Biological Energy Transduction

Manuela M. Pereira

Project 1

Energy Transduction by Complex I from Respiratory Chains

Complex I from respiratory chains couples the NADH:quinone oxidoreduction to charge translocation across the membrane, which contributes to the build up of an electrochemical potential. The dissipation of this potential through the ATP synthase is used for the synthesis of ATP. Complex I deficiencies have been shown to be implicated in several pathologies, namely neurodegenarative diseases such as Parkinson and Dystonia disorders.

The present proposed project will address the functional role of the membrane subunits of complex I, namely the Na^+/H^+ antiporter modules, Nqo12, 13 and 14 by investigating their proton and sodium transports. Specifically we aim 1- at functionally and structurally characterizing the three different modules; 2- at investigating the operation of these modules individually or integrated in a consortium as the related Mrp antiporters seem to operate; 3-at identifying the architectural features (single amino acid residues or secondary structure motives) responsible for sodium and/or proton conduction; and 4- kinetically studying the ion transports. In particular, these modules expressed in *E.coli* will be reconstituted in liposomes or planar membranes in order to determine the kinetic parameters of Na^+ and H^+ translocations, the Na^+/H^+ stoichiometry and Na^+ binding constants. Addressing these questions will definitively contribute to the knowledge of complex I, a fundamental enzyme in bioenergetics and thus in all metabolism.

Project 2

Structural and functional investigation of type II NADH:quinone oxidoreductases

Type II NADH:quinone oxidoreductases (NDH-II) or alternative NADH dehydrogenases are membrane associated enzymes involved in respiratory chains. In opposite to the other respiratory complexes, which are transmembrane oligomeric enzymes having several prothetic groups, NDH-II are non-transmembrane monomeric enzymes with a molecular mass around 50 kDa and having FAD as the only prosthetic group. NDHs-II have been suggested to be used in gene therapy correcting NADH:quinone oxidoreductase activity in pathologies with malfunctioning complex I, such as neurodegenerative disorders. The project aims to investigate NDH-II catalytic mechanism and intermediates, and the interaction with the substrates. It is also a goal to recognize the structural elements/motives determinants for catalysis and substrate interaction. A multidisciplinary approach will be performed using a wide range of biochemical and biophysical techniques.

Metalloproteins and Bioenergetics Laboratory

Miguel S. Teixeira, Manuela M. Pereira

Project 3

Functional characterization of haem-copper oxygen reductases

Haem copper oxygen reductases are the widest spread enzymes involved in aerobic respiratory chains, in eukarya, bacteria and archaea. However, both the catalytic mechanism for oxygen reduction and its coupling to proton translocation remain to be fully understood.

With this project we expect to contribute to the elucidation of the electron-transfer, catalytic and proton translocation mechanisms in haem copper oxygen reductases, exploring the natural diversity of this family of enzymes. A multidisciplinary approach will be performed using a wide range of biochemical and biophysical techniques.