# Earliest stages of Photosystem I assembly in the cyanobacterium *Synechocystis* sp. PCC 6803

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Despite extensive structural characterization of Photosystem I (PSI), its biogenesis remains poorly understood; we investigate its early assembly intermediates in *Synechocystis sp.* PCC 6803. Using mutants expressing FLAG-tagged PsaA or PsaB individually and FLAG-tagged PsaA in the presence of PsaB, we isolated PSI assembly intermediates and the whole PSI complex for comparative analysis. The FLAG-PsaA subunit formed a stable homodimer with PsaK1, while FLAG-PsaB formed a less stable, heterogeneous homodimer. Both complexes bound pigments and associated with known and novel assembly factors. Cryo-EM at 1.9 Å resolution revealed the PsaA-PsaK1 homodimer structure, resembling ancestral type I reaction center. Both of the homodimeric complexes lacked charge-separation activity, showing singlet-to-triplet Chl conversion quenched by carotenoids. A PSI mutant lacking PsaC (FLAG-PSIΔC) accumulated more monomeric PSI, with PsaC, PsaD, and PsaE absent, while PsaF, PsaK1, and partially PsaL persisted. The 1.83 Å cryo-EM structure of FLAG-PSIΔC revealed that PsaC is essential for assembling cytoplasmic subunits, proper $F_X$ cluster formation, and trimerization via PsaL-PsaD interactions. These findings propose a modular PSI assembly mechanism in which small transmembrane subunits can associate early or concurrently with cytoplasmic components, revising the traditional sequential model and supporting a shared evolutionary origin with Photosystem II.