Towards Structure Determination of the of the Photosynthetic Supercomplex to understand the Regulation of sustained Cyclic Electron Flow (CEF) in the photopsychrophile *Chlamydomonas* sp. UWO241

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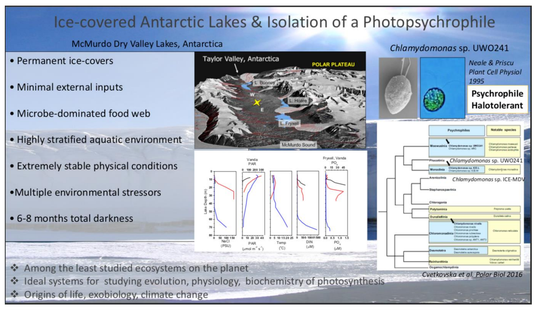
Overall research goals:

This abstracts reports on the structural aspects of the project, which aims to determine the structure of the supercomplex with Cryo-EM. The overall goal of this project is to describe the function of sustained CEF and assembly of the PSI supercomplex in the Antarctic psychrophile Chlamydomonas sp. UWO241 (UWO241) and the model Chlamydomonas reinhardtii acclimated to long-term salinity stress. Major objectives are: 1) determine the functional role of sustained CEF and impacts on downstream carbon metabolism in UWO241 acclimated to variable environmental stressors; 2) dissect the structure of the UWO241 PSI supercomplex through proteomic and structural studies; 3) determine whether C. reinhardtii utilizes “UWO-like” supercomplexes to support sustained CEF during long-term stress acclimation. Outcomes of this project will support research focused on meeting future energyand food needs by advancing our understanding of how e xtremophilic phototrophs use sustained CEF and rewired carbon metabolism to survive long-term exposure to environmental stressors, such as excessive light, low temperature, and high salinity. The work at ASU is focused on determining the high-resolution structures of the PSI supercomplex in the Antarctic Chlamydomonas sp. UWO24 by single-particle cryo electron microscopy (cryo-EM). We aim to gain detailed information on the interaction of the proteins and cofactors in the supercomplex at molecular resolution. The goal is to identify the key amino acids in the formation and stabilization of the supercomplex including details on the cofactor interactions that will reveal the electron transfer pathways that drive the cyclic electron transfer in the supercomplex.



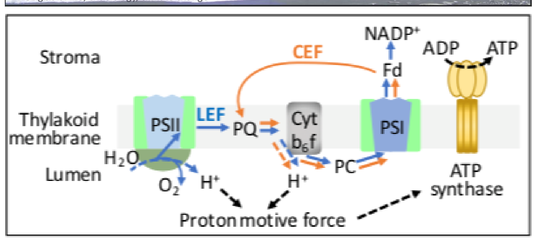
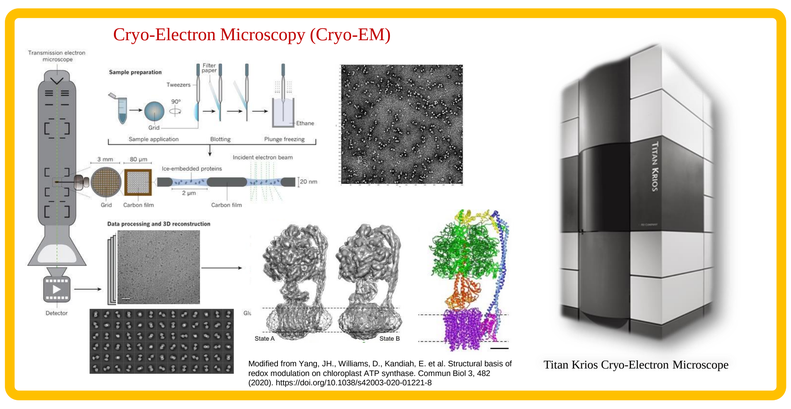


Figure 1. Linear and cyclic electron flow (LEF and CEF) both contribute to transthylakoid proton motive force.
Blue and orange solid lines denote electron transports in LEF and CEF, respectively. Blue and orange dashed lines denote proton pumping from stroma to lumen by LEF and CEF, respectively. Black dashed lines denote proton translocation from lumen to stroma through ATP synthase to make ATP.
**PSII and PSI, photosystem II and I; Cyt b6f, cytochrome b6f; PQ, plastoquinone; PC, plastocyanin; Fd, ferredoxin.



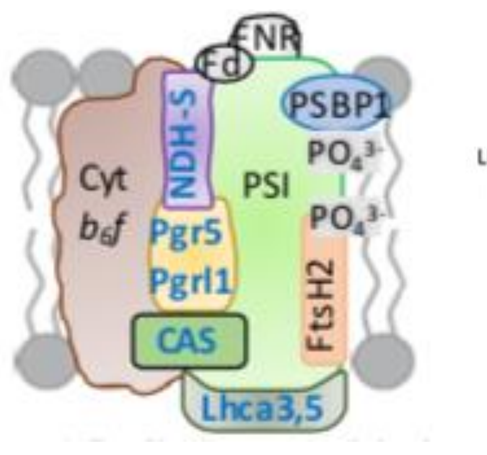


Figure 2. Preliminary model of subunit compositions of the UWO241 PSI supercomplex. Model is based on analysis of preliminary proteome data in this proposal and Szyszka et al. [2]. Blue letters indicate additional new components identified by preliminary data in this proposal. The model does not predict the actual positions of the subunits. Note presence of novel phosphoproteins, PsbP1 and FtsH2. CAS, a chloroplast Ca2+ sensor protein.

