



GÖTEBORGS UNIVERSITET

AQUAPORINS

PRODUCTION OPTIMIZATION AND CHARACTERIZATION

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ABSTRACT

Aquaporins are water facilitating proteins embedded in the cellular membranes. Such channels have been identified in almost every living organism – including humans. They are vital molecules and their malfunction can lead to several severe disorders. An increased understanding of their structure, function and regulation is of utmost importance for developing current and future drugs.

The first problem to overcome is to acquire the proteins in sufficient amounts to enable characterization. To achieve this, proteins are often produced in a host organism. One of the most successful hosts for recombinant overproduction is the yeast *Pichia pastoris*. Using this yeast we could obtain exceptional yield of aquaporin 1, whereas some others were below the threshold needed for successful subsequent characterization. In this process, we have established methods allowing fast and accurate determination of the initial production yield. Furthermore, we optimized the yield for low producing targets, enabling studies of proteins previously out of reach, exemplified with human aquaporin 4.

Characterization has been performed on aquaporins obtained in sufficient quantities, and the functionality of aquaporin 1, 5 and 10 has been assessed. Furthermore, a glycosylation was found to stabilize the aquaporin 10 tetramer although only a minority of the monomers were modified. Moreover, we used protein crystallography to determine the three dimensional structure of a hAQP5 mutant, providing insight into regulation of the protein by trafficking.

Taken together, these results provide insight into factors directing high production of eukaryotic membrane proteins. The subsequent characterization, including functional and structural determination, reveals new knowledge about aquaporin activity and regulation.