverse role of TNF highlights its complex involvement in immune responses. Interestingly, TNF blockade has been shown to overcome resistance to anti-PD-1 therapy in melanoma (Steinshamn et al. 1996), indicating a potential cross-talk between TNF signaling and immune checkpoint pathways. The abundance of *C. albicans* in the CB group also positively correlated with cytotoxic TD lymphocytes, suggesting activation of an antifungal immune response in this group (Aybay and Imir 1996).

In the NB group, different patterns were observed for *C. albicans*, including positive correlations with lymphocyte subsets responsible for early antibody response, plasmablasts, and late memory B cells, as well as regulatory T cells. The percentage of late memory B cells also negatively correlated with OS and PFS and positively with serum LDH level and lack of benefit from anti-PD-1 therapy in the BT group, suggesting it is a marker of poor prognosis in melanoma. Moreover, in the NB group, *C. albicans* negatively correlated with the percentage of Mo CD273+ (PD-L2) monocytes and positively with Mo CD274+ (PD-L1) monocytes. Notably, paired sample analysis showed that in the NB group, an increase in *C. albicans* was correlated with a decrease in total T cells, PD-1-expressing T cells, PD-1 and CD152-expressing T cells, CD185-expressing cytotoxic T cells, and CD273+/CD274+ monocytes. Simultaneously, the *C. albicans* increase was associated in this group with an increase in CD273+ monocytes and PD-1-/CD152- T cells. It is known from the literature that PD-L1 negatively regulates host antifungal immunity to *C. albicans* infection by inhibiting the release of neutrophils from the bone marrow (Y. Yu et al. 2022). This mechanism, observed in both murine and human neutrophils, involves activation of Dectin-1 by fungal β-glucans, which induces PD-L1 expression. Elevated PD-L1 levels regulate neutrophil trafficking by modulating autocrine secretion of the chemokines CXCL1 and CXCL2. This regulation hinders neutrophil release from the bone marrow into circulation, exacerbating

C. albicans infection. The differences in immune response between NB and CB patients suggest a potential link between immune responses to fungal infections such as *C. albicans* and the effectiveness of immunotherapeutic strategies in cancer treatment.

Regarding *S. cerevisiae*, in NB patients I observed a negative correlation with PD-L2-expressing monocytes and a positive correlation with CD3-expressing CD4/CD8 T cells. Furthermore, in the NB group, paired analysis showed a positive correlation between changes in *S. cerevisiae* and CD273-/CD274- T cells, and a negative correlation with T cells expressing PD-L2, PD-L1, CD4-/CD8-, immature activated B cells, plasma cells among B cells, and late memory B cells. This result may suggest that in patients not benefiting from anti-PD-1 therapy (NB), a decrease in *S. cerevisiae* may be associated with an increase in PD-L1, PD-L2, and lymphocytes related to shorter progression-free and overall survival, as well as an increase in serum LDH levels.

Another interesting fungal species with antioxidant and antitumor properties (Y. Yang et al. 2022), *D. hansenii*, showed a positive correlation in my study with monocytes expressing PD-L1 and PD-L2 in CB patients. Literature reports indicate that oral administration of this yeast improved innate immune responses in mice by increasing the expression of pro-inflammatory cytokine genes (IFN-γ, IL-6, and IL-1β) after *E. coli* challenge (Angulo et al. 2023). In another animal model, *D. hansenii* exhibited immunostimulatory effects on leukocytes via β-glucans (Angulo et al. 2019). In this case, oral administration of *D. hansenii* CBS 8339 stimulated immune responses, antioxidant factors, and the expression of immune-related signaling pathway genes within a short time frame. These findings suggest that *D. hansenii* is a promising candidate for further research on enhancing the efficacy of immunotherapy.