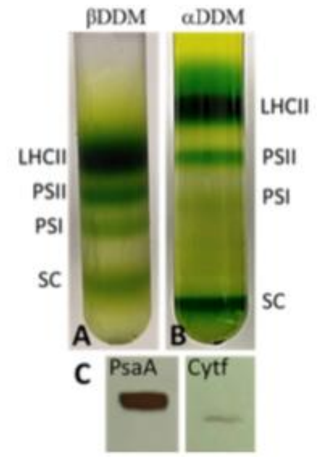


Figure 3. We can isolate stable PSI supercomplexes from UWO241. A,B Thylakoids were isolated from 1L of high salinity- grown UWO241 cultures, treated with the phosphatase inhibitor, NaF, and were solubilized in either aDDM or βDDM prior to sucrose density ultracentifugation. SC, PSI supercomplexes. **C.** UWO241 supercomplex contains both PSI and Cytb6f. Immunoblots of PsaA and Cytf from aDDM-solubilized supercomplex band (ie. from panel B ‘SC’ band).

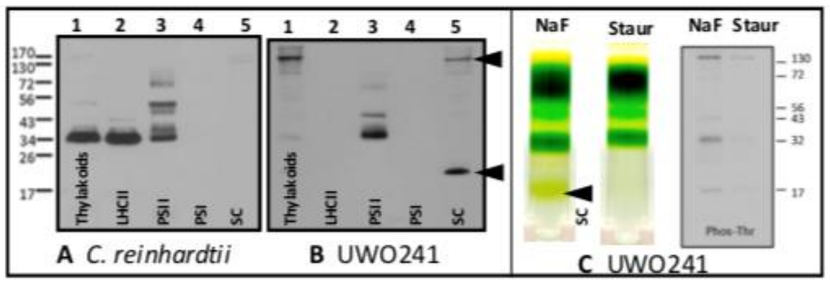


Figure 4. UWO241 supercomplex assembly requires protein phosphorylation. A,B Phosphoprotein profiles of thylakoid proteins and fractions isolated by sucrose density centrifugation. Lanes: 1-thylakoids, 2- LHCII, 3-PSII, 4-PSI, 5-PSI supercomplex, SC. Arrows indicate novel phosphoproteins in the UWO241 supercomplex. **C.** Sucrose gradients (left) and immunoblots (right) of UWO241 thylakoids treated with the phosphatase inhibitor, NaF, or the kinase inhibitor, stauroporine (Staur). UWO241 cultures were grown at 8oC /150 µmol m-2 s-1, 700 mM NaCl. *C. reinhardtii* cultures were grown at 29oC /150 µmol m-2 s-1, 0.43 mM NaCl. All immunoblots were probed with anti-phospho-threonine antibodies. (modified from Szyszka-Mroz *et. Al.*, *Plant Physiology* **2015**).

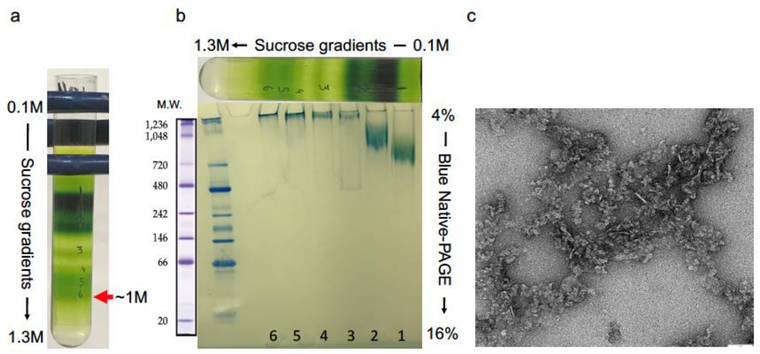


Figure 5. Characterization of the Chlamydomonas sp. UWO241 supercomplex isolated with Mg^{2+}, no EDTA added. a) Supercomplexes were purified by centrifugation using a sucrose gradient where the faint green band of the supercomplexes is indicated by the red arrow at approximately 1 M sucrose. **b)** Six collected fractions of sucrose gradients were analyzed by 4-16% Blue Native-PAGE. **c)** Negatively-stained electron micrograph of supercomplexes, collected at 1 M sucrose, after removal of sucrose using a G-50 desalting column. The image shows protein aggregation on the gird, which explained the reason of why supercomplexes were stuck in top of Blue Native-PAGE. The aggregation could have occurred when the isolated thylakoid membranes prepared at Miami U were frozen for shipment to ASU. It was concluded that frozen pre-prepared thylakoid membranes at Miami University were not suitable for cryo-EM analysis, and we concluded that the supercomplex must be freshly-prepared from cells onsite at ASU prior to cry-EM sample preparation.

