

1. Objective

The overall goal of this proposal is to understand at molecular level how cytochrome  $bc_1$  (mitochondrial complex III), one of common bioenergetic enzymes, contributes to regulation of energy conversion and generation of free radicals. This enzyme is likely to be an important point at which the regulation takes place because it is the only point of connection between the two reservoirs of diffusible electron carriers that connect various complexes to a functional chain within which energy conversion takes place. In addition, the regulatory function of the enzyme may be associated with the fact its cycle, quite uniquely, is based on operation of two catalytic sites working in opposite direction, thus the enzyme may work according to a negative feedback mechanism.

2. Basic research to be carried out

To identify molecular elements of regulation we will undertake in-depth analysis of intermediate states of reactions taking place at the catalytic sites and their influence on operation of the enzyme. This will involve spectroscopic analyses and computer modeling. Our subject of study will be cytochrome  $bc_1$  from purple photosynthetic bacterium *Rhodobacter capsulatus*, in the form of native enzyme and site-directed mutants, as well as complex III isolated from mitochondria. A comparison of bacterial and mitochondrial enzyme will be undertaken to establish which of the identified molecular elements of regulation are universal for both eukaryotic and bacterial systems and which are specific to one of them, and whether mitochondrial enzyme acquired elements of adaptation to the presence of molecular oxygen.

From a broader perspective, the project addresses fundamental questions related to redox reactions, molecular mechanism of operation of catalytic sites, formation/stabilization of radical intermediate states, physical nature of interaction between metal centers in proteins, dynamics of protein-protein interactions, possibilities to design catalytic centers with modified physico-chemical properties. This extends beyond description of cytochrome  $bc_1$  mechanism but is relevant to other natural and de-novo designed proteins, particularly those harboring active metal centers.

3. Reasons for choosing the research topic

Living organisms use energy conserving systems to power cellular metabolism. This involves electron transfer taking place within the electron transfer chains consisting of large membranous protein complexes connected functionally by small diffusible electron carriers. The project addresses an issue of how the operation of these systems is regulated. Regulation is important as it enables the systems to respond to changes in energetic demand and/or conditions of stress (for example sudden shortage of oxygen in hypoxia). It is believed that regulation is associated with changes in the rate of electron flux through the chains and also with changes in the amounts of released free radicals, however the mechanisms of regulation remain unknown. Several significant discoveries made over the last few years and the preliminary data provided framework with which attractive new concepts for regulatory function of the enzyme can now be formulated and explored. Answering the questions addressed in this project is likely to provide textbook knowledge contributing to our general understanding of redox homeostasis in living cells.