

Can *Arabidopsis thaliana* Rubisco incorporate distinct SSu isoforms?
Master degree project / Research training / Internship

We are looking for an ambitious student with an interest in molecular biology, synthetic biology, protein purification and carbon fixation for a project to answer a fundamental question about the biogenesis of nature's carbon fixing enzyme: Rubisco.

The CO₂-fixing enzyme ribulose-1,5-carboxylase/oxygenase (Rubisco) represents the major point of carbon entry into the biosphere. Rubisco has a slow catalytic rate and exhibits poor substrate specificity such that Rubisco catalysis often limits the growth rate of photosynthetic organisms. Rubiscos from higher plants are comprised of eight catalytic large- (LSu, *rbcL* gene) and eight auxiliary small- (SSu, *rbcS* gene) subunits, which form a L₈S₈ hexadecamer.

The nucleus of higher plants encodes an *rbcS* multigene family that may provide the opportunity for differential SSu expression in response to a range of intrinsic and extrinsic cues. All higher plants have one LSu-encoding gene located in the plastome, and a variable number (depending on species) of nuclear-encoded SSu isoforms.

It is not known whether different SSu isoforms can bind the same L₈ core. On the surface, this seems like a minor detail, but it represents an important and fundamental question underlying Rubisco assembly in higher plants. All crystal structures that have been published are pure with respect to their relevant SSu isoforms (see: Vålegård et al., 2018), but whether or not this represents what occurs in nature, or is simply an artifact of crystallisation remains unclear.

This project will address this question. Our preliminary results are exciting, and we need a motivated student to perform full characterisations and construct additional controls to strengthen the findings. In this project you will (i) generate a new control expression plasmid (ii) transform this plasmid into *E. coli*, (iii) perform affinity chromatography to purify Rubisco (from your new, as well as a large number of previously constructed expression plasmids), and (iv) characterise these using PAGE and immunoblot techniques.

If you find this project interesting, please contact Laura Gunn:

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