Spectroscopic Evaluation of Orange Carotenoid Protein (OCP)-Mediated Fluorescence Quenching of Light Harvesting Antenna Phycobilisome in Cyanobacteria with Highly Accumulated Iron Stress Inducible Protein A (IsiA)

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Many cyanobacteria utilize the orange carotenoid protein (OCP) to bind the phycobilisome (PBS), dissipating excess absorbed light energy as heat. The canonical model of this non-photochemical quenching mechanism emphasizes PBS coupled to both photosystems (PSI, PSII), yet it remains unclear how OCP functions under nutrient stress. Under iron limitation and stress conditions, Synechocystis sp. PCC 6803 accumulates the CP43 homolog, IsiA, that assembles into oligomeric rings around PSI or forms independent aggregates, raising the question of whether OCP-mediated quenching extends to non-canonical PBS-IsiA-PSI supercomplexes. Here, we combined steady-state and time-resolved fluorescence (TRF) spectroscopy at room temperature and 77K with whole-cell analyses of Synechocystis sp. PCC 6803 wild type, OCP-deletion, and OCP-overexpression strains. Distinct fluorescence bands were attributable to PSII (~693 nm), PSI (~724 nm), and IsiA (~688 nm), indicating that under these stress conditions, PBS can coexist with both PSII and IsiA. Blue-light activation and TRF demonstrated that OCP quenching acts by depleting the amplitude of APC660/680-associated components rather than altering decay lifetimes, revealing that OCP remains an effective photoprotective mechanism, even upon IsiA accumulation. Moreover, OCP-overexpression cells enhanced IsiA quenching compared to wild type, showing a quantitative link between OCP abundance and IsiA suppression. Together, these results reveal that OCP-mediated quenching remains robust when antenna architecture is remodeled by stress conditions, extending its functional role to IsiA-containing supercomplexes and highlighting the adaptability of light-harvesting systems in fluctuating environments.

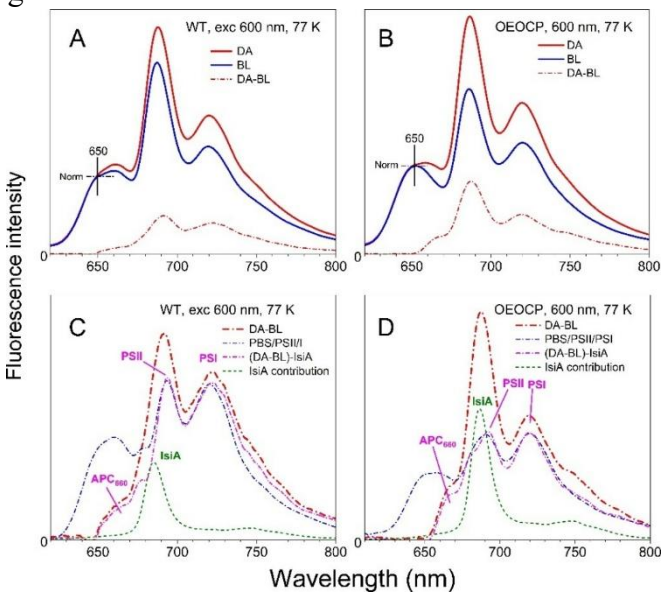

Figure 1. Blue-light induced difference spectra of WT and OEOCP *Synechocystis* 6803 at 77 K after PBS excitation (600 nm). (A–B) Fluorescence emission and difference spectra. (C–D) Spectral reconstructions showing contributions from IsiA, APC660, PSII, and PSI.