Time-Resolved Single-Frequency IR-Spectroscopy on Photosystem II

IGK Report 2020

Sarah M. Mäusle, Aiganym Abzaliyeva, Paul Greife, Philipp S. Simon, Rebeca Perez, Yvonne Zilliges, Richard J. Debus, Holger Dau

Freie Universität Berlin

sarah.maeusle@fu-berlin.de

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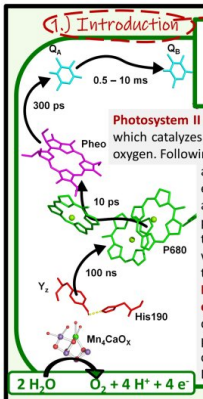
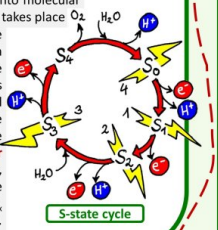
1.) Introduction

Photosystem II: The Site of Oxidative Water Splitting

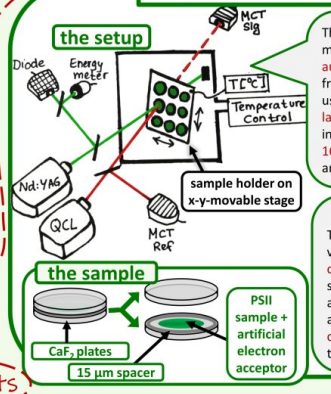


Photosystem II (PSII) is a large protein complex in **plants** and **cyanobacteria** which catalyzes the oxidative splitting of two water molecules into molecular oxygen. Following the absorption of a photon, charge separation takes place at the **special chlorophyll unit P680**. The free electron is transferred towards the electron acceptor side and reduces the mobile plastoquinone Q_B, which is exchanged once it has taken up two electrons. The hole at P680 is filled with an electron donation of the redox-active tyrosine Y_z, which in turn is reduced by the **Mn_4CaO_x complex**. After accumulating **four oxidative equivalents** at the Mn_4CaO_x cluster, dioxygen (O_2) is formed and released from the protein. The intermediate states of the $Mn4CaO_x$ cluster are referred to as the S-states (S_0 to S_4). Dark-adapted PSII is mostly in the S_1 state.

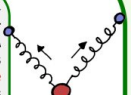


2.) Method

Single-Frequency IR Spectroscopy

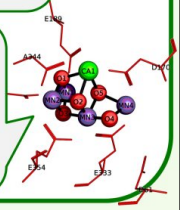


The setup is build around an x-y-movable sample holder, allowing for automated sample exchange. A frequency-doubled Nd:YAG laser is used for excitation; a quantum cascade laser (QCL) is used as a continuous infrared (IR) source, tunable between 1650 and 1300 cm^{-1}. MCT detectors are used to measure the IR absorption.



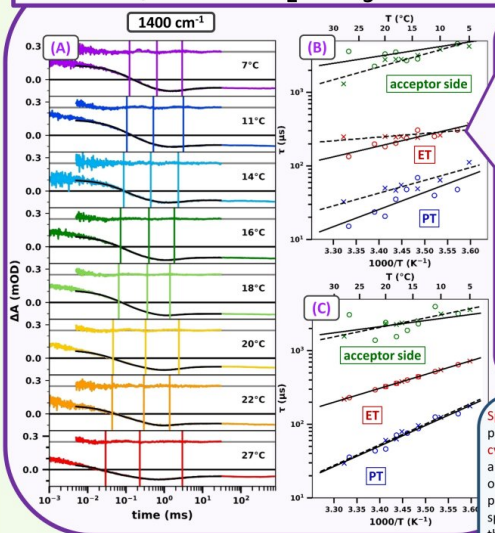
IR on PSII

The symmetrical stretching vibration of the free carboxylate groups of several amino acid residues around the Mn_4Ca cluster are sensitive to the S-state cycle transitions and can be tracked around 1400 cm^{-1}.



