Extremophilic archaea bioenergetics: structure and function studies of the newly discovered homologues of respiratory complex I

The Archaea are unicellular prokaryotes recognised as a third domain of life almost 50 years ago. They are often recognised as extremophiles, capable of thriving in some of the most inhospitable places of the Earth. However, thanks to the growing interest and numerous recent studies, we know that they are ubiquitous in a range of environments including soil, aquatic sediments or even the respiratory and gastrointestinal tracts of animals. Still, very little is known about archaeal bioenergetics, a group of processes that are responsible for the production of useful energy that keeps cells alive. We lack basic knowledge about the enzymes involved in these processes, both in terms of their structure and function. Archaea is a diverse group and many species exhibit a high metabolic versatility, with unknown impact on the ecosystem. In these terms, they are a dark matter of the microbiology. Therefore, it is crucial to understand their respiratory processes and provide a contemporary description of each enzyme involved.

In many aerobic organisms and in mitochondria, the complex I (NADH:ubiquinone oxidoreductase) is a key respiratory enzyme that couples the NADH oxidation to quinone reduction. This activity is joined with a proton transfer across the membrane, creating the proton gradient which then contributes to the generation of the so-called proton-motive force. The proton-motive force is then spent on ATP production, which is an important compound used as a molecular currency to perform work at the molecular level. Based on previous studies, Archaea were believed to be devoid of complex I homologue. Nevertheless, later genomic analyses confirmed that some of the archaeons possess a ferredoxin:ubiquinone oxidoreductase (NDH) complex, which is similar to the one found in chloroplasts and cyanobacteria. However, recently we have discovered that a thermoacidophilic archaeon, *Sulfolobus acidocaldarius*, comprises an even more unusual homologue of respiratory complex I. This novel type of NDH comprises two additional parts, a putative 2-oxoacid:ferredoxin oxidoreductase (OFOR) and an indole-3-glycerol phosphate synthase.

Herein, we would like to further study the archaeal NDH complexes. We suspect that this unusual NDH architecture can occur in other archaeal lineages. Therefore, we will isolate the NDH from another, evolutionary distant species, *Haloferax volcanii*, and compare its subunit composition to the NDH from *Suflolobus*. Moreover, we will conduct structural and functional studies on enzymes isolated from both species, probing the interaction with ferredoxins, small proteins which serve as carriers for the electrons in the cells. We propose that the novel type of NDH couples the Krebs cycle to the respiratory chain via 2-oxoacids. To test and confirm our hypotheses, we will use contemporary methods of biochemistry, electrochemistry, electron paramagnetic resonance spectroscopy and electron cryomicroscopy (cryo-EM). Within the project, we would like to implement and improve unusual and novel methods, such as preparative native electrophoresis or cryo-EM of redox poised samples.

We hope that the possible outcome of this project will mark significant progress in our understanding of the aerobic respiration of Archaea. Moreover, as both model organisms are extremophiles, the project can provide us with mechanistic details unseen in mesophilic systems and help us learn how organisms adapt to hostile environments. It will also enrich our knowledge of respiratory complex I with the novel, evolutionary background.