

## **Absolute quantification of clock proteins reveals a quantitative engineering path for tuning the Arabidopsis circadian oscillator and output pathways**

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The circadian oscillator controls a myriad of process in plants. Modulating the clock could be an important synthetic biology approach for increasing crop performance. However, the complexity of the system requires advanced mathematical modelling. The current models present real time units and have realism regarding biochemical representation, though they lack absolute units on the mass scale. Therefore, we quantified protein numbers by generating Nano Luciferase reporter lines for key clock proteins, allowing determination of average absolute numbers/cell. Data integration and careful mathematical modelling suggests that the measurements are reasonable. Furthermore, the theory developed for assessing these quantifications provides a path for extending the modelling of output pathways for CCA1 and LUX using DNA sequence information and absolute protein numbers. This is achieved by exploiting the use of Protein Binding Microarrays and Surface Plasmon Resonance. The use of this type of approach can be extended to 1001 genomes data, with a first exploration using GI a key clock and flowering regulator. The approach presented here therefore provides a path for linking DNA sequence to clock dynamics. In principle, blending the new model with genome editing tools could potentially allow clock and output dynamics to be tuned quantitatively.