The eukaryotic cell cycle is controlled through series of checkpoints that ensure the fidelity of cell division. These checkpoints prevent progression to the next phase of the cycle if the previous phase has not been satisfactorily completed. Once appropriate conditions have been met, temporal controlled degradation of key cell division proteins ensures a correct onset of the different cell cycle phases and exit from the cell division program. In the light of the cell cycle, the Anaphase Promoting Complex/Cyclosome (APC/C) is an important mega Dalton size multi-subunit E3 cullin-RING ligase, marking targets for degradation by the 26S proteasome. Highly conserved among eukaryotes, the fully assembled APC/C of yeast and humans have been extensively characterized biochemically and structurally thereby providing a detailed description of subunit assembly as well as a molecular mechanism for ubiquitination by this macromolecular machine.

In this PhD, I demonstrate the cloning, expression and purification of various A. thaliana APC/C complexes as well as ERF115. Co-expressing of the different APC/C subunits from Arabidopsis thaliana was successful in insect cells using the MultiBac expression system. Using a combination of negative stain electron microscopy and in vitro ubiquitination assays, the A. thaliana APC/C was characterized in terms of structure and function. My results indicate that the sample composition looks promising but additional factors might be required to activate the APC/C. To gain insights into assembly of the APC/C, the platform subunit APC4 was separately expressed, purified and characterized. Native mass spectrometry and gel filtration experiments revealed that APC4 forms homodimers and a solution model was generated using SAXS. These results will provide insights in the QC-based stem cell recovery mechanism, thereby further elucidating the understanding behind longevity in plants.