Adding a fourth dimension to protein structures: intermediates, perturbations and movies.

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Determining directly the structure of functional intermediates is a task considered unattainable by crystallography, but recent developments have allowed achieve this goal to address questions about 3D structure and dynamics. Only in a few cases perturbations can be applied to protein crystals rapidly and homogeneously in order to obtain “molecular movies”. We used single-bunch Laue diffraction to study with ns time resolution the conformational changes 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 wt Mb, CO dissociation induces an immediate out of plane motion of the 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. The extended dynamics of the globin’s conformational changes is in agreement with the idea that the protein populates different conformational substates [1]. 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 μs, consistently with time-resolved spectroscopy [2]. These results 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. We are currently extending this approach by studying the rebinding structural dynamics of hemoglobin.

[1] Frauenfelder, H., Sligar, S.G. and Wolynes P.G. (1991) *Science* **254**,1598-1603.

[2] Bourgeois D, Vallone B, Schotte F, Arcovito A, Miele AE, Sciara G, Wulff M, Anfinrud P, Brunori M. (2003) Proc Natl Acad Sci U S A. **100**, 8704-9.