



VIRTUAL BIOLOGY COLLOQUIUM

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Hosted by A/P Lu Gan

CO₂ fixing droplets- biochemical studies into the Rubisco condensates that enhance microalgal photosynthesis

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About the Speaker

I obtained my PhD at the Australian National University in Canberra. In the group of Prof. Spencer Whitney I used the emerging technique of directed evolution to alter Rubisco's properties. This was followed by a postdoctoral fellowship at the Max-Planck Institute of Biochemistry in Martinsried, Germany. With Prof. Manajit Hayer-Hartl I provided the first mechanistic model of Rubisco activase function. In 2012 I joined NTU as a Nanyang Assistant Professor. My group's research focusses on mechanistically dissecting the molecular machinery that autotrophs have evolved to mitigate the problematic properties of the carboxylase Rubisco. In Singapore we have discovered and characterized convergently evolved Rubisco activases and carbonic anhydrases. A critical Rubisco enhancement strategy has emerged to involve the liquid-liquid phase separation of the enzyme into biomolecular condensates. The detailed study of these processes using biochemical reconstitution is taking up more and more of our time.

The slow kinetics and poor substrate specificity of the key photosynthetic CO₂-fixing enzyme Rubisco have prompted the repeated evolution of Rubisco containing compartments known as pyrenoids in algae and carboxysomes in prokaryotes. Inside these compartments actively transported bicarbonate is converted into CO₂ gas, which saturates the carboxylase with its substrate. The pyrenoid of the model green algae *Chlamydomonas reinhardtii* has recently been demonstrated to behave as a liquid non-membraneous compartment. Using pure components we have shown that algal Rubisco and the disordered tandem repeat protein EPYC1 are necessary and sufficient to phase separate by complex coacervation. The droplets exhibit mixing behavior similar to that reported in vivo. The phase-separated Rubisco was catalytically functional. Pyrenoids are ubiquitous and have evolved multiple times convergently. Diatoms are responsible for up to half of marine photosynthetic carbon fixation. Using chemical cross-linking and co-immunoprecipitation we have identified a largely disordered repeat protein (Pyrenoid factor 1-PF1) that localizes to the diatom pyrenoid in vivo. Full length PF1 and single repeats specifically bind diatom Rubisco. Unlike EPYC1, PF1 phase separates homotypically into condensates that recruit Rubisco. Our findings will inform the introduction of microalgal CO₂ pumps into plants.

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