Extremophilic archaea bioenergetics: respiratory oxidoreductases structure and function in *Sulfolobus acidocaldarius*

Conversion of matter and energy is one of the fundamental processes that characterize living organisms, required to maintain their homeostasis and to copy and spread the carried genetic information. In cells, these processes have to be efficient and safe. The way in which this is achieved is universal among the tree of life. The energy is stored in keeping the concentrations of ATP, ADP and P_i far from their equilibrium concentrations, and ATP serves as an energy carrier, used to perform work. Several ways of ATP synthesis exist, one of the most important of them is associated with operation of respiratory chains. Respiratory chains are sets of membrane-embedded redox enzymes, which convert the energy stored in various substrates to generate proton motive force by coupling the flux of electrons through their cofactors to the transport of protons across the biological membrane. The produced proton motive force is used for ATP production by ATP-synthase.

Today, our knowledge on the organisation of respiratory chains in bacteria and mitochondria of eukaryotic organisms can be considered as substantial. Advances in spectroscopy and development of novel tools, such as cryogenic electron microscopy, allowed us to gain insight to the function of respiratory enzymes on sub-atomic and molecular level and to their supramolecular organisation into larger assemblies, known as supercomplexes. However, still little is known about archaeal counterparts of respiratory enzymes. In some cases of extremophilic Archaea, the composition of respiratory chains is entirely different from the known systems and so the way of how they operate is a mystery.

Sulfolobaceae are members of Archaea domain known to populate extremely hostile environments, such as hot solfataric lakes in areas of volcanic activity. They were shown to possess many unique enzymes responsible for energy conversion, which exhibit both divergences and homologies to their bacterial and mitochondrial counterparts. **The purpose of this research project is to unravel the molecular structure and function of respiratory supercomplexes in *Sulfolobus acidocaldarius***, which was recently proposed as a novel model organism. Project is going to focus on the unusual succinate dehydrogenase and the terminal cytochrome-oxidase supercomplexes that occur in *Sulfolobus*.

To achieve the goal stated above, modern spectroscopic and biochemical techniques will be employed, such as continuous wave and pulse electron paramagnetic spectroscopy, freeze-quenching, redox poising, time-resolved diode-array spectrophotometry, polarographic measurements of respiration combined with fiber-optics spectroscopy and native protein electrophoresis. Combination of electron paramagnetic spectroscopy techniques with freeze-quenching and redox poising allows to detect and describe redox properties of paramagnetic cofactors and reaction intermediates present in protein samples. Kinetic measurements with use of diode-array spectrophotometry, polarographic oximetry at various experimental conditions will be applied to predict the electron transfer pathway and sequence. Native electrophoresis methods, combined with denatured-state electrophoresis and spectroscopic methods will be used to detect, identify and describe higher-order molecular assemblies of large protein complexes and supercomplexes. Cryogenic electron microscopy will be employed to determine the molecular structure of two of the studied enzymatic complexes.

The research project is expected to elucidate the molecular structure of the cofactor chain and the electron transfer path within the archaeal succinate dehydrogenase, the enzyme which couples the Krebs cycle and the respiratory chain. Moreover, it will provide new insight into molecular assembly and mechanism of action of large respiratory cytochrome-oxidase supercomplexes. In a broad context, the project will provide us with a new knowledge on *Sulfolobaceae* respiratory chain, and possibly the mechanistic details unseen in mesophilic systems, which can help us to better understand the evolutionary roots of respiratory systems, i.e. how they adapt to environmental conditions. From this perspective, *Sulfolobaceae* can also pose as an interesting subject for astrobiologists, as the conditions in which they thrive can be possibly found in the middle parts of the Venus' atmosphere or in presumably still active volcanic areas of Mars.