**Report:** Structural dynamics: time resolved diffraction of photolytic intermediates of myoglobin and hemoglobin mutants. Eperiment MX8 (7-16 October 2002)

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INTRODUCTION: Biological function involves dynamics of structures, which can be followed by a wealth of biochemical and biophysical techniques. Determining directly the structure of functional intermediates is a task which was considered unattainable by crystallography, but recent developments have allowed achieve this goal to address significant questions about 3D structure and dynamics states. A successful approach has been the trapping of intermediates in crystals, by physical or chemical techniques. Only in a few cases perturbations can be applied to protein crystals rapidly and homogeneously in order to obtain "molecular movies" following the evolution of protein structure in time by Laue X-ray diffraction, yielding information of general interest on the structural dynamics of proteins at large.

MATERIALS AND METHODS: Single bunch Laue data (1) were collected on the ID09 beamline of the ESRF, Grenoble, France, using a single line undulator. Five 'excited' data sets were collected, corresponding to delay times between laser excitation and x-ray probing from 3.2 ns to 3 ms. Mb-YQR CO liganded crystals were photolyzed with 630 nm, 2.2 ns pulses generated by a Nd:YAG laser. Diffraction data were processed in a fully automated way (2) after a single frame was indexed manually with Lauegen (3), significant diffraction was recorded up to 1.5-1.6 Å.

RESULTS: We used single-bunch Laue diffraction to study with nanosecond time resolution the conformational changes occurring in crystals of a triple mutant of sperm whale myoglobin (L29Y, H64Q, T67R; denoted "Mb-YQR") upon rupture of the Fe-CO bond by laser photolysis. Outstanding crystal quality, high level of photolysis, optimisation of the ESRF ID09 beamline and efficient data processing allowed to obtain complete data sets to 1.55 Å resolution from 3ns to 3ms after photolysis. As already observed for wild-type myoglobin, CO dissociation induces an immediate motion of the iron out of the heme plane as well as bending of the heme pyrrole ring C towards the distal pocket. However, a number of novel features were discovered. Immediately following dissociation, Y29 swings towards the CO binding location to fill the vacant space. Remarkably, the rotation of Q64 to establish a hydrogen bond with Y29 extends to the microsecond timescale, dragging the whole of helix E towards its position in the deoxy state of the protein. On this timescale, other significant motions of residues and water molecules are identified on the distal site whereas a transient, weak occupation of the "xenon 1" cavity is observed on the proximal site, presumably due to CO still trapped in the matrix. Our observation of asynchronous internal motions in Mb may be taken as the first direct evidence for the complex potential energy surface of a protein, as analysed by Frauenfelder et al. (4). The extended dynamics of the globin's conformational changes is in agreement with the idea that the protein populates different conformational substates. The time course of the 3D structural changes indicates that we have unveiled the conformational relaxation of the globin which may begin in the sub-ns time regime with bending of the heme, but extends over several orders of magnitude in time towards microsec, consistently with time-resolved spectroscopy (5, 6). These results significantly advance our understanding of the conformational relaxation dynamics of myoglobin, and provide the first structural evidence of their extended nature in time, as discovered in the past by time resolved spectroscopy. These results were published in PNAS (7) and considered as a breakthrough in the field by H. Frauenfelder et al. on a commentary paper (8).

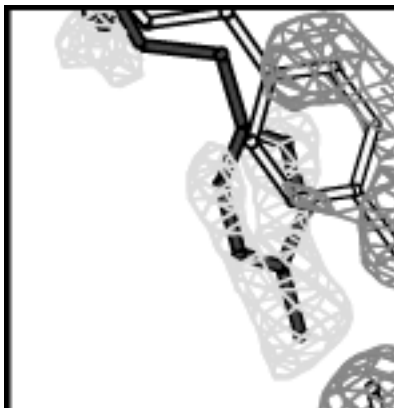

Fig. 1. Difference Fourier maps of the active site of Mb-YQR at 316 ns laser/X-ray pulse time delay. Negative density (dark grey) and positive density (light grey) of the difference map [Mb*–MbCO] are contoured at 3.5 σ.

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