A major challenge of structural investigations of metalloproteins at synchrotrons is the damaging effects of radiation exposure. Even small X-ray doses can reduce or initiate reactions at metal centers modifying the active site. For example, in-situ visible absorption spectroscopy measurements have demonstrated that the heme/copper active site in oxidized ba3 cytochrome oxidase (ba3) is compromised during a single X-ray diffraction exposure. The use of ultrashort X-ray pulses at LCLS provides a means to measure high resolution diffraction before these damage processes occur. To this end, experiments were conducted at LCLS using large multiple crystals (> 50 µm) of ba3, hydrogenase and myoglobin. Crystals were mounted in ‘grids’ or loops and flash frozen. The grids hold up to 75 crystals in known locations and are compatible with the Stanford Automounter used to exchange them. Following a semi-automated grid alignment procedure, a fully automated routine was used to position each crystal and collect a series of diffraction images and the Blu-Ice/DCS control system that coordinated with the LCLS EPICS system and XPP DAQ software. Single femtosecond X-ray pulses produced a ‘damage free’ still diffraction image from each crystal. To provide additional information about crystal orientation, a series of pseudo-oscillation images were collected +/− 5.5 degrees spanning the orientation of the still image. For each one degree oscillation image the crystal was exposed to 120 attenuated X-ray pulses. A hard X-ray spectrometer was used to measure the energy spectrum of each individual X-ray pulse. The details and results of these experiments will be presented.

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