Abstract in English

Ferroptosis emerged in 2012 as a form of programmed cell death, different from known cell death pathways. It is triggered by iron-mediated lipid oxidation. Cells undergoing ferroptosis demonstrate significant mitochondrial alterations, including cellular shrinkage, increased membrane density, and reduced mitochondrial complexity.

The process is intricately controlled by iron metabolism dynamics and thiol regulation, creating a complex biochemical environment that drives extensive membrane lipid peroxidation. Ferroptosis has been associated with the pathogenesis of chronic degenerative diseases and injuries of the brain, and other organs.

This dissertation explores the molecular mechanism of ferroptosis regulation triggered by the protein-protein complex, 15-lipoxygenase-1 (15LOX-1), and phosphatidylethanolamine (PE)-binding protein 1 (PEBP1) in the presence of the substrate stearoyl/arachidonoyl-PE (SAPE) and a biological membrane model. The findings are detailed across three articles A, B, C.

In *Article A*, we employed computational structural modeling techniques, such as molecular docking, to study the complex formation between 15LOX-1 and PEBP1. We conducted molecular dynamics (MD) simulations to investigate conformational changes within the 15LOX-1/PEBP1 complex induced by a membrane. The study reveals that the association of the 15LOX-1/PEBP1 complex with cellular membrane facilitates access to the catalytic site for specific substrates, particularly SAPE. This substrate binding stabilizes the complex and enhances its catalytic activity. Additionally, the performed mutagenesis studies, both computational and experimental, observed the 15LOX-1/PEBP1 complex dissociation upon P112E mutation in PEBP1.

In *Article B*, we described the development of a new tool WatFinder – a part of ProDy framework to identify and visualize protein-water contacts. The tool detects and visualizes critical protein-water interactions, including water bridges and clusters. WatFinder processes ensembles of biomolecular structures, and snapshots from MD trajectories and provides statistical insights into the duration and frequency of water interactions. Accurate evaluation of protein-water interactions is vital for understanding biophysical processes. In *Article C* we investigate oxygen channels localized in 15LOX-1. It was shown that the membrane and substrate SAPE binding enhances oxygen access to the 15LOX-1/PEBP1 catalytic site. Conserved residues in the LOX

family indicate an evolutionary adaptation for oxygen transport through two tunnels, identified through MD, that connect surface regions to the catalytic center, aiding oxygen transport. Using the WatFinder tool, water and oxygen clusters within the 15LOX-1/PEBP1 complex were analyzed. We postulate that water clusters may facilitate oxygen transport. An analysis of 140 PDB structures from the lipoxygenase family highlights the importance of water clusters, which likely stabilize O_2 transport. Oxygen cluster analysis revealed two oxygen binding sites in the active site of 15LOX-1: one that supports single oxidation and another that may facilitate double oxidation. This study also incorporates lipidomics experiments. The results from computational biophysics approaches at the molecular/all-atoms level.

In summary, the results from structural modeling and computational biophysics approaches applied in this dissertation, and complemented by experimental studies conducted by US collaborators, reveal potential molecular mechanisms of ferroptosis regulation linked to the 15LOX-1/PEBP1 complex. These mechanisms include, among others, the structural details of the complex formation, the influence of the membrane environment, substrate acquisition processes, and the effect of substrate binding on O_2 association.