



GÖTEBORGS UNIVERSITET

**PRODUCTION AND CHARACTERISATION OF
AQUAPORINS AND PROTON-TRANSLOCATING
TRANSHYDROGENASE**

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Akademisk avhandling för filosofie doktorsexamen i biokemi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentliggöras fredagen den 6 december, 2013, kl. 09:30 i hörsal: Ivan Ivarsson, Institutionen för Kemi och Molekylärbiologi, medicinaregatan 3, Göteborg.

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Dissertation Abstract

Water transport in eukaryotic cells is a highly regulated and fine-tuned process. Water channel protein known as aquaporins (AQPs), constitute the main cellular water transport system, preserving water homeostasis by maintaining specific selectivity-mechanisms. Dysfunctional AQPs induce a wide variety of diseases in humans thereby enhancing the clinical significance of structural and functional knowledge.

Macromolecular structural research requires large amounts of pure, stable protein to initiate crystallization trials. To achieve this, genetic engineering and overproduction systems such as the methylotrophic yeast *Pichia pastoris* (*P. pastoris*) are employed. The next step, crystallization, involves arrangement of the macromolecule in a repetitive fashion. Once crystals have been obtained, these are exposed by synchrotron radiation (X-rays), producing a diffraction pattern. This reciprocal representation of the arrangement of atoms in the unit cell is converted back to real space by a Fourier transform, which generates a atomic model of the protein. This thesis is based on a comparative study of the production levels of all human AQPs, an production and purification analysis of eukaryotic transhydrogenases, and a structural and functional investigation of the spinach AQP SoPIP2;1 with associated mutants.

The human AQPs produced in the study displayed a considerable variety in production yield. Although the production yield seemed to depend on multiple factors, a correlation could be drawn between the extent of protein inserted into the membrane and phylogenetic relationship, providing further insight into eukaryotic membrane protein production. Furthermore, zebrafish transhydrogenase was successfully produced in *P. pastoris*, but although the production yield was sufficient, further optimisation of purification conditions is required in order to obtain sample suitable for crystallization. Finally, crystal structures and water transport assays of SoPIP2;1 phosphomimicking mutants as well as of SoPIP2;1 in complex with mercury have given novel insights into the mechanism of plant AQP gating.

Keywords: aquaporins, transhydrogenase, membrane proteins, X-ray crystallography, structure, protein production, overproduction.

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