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Time-resolved Crystallographic Studies of a Cooperative Dimeric Hemoglobin. W.E. Royer¹, J. E. Knapp¹, R. Pahl², V. Srajer², ¹Dept. of Biochemistry and Mol. Pharm., Univ. of Massachusetts Med. School, Worcester, MA 01605, ²Dept. of Biochemistry and Mol. Biol. and CARS, Univ. of Chicago, Chicago, IL 60637.

Despite the availability of static structures of different states in a number of allosteric proteins, information about the kinetic pathway between such alternate states is limited. We have carried out nanosecond time-resolved diffraction experiments on single crystals of *Scapharca* dimeric hemoglobin, a protein whose alternate states show strong functional differences, despite relatively localized transitions that are compatible with the crystal lattice. Within 5ns of the photolytic release of ligands, an intermediate forms as R-state protein subunits respond to the presence of unliganded heme groups. Transition to this intermediate involves structural changes in the heme groups, neighboring residues and interface water molecules. The intermediate changes very little during the ns time-domain and lays a foundation for apparently concerted tertiary and quaternary structural changes that occur on a microsecond time scale and are associated with the transition to a low affinity T-state structure. Persistence of a T-state structure even after ligands rebind suggests a slow T to R transition that may result from the greater dimeric stability in the T-state.