To date, studies on the gut microbiome in the context of immunotherapy have focused primarily on bacteria, leaving the role of gut fungi largely unexplored. My work is the first to provide detailed data on correlations between specific fungal species and lymphocyte subpopulations during anti-PD-1 therapy, filling a critical gap in knowledge about the impact of the mycobiome on immune responses in cancer. The identified patterns suggest that changes in the gut mycobiome may be associated with the immune response to anti-PD-1 therapy, which could have significant implications for personalized treatment strategies. In particular, analysis of immune response differences between CB and NB groups enabled the identification of potential biomarkers of therapy efficacy, such as specific correlation patterns between lymphocytes and *M. restricta* or *C. albicans*. Furthermore, incorporating temporal dynamics (BT vs. T3) allowed us to understand how anti-PD-1 therapy influences mycobiome–immune system interactions, an area previously underexplored. My findings also shed light on the molecular mechanisms underlying heterogeneous responses to immunotherapy. For instance, the demonstrated role of PD-L2 (CD273) and PD-L1 (CD274) in interactions between gut fungi and lymphocytes raises new questions about the competition of these ligands with anti-PD-1 antibodies, which may explain resistance to treatment in some patients. These results are consistent with literature showing that PD-L2 has a stronger binding affinity to the PD-1 receptor than PD-L1 L1 (Miao et al. 2021), potentially hindering the therapeutic activity of anti-PD-1 antibodies.

REVIEW OF THE ROLE OF THE MYCOBIOME IN LIVER DISEASES

In the review paper *"The emerging role of the gut mycobiome in liver diseases"* (**H5**), I gathered and analyzed publications concerning the role of the gut mycobiome in the pathogenesis of liver diseases, emphasizing its significance in immunological and metabolic mechanisms. In this work, I examined various liver conditions such as alcohol-associated liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), primary sclerosing cholangitis (PSC), cirrhosis, and hepatocellular carcinoma (HCC). The

analysis of the available literature indicated that the gut mycobiota may play a role in all of these conditions.

In studies on ALD, mice treated with ethanol exhibited fungal overgrowth in the gut and elevated blood β-glucan levels. Moreover, administration of antifungal drugs alleviated ethanol-related liver disease symptoms (A.-M. Yang et al. 2017). One proposed explanation was the induction of liver inflammation by β-glucan, which activates the pro-inflammatory cytokine IL-1β via a signaling cascade (Kankkunen et al. 2010). On the other hand, another study showed that 1,3-β-glucan from *Wolfiporia cocos* had a protective effect, reducing inflammation and liver steatosis (S. Sun et al. 2020). In patients with alcohol use disorder (AUD) and ALD, an increased abundance of various fungal genera and species, including *Candida*, particularly *C. albicans*, was observed (Chu et al. 2020; A.-M. Yang et al. 2017; Lang et al. 2020; Hartmann et al. 2021). The negative effects of *C. albicans* were attributed to candidalysin, a peptide secreted by this fungal species that directly causes hepatocyte death and liver damage.

Regarding NAFLD, although there are some reports on the role of the gut mycobiota in this disease, most studies focus on its association with obesity, a condition closely linked to NAFLD. In line with my earlier research (**H2**), obese individuals exhibit an altered gut mycobiome composition, including increased levels of *Candida*, *Nakaseomyces*, and *Penicillium* species (Hoffmann et al. 2013; Morales, Brignardello, and Gotteland 2010; Borges et al. 2018), and generally elevated levels of yeast fungi (Borges et al. 2018). Additionally, *Candida parapsilosis* was recently identified as a critical commensal fungus associated with diet-induced obesity in mice (S. Sun et al. 2021). Studies examining the relationship between fungal gut composition and anthropometric or metabolic parameters related to obesity found that the relative abundance of certain fungi was linked to obesity and its metabolic complications, including insulin resistance, dyslipidemia, blood pressure, and inflammation (Mar Rodríguez et al. 2015). However, even though some associations between gut mycobiota and obesity exist, it remains unclear whether these fungal alterations contribute to the development of obesity or merely result from dietary habits, such as high carbohydrate or fat intake, leading to weight gain.

Regarding NAFLD, the pro-inflammatory effect of obesity-associated mycobiota may be one of the factors contributing to gut barrier dysfunction, further exacerbating hepatic inflammation. Literature also lists fungal genera whose increased or decreased abundance is associated with NAFLD and non-alcoholic steatohepatitis (NASH). Among fungal species potentially linked to NAFLD and its more severe stages, *C. albicans* has been highlighted due to increased systemic immune responses (Demir et al. 2022). Interestingly, these changes were observed particularly in non-obese patients, pointing to previously unreported features of non-obese NAFLD phenotypes. Although one study using a mouse model of diet-induced steatohepatitis showed that treatment with the antifungal amphotericin B reduced liver damage, this effect may be related to its influence on cholesterol and TLR pathways, which are directly implicated in NASH and liver fibrosis.

