A functional assay for aquaporins

Water is essential for life. A good distribution of water is required between the cell and its surroundings and also between the subcellular compartments for the cell to survive. This water distribution is facilitated by water channel proteins called aquaporins, which are embedded in most biological membranes.

In this diploma, the main goal was to optimize a water transport assay based on the shrinkage of *Pichia pastoris* spheroplasts. Prior to the measurements, the cell wall was destabilized using β-mercapto-ethanol and the spheroplasts were subjected to an osmotic gradient, which resulted in the shrinkage of the spheroplasts. The change in light scattering due to the shrinkage was measured using stopped-flow spectroscopy. The density of the cells, the osmolality of the buffers and the preparation of the spheroplasts were analyzed, evaluated and optimized.

It was found that, to obtain a good curve quality, the density of the spheroplasts has to be high, the concentration of β-mercapto-ethanol has to be around 1 µl per ml initial cell culture and the osmotic gradient showed good results at 0.4 M.

This functional assay was then used for inhibitor studies of human aquaporin 1, which showed to be problematic. It was successfully used for functional studies on the regulation of the yeast aquaporin Aqy1.

In preparation for structural studies using X-Ray diffraction, a mutant of the yeast aquaporin Aqy1 was successfully purified. The structural data is expected to yield further information on the regulation and function of this water channel.

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Aim and background
Aquaporins are proteins embedded in the membrane and regulate the water flow across biological membranes. These membrane bound proteins are found in all living organisms, from bacteria to fungi and plants to mammals. A good distribution of water is required between the cell and its surroundings and also between the subcellular compartments for the cell to survive. It has been shown that the aquaporins are involved in diseases like high blood pressure, Sjögren’s syndrome, diabetes and cancer. Many studies have shown that there is a connection between high levels of human aquaporin 1 (hAQP1) and regeneration as well as distribution of certain cancer species. By finding specific inhibitors for hAQP1 could decrease the distribution of the cancer cells.

The purpose in this diploma work is to optimize a method to analyze how fast aquaporins transport water, using a cell-based assay in combination with stopped-flow spectroscopy.

The water transport assay of spheroplasts is based on the shrinkage of methylotrophic yeast cells, called *Pichia pastoris*. The water flow out of the spheroplast, which shrink when exposed to a hypertonic solution. Stopped-flow spectroscopy was used to trace the shrinkage over time.

Different parameters were to be optimized. These are as follows: the osmotic gradient and different steps in the sample preparation.

Additional information about the regulation and function of biomolecule, can be obtained by analyzing their three-dimensional structure. In order to do this, the protein was purified, as the first step to protein crystallization and additional functional studies.

Conclusions
To study the regulation of the yeast Aqy1 the mutant S107D of the protein was successfully purified. This protein is later on to be crystallized for structural studies and is expected to show an open conformation, as opposed to the wild type yeast aquaporin.

As a valuable tool for the functional characterization of aquaporins in general, a water transport assay based on *Pichia pastoris* spheroplasts was optimized. This assay was further used for inhibitor studies, where finding inhibitors can be considered the first step in the drug discovery process. The assay showed good curve quality after optimization, even though it is suggested to run samples in duplicates or triplicates to even out the variance of biological samples.

The assay was also used to determine the water transport of different yeast aquaporin mutants. The highest water conductivity was obtained for the mutant that represented an artificially phosphorylated form at Serine 40 (S40D), indicating that it might be involved in channel regulation.

Adapting the assay for inhibitor studies on human aquaporin 1 (hAQP1) showed that inhibition can be observed using the assay, but that using compounds dissolved in DMSO might be a problem due to the sensitivity of the yeast cells towards DMSO. To resolve this, different option might be explored in the future, like e.g. using a different solvent, lower concentrations of the inhibitors.
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