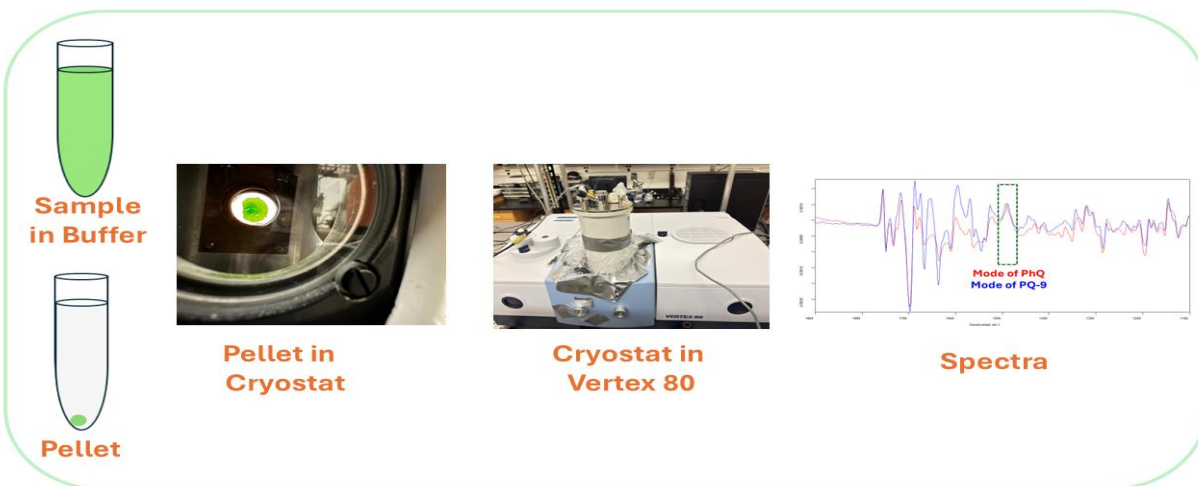


# Light-Induced FTIR Difference Spectroscopy for the Study of the Cofactors in Photosystem I.

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Fourier-transform infrared (FTIR) difference spectroscopy (DS) is widely used to study the structure and binding environment of electron transfer cofactors in photosynthetic complexes. Here, photo accumulated FTIR DS has been used to examine the photo-oxidation of the primary electron donor, P700, in Photosystem I (PSI) from *Synechococcus* sp. PCC 7002 (S7002) and in *menB*<sup>-</sup> mutant PSI from *Synechocystis* sp. PCC 6803 (S6803). In PSI the secondary radical pair state, P700<sup>+</sup>A<sub>1</sub><sup>-</sup>, forms within ~50 ps after excitation and decays within ~300 ns at room temperature, or ~300 μs at 77 K, making it inaccessible to photo accumulation FTIR DS methods. To overcome this limitation, we used time-resolved step-scan FTIR DS to study the short-lived A<sub>1</sub><sup>-</sup> state in PSI from S7002 and in *menB*<sup>-</sup> mutant PSI from S6803. Photo accumulated FTIR difference spectra reveal distinct features of P700<sup>+</sup> formation, while transient A<sub>1</sub><sup>-</sup> signals provide complementary insight into electron transfer recombination reactions. Together, these spectra offer information on cofactor binding and redox chemistry, enabling comparison of PSI variants. Preliminary data show reproducible signals associated with the quinones and chlorophyll molecules that act as donors and acceptors, forming the basis for a more detailed kinetic and structural analysis.



Graphical Abstract