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Report:

Myoglobin is a small (18 kD) globular heme protein found in striated muscle cells. Its function is to increase a vertebrate's aerobic capacity by taking up molecular oxygen from blood during rest and delivering this dioxygen to mitochondria during muscle contraction when blood flow through capillaries is restricted. Its ferrous form can also bind carbon monoxide and nitric oxide, produced *in vivo* as messengers for regulating blood pressure, platelet aggregation, and neurotransmission. Renewed interest in this protein has been due to the expression of recombinant myoglobin in *Escherichia coli*, the development of ultrafast laser photolysis techniques to study myoglobin function, and the evolution of even more brilliant (synchrotrons) X-ray sources to quickly capture the structure of protein intermediate states via Laue diffraction.

Under laser photolysis, the myoglobin's heme iron - diatomic gas ligand bond is disrupted by an intense excitation pulse, producing free ligand and pentacoordinate heme inside the pocket of the protein. The ligand can then either rebind rapidly by an intramolecular process or migrate out into the solvent. Two kinetic phases for the geminate rebinding are seen in nanosecond time courses. Originally these two phases were assigned to ligands very close to the iron atom, i.e. contact pairs, and to ligands farther away in the protein matrix, respectively. Current thinking (Olson and Phillips,

1996) is that the first state represents a number of ligand positions in the distal pocket with differing rates of approach to the iron atom. The second nanosecond state approximates the rapid interconversion of the ligand between these different positions. These questions and more on the state and trajectory of the photolysed ligand await structural answers from X-ray diffraction studies.

Toward this end, Schlichting *et al.* (1994) published the crystal structure of photolysed carbonmonoxy myoglobin (Mb* CO) at 20 K which revealed the position of the non-covalently bound ligand frozen in a kinetic intermediate state. A similar study was reported by Teng *et al.* (1994) at 40 K. The importance of the Mb*CO structure has been summarized by Boxer and Anfinrud (1994). However, the question of whether this intermediate is authentic or an off-pathway artifact remains. Recently Srajer *et al.* (1996) published an X-ray Laue crystallographic investigation with nanosecond time resolution of the structural changes which occur in carbonmonoxy myoglobin after photolysis at room temperature. Their results revealed relaxation of the heme and protein in response to ligand photodissociation and proved that nanosecond time-resolved macromolecular crystallography is feasible.

By utilizing the single pulse mode and the brilliance of the European Synchrotrons Radiation Facility coupled with ultrafast flash photolysis, we were able to obtain Laue diffraction data of the carbon monoxide photodissociation from recombinant wild type myoglobin over a time period ranging from nanoseconds to milliseconds. These experiments should reveal the time-resolved structures of the carbonmonoxy wild type myoglobin photoproducts. We are currently indexing the data frames using new algorithms for integration and profile fitting of the diffraction intensities. Each of the 15 time points taken of the structure of Mb*CO will then be refined, taking into account the extent of photolysis, leaving us with a physical map over time of the ligand pathway after bond cleavage. The next steps should be the acquisition of more and different time points to obtain a smoother structural map for both wild type and kinetically interesting mutant myoglobin. This imaging lies just beyond traditional crystallography much like a video gives more information than a still picture.