Time-resolved X-ray emission spectroscopy and serial femtosecond crystallography studies on the transient S4 state in Photosystem II

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Overall research goals:

Photosystem II (PSII), was isolated and crystallized from thermophilic cyanobacterium TS elongatus BP-1. We collected time-resolved X-ray emission spectroscopy (TR-XES) and serial femtosecond X-ray crystallography (SFX) data at the MFX beamline at LCLS. PSII contains an Oxygen Evolving Complex (OEC) and using SFX paired with TR-XES we were able to better investigate the structural and quantum changes of the OEC and its oxidation states; with a special focus on the transient S4 -> S0 state during photo-oxidation. We also measured the XES spectra for Mn-model compounds like Dragon and Pegasus, which represent the oxidation state of the OEC throughout the S-cycle. PSII microcrystals were embedded into a PEG 8 million solution for sample injection, giving us heightened background/noise. Due to the nature of time-resolved studies and the SFX pulse system, every XFEL pulse that does not hit a crystal will produce extensive noise and no single. We developed a data pipeline that allows us to pair SFX and TR-XES data in order to accurately visualize the PSII XES spectrum more effectively and efficiently by reducing the noise from the XES sensor and the computational requirements (time and space). This pipeline was able to process raw TR-XES data, communicate with SFX sensors to determine the exact timestamps of when a PSII crystal was hit, average out the non-hit/background timestamps to remove the noise, and finally visualize the XES spectrum. We obtained a proposed XES spectrum for the transient S4 -> S0 state that suggests the PSII OEC can achieve an oxidation state >4+ for a few femtoseconds which has never been observed before.

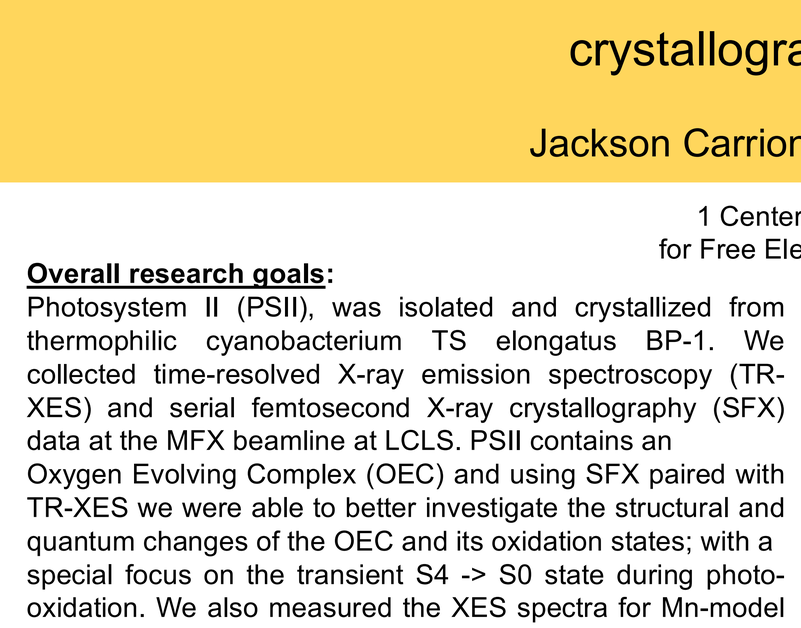

Figure 1. S-Cycle in the PSII OEC and Mn-model compounds 'Dragon' and 'Pegasus'.
a.) From the initial 'Dark' state, consisting of +3, +3, +4, +4 Mn atoms, PSII requires 3 flashes to reach the S4 state where each flash converts a +3 Mn atom into +4. We hypothesize to see a rare +5 oxidation state in one of the Mn atoms in the OEC. **b.)** Below we can also see Mn-model compounds 'Dragon' and 'Pegasus' that represent the S3/S4 and S0/S1 state, respectively.

