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High-level production of human aquaporins in yeast

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Aquaporins are water facilitating proteins embedded in the cellular membranes. Such channels have been identified in almost every living organism – including humans. These proteins are vital molecules and their malfunction can lead to several severe disorders and diseases. Hence, an increased understanding of their structure, function and regulation is of the utmost importance for developing current and future drugs. Heading towards this goal, the first problem to overcome is to acquire the proteins in sufficient amounts to enable functional and structural characterization. Using a suitable host organism, large amounts of target molecules can possibly be produced, but for membrane proteins limitations are frequently encountered. In the work described here, we have produced the 13 human aquaporins (hAQPs) in one of the most successful hosts for recombinant overproduction of eukaryotic proteins; the yeast *Pichia pastoris*, in order to explore the underlying bottleneck to a successful membrane protein production experiment. Here we present exceptional yield of hAQP1, whereas some other hAQPs were below the threshold needed for scaled up production. In the overproduction process, we have established methods for efficient production screening as well as for accurate determination of the initial production yield. Furthermore, we have optimized the yield of low producing targets, enabling studies of proteins previously out of reach and evaluated the glycosylation of a specific target. Taken together, our results present insight into factors directing high production of eukaryotic membrane proteins together with suggestions on ways to optimize the recombinant production in the yeast *P. pastoris*.

Biography

Kristina Hedfalk completed his PhD in 2002 from Chalmers University of Technology, Sweden, and postdoctoral studies from University of Gothenburg, Sweden, in collaboration with Aston University, Birmingham, United Kingdom. . She has published 38 papers in reputed journals of which four have been cited more than 100 times.

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