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

Project title:

Computational insights into the oxidized lipid-mediated modifications of membrane and proteins occurring during ferroptosis

1.1. Project (main) goals

- To identify proteins most susceptible to modifications by oxidized lipids (lipid-derived reactive oxygen species, lipid ROS) and to develop a computational pipeline for assessing the impact of lipid ROS on protein dynamics and function.
- To develop advanced computational models to simulate oxidized lipids in the membrane system, providing insights into the molecular basis of oxidized lipids on membrane properties and behavior.

1.2. Outline

The dissertation is to address the effect of oxidized lipids on proteins and membrane functionality. Oxidized lipids are generated via an enzymatic reaction that is triggered by a protein complex of 15-lipoxygenase and PE-binding protein 1, and is a part of ferroptosis, a regulated cell death mechanism in which the accumulation of oxidized lipids appears. Those lipids can undergo further modifications into secondary products. Both products can interact with proteins and form so-called protein adducts by covalently bonding their structures to electrophilic residues. Additionally, their interaction with the membrane changes membrane integrity, leading to membrane disruption. The doctoral thesis aims to identify proteins that are more susceptible than others to the effects of oxidized lipids by preparing an innovative computer approach allowing for their identification. It will involve screening biological databases with protein structures and the development of an analysis determining the possible protein-adduct formation sites, along with advanced computational analysis of their impact. It will require several computational approaches, such as molecular docking, elastic network models (ENMs), and programming skills. An additional aspect of the PhD is checking the impact of various oxidized lipids and their secondary product on membrane dynamics and integrity. Selected types of oxidized lipids and their secondary products will be used to study this aspect using all-atom molecular dynamics simulations. The project will be conducted in collaboration with an experimental group from the University of Pittsburgh, USA.

1.3. Work plan

A. Mastering the use of Advanced Search in biological databases for protein spatial structures using Python.
B. Mastering various computational tools available in the ProDy API (molecular docking, ENMs, ESSA, and more).
C. Developing an automated process for preparing proteins and selected oxidized lipids for molecular docking.
D. Analyzing proteins in biological databases and establishing criteria for selecting the most susceptible proteins for adduct modifications.
E. Conducting molecular dynamics simulations with oxidized lipids and membranes.

1.4. **Literature** *(max. 7 listed, as a suggestion for a PhD candidate preliminary study)*

- A Amoscato, et. al, Formation of protein adducts with Hydroperoxy-PE electrophilic cleavage products during ferroptosis, Redox Biol, 63 (2023) 102758.
- L Milkovic, et al. **,** The 4-hydroxynonenal–protein adducts and their biological relevance: are some proteins preferred targets? Antioxidants,12 (2023) 856.
- A Altomare, et al., Lipid peroxidation derived reactive carbonyl species in free and conjugated forms as an index of lipid peroxidation: limits and perspectives, Redox biology 42 (2021) 101899.
- N Moldogazieva, et al., Lipid peroxidation: Reactive carbonyl species, protein/DNA adducts, and signaling switches in oxidative stress and cancer, Biochemical and Biophysical Research Communications 687 (2023) 149167.
- Y Shabanpour, et al., Protein-free domains in native and ferroptosis-driven oxidized cell membranes: a molecular dynamics study of biophysical properties and doxorubicin uptake, Frontiers in Molecular Biosciences (2024) 11 1494257.
- S Zhang, et al., ProDy 2.0: Increased Scale and Scope after 10 Years of Protein Dynamics Modelling with Python, Bioinformatics 37 (2021) 3657-3659.

1.5. Required initial knowledge and skills of the PhD candidate

- Experience in computational techniques such as molecular dynamics simulations, molecular docking, elastic network models, or related methods
- Basic programming skills, with preference for proficiency in Python
- Good command of spoken and written English
- Strong motivation for research and ability to work effectively in a team
- Experience with virtual screening, molecular visualization tools (e.g., VMD or PyMol), or the ProDy package is considered an advantage

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

- Proficiency in computational biophysics methods, such as molecular docking and all-atom molecular dynamics simulations (including protein–ligand and membrane systems)
- Development of advanced programming skills, particularly in Python
- Practical experience with computational tools, including the ProDy package
- In-depth knowledge of biological databases (e.g., PDB, UniProt)
- Training in elastic network models
- Skills in parametrization of non-standard ligands for molecular dynamics simulations
- Ability to design, conduct, and analyze computational experiments
- Competence in graphical visualization of biomolecular systems using advanced software (e.g., VMD, PyMol)
- Development of scientific communication skills, including scientific writing, proper use of domain-specific terminology, and preparation of publications
- Presentation of research results at scientific conferences