Plants and algae have a great capacity to acclimate themselves to changing environments. We are interested in these acclimation processes, and how they efficiently yet safely harness sunlight for photosynthesis under fluctuating light conditions in particular. Using a model green alga, we are studying the molecular mechanisms underlying photoacclimation of photosynthetic machinery. We are also applying knowledge obtained in the studies of this model green alga to various phytoplankton, including a symbiotic dinoflagellate known to associate with coral and sea anemones, to explore how environmentally important photosynthetic organisms thrive in their ecological niche.

I. Amphipol-associated purification method for the highly active and stable photosystem II

Photosystem II (PSII) splits water and drives electron transfer to plastoquinone via photochemical reactions using light energy. It is surrounded by light-harvesting complex II (LHCII) to form the PSII-LHCII supercomplex. However, a complete characterization of its structure and function is hampered due to instability of the complex in the presence of detergent. To overcome this problem, we developed a new procedure for purifying the PSII-LHCII supercomplex of Chlamydomonas reinhardtii by employing amphipol A8-35 (Watanabe, A., et al. FEBS Lett. 593: 1072-1079). The obtained supercomplexes showed little LHCII dissociation even 4 days after purification. Oxygen-evolving activity was retained within amphipol when the extrinsic polypeptides were kept associated by betaine. Electron microscopy revealed that this method also improved structural uniformity and that the major organization was C$_2$S$_2$M$_2$L$_2$.

II. Structure of light harvesting for photosystem II in green algae

In C. reinhardtii, LHCII molecules associate with PSII to form various supercomplexes, including the C$_2$S$_2$M$_2$L$_2$ type; the largest PSII–LHCII supercomplex in algae and plants that is presently known. We reported high-resolution cryo-electron microscopy (cryo-EM) maps and structural models of the C$_2$S$_2$M$_2$L$_2$ and C$_2$S$_1$ supercomplexes from C. reinhardtii (Figure 1) (Sheng, X., Watanabe, A., et al. Nature Plants, 5: 1320-1330). The C$_2$S$_1$ supercomplex contained an LhcbM1–LhcbM2/7–LhcbM3 heterotrimer in the strongly associated LHCII, and the LhcbM1 subunit assembled with CP43 through two interfacial galactolipid molecules. The loosely and moderately associated LHCII trimers interacted closely with the minor antenna complex CP29 to form an intricate subcomplex bound to CP47 in the C$_2$S$_2$M$_2$L$_2$ supercomplex. A notable direct pathway was established for energy transfer from the loosely associated LHCII to the PSII reaction centre, as well as several indirect routes. Structure-based computational analysis concerning the excitation energy transfer within the two supercomplexes provided detailed mechanistic insights into the light-harvesting process in green algae.

III. Regulation of light-inducible photoprotection mechanism, qE, in green algae

Light is essential for photosynthesis, but the amounts of light that exceed an organism’s assimilation capacity can result in oxidative stress and even cell death. Plants and microalgae have developed a photoprotective response mechanism, qE, that dissipates excess light energy as thermal energy. In the green alga C. reinhardtii, qE is regulated by light-inducible photoprotective proteins. However, the pathway from light perception to qE until now has remained unknown. We discovered that the transcription factors CONSTANS and Nuclear transcription Factor Ys (NF-Ys) form a complex that governs light-dependent photoprotective responses in C. reinhardtii (Tokutsu, R., et al. Nature Commun., 10: 4099). The qE responses did not occur in CONSTANS or NF-Y mutants. The signal from light perception to the CONSTANS/NF-Ys complex was directly inhibited by the SPA1/COPI-dependent E3 ubiquitin ligase. This negative regulation mediated by the E3 ubiquitin ligase and the CONSTANS/NF-Ys complex was common to photoprotective response in algal photosynthesis and flowering in plants.
IV. Green fluorescence from corals attracts symbiotic algae *Symbiodinium*

Reef-building corals form an obligate symbiotic relationship with dinoflagellates from the family Symbiodiniaceae. Most coral species recruit algal symbionts from the environment. However, it has remained unknown how they encounter each other. We focused on green fluorescence protein (GFP)-associated fluorescence, which is commonly seen in coral (Figure 3), and examined whether it attracts motile symbiotic algae. We first examined their phototaxis behavior and found that symbiotic algae show positive and negative phototaxis mostly toward strong blue and weak green light, respectively. Attraction shown by algae towards green fluorescence was observed by using both a live coral fragment and an artificial green-fluorescence dye, but only under blue light conditions i.e. the wavelength that induces green fluorescence. We also showed that traps painted with a green fluorescent dye attracted symbiotic algae in the field. Our results revealed a novel biological signaling mechanism between the coral host and its potential symbionts (Aihara, Y., et al. Proc. Natl. Acad. Sci. USA 116, 2118-2123).

**Publication List:**


[Original paper (E-publication ahead of print)]