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Human aquaporins:
Production, Characterization and Interactions

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Abstract

Membrane proteins are essential components of the cell and responsible for cell communication with the outside environment and transport across the membrane. Water transport is facilitated by aquaporins, which are water selective transmembrane pores that serve to maintain cell homeostasis. Aquaporins have been well characterized in terms of structure and function and a broad variety of cell based assays have given insight into their mechanism of regulation, including membrane localization and conformational changes. High-resolution structural information on aquaporins has emerged mainly using X-ray crystallography, which require large quantities of pure and homogenous protein.

This thesis presents the importance of codon optimization and clone selection in the first step of the pipeline to obtain high yields of recombinant membrane protein. This, in turn, enables biochemical characterization of the protein of interest. One such target, the human aquaporin 10, was found to be glycosylated in *P. pastoris*, increasing the protein stability *in vitro* but without any measurable impact on function.

Aquaporin function is regulated by both physiological signals and interactions with other proteins. The regulation of the plasma membrane abundance of hAQP5 shows that three independent mechanisms – phosphorylation at Ser156, protein kinase A activity and extracellular tonicity – work in synergy to fine-tune the fraction of membrane localized protein. Furthermore, an overview of the literature of AQP protein:protein interactions reveal that the C-terminus is the most diverse sequence between aquaporins and that the majority of the known interactions map there.

Obtaining high-resolution structural information of protein:protein complexes is one of the future challenges in structural biology. We developed a novel method for the characterization and purification of membrane protein complexes using hAQP0 and calmodulin as the proof-of-principle interaction partners. Our approach combined bimolecular-fluorescence complementation to characterize the interaction and fluorescence detection to detect the complex throughout purification. This resulted in a versatile method to purify intact protein complexes in enough yields for crystallization, potentially facilitating future structural determination by X-ray crystallography or electron microscopy.