Research on PSC points to the “leaky gut” hypothesis as well as fungal dysbiosis playing a role in its pathogenesis. Studies also emphasize the connection between PSC and inflammatory bowel disease (IBD), with approximately 75% of PSC patients also having IBD (Loftus et al. 2005). Variants in *CARD9*, a protein involved in innate antifungal immunity, have been found to increase susceptibility to both PSC and IBD (X. Li, Shen, and Ran 2017). High levels of ASCA antibodies against *S. cerevisiae* are present in PSC patients (Muratori et al. 2003), while the presence of *Candida* in bile is associated with worse prognosis (Rudolph et al. 2009). The effect of *Candida* may be related to the induction of a Th17 response (Katt et al. 2013), which may exacerbate PSC, as confirmed by elevated Th17 levels in patients with this disease (Nakamoto et al. 2019). Other studies show increased *Sordariomycetes* and *Exophiala* levels and decreased *S. cerevisiae* levels in PSC (Lemoinne et al. 2020), where *S. cerevisiae* may play a protective role through IL-10 (Sokol et al. 2017), while *Exophiala dermatitidis*, a fungal

species from the *Exophiala* genus, can cause infections that mimic PSC and damage bile ducts (K. H. Hong et al. 2009; Oztas et al. 2009). An increased fungal-to-bacterial ratio (ITS2/16S) and disrupted correlation networks between the two kingdoms have also been observed in PSC patients, confirming mycobiome alterations, including increased *Candida* (Lemoinne et al. 2020).

Studies also point to a potential role of the gut mycobiota in liver cirrhosis, which represents an advanced stage of liver scarring due to chronic damage. The Bacteroidetes/Ascomycota ratio changes with disease severity, and its lower value correlates with fewer hospitalizations and can predict 90-day hospitalization independently of other factors (Bajaj et al. 2018). Fungal diversity decreases in patients with cirrhosis and is inversely correlated with the Model for End-Stage Liver Disease (MELD) score. Notably, *C. albicans* was detected in about 20% of stool samples from cirrhotic patients undergoing rifaximin treatment (Zullo et al. 2012). The situation differs in HBV-related cirrhosis, where an increase in fungal diversity and abundance is observed, including species such as *Candida* (*C. albicans, C. parapsilosis, C. krusei*), *Aspergillus*, and *Saccharomyces* (Mou et al. 2018; Cui, Morris, and Ghedin 2013; Guo et al. 2010; Chen et al. 2011). According to some studies, cirrhosis increases the risk of esophageal candidiasis (Ou et al. 2014), although the causal relationship remains unclear. It is also worth noting that statistics show that *Candida* infections and associated candidiasis pose a serious threat, with a 55% mortality rate and a 4.4-fold increased risk of death in patients with liver cirrhosis (Verma et al. 2022).

With regard to HCC, a review of the available literature indicates its multifactorial pathogenesis, with HBV, HCV infections and NASH being among the key risk factors (Chidambaranathan-Reghupaty, Fisher, and Sarkar 2021). However, studies show that the gut microbiome of patients with HCC differs from that of healthy individuals and may correlate with disease (Ponziani et al. 2019; Ren et al. 2019). Aflatoxin B1 (AFB1), a toxic metabolite produced by fungi of the *Aspergillus* genus, plays a particularly important role in HCC pathogenesis, as it can lead to mutations in the *Tp53* tumor suppressor gene (Hussain et al. 2007; Bressac et al. 1991; Kew 2013; Hamid et al. 2013). There is also evidence that AFB1, in combination with chronic HBV infection, significantly increases carcinogenic risk by impairing DNA repair mechanisms and stimulating uncontrolled proliferation of hepatocytes (Allen et al. 1992; Turner et al. 2000; Qian et al. 1994). Mycobiome studies in HCC patients also revealed reduced fungal diversity and increased abundance of *C. albicans*, suggesting a potential link with tumor development (Z. Liu et al. 2022). Furthermore, in the serum of HCC patients and in animal models colonized with *C. albicans*, several metabolic alterations were found, including increased concentrations of L-carnitine and L-acetylcarnitine (L. Zhou et al. 2012; Fujiwara et al. 2018). However, their role in HCC remains ambiguous – they may either reflect increased energy demand of tumor cells or exert protective effects against disease progression (Ishikawa et al. 2014; Z. Liu et al. 2022). In addition, it has been shown that the NLRP6 receptor, which regulates immune responses to microorganisms, is crucial for the development of *C. albicans*-induced HCC (Levy et al. 2015; R. Li and Zhu 2020). This discovery may open new therapeutic opportunities by targeting mycobiota-related signaling pathways in HCC treatment.