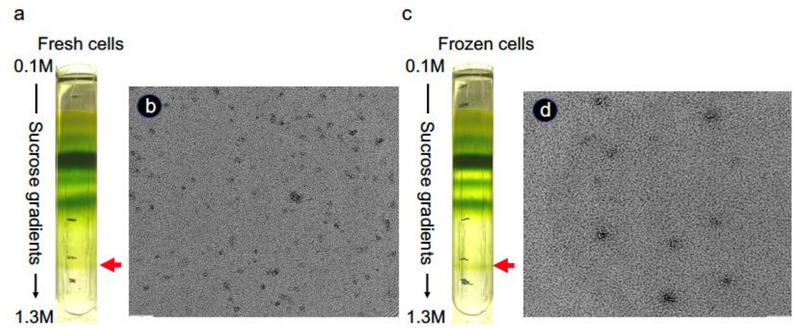


Figure 6. Characterization of the Chlamydomonas sp. UWO241 supercomplex. isolated from fresh cells and frozen cells, respectively. **a)** Supercomplexes were isolated from fresh cells by centrifugation using a sucrose gradient where the faint green band of the supercomplexes is indicated by the red arrow at approximately 1 M sucrose. **b)** a negatively-stained electron micrograph of supercomplexes isolated from fresh cells, collected at the gradient of approximate 1 M, after removal of sucrose using a G-50 desalting column. **c)** Supercomplexes were isolated from frozen cells by centrifugation using a sucrose gradient where the faint green band of the supercomplexes is indicated by the red arrow at approximately 1 M sucrose. **d)** a negatively-stained electron micrograph of supercomplexes isolated from frozen cells, collected at the gradient of approximate 1M, after removal of sucrose using a G-50 desalting column.

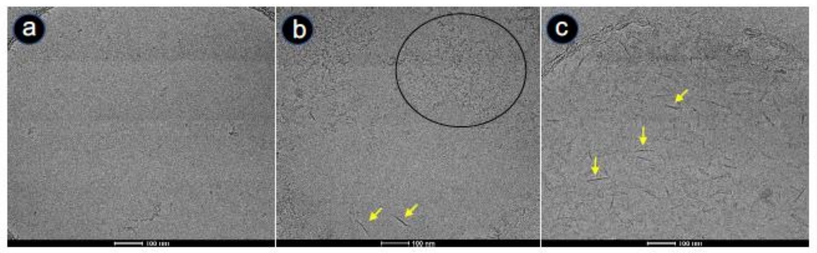


Figure 7. Cryo-EM micrographs of the Chlamydomonas sp. UWO241 supercomplexes. Supercomplexes were isolated from frozen cells by centrifugation using a sucrose gradient at approximately 1 M sucrose. Then, cryo-EM grids were prepared immediately after removal of sucrose. **a)** No particles were found in most of the holes of cryo-EM grids for both samples. **b)** Supercomplexes were isolated from cells shipped at 4C to ASU, very few long skinny needle particles (yellow arrow) were observed, which we hypothesize to be a side-view of the supercomplexes. c) Supercomplexes were prepared from frozen cells shipped to ASU using the method described previously and then concentrated using a 100K Amicon concentrator. Many more long skinny needle particles (yellow arrow) were observed.

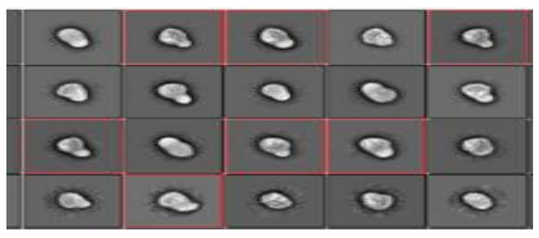
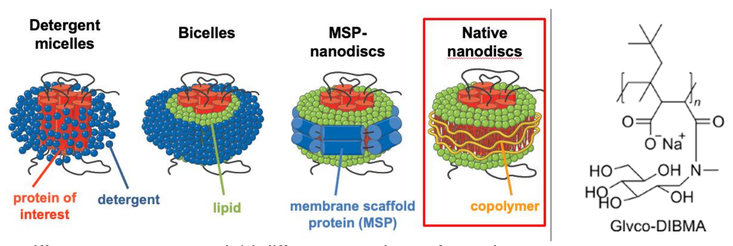


Figure 8. 2D Classification of isolated particles highlighting potential supercomplexes. Utilizing standard RELION 4.0 workflows for micrograph preprocessing, the motion and CTF corrected images were then subject to template free LoG-Based autopicking to find all potential particles. 2D classification was used to further refine the particle stack and ensure only structural features found in the supercomplex.

Future plan:

Since detergent micelles are relatively poor membrane mimetics, that may remove surrounding lipids (annular lipids), which may be critical for structure/function and have lower lateral pressure vs. lipid bilayer, **native nanodiscs** (Glycon-DIBMA) is an alterative approach to allow detergent-free extraction of membrane proteins from the membrane and maintain membrane proteins in their **native lipids**.



Different systems can yield different protein conformations
→ different structures/biology

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