NANYANG TECHNOLOGICAL UNIVERSITY

School of Biological Sciences

Reg. No. 200604393R

**Research Theme: Photosynthesis – past and future**

**Research Project Title: Microalgal $CO_2$ superchargers**

**Principal Investigator/Supervisor: A/P. Oliver Mueller-Cajar**

**Co-supervisor/ Collaborator(s) (if any):**

## Project Description

### a) Background:

The vast majority of photosynthetic organisms utilize the slow and sluggish enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) to capture the greenhouse gas $CO_2$ and convert it into usable sugars. In most microalgae the short-comings of this enzyme has been compensated for by the evolution of additional protein machinery that collectively functions to actively transport $CO_2$ towards the active site of Rubisco, resulting in saturation of the enzyme with its substrate. A critical component of this system is the sequestration of Rubisco into a micron-sized membraneless compartment inside the chloroplast stroma, known as the **pyrenoid**. These compartments form by the process of liquid-liquid phase separation, a mechanism towards assembling compartments without the requirement for membranes. This concept is familiar from the formation of oil droplets in a salad vinaigrette. It is currently one of the most exciting areas of biology, and studying the behavior of the pyrenoid provides fertile experimental access to this field.
We use biochemistry to study how alga enhance their photosynthetic efficiency, and at the same time aim to provide information of general scientific importance to the biological community.

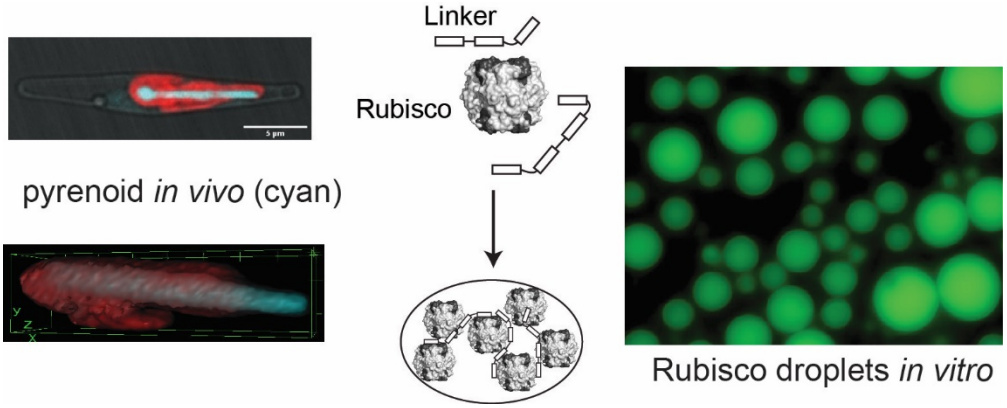
**References:**
**Wunder et al. (2018)** https://www.ncbi.nlm.nih.gov/pubmed/30498228
**Mueller-Cajar (2017)** https://www.ncbi.nlm.nih.gov/pubmed/28580359
**Dolgin (2018)** https://www.nature.com/articles/d41586-018-03070-2

**Rubisco droplet movie from Wunder 2018:**
https://static-content.springer.com/esm/art%3A10.1038%2Fs41467-018-07624-w/MediaObjects/41467_2018_7624_MOESM4_ESM.mov

### b) Proposed work:

We have achieved the reconstitution of pyrenoid-like droplets using pure proteins from both the model alga Chlamydomonas reinhardtii (Wunder 2018) and a marine alga, the diatom Phaeodactylum tricornutum (unpublished). Currently we are working with Rubisco and a Rubisco linker protein from these organisms. However, ~100 additional proteins (mostly of unknown function) are known to be found in the pyrenoid at low levels. Key questions to address now regard an increase of complexity, where we will add additional components that are part of the pyrenoid and study their biochemistry in the context of the droplets. Techniques range from biochemical assays (e.g. enzymatic and binding) to the incorporation of structural technologies (e.g. cryo-EM) if needed. The concept is somewhat like playing molecular legos,

we will continue adding pure components (and their mutants) to increase complexity and perform detailed experiments to study the behavior of the pyrenoid in vitro. We will also test the behavior of newly discovered proteins in vivo, by expressing fluorescently tagged protein in the alga, followed by confocal microscopy.




**Supervisor contact:**

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**SBS contact and how to apply:**

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Please apply at the following:

http://admissions.ntu.edu.sg/graduate/R-Programs/R-WhenYouApply/Pages/R-ApplyOnline.aspx