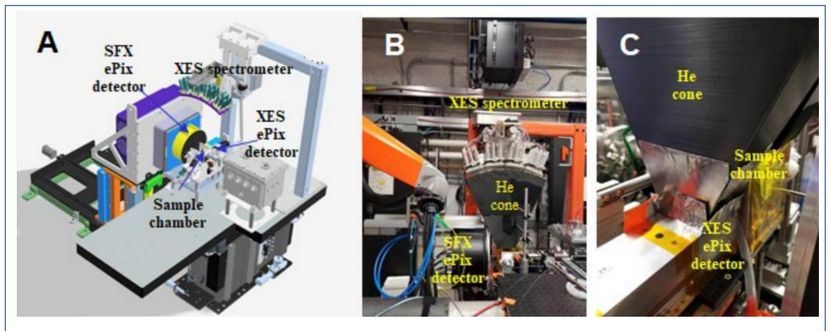

Figure 2. Setup at MFX beamline for parallel TR-SFX and XES data collection on Photosystem II at beamtime LW95 & LY00

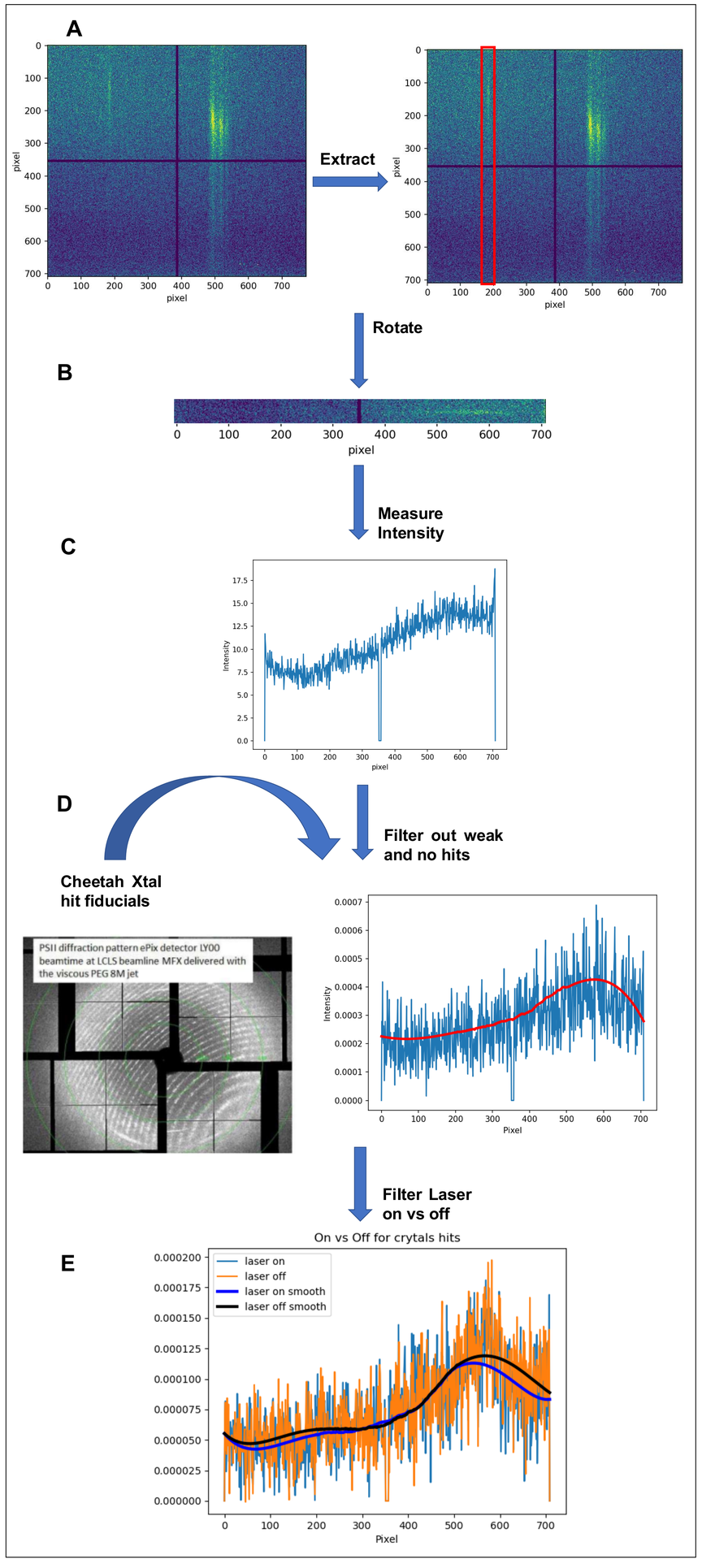

Figure 3. TR-XES and SFX data pipeline for LY00 run 136, 137, & 139.
a) Output from the XES ePix100 detector can be seen as a 700x700 matrix and from past experiments we know the Mn signal is contained in the region between 180 and 220 so that section of the detector is extracted for further evaluation. **b)** Due to PSII only containing 4 Mn atoms (~ 6-7 uM) the signal is very low and we see increased noise as the pixel numbers increase due to detector issues. **c)** Visualize the Kβ XES spectrum by graphing the pixels based on intensity and here we can see a logistic curve which indicates the noise at the top of the sensor is too strong compared to the Mn signal. **d)** Use Cheetah's hit finding software to find femtosecond level fiducials of quality PSII crystal hits and only process the XES events at those timestamps. **e)** Filter and compare laser on vs laser off spectra to attempt and visualize the transient S4 state. A peak slightly shifted to the left would indicate a higher oxidation state.

