ESRF	**Experiment title:** Probing the structural dynamics of myoglobin using a site-specific iodine-labeling scheme	**Experiment number**: SC-4426
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Report:

We performed the experiment "Probing the structural dynamics of myoglobin using a site-specific iodine-labeling scheme" using the ID09B beamline. We prepared wild type myoglobin (Mb) and Mb mutants with two (K147C, D60C) and four (K147C, D60C, A22C, G5C) surface cysteines, respectively, and labeled the mutant proteins with iodine. The unlabeled and iodine-labeled Mb proteins were dissolved in 100 mM 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 a previous visit, we obtained preliminary data for two iodine-labeled Mb mutants to test the iodine-labeling scheme. During this beamtime, we tested additional Mb mutants to increase the information gained from iodine-labeling and to confirm the reproducibility of the experiment. We focused on and measured the X-ray scattering data of the samples at six time points (10 us–3.16 ms) to obtain high signal/noise ratios for late intermediates.

To extract late intermediate-associated species curves, we collected diffraction data at -5 ns, 10 us, 31.6 us, 100 us, 1 ms, 10 ms, 17.8 ms, and 31.6 ms. We used the typical pump-probe setup installed at ID09B. The reaction was initiated by a 532-nm wavelength laser pulse (150 uJ power, 1–3 mJ/mm^2 energy density). After excitation, the structural change of sample was probed using hard X-ray pulses (E_{photon} = 18.0 keV). Diffraction patterns were collected using a Rayonix charge-coupled device camera. The data were processed to yield difference X-ray solution scattering curves. Figure 1 shows the difference curves of wild type Mb and iodine-labeled (red) and unlabeled (black) Mb mutants. Clear differences in features were observed for the swMb mutants before and after iodine labeling.

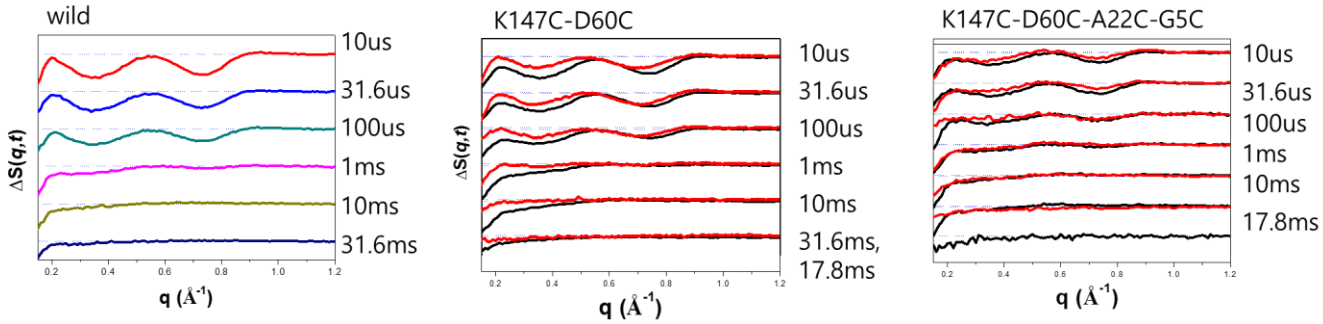

Figure 1 Time-resolved difference X-ray solution scattering curves of wild type Mb and unlabeled (black lines), and iodine-labeled (red lines) Mb mutants at each time point

Collected scattering data were analyzed to extract late intermediate-associated species curves using singular value decomposition and principal component analysis. Then, we compared the iodine-labeled and unlabeled results in the both samples (Fig. 2). The results also demonstrate different features between the iodine-labeled and unlabeled Mb mutants. Therefore, we conclude that this variation is due to the scattering contribution from the two and four iodine atoms bound to the surface cysteine residues, respectively.

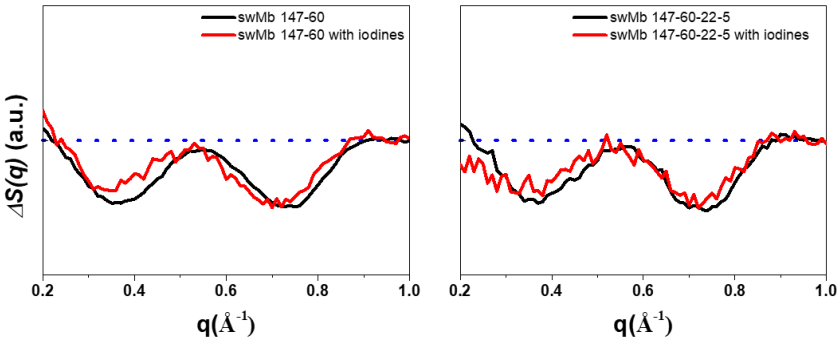

Figure 2 Intermediate-associated species curves of unlabeled (black line) and iodine-labeled (red line) Mb mutants

We are currently using these data to analyze Mb structural changes using Molecular Dynamic simulations. We expect that combining data from iodine-labeled and unlabeled proteins will increase the information gained for structural changes. Accumulating a series of one-dimensional scattering curves using this experimental scheme with various labeling sites would also increase the structural information.

In conclusion, we have collected and analyzed time-resolved X-ray solution scattering data of iodine-labeled Mb mutants to investigate their structural dynamics. The results show that there are indeed detectable changes before and after labeling. Successful analysis of the data will reveal the utility of the iodine-labeling method to amplify the structural information from solution scattering data. This will allow the analysis of intermediate protein structures with high reliability.