

# Electric-field-stimulated protein mechanics to study structural changes in yeast aquaporin AQY1

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Aquaporins are membrane channel proteins that maintain water homeostasis by regulating the transport of water and small molecules across biological membranes. Their activity is controlled at many different levels, with rapid changes in water transport activity achieved through gating, where the pore transitions between open and closed states. Gating is triggered by external stimuli such as ligand binding, in response to pressure, pH changes, or voltage. In addition, aquaporins must prevent proton transport via the Grotthuss mechanism, in which protons move rapidly through hydrogen-bonded water networks. This selectivity is due to the formation of a positive electrostatic potential in the conserved Asparagine-Proline-Alanine (NPA) motif located near the center of the pore <sup>[1]</sup>. This acts as the main barrier against proton transport.

In this study, we focus on the gated eukaryotic water channel AQY1, which is the sole and well-studied aquaporin of the yeast *Pichia pastoris*. The hypothesis that static electric fields within the pore prevent proton transport will be tested by applying strong electrical fields (as described in Hekstra et al. <sup>[2]</sup>) across single crystals of AQY1. The resulting structural response is probed using time-resolved X-ray diffraction, followed by a symmetry-based analysis, where we will compare internal difference maps of molecules exposed to opposite electric field directions. By following the rearrangements of the water molecules in response to the applied electric fields, we aim to directly observe conformational changes and isolate electric-field-induced structural differences.

This study will provide insights into the dynamic structural mechanisms underlying aquaporin gating and selectivity and will offer a deeper understanding of how aquaporins prevent the transport of protons across biological membranes.

<sup>[1]</sup> Fischer G, et al., “Crystal structure of a yeast aquaporin at 1.15 angstrom reveals a novel gating mechanism.” *PLoS Biol.* (2009)

<sup>[2]</sup> Hekstra DR, et al., “Electric-field-stimulated protein mechanics”. *Nature* (2016)