Analyzing the literature on gut mycobiota and liver diseases, I also examined the potential protective role of fungi as probiotics. The most widely described species in the literature is *S. cerevisiae* var. *boulardii*. This species has beneficial effects in treating gastrointestinal diseases and may modulate the gut microbiome as well as alleviate liver damage, hepatic steatosis, and chronic inflammation (L. Yu et al. 2017; Everard et al. 2014; Ansari et al. 2021). As a probiotic, it is used in the prevention and treatment of NAFLD. Animal studies have shown that oral supplementation reduces body weight, fat content, liver steatosis, and inflammation (Everard et al. 2014). Moreover, *S. boulardii* influences gut microbiota composition by reducing levels of *Escherichia coli* and *Bacteroides* in animals with NAFLD (Y. T. Liu, Li, and Wang 2016). Similar protective properties are also shown by macrofungi such as *Agaricus bisporus* (Huang et al. 2016; Y. Liu et al. 2018; S. Li et al. 2017) and *Pleurotus ostreatus* (Duan et al.

2020), whose polysaccharides reduce serum ALT and AST levels, limit hepatocyte damage, inflammation, and oxidative stress, and improve lipid metabolism. The literature also emphasizes that fungal probiotics should be used with caution, especially in immunocompromised patients. There are reports of *S. cerevisiae* fungemia following its use in individuals with *Clostridium difficile*-associated colitis and in neutropenic patients (Santino et al. 2014; Cesaro et al. 2000), highlighting the need for further studies on the safety of mycobiota-based therapies.

POTENTIAL

My research holds significant potential both scientifically and clinically.

First, it contributes to the development of new standards for fecal metagenomic studies (**H1**), which improves the reproducibility of results and enables better comparison of data across different studies. The standardization of research protocols in metagenomics is crucial for increasing the reliability of findings, especially when analyzing populations of microorganisms other than bacteria, such as fungi, which constitute only a small fraction of the microbiome but may have significant biological relevance. The lack of uniform guidelines regarding isolation, sequencing, and data analysis methods leads to difficulties in interpreting and comparing results between studies. My work allows the identification of key factors affecting methodological effectiveness and helps develop recommendations for optimal research strategies, which is particularly important in clinical applications, where reliability and reproducibility directly impact therapeutic decision-making.

Second, this research provides evidence that although gut-residing fungi constitute a minor component of the gut microbiota, they may play a crucial role in maintaining organismal homeostasis and in the etiology and progression of diseases, including cancer (**H2**-**H5**). My analyses show that the gut mycobiota is not merely a passive component of the microbiome but may actively influence the host's immune response. Notably, the studies on the role of gut mycobiota in modulating the response to anti-PD-1 therapy in melanoma patients reveal its substantial scientific and clinical potential. I demonstrated that the presence of specific fungal species, such as *M. restricta* and *C. albicans*, correlates with poor outcomes of anti-PD-1 therapy (**H3**). Importantly, the pro-inflammatory roles of these species are also observed in other conditions, such as alcoholic liver disease (ALD) and hepatocellular carcinoma (HCC) (**H5**), suggesting their potential use as universal biomarkers of immune response.

Third, my research indicates a possible protective role of particular fungal species, such as *S. cerevisiae* and *D. hansenii*, which may support beneficial immune responses and act as microbiome modulators (**H3**-**H5**). These findings open the door to using mycobiota modulation as a therapeutic strategy to enhance the efficacy of cancer immunotherapy and other treatments related to immune regulation. The concept of microbiome modulation as a support for oncological therapies is gaining increasing importance in the context of precision medicine, and my research provides new data that may aid in identifying specific mycobiota-targeted interventions.

From a clinical perspective, the findings may contribute to optimizing cancer therapies through the implementation of new diagnostic and therapeutic strategies that take the composition of a patient's gut mycobiota into account. The identified correlations between specific fungal species and populations of circulating lymphocytes highlight a complex network of interactions between the gut mycobiota and the immune system (**H4**). This emphasizes the need for a holistic approach to microbiome analysis, including not only bacteria but also fungi and their impact on the effectiveness of immuno-oncological treatments and other immune-related disorders. The results of the literature review on the role of gut mycobiota in liver diseases (**H5**) suggest that its impact on the immune system extends beyond cancer and may also play a role in other chronic inflammatory conditions.