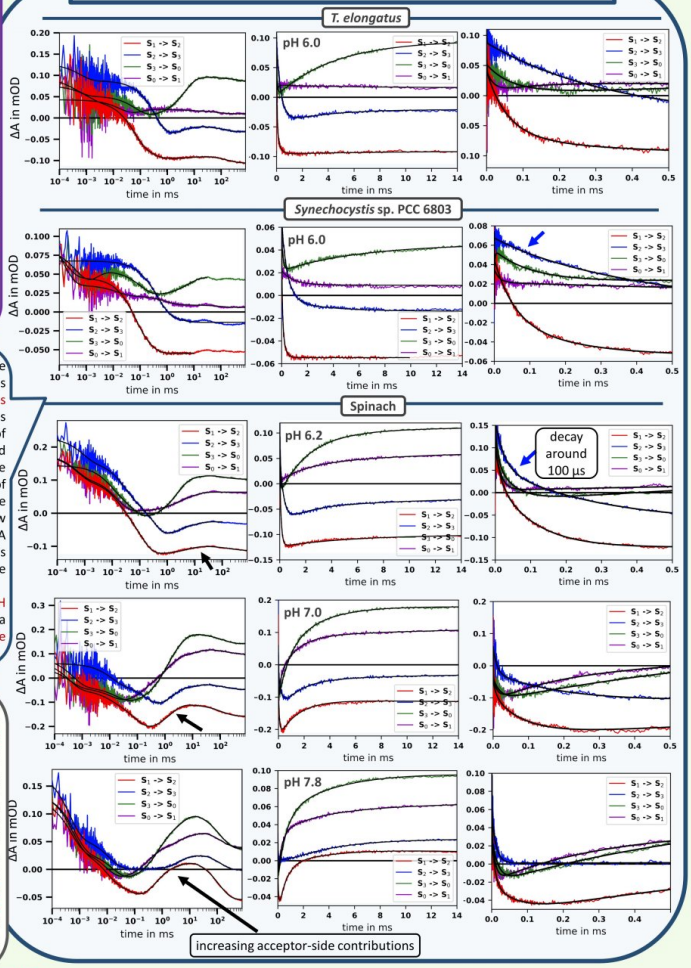
3.) Results

Spinach PSII: Activation Energies of Two Steps of the $S_2 \rightarrow S_3$ Transition



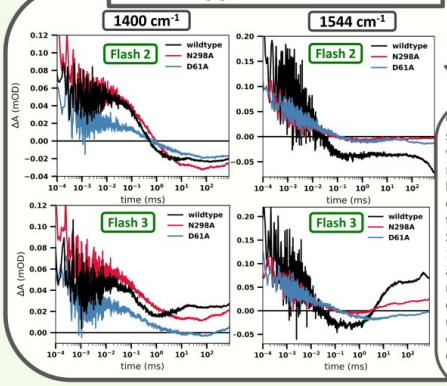
Transients were measured at 1400 cm^{-1} and 1395 cm^{-1} at various temperatures (5°C – 27°C). We restricted the fit to the area from 5 µs to 30 ms with two decaying and one rising component. The resulting Arrhenius plots varied for the two wavenumbers (B). When however constraining the slower decaying time constant to literature values for electron transfer (ET) of $S_2 \rightarrow S_3$ (C), the resulting activation energy (E_a) of the faster phase was identical for both wavenumbers. The fast phase was assigned to a proton transfer (PT). Results published in Ref. [1].

Comparing PSII Transients from Different Samples at 1400 cm^{-1}



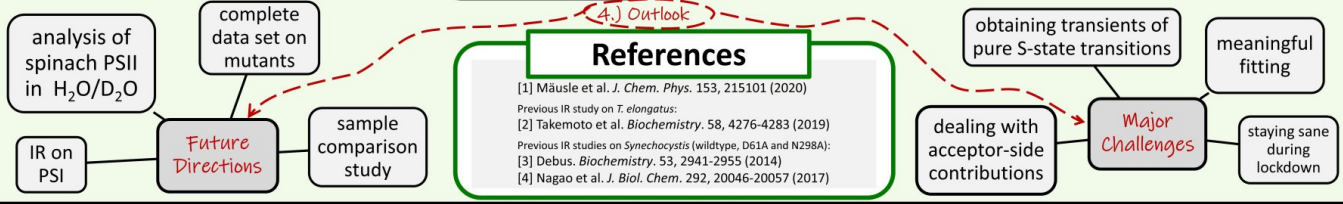
Spinach PSII membrane particles as well as cyanobacterial core complexes are commonly used for studies of the water-splitting step of photosynthesis using infrared spectroscopy. We demonstrate that transients at 1400 cm^{-1} of the different organisms, while overall of similar shape, show some significant differences: A fast decaying phase in $S_2 \rightarrow S_3$ is mostly absent in the core complexes.
Furthermore, changing the pH value in spinach PSII has a strong impact on acceptor-side contributions.

Cyanobacterial PSII: Wildtype vs. Mutants



Core complexes from Synechocystis sp. PCC 6803 were used to study wildtype PSII as well as PSII with point mutations, as it may help to identify key players around the $Mn4CaO_x$ cluster and help the understanding of the water oxidation mechanism. In $S_2 \rightarrow S_3$, D61A shows a slowed decay at 1400 cm^{-1}. At 1544 cm^{-1} this phase is absent – also in the wildtype – and replaced with a faster decay. At 1400 cm^{-1}, the rising phase around 5 ms (ET) in the wildtype is slowed down extremely in both mutants. The 1544 cm^{-1} data also suggest that the ET is slowed down, but less drastically.

4.) Outlook



References

[1] Mäusle et al. *J. Chem. Phys.* 153, 215101 (2020)

Previous IR study on *T. elongatus*:

[2] Takemoto et al. *Biochemistry*. 58, 4276-4283 (2019)

Previous IR studies on *Synechocystis* (wildtype, D61A and N298A):

[3] Debus. *Biochemistry*. 53, 2941-2955 (2014)

[4] Nagao et al. *J. Biol. Chem.* 292, 20046-20057 (2017)