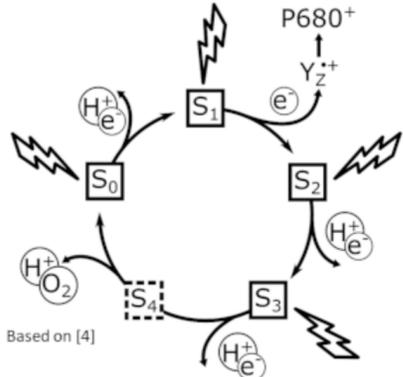
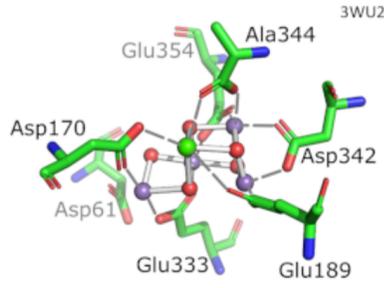


## Photosystem II

In the light-driven reaction cycle of PSII, two 'substrate' water molecules are oxidized resulting in the release of O<sub>2</sub> at the Mn<sub>4</sub>CaO<sub>5</sub> cluster. Driven by a sequence of light flashes, the OEC cycles through its four semi-stable S-state intermediates

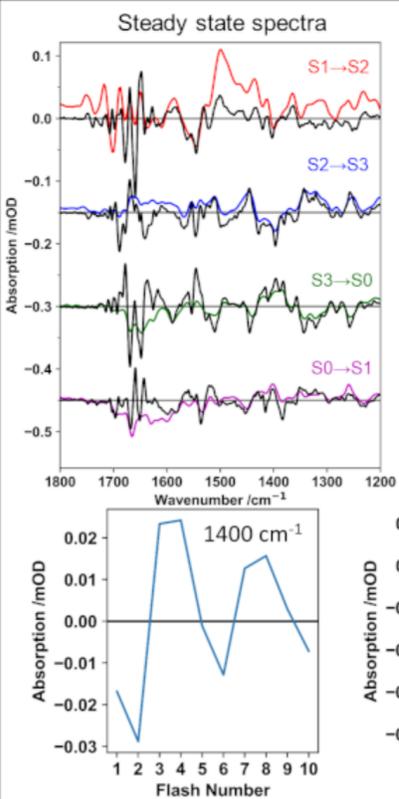
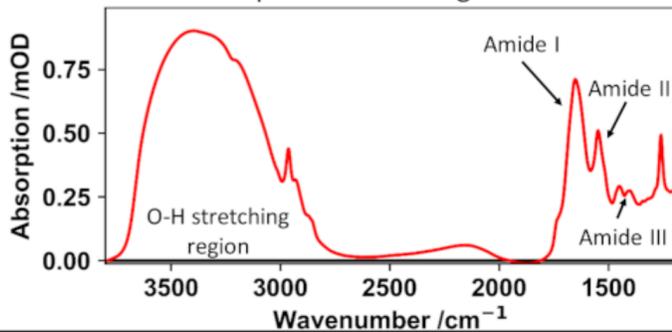


The processes associated with the sequential oxidation of the Mn<sub>4</sub>CaO<sub>5</sub> induces changes in the protein structure. These effects can be observed using a variety of techniques, including infrared (IR) spectroscopy.



## IR on Photosystem II

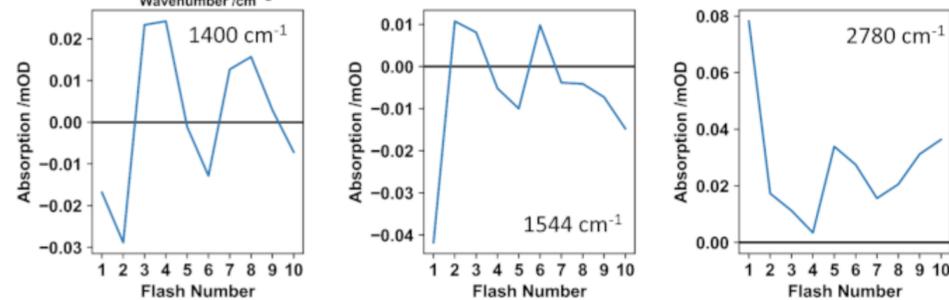
The semi-stable intermediates have been extensively investigated using Fourier transform IR (FTIR) spectroscopy [1] and recently, measurements have been performed using time resolved IR methods [2], [3].