Moreover, my research aligns with the broader context of precision medicine, highlighting the need to integrate microbiological and clinical data to develop innovative diagnostic and therapeutic methods. The use of a multimodal approach, combining metagenomic, immunological, and clinical data, can provide deeper insights into the mechanisms of treatment response and support the development of more personalized therapeutic strategies, which is a key direction in the advancement of modern oncology and immunology.

DISCUSSION OF OTHER SCIENTIFIC AND RESEARCH ACHIEVEMENTS

My scientific interests have always been focused on the application of bioinformatics tools to analyze complex biological processes, and subsequent stages of my academic career have allowed me to further develop this path through increasingly interdisciplinary research projects. My work has combined theoretical and practical aspects – from the development of algorithms and analytical pipelines to their application in biomedical research – which enabled me to gain broad experience and contribute to the advancement of modern methods for analyzing biological data.

In the past, I worked on developing computational methods for the analysis of RNA molecule structures. During my master's studies at the Faculty of Biology at Adam Mickiewicz University in Poznan, under the supervision of Prof. Janusz Bujnicki, I created a tool called RNAmap2D, which enables visualization and analysis of contact and distance maps in nucleic acid molecules as well as their complexes with proteins and ligands. RNAmap2D not only generates contact maps but also allows the classification of base pairs into one of twelve base-pair families and the identification of stacking interactions, distinguishing among four stacking classes. RNAmap2D also makes it possible to compare contact maps between different RNA structures, e.g., native vs. mutated. The results of this work were published in paper **N1**.

Continuing my efforts to improve the understanding of RNA structure and function, at the Faculty of Computing of Poznan University of Technology under the supervision of Prof. Jacek Błażewicz, I participated in the development of a novel approach to evaluating RNA secondary structures. This method accounted for both canonical and non-canonical base pairs, classifying them accordingly. The approach involved predicting the 3D structure of RNA based on its sequence or canonical-only secondary structure and then deriving an extended secondary structure – including non-canonical base pairs – from atomic coordinates. The implemented method showed better accuracy in identifying non-canonical base pairs compared to existing approaches at the time. These results were published in paper **N3**.

My research on structural aspects of RNA molecules also included collaborations with scientists from CIC bioGUNE in Spain and the University of Luxembourg. Using *in silico* methods, I identified a sequence and structural signal in the 3′-UTR of mRNA that directs RNA molecules into extracellular vesicles (exosomes). My findings were experimentally validated, confirming the reliability of the bioinformatics approach. My presentation on this topic won the award for Best Oral Presentation at the 2014 Polish Bioinformatics Society conference. The results were also published (**N2**).

Developing tools and methods for understanding the structure of biological macromolecules significantly contributes to our knowledge of their biological functions. In particular, the methods I developed may be useful in identifying potential drug candidates. My research in this area has resulted in three publications in reputable scientific journals (**N1-N3**).

During my PhD studies, I pursued research related to the RNA World Hypothesis (**N4-N7**), which I continued after obtaining my doctorate at Poznan University of Technology (**N8**, **N9**). I conceptualized and developed a multi-agent system model that enabled simulations representing the behavior of model

RNA molecules (**N6**). The system I developed was the first algorithm allowing simulation of complex reaction-diffusion systems while incorporating physical properties of modeled molecules, such as their shape, size, and packing density observed in cells. Computational experiments led to the hypothesis that the separation of functions into a genetic information carrier (DNA) and a catalyst enabling efficient replication (polymerase proteins) was an essential evolutionary step in the emergence of more complex living systems. Furthermore, the developed methodology and algorithm can be applied to simulate other biological systems.

I also conducted a literature-based study on hypercycle theory in light of recent experimental findings, synthesizing the state of knowledge in the field (**N4**). The resulting article became part of the Topic Pages initiative in *PLOS Computational Biology* (**N7**), aimed at enriching Wikipedia's resources in computational biology while recognizing authors for their scientific contributions. Articles in this series are peer-reviewed and subsequently published in both the journal and an editable version on Wikipedia. Additionally, my article was featured in the "Did you know…" section of Wikipedia's homepage, significantly increasing its reach - over 12,000 people viewed it in a single day. Thanks to this initiative, my work on hypercycle theory, a key topic at the intersection of biology and mathematics, reached a wide audience and contributed to a reliable, accessible online knowledge base.

