Göteborg, November 2020

A Holistic View on Aquaporins:

Production, Structure, Function and Interactions

Akademisk avhandling

Som för avläggande av filosofie doktorsexamen i Naturvetenskap, Göteborgs universitet kommer att offentligen försvaras i Carl Kylberg, Medicinaregatan 7, den 20.11.2020, klockan 10:00

av Florian Schmitz

Fakultetsopponent:
Professor Per Amstrup Pedersen
University of Copenhagen, Copenhagen

Avhandlingen baseras på följande delarbeten

I. **Schmitz F**, Luthman K, Jarho E, Hedfalk K, Seifert T (2020) Efficient production of pure and catalytically active SIRT2 in *Pichia pastoris*. *Manuscript*

II. Zeng J, **Schmitz F**, Isaksson S, Arbab O, Andersson M, Sundell K, Eriksson L, Swaminathan K, Törnroth-Horsefield S, Hedfalk K (2020) Novel structural mechanism of extracellular gating of aquaporin from the fish climbing perch (*Anabas testudineus*). *Manuscript*

III. Wang H, Schoebel S, **Schmitz F**, Dong H, Hedfalk K (2020) Characterization of aquaporin-driven hydrogen peroxide transport. *Biochimica et Biophysica Acta* (BBA)-Biomembranes, 1862(2), 183065.

IV. Wang H, Schoebel S, **Schmitz F**, Dong H, Hedfalk K (2020) Quantitative analysis of H_2O_2 transport through purified membrane proteins. *MethodsX*, 7, 100816.

V. **Schmitz F***, Glas J*, Neutze R, Hedfalk K (2020) High-throughput screening combining bimolecular fluorescence with flow cytometry reveals constructive membrane protein complex formation. *Manuscript*

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INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

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Florian Schmitz

Institutionen för kemi och molekylarbiologi, Göteborgs universitet, Sverige, 2020.

Abstract

Aquaporins are specialised membrane proteins, which regulate the water homeostasis of cells. In eukaryotic organisms, this process is tightly regulated, and aberrations in aquaporin functionality lead to severe pathologies in humans. The aim of this thesis is to shed light on the aquaporin function and regulation, both as individual protein targets and in the cellular context, as well as exploring various applications for human aquaporin 4, specifically. A wide range of biochemical methods have been applied, ranging from the importance of robust protein production methods, for targets as well as for their complexes, to functional and structural characterization.

For biochemical characterization and structural analysis, large amounts of pure, homogeneous and stable recombinant protein are needed. The methylotrophic yeast *Pichia pastoris* was utilized for the overproduction of the soluble protein Sirtuin2, an indirect up-regulator of Aquaporin4 in humans. The highest yet-reported yield of the protein (40 mg/l) was achieved, facilitating modulation trials of the potential drug target. The *P. pastoris* overproduction system was also employed for the expression of human AQP4, facilitating new research applications, such as improved Neuromyelitis Optica diagnosis, and a better understanding of the intermolecular binding between the monomeric subunits.

In addition, the novel structural characteristics of AQP1 from the fish *Anabas testudineus* were studied in this thesis and key residues responsible for the molecular mechanisms for osmoregulation were identified by mutational analysis combined with functional studies. By combining stopped-flow assays and molecular dynamics simulations, a novel extracellular gating mechanism could be elucidated for this particular aquaporin isoform, being less efficient in water transport than AQP4 and phosphorylation of Tyrosine 107 leads to a closed conformation involving loop C.

Functional studies were also performed for the development of a new method for testing the transport specificity of aquaporins regarding hydrogen peroxide. The transport rate can be standardized in relation to protein quantity, resulting in a more accurate determination of transport rates as compared to cell growth assays.

Interactions between proteins are difficult to evaluate, but using bimolecular fluorescence complementation, membrane protein complexes could be quantified and *screened in* vivo in a high-throughput manner. During the course of this work, we standardized sample preparation and defined criteria which allow the discrimination between constructive and random interactions. Taken together, the results presented in this thesis lay the fundament for future screening for novel interaction partner using a cDNA library, a method that is not limited to aquaporins.

Keywords: aquaporin, membrane protein, protein interaction, functional studies

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