S-state difference FTIR spectra of photosystem II, deconvolution, simulation, and time-resolved step-scan experiment

*P. Chernev[1], J. Menzel[1], B. Süss[1], M. Görlin[1,2], H. Dau[1]

[1]*Freie Universität - Berlin, Fachbereich Physik, Berlin, Germany*
[2]*Technische Universität Berlin, Berlin, Germany*

In photosynthetic water oxidation, the coupling of the redox chemistry to proton movements is of prime interest but still not fully understood. To track protonation-state changes, Fourier-transform infrared (FTIR) difference spectroscopy can be applied which is especially sensitive to protonation/deprotonation structures and hydrogen-bond interactions, which gives information that is complementary to X-ray crystallography and is essential for the understanding of the molecular mechanisms of water oxidation. The application of ns laser flashes that induce the transition of Photosystem II through the S-state cycle yields FTIR difference spectra that are however a mixture of signals coming from different S-states due to misses. Additionally, the spectra contain a multitude of bands that are not well separated and overlap significantly. Here we report a method that yields difference spectra that are corrected for the mixing of the S-states, and a simulation approach that allows us to correctly assign peak frequencies and amplitudes to the different S-state transitions. The effect of the measurement resolution and of the window function applied prior to Fourier transformation is discussed, giving us an estimate of the intrinsic parameters of the spectral bands from the parameters observed in the experimental spectra.

To investigate the temporal development of the protonation-state changes after each laser flash, we performed a time-resolved step-scan FTIR experiment on Photosystem II membrane particles from spinach with a time resolution of 12.5 µs, giving us a complete picture of the mid-infrared absorption region and allowing us to follow the proton movements through the S-state cycle. The laser-induced heating of the sample causes an artifact in the spectra, which complicates the interpretation of the data in both step-scan FTIR and single-wavelength IR experiments.