Focus of the PhD-Project (Prof. Dr. C. Roy D. Lancaster)

NOX5 – structure and function of a Ca^{2+}-dependent NADPH oxidase

The research group has a long-standing interest in the structural and functional characterization of dihaem cytochrome-*b*-containing membrane proteins [1-8]. Examples of human dihaem cytochrome-*b* proteins are the catalytic subunits of superoxide-generating NADPH oxidases. The NADPH Oxidases (NOX) are a family of enzyme complexes with each member having the transmembrane catalytic NOX or DUOX subunit. The NADPH oxidase (NOX) family has seven members, NOX 1–5 and the DUOX 1 and 2. They are oxidoreductases which produce superoxide by transmembrane electron flow from NADPH to molecular oxygen. The NADPH oxidase (NOX2) was originally identified as a key component of human innate host defence. In phagocytes, this enzyme complex is activated to produce superoxide and its derivatives which destroy micro-organisms Homologs of NOX2 are present in various non-phagocytic cells. Here, superoxide and its derivatives are implicated in the regulation of a variety of physiological functions, e.g. oxygen detection, blood pressure regulation, activation of lymphocytes and the fusion of sperm and egg cells. The focus of the PhD project is on NOX5. This member is genetically the most distinct from the other NOX enzymes. NOX5 has four calcium binding EF- hand domains located in an N-terminal domain. Calcium activates the enzyme by inducing a conformational change and subsequent interaction between the N-terminal domain and the C-terminal domain. Apparently, this causes transmembrane electron flow and superoxide production. To understand the mechanism of action of NOX5, it is crucial to obtain the three dimensional structure. To achieve this, large-scale heterologous production and purification of various NOX5 constructs will be carried out for further characterization and crystallization.

Methods:

Various molecular biology techniques; protein chemistry, construction and purification of recombinant proteins, enzymology, membrane protein crystallization, protein crystallography

Selected publications:

(for a complete list see: http://saarland.structural-biology.eu/publikationen)

[1] Lancaster, C.R.D., Kröger, A., Auer, M., Michel, H. (1999) Structure of fumarate reductase from *Wolinella succinogenes* at 2.2 Å resolution. **Nature 402**, 377-385.

[2] Gross, R., Pisa, R., Sänger, M., Lancaster, C.R.D., Simon, J. (2004) Characterization of the menaquinone reduction site in the diheme cytochrome *b* membrane anchor of *Wolinella succinogenes* NiFe-hydrogenase. **J. Biol. Chem. 279**, 274-281

[3] Haas, A.H., Lancaster, C.R.D. (2004) Calculated coupling of transmembrane electron and proton transfer in dihemic quinol:fumarate reductase. **Biophys. J. 87**, 4298-4315.

[4] Lancaster, C.R.D., Sauer, U.S., Gross, R., Haas, A.H., Graf, J., Schwalbe, H., Mäntele, W., Simon, J., Madej, M.G. (2005) Experimental support for the “E-pathway hypothesis” of coupled transmembrane and H+ transfer in dihemic quinol:fumarate reductase. **Proc. Natl. Acad. Sci. U. S. A. 102**, 18860- 18865.

[5] Mileni, M., MacMillan, F., Tziatzios, C., Zwicker, K., Haas, A., Mäntele, W., Simon, J., Lancaster,C.R.D. (2006) Heterologous production in *Wolinella succinogenes* and characterization of the quinol:fumarate reductases from *Helicobacter pylori* and *Campylobacter jejuni*. **Biochem. J. 395**, 191-201.

[6] Madej, M.G., Nasiri, H.R., Hilgendorff, N.S., Schwalbe, H., Lancaster, C.R.D. (2006) Evidence for transmembrane proton transfer in a dihaem-containing membrane protein complex. **EMBO J. 25**, 4963-4970.

[7] Madej, M.G., Müller, F.G., Ploch, J., Lancaster, C.R.D.(2009)
Limited reversibility of transmembrane proton transfer assisting transmembrane electron transfer in a dihaem-containing succinate:quinone oxidoreductase.
Biochim Biophys Acta - Bioenergetics 1787, 593-600

[8] Cenacchi, L., Busch, M., Schleidt, P.G., Müller, F.G., Stumpp, T.V.M., Mäntele, W., Trost, P., Lancaster, C.R.D. (2012) Heterologous production and characterisation of two distinct dihaem-containing membrane integral cytochrome b_{561} enzymes from *Arabidopsis thaliana* in *Pichia pastoris* and *Escherichia coli* cells. **Biochim. Biophys. Acta - Biomembranes**, accepted, published online: http://dx.doi.org/10.1016/j.bbamem.2011.10.030