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# Plant aquaporin regulation:
## Structural and functional studies using diffraction and scattering techniques

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ABSTRACT

Water is the basis for life as we know it. It is only logical then that all organisms have evolved specialized proteins, aquaporins, that regulate water flow across their membranes. Plants, which are immobile, depend more on their environment and also use water flows to move, to breath, and to grow. This is reflected by the much more diverse set of aquaporins plants facilitate. These work in cohort to tightly control the water flow throughout the plant.

The aim of this thesis has been to deepen the understanding of a spinach leaf aquaporin, SoPIP2;1 and to develop new tools for structural studies of membrane proteins. We have studied how the SoPIP2;1 function is modulated by pH, calcium and mercury using X-ray crystallography and water transport assays in proteoliposomes. We elucidated the pH gating mechanism, discovered an additional binding site for calcium, found an unusual activating effect of mercury and hypothesized a novel mechanism by which this occurs.

We have also used X-ray scattering techniques for structural studies of SoPIP2;1 in solution, thereby circumventing the need for crystallisation. Using WAXS we studied the calcium-induced structural changes of SoPIP2;1 in detergent micelles. However, solvation in detergent micelles is a problem in many ways, both for the protein and for many research tools. To deal with this we explored the nanodisc system, which is a solubile discoidal bilayer in which membrane proteins can be reconstituted – thus creating a homogenous population of soluble membrane proteins without the need for detergent. We then used this tool to extract useful structural data from SoPIP2;1 using SAXS/SANS.