I also participated in research verifying the potential for constructing a computational "life probe" for identifying biological genetic sequences (**N8**). Proposed decision algorithms based on compression and Levenshtein distances were tested in the context of the RNA World Hypothesis. The studies showed that both metrics could be effectively used to build such life probes, while also differing in several characteristics. In particular, sequence fragments associated with replication demonstrated higher discriminatory power than sequences with other molecular functions.

Collaborating with physicists from the Nicolaus Copernicus University in Torun, I extended my research on the origin of life to modern *ab initio* methods based on quantum theory (**N9**). Car-Parrinello molecular dynamics simulations were used to evaluate the role of encapsulating simple inorganic compounds and ions within montmorillonite (MMT) nanocompartments. The research demonstrated that the presence of MMT significantly increased the likelihood of new molecule formation. Atom-atom radial distribution function analysis suggested that Ca^{2+} ions in MMT cavities might play a significant role in the initial stages of complex molecule formation.

My research on the RNA World Hypothesis has broadened our understanding of abiogenesis and life itself. This work resulted in six publications in reputable scientific journals (**N4-N9**) and a doctoral dissertation that was awarded the Best PhD Thesis in Bioinformatics in 2018 by the Polish Bioinformatics Society. Furthermore, I received the NCN Etiuda grant, which enabled me to complete a research internship at the University of Luxembourg. My work was also recognized by the Foundation for Polish Science, which awarded me the START scholarship in 2019, an award granted annually to one hundred of the most promising young scientists in Poland.

In collaboration with physicists, chemists, biologists, and medical doctors, I also participated in several interdisciplinary research projects, where I contributed my expertise in selecting analytical methods, designing bioinformatics pipelines, and interpreting results of the analysis. For example, in cooperation with a group from the Poznan University of Medical Sciences, we analyzed two miR-125a polymorphism variants in the context of breast cancer. Our study showed that the investigated variants can promote different RNA folding patterns, leading to alternative protein binding and reduced expression of miR-125a in breast cancer (**N11**). In another internal collaboration, I participated in a project investigating the genetic aspects of basal cell carcinoma. Analyses performed using a dedicated computational pipeline revealed numerous alterations in non-coding gene regions, which may contribute to diagnostics and the development of new cancer therapies (**N12**). Additionally, I developed an

innovative bioinformatics protocol and computational pipelines enabling the analysis of 3′UTR ends based on high-throughput sequencing data. This made it possible to study the significance of non-templated 3′ ends of L1 retrotransposons and the interaction between 3′ and 5′ L1 ends (N13). A publication based on these analyses was nominated in 2025 for the ICHB PAS Director's Award for the best experimental article published in 2024. I was also the originator and supervisor of efforts aimed at systematizing knowledge in the field of RNA structure prediction tools based on machine learning, particularly focusing on recent advances in deep learning (DL). As a result of these efforts, a review article was developed under my supervision (**N14**).

I also participated in several projects: "Regional COVID-Hub", "ECBiG – European Centre for Bioinformatics and Genomics – MOSAIC", and the "Genomic Map of Poland" (GMP) within the "ECBiG – European Centre for Bioinformatics and Genomics" initiative. In the MOSAIC project, I was responsible for developing methods for the integration and analysis of multidimensional biomedical data, as well as testing and validating the created solutions. In the "Regional COVID-Hub" project, I worked on implementing bioinformatics tools for processing and analyzing epidemic data and on the implementation of data visualization methods. In the GMP project, I was part of the team developing bioinformatics tools for large-scale genome analysis, with my work focusing in particular on retrotransposons.

In the context of external collaborations, I served as project leader in two research and development projects funded by BRIdge Alfa, concerning the use of artificial intelligence in the diagnosis of eye diseases. The developed algorithms enable disease identification based on image analysis, supporting the diagnostic process. These studies contribute to the advancement of applying cutting-edge technologies, such as artificial intelligence, in medicine, improving diagnostic accuracy and supporting healthcare.

