MS2-02 **Taking Snapshots of Photosynthetic Water Oxidation: Towards Time-Resolved X-ray Spectroscopy and Crystallography.** Junko Yano,[a] Jan Kern,[a,b] Rosalie Tran,[a] Roberto Alonso-Mori,[b] Uwe Bergmann,[b] Vittal Yachandra,[a] *[a]Physical Biosciences Division, Lawrence Berkeley National Laboratory,USA, [b]SLAC National Accelerator Laboratory, USA*
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Oxygen, that supports all aerobic life, is abundant in the atmosphere because of its constant regeneration by photosynthetic water oxidation, which is catalyzed by a Mn4CaO5 cluster in photosystem II (PS II), a multi subunit membrane protein complex.[1] Although the structure of PS II and the catalytic intermediate states have been studied intensively over the past several years, an understanding of the sequential chemistry of light absorption and water-oxidation requires a new approach beyond the conventional steady state crystallography and X-ray spectroscopy at cryogenic temperatures. The femtosecond X-ray pulses of the free-electron laser allows us to outrun X-ray damage at room temperature, and the time-evolution of the photo-induced reaction can be probed using a visible laser-pump followed by the X-ray-probe pulse. XFELs can be used to simultaneously determine the light-induced protein dynamics using crystallography and the local chemistry that occurs at the catalytic center using X-ray spectroscopy under functional conditions. We have shown that PS II microcrystals can be used to obtain XRD data at room temperature using the short <50 fs X-ray pulses at the LCLS. This prototypical study serves as the basis for future time-resolved measurements and more detailed investigations on the intermediate states of PS II as it demonstrates that the probe before destroy approach is feasible for this system.

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Keywords: photosynthetic proteins; oxygen evolution; X-ray spectroscopy

MS2-03 **5-Dimensional Crystallography**
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Time-resolved crystallography [1] unifies structure determination and kinetics. Recent upgrades at existing synchrotrons [2] offer the opportunity to rapidly collect a large number of time points covering a comprehensive kinetic time-series from picoseconds to seconds and longer at ambient temperatures. The kinetic analysis is driven by the singular value composition [3]. All time-points in a time-series can be collected rapidly from a single crystal. This provides the opportunity to augment the 4D time-resolved X-ray data with another dependency, which could be temperature [4], pH [5], X-ray dose [6] or pressure. Crystallography becomes 5-dimensional [4]. The holy grail of any time-resolved experiment in biochemistry, however, is the ability to investigate the transient kinetics of non-reversible reactions in biologically and pharmaceutically important enzymes. With enzyme solutions rapid mixing with substrate using the stopped-flow technique is commonly used to spectroscopically identify intermediates on the sub-second time scale. In macroscopic crystals, diffusion times are typically in the seconds to minute range, which is too slow for most enzymatic reactions. This discourages rapid-mixing experiments with crystals. Initially inactive, caged substrates have to be used that are already present in the crystal and can be activated by ultra-short light pulses. With small micro-crystals or nano-crystals, however, micro-second diffusion times become possible. This enables stopped-flow type approaches even with crystals. After mixing with substrate, the nano-crystals are rapidly injected into the X-ray beam. Scattering patterns are collected using serial crystallography with the diffract-and-destroy approach at an X-ray Free Electron Laser (XFEL) [7]. If this approach can be routinely used, the XFEL becomes the ultimate machine for five-dimensional, real-time and ambient temperature structural enzymology.

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Keywords: five-dimensional crystallography; structural enzymology; serial crystallography