ESRF	**Experiment title:** Probing structural dynamics of myoglobin using site-specific iodine labeling scheme	**Experiment number**: SC-3760
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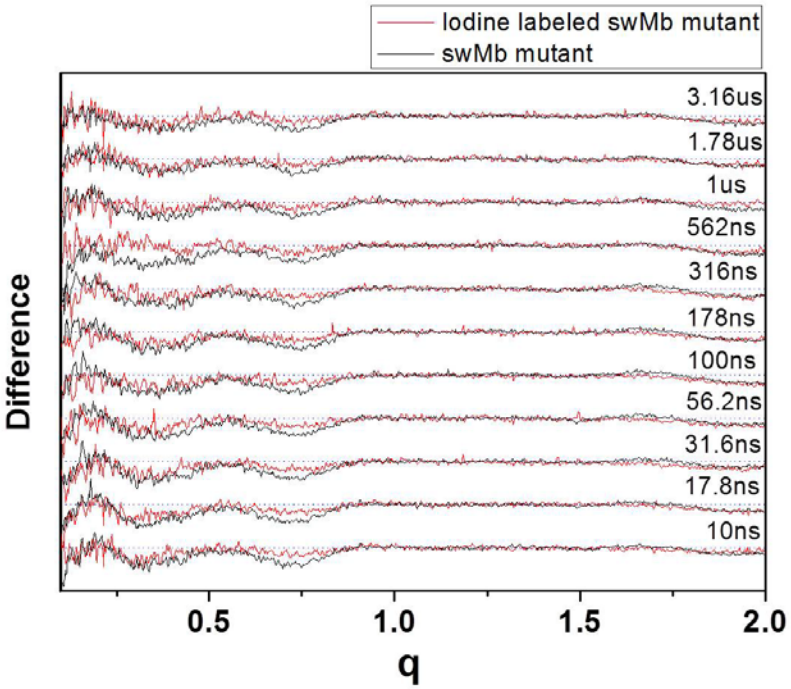
Report:

We performed an experiment on 'Probing structural dynamics of myoglobin using site-specific iodine labeling scheme' at ID09B beamline. We prepared a myoglobin mutant with two surface cysteines to label two iodine atoms. The iodine-labeled myoglobin molecules were dissolved in 100mM of sodium phosphate buffer (pH 7.0) to a final concentration of 8 mM. The sample solution was sealed in a quartz capillary and mounted on the goniometer.

In the previous visit, we obtained a preliminary data for these samples only for a few time points (from 10ns to 3.16ms) for testing heavy atom labeling scheme. In this beamtime, we tested the reproducibility of the experiment. Furthermore, we measured the difference X-ray scattering data of the samples with more time points (from 10ns to 3.16ms, four time points/decade) including several points which were not covered in the previous beamtime.



curves of the swMb mutant (black line) and iodine labeled swMb mutant (red line) at each time delays

In order to investigate the reaction mechanism in a broad time range and to extract intermediate-associated species curves, we collected diffraction data at the following 32 time points; -5ns, 100ps, 178ps, 316ps, 562ps, 1ns, 1.76ns, 3.16ns, 5.62ns, 10ns, 17.8ns, 31.6ns, 56.2ns, 100ns, 178ns, 316ns, 562ns, 1us, 1.78us, and 3.16us, 5.62us, 10us, 17.8us, 31.6us, 56.2us, 100us, 178us, 316us,

562us, 1ms, 1.78ms, and 3.16ms.

We used a typical pump-probe setup installed at ID09B. The reaction was initiated by 532 nm wavelength laser pulse (150uJ power, 1mJ/mm^2 energy density). After the excitation, the sample reaction was probed by using hard X-ray pulses (E_{photon} = 18.0 keV). Diffraction patterns were collected using FReLoN CCD. The data were processed to yield time-resolved difference X-ray solution scattering curves. Figure 1 shows the comparison of the difference curves of the iodine-labeled myoglobin mutant (red) and the unlabeled myoglobin mutant (black). Compared to the data obtained from the myoglobin mutant prior to iodine labeling, the data from iodine-labeled samples clearly shows a different feature at q = 0.3 Å^{-1} and q = 0.75 Å^{-1}.

Collected scattering data have been analyzed to extract their structural dynamics using singular value decomposition, principal component analysis and rigid-body MD simulations. We expect that the combination of the data from iodine-labeled molecule with the data from unlabeled mutant can increase the information content. Additionally, accumulating a series of 1D scattering curves by using these experimental scheme for various labeling sites would increase the structural information.

In conclusion, we collected and analyzed time-resolved X-ray solution scattering data of iodine-labeled myoglobin at various time points to investigate structure dynamics of the myoglobin mutant. A successful analysis of the data will reveal the applicability of iodine-labeling method for the purpose of amplifying the structural information of solution scattering data.