PRESENTATION OF SIGNIFICANT SCIENTIFIC OR ARTISTIC ACTIVITY CARRIED OUT AT MORE THAN ONE UNIVERSITY, SCIENTIFIC OR CULTURAL INSTITUTION, ESPECIALLY AT FOREIGN INSTITUTIONS

I have conducted my scientific work at three institutions:

A. Before obtaining my doctoral degree

- Adam Mickiewicz University in Poznań (2008–2010): **N1** – research carried out during my MSc studies at the Faculty of Biology.
- Poznan University of Technology (2012–2018): **N2-N7** – research conducted during my PhD studies at the Faculty of Computing.
- Institute of Bioorganic Chemistry, Polish Academy of Sciences (2016–2020): **N5-N7** – work as a research assistant in the Department of Structural Bioinformatics.

B. After obtaining my doctoral degree

- Poznan University of Technology (2018): **N8–N11** – work as a senior research and technical specialist at the Institute of Computing Science.
- Institute of Bioorganic Chemistry, Polish Academy of Sciences (2020–present): **H1–H5**, **N12–N14** – work as an assistant professor in the Laboratory of Bioinformatics.

Throughout my scientific career, I have also participated in national and international collaborations.

Before obtaining my PhD:

- I collaborated with Prof. Antonio del Sol (University of Luxembourg) and Prof. Juan Manuel Falcon-Perez (Center for Cooperative Research in Biosciences, CIC bioGUNE, Spain). This collaboration focused on investigating mechanisms of mRNA sorting into extracellular vesicles (EVs) in hepatic cell models. My contribution involved the use of bioinformatic tools to identify a novel 12-nucleotide-long sequence significantly enriched in mRNAs accumulating in EVs. Experiments conducted by our Spanish partner using luciferase reporters containing this 3′-UTR motif confirmed that the sequence facilitates mRNA packaging into EVs in a hepatic cell model. Our work contributed significantly to understanding mRNA sorting mechanisms in intercellular communication and resulted in one scientific publication (**N2**).

After obtaining my PhD:

- Since 2024, I have been collaborating with Dr. hab. Bartłomiej Budny (Poznań University of Medical Sciences) and the Greater Poland Center of Pulmonology and Thoracic Surgery. I am responsible for the bioinformatic analysis of sequencing data from lung cancer patients and for identifying novel genetic risk factors and potential therapeutic targets. This research is currently ongoing.
- Since 2022, I have maintained ongoing collaboration with Prof. Andrzej Mackiewicz and Prof. Mariusz Kaczmarek (Department of Cancer Immunology, Poznań University of Medical Sciences and Greater Poland Cancer Centre), as well as Prof. Jacek Mackiewicz (Institute of Oncology, PUMS). This collaboration resulted in two publications (**H3** and **H4**), in which I analyzed the gut mycobiome of advanced-stage melanoma patients and its association with immunotherapy response.
- Since 2020, I have been collaborating with Prof. Marcin Schmidt (Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences). This work has resulted in four publications (**H1-H4**) addressing various aspects of gut microbiota research, including standardization of profiling procedures, analysis of the gut mycobiome in the Polish population, its relation to diet and lifestyle, and differences between healthy individuals and melanoma patients.
- I collaborated with Prof. Wiesław Nowak (Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University in Torun). The research focused on the catalytic properties of minerals such as montmorillonite (MMT) under prebiotic conditions. My role involved the critical analysis of ab initio Car-Parrinello molecular dynamics simulations, focusing on the reactivity of gaseous components, the influence of local electric fields, and the presence of Ca^{2+} ions on molecular formation under nanoconfinement. This collaboration resulted in one publication (**N9**).
- I collaborated with Dr. Tomasz Lehman and Prof. Paweł Jagodziński (Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences). Our work focused on the rs12976445 polymorphism and its potential effect on miR-125a expression, a biomarker in various cancers. I analyzed clinical data from 175 breast cancer patients and 129 controls and interpreted *in silico* modeling results, which showed that the presence of uridine (U) in pri-miR-125a could decrease binding to NOVA1 and HNRNPK proteins. I also demonstrated that U/C variants may alter RNA folding and protein interactions, possibly leading to reduced miR-125a expression in breast cancer. This collaboration resulted in one publication (**N11**).

Scientific internships completed before obtaining the PhD:

After graduating with an MSc in Biology at AMU:

- From November 2016 to February 2017, I completed a three-month internship at the University of Luxembourg under the Etiuda scholarship. My supervisor was Prof. Pascal Bouvry. During this internship, I gained hands-on experience with high-performance distributed computing systems (HPC), a skill I continue to use in my daily work analyzing large-scale multi-omic datasets.
- In 2012, I was admitted to the PhD program at the Faculty of Computing, Poznan University of Technology, under the supervision of Prof. Jacek Błażewicz. I defended my doctoral thesis with distinction in 2018. The research conducted during my PhD resulted in several publications (**N4-N9**). During my doctoral studies, I was also involved in two research grants: the OPUS project "RNApolis – methods and algorithms for RNA structure modeling and analysis" (2016–2017) and the OPUS project "Computational models and methods in cell biology" (2012–2015), resulting in three publications (**N3**, **N9**, **N11**).