## First results

Simple spinach membrane particle measurement

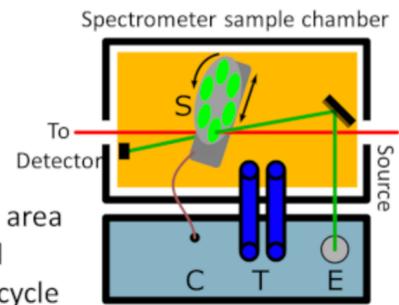
- 15 °C, pH 6.2, 1 Hz flash spacing
- 8 cm<sup>-1</sup> resolution
- 5 minutes worth of data per spectrum
- Compared to 10 °C, 4 cm<sup>-1</sup> (black spectra) from previous setup
- Significant agreement in spectra
- Flash patterns show period of four oscillations



## A new setup: Vertex 80v

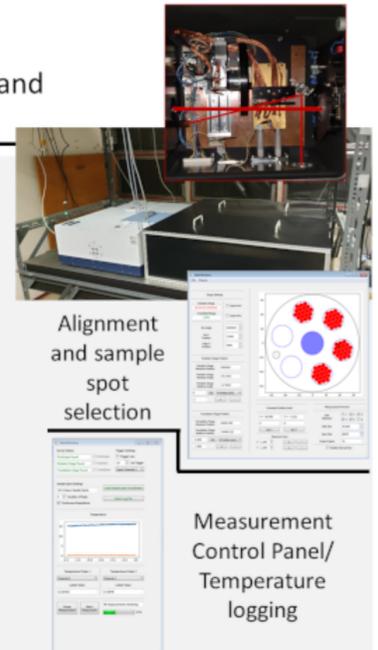
**The Plan:** Modification to Bruker Vertex 80v

- Avoid extended sample chamber
- Use sample wheel to reduce footprint
  - Φ,x for sample positioning
- Use 35 mm CaF<sub>2</sub> plates to increase sample area
- Thermostatic bath for temperature control
- 532 nm excitation laser for driving S-state cycle
- Retain vacuum capability of spectrometer
- Increase spectral range to 5000 -1200 cm<sup>-1</sup>
- Communication between spectrometer and setup, automated sampling