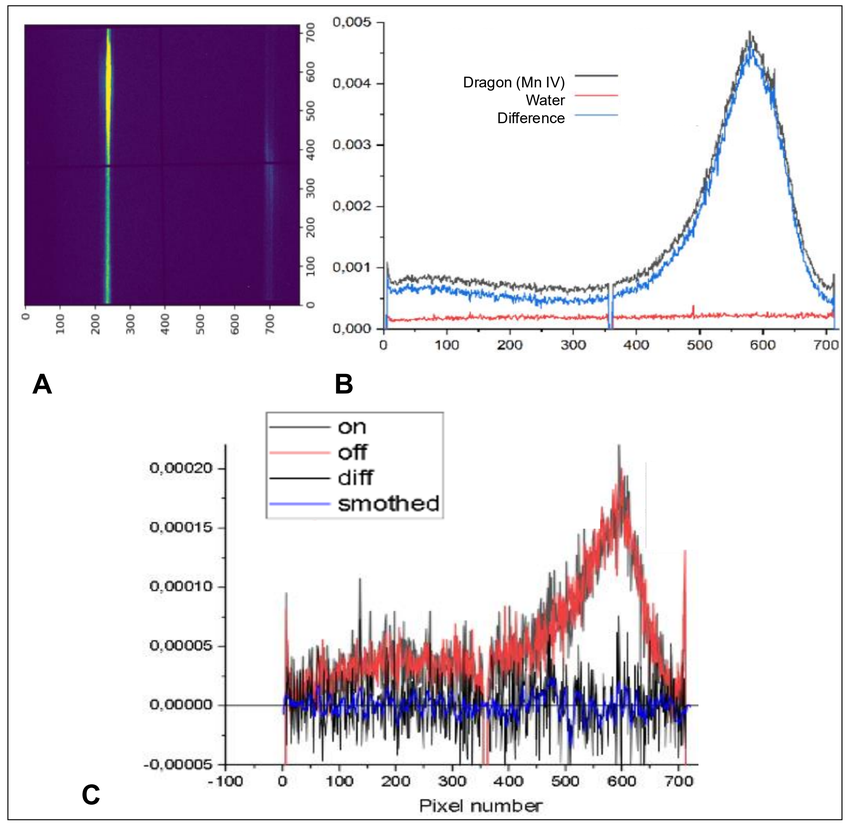

Figure 4. TR-XES for LW95 run 238 - 243.
a. Output from the XES ePix100 detector showing a very strong Mn single with minimal background/noise. ePix100 detector was shifted during the LW95 beamtime resulting in the Mn signal is contained in the region between 220 and 250 **b**. Kβ XES spectrum from 150mM Dragon (Mn IV) with a water background. Very similar methods were used to visualize the Kβ XES spectra as mentioned before without the SFX data integration. **c**. The Kβ XES spectrum for laser on and laser off events averaged over 6 runs. We can see the difference, represented by the black line, shows minimal differences between laser on vs laser off which suggests two possible outcomes, the transient S4 state has the same oxidation state as the S3 state (Mn IV) or due to XFELs being able to produce extremely strong photons, we cannot observe the higher oxidation state due to varying pulse durations and the Mn cluster is being destroyed during the longer pulse durations.

Future Plan:

As seen below, every XFEL pulse can have a slight deviation from the desired pulse duration and new XTCAV sensors in LCLS allow users to track the distribution of pulse duration throughout the experiment. XFEL scientist have learned how to control this variance and reduce the range from 0-40 fs to 0-20 fs. This can allow users to add an addition step in the pipeline that filters out XES signal that corresponds to an event with a longer pulse duration, hopefully resulting in a Kβ XES spectrum that accurately represents the S4 state only a few femtoseconds after being hit with the XFEL beam and reduce signal from damaged Mn atoms.