During my MSc studies in Biology at AMU:

- I spent the first semester of my third year on a Sokrates-Erasmus exchange program at the University of Milan, Italy. There, I interned in the Laboratory of Bioinformatics, Evolution and Comparative Genomics under the supervision of Prof. Giulio Pavesi. I worked on identifying transcription factor binding sites (TFBS) and applying supervised and unsupervised clustering methods to analyze gene regulatory regions for transcription prediction. This internship introduced me to computational biology and bioinformatics tools and helped me decide to pursue a scientific career in bioinformatics.

PRESENTATION OF TEACHING AND ORGANIZATIONAL ACHIEVEMENTS AS WELL AS ACHIEVEMENTS IN POPULARIZATION OF SCIENCE OR ART

TEACHING ACHIEVEMENTS

After obtaining the doctoral degree:

- I prepared and delivered lectures for the Medical University of Lublin in the course "Basics of Bioinformatics" for the specialization in Medical Laboratory Genetics (02/2025).
- To date, I have supervised two PhD students:
    - Mr. Michał Budnik, pursuing an implementation doctorate at the Faculty of Computing, Poznan University of Technology in cooperation with a private company (**N1**).
    - Mr. Maciej Michalczyk, conducting his PhD under the supervision of Prof. Anna Philips, as part of her OPUS grant aimed at investigating the relationship between different fractions of airborne particulate matter and the composition of microorganisms present in the air.
- I reviewed the master's thesis entitled "Analysis and Identification of Protein Domains with a High Degree of Sequence Diversity", Bioinformatics program, Poznan University of Technology / Adam Mickiewicz University, 2018.
- I supervised an Erasmus exchange intern.

During the doctoral studies:

- I supervised two master's theses:
    - Mr. Jarosław Synak, thesis topic: "*Methods for Efficient Simulation of Multi-Agent Systems Based on Cellular Automata*", Poznan University of Technology, Computer Science, 2018.
    - Mr. Amadeusz Juskowiak, thesis topic: "*Solving Quadratic Assignment Problem Using Algorithm Inspired by Physarum polycephalum*", Poznan University of Technology, Computer Science, 2016.

  I consider a particular teaching achievement that after defending his thesis, Mr. Jarosław Synak chose to continue his studies at the doctoral level.
- I supervised two bachelor's/engineering theses.

ORGANIZATIONAL ACHIEVEMENTS

- During my doctoral studies, I served as the Chairperson of the Doctoral Student Council of the Faculty of Computing and represented doctoral students in the Faculty Council of the Faculty of Computing.
- Organization of the historical picnic event "Breakfast with the General" held at the Palace in Turwia, property of ICHB PAN.

AWARDS, DISTINCTIONS, AND SCHOLARSHIPS

- Nomination for the ICHB PAN Director's Award for the best experimental publication published in 2024 (**N13**), awarded in 2025.
- Nomination for the ICHB PAN Director's Award for the best experimental publication published in 2023 (**H2**), awarded in 2024.
- START scholarship from the Foundation for Polish Science (FNP) for 100 outstanding young scientists, 2019.
- Distinction by the Polish Bioinformatics Society for the best doctoral thesis defended in 2018.
- Doctoral degree with distinction, 2018.
- Etiuda scholarship (NCN) for the project "Bioinformatics methods for modeling and verification of the RNA World hypothesis" (2016/20/T/ST6/00395), 2016.
- Best oral presentation at the Polish Bioinformatics Society (PTBI) Conference, 2014.
- Scholarship for the best doctoral students at Poznań University of Technology, 2013–2018.
- Scientific scholarship of the Faculty of Computing, Poznań University of Technology (doctoral studies), 2012–2018.
- Dean's award of the Faculty of Biology, Adam Mickiewicz University, for excellent academic results, 2008.
- Scientific scholarship of the Faculty of Biology, Adam Mickiewicz University (undergraduate and master's studies), 2006–2010.

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