## 1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

**Project title:** Investigation of the specific PEBP1 interactions upon involvement in different cell death programs.

### 1.1. Project goals

- To characterize the unknown complex of PEBP1-analog/15-lipoxygenase in *P. aeruginosa and its* regulatory mechanisms.
- To identify different partners of PEBP1 and study their interactions.
- To develop *in silico* new inhibitors which will stop ferroptotic cell death signal initiated by the PEBP1-analog/15-lipoxygenase complex of *P. aeruginosa* in the pathology and pneumonia and cystic fibrosis.

### 1.2. Outline

Ferroptosis is a newly found cell death program that is characterized by the accumulation of lipid peroxides. It is connected with different kinds of diseases and can be initiated by different species. We have shown that gram-negative bacteria *P. aeruginosa* induce ferroptosis using an enzyme called 15-lipoxygenase in the pathology of pneumonia and cystic fibrosis [1]. According to recent studies, *P. aeruginosa* is able to hijack the ferroptotic cell death program and enhance gut epithelial damage to facilitate infection, leading to multiple organ failure, sepsis, and death. The human form of 15-lipoxygenase creates a complex with PEBP1, which is not present in *P. aeruginosa* [1], to initiate the production of the ferroptotic cell death signal [2].

In the SONATA BIS research project, funded by the NCN, we aim to conduct comprehensive computational and experimental studies to gain deeper insights into PEBP1 functionality. PEBP1 is an important regulatory protein involved in various forms of regulated cell death processes, such as ferroptosis, necroptosis, and autophagy [3]. By comprehending the mechanisms underlying PEBP1's actions, we can discover PEBP1-analog for 15-lipoxygenase in *P. aeruginosa*. It will enable the investigation of mechanisms utilized by P. aeruginosa in the pathology of pneumonia and cystic fibrosis, ultimately leading to the design of new inhibitors - potential therapeutic agents.

The main goal of the present research project is to find PEBP1-analog for *P. aeruginosa* using computational and experimental approaches to obtain a reliable computational model of the complex for drug design studies. The promising outcome of the studies will be shared with the US collaborators for biochemical verification of our computational predictions.

### 1.3. Work plan

1) Literature screening to find PEBP1 partners.
2) Mastering bioinformatics skills, Python programing skills, and molecular dynamics.

3) Bioinformatics studies to find proteins from other species that will have similar functions, 3D structures or biding motifs as PEBP1.

4) Mastering mass spectroscopy measurements (internship in US collaborators) and performing experiments that will confirm/exclude the involvement of the results from task #3.

5) Based on knowledge from tasks #1-2 performing molecular dynamics simulations for PEBP1 and its partners (including LOX).

6) Based on task #4, finding potential PEBP1-anaglog for *P. aeruginosa*, and preparing computational model of the complex. Verifying the model.

7) Inhibitory studies of the complex to select the most promising compounds that will be tested in biochemical studies by the US collaborators.

### 1.4. Literature


1. H. Dar, Y. Tyurina, K. Mikulska-Ruminska, et al., *Pseudomonas aeruginosa utilizes host polyunsaturated phosphatidylethanolamines to trigger theft-ferroptosis in bronchial epithelium*. J. Clin. Invest. 128 (2018) 4639-4653.
2. S. Wenzel, Y. Tyurina, J. Zhao, C. Croix, G. Mao, V. Tyurin, T. Anthonymuthu, A. Kapralov, K. Mikulska-Ruminska, et al., *PEBP1 Wardens Ferroptosis by Enabling Lipoxygenase Generation of Lipid Death Signals,* CELL 171 (2017) 628-641.
3. A. M. Lamade et al., Inactivation of RIP3 kinase sensitizes to 15LOX/PEBP1-mediated ferroptotic death, Redox Biol. 50 (2022) 102232.


### 1.5. Required initial knowledge and skills of the PhD candidate

- Analytical thinking
- Eager to learn and focus on the goals
- Skills in programming, preferably in Python
- Skills in mass spectroscopy techniques
- Good understanding of physics, biology and chemistry
- Involvement in scientific work

### 1.6. Expected development of the PhD candidate's knowledge and skills

- Improving knowledge in biophysics, bioinformatics and computer science.
- Improving experimental skills in mass spectroscopy and lipidomics techniques.
- Improving knowledge of protein database services and other computational tools and methods.
- Improving programming skills in Python programming language.
- Improving English proficiency.
- Networking - making new connections with other scientist in Poland and US.