Among the most important biochemical pathways for the acquisition of energy by living organisms are cellular respiration and photosynthesis. One of the enzymes involved in these processes are membrane proteins belonging to the cytochrome bc family, which use the energy stored in electron transporters (electron donors and acceptors) to actively transport protons across the cell membrane. In this way, cytochromes bc contribute to building the proton motive force available for various life-sustaining biochemical processes. A particularly intriguing reaction associated with the activity of these enzymes is the so-called quinone-dependent electron bifurcation. It involves splitting electron transport pathways from the donor, which are quinones, into two separate branches of acceptors (cofactors) that are integral components of these enzymes. This process is extremely efficient while minimizing the risk of side reactions that can reduce the efficiency of energy conversion and can be a source of harmful reactive molecules that contribute to cell degradation. The bifurcation reaction itself in a broader context is of great importance in regulating bioenergetic processes and maintaining the redox balance of cells, and mutations impairing this process are the cause of severe genetic metabolic diseases. Moreover, targeted inhibitors of the site where this reaction occurs are of great importance in combating fungal infections or parasites such as malaria spores. By the fact that the bifurcation reaction is the central point of quinone-based cellular energy conversion, understanding it is one of the greatest challenges of modern bioenergetics.

Despite many previous studies on bifurcation, its molecular and quantum mechanism has not been understood. This has resulted in the consolidation of two mutually exclusive reaction models, each of which faces its own interpretation problems in the context of existing experimental data. In addition, a number of results appear to be partially incompatible with each other. This causes interpretive problems and requires the use of certain assumptions that may not necessarily be valid. Current breakthroughs in research capabilities at the molecular and quantum levels are opening up new ways for understanding the phenomenon of this remarkable reaction of great importance in the world of living organisms.

Several of our recent works have led to the discovery of new transition states (so-called metastable states) associated with the bifurcation process in cytochrome bc_1, and new available techniques such as quantum computing and cryo-electron microscopy (cryo-EM) methods allow us to conduct in-depth studies on the molecular and quantum mechanism of catalysis of these proteins. In this project we plan to conduct studies using these two techniques in combination with low-temperature electron paramagnetic resonance (EPR) spectroscopic measurements and optical and electrochemical methods on a bacterial model of cytochrome bc_1 derived from *Rhodobacter capsulatus*.

The project plans studies related to the kinetics of transition state formation and disappearance using a series of mutations that alter electron affinity of cofactors and modify postulated proton transfer pathways. The interactions of transition forms, generated by natural and synthetic semiquinones, with the environment of amino acid residues will be investigated, and the effect of the movement of the domain of one of the subunits of this protein on the internal proton environment affecting the equilibrium of transition states will be studied. A spectroscopic approach using pulsed EPR and temperature dependence of the equilibrium change between reaction steps will be used. Structural data obtained from cryo-EM will be used to construct appropriate models on which quantum calculations will be carried out to determine probable reaction paths depending on changing structural and functional conditions. The results of the quantum calculations will be compared with data obtained from spectroscopic measurements in order to verify the correctness of the calculations in terms of their agreement with experiment. An additional task will be to analyze the effect of changing the spin states of cofactors (b hemes) on electron transfer processes in light-activated systems, in kinetic measurements on isolated proteins, and on the establishment of thermal equilibrium in stationary states at different values of the external redox potential.

In summary, the goal of the proposed research is to understand the universal molecular mechanism involved in the catalysis of quinones by cytochromes bc taking into account quantum effects. We expect that this will contribute to the understanding of the efficiency of catalysis which is important in the context of research on other bioenergetic enzymes that act on quinone-based electron transporters. In the somewhat longer term, understanding the mechanisms of this reaction will cause the development of the next stage of research, in which it will be possible to construct new synthetic enzymes catalyzing new redox reactions based on solutions existing in nature.