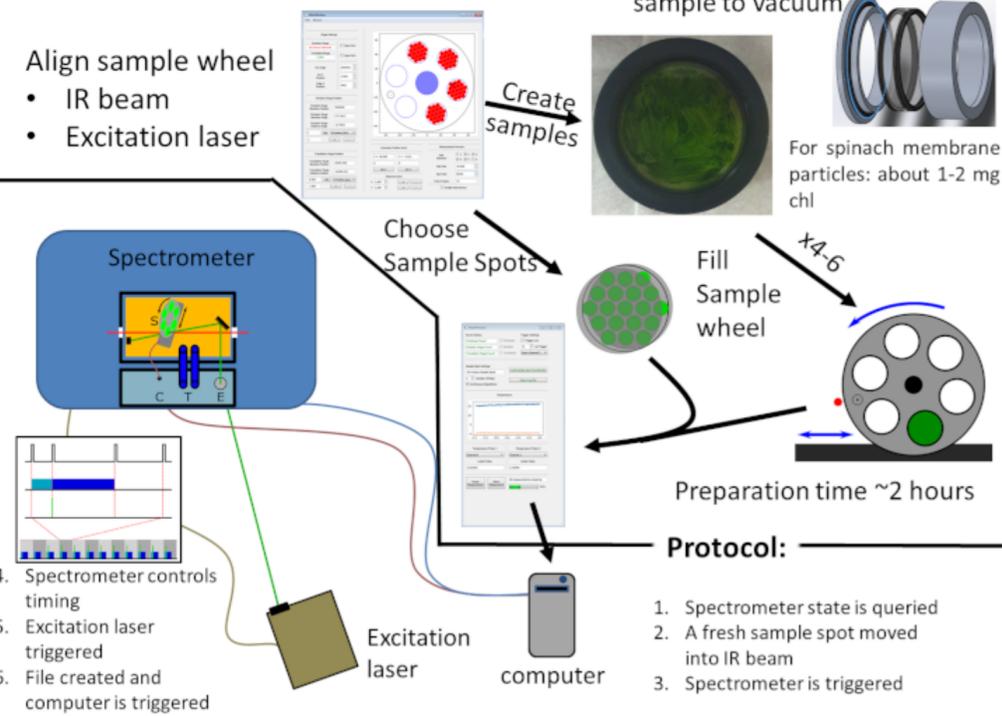


**The Reality:** Mostly realized...

- Sample wheel works well.
- Temperature control needs further work
  - Limited to 15 °C
  - Underestimated black body radiation of sample compartment
- Vacuum capable
- Many setbacks due to spectrometer
  - Firmware problems (Solved)
  - Software problems (Solved)
  - Hardware problems (Solved)
- Self written software communicates with Bruker Opus software



**The Measurement:** A rough overview



## Conclusion

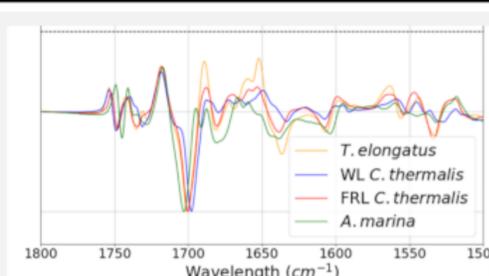
- Finally reliable measurements
- Critical bugs fixed
- Agreement with previous measurements
- Integration with OPUS allows flexibility
- Temperature control not good enough

## Outlook

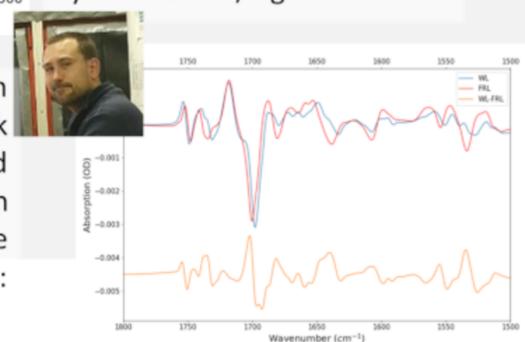
- Improve above measurement with more repetitions
- Measure under different conditions, buffer, pH, temperature, flash spacing, core complexes, etc...
- Install 'Chernobyl' dome to shield setup from black body radiation

## PS I core complexes

Masters' thesis of Viktor Eichmann in the AG Nürnberg, examining the effect of far red light on the growth of cyanobacteria/algae.



Installed red 632 nm cw excitation laser into setup to compare dark and illuminated PSI. Showed significant differences between species, but also a difference based on growth condition: WL/FRL



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[1] Debus, R. J. (2015). *Biochimica Et Biophysica Acta (BBA) - Bioenergetics*, 1847(1), 19-34.  
[2] Sakamoto, H. et al (2017). *J. Am. Chem. Soc. (JACS)*, 139, 2022-2029  
[3] Mäusle, SM. Et al (2020). *Journal of chemical physics*, 153, 10.1063/5.0027995  
[4] Klauss, A. et al. (2012). *Proc. Natl. Acad. Sci. U.S.A.*, 109(40), 16035-16040.