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Report:

We have determined the most useful protocol to perform the photolysis experiment on our crystals at room temperature. Moreover we succeded to set up the laser timing from nanosecond to microsecond. We collected 19 data set on one single crystal, whose spectrum was measured before and after data collection, using the facilities of the cryobench laboratory in collaboration with Dr. Dominique Bourgeois, in order to assign the initial rate of MetMb with respecto to Mb CO-bound. The time range explored spanned from 3ns to 3 microsecond delay. Other 7 data sets were collected spanning a narrower time range on another single crystal, indeed checked for the content of MetMb by means of microspectrophotometer measurements.
Data were reduced, scaled and refined to 1.7Å successfully by Dr. Bourgeois at ESRF, showing the CO bound to the mutant Mb YQR photolysed and escaped from the pocket to the bulk in the time range explored. The percentage of photolysis was consistent with the amount of CO-bound Mb. Moreover in the difference maps was clear the displacement of the Fe from the hem plane, the tilting of the heme itself and some more major movements of distal residues of helix E and of helix F.
We have also exposed a crystal of a mutant of human Hb (HbαβYQ) in the liganded T-state. Indeed preliminary data showed that time resolved crystallography could be effectively successfull on this particularly interesting mutant at room temperature. The single crystal tested diffracted to 1.6Å resolution and the spectrum was consistent with a liganded species.