Supporting Information

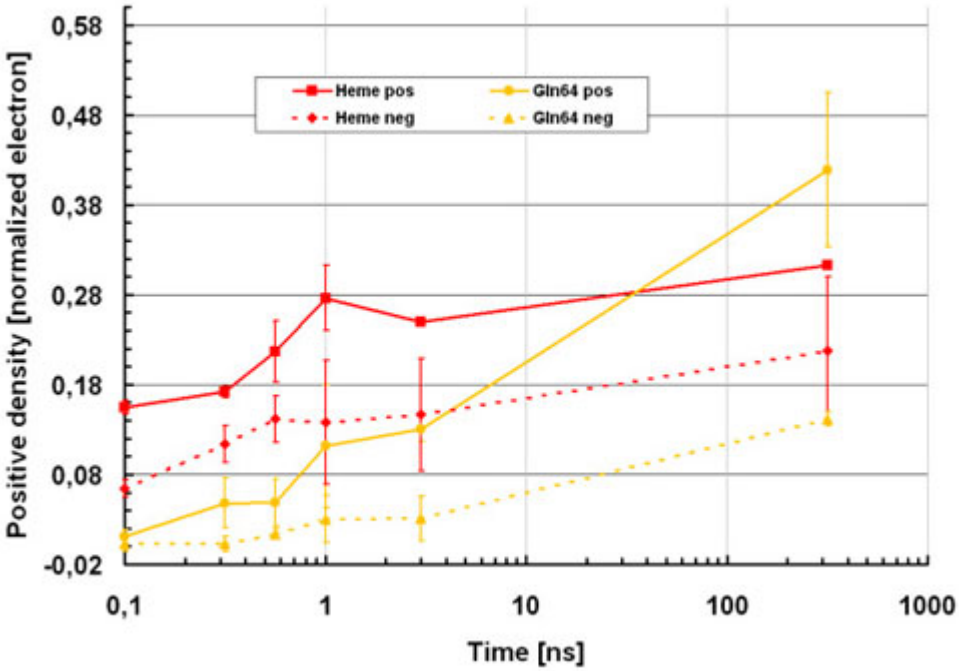

Fig. 4. Time dependence of difference electron density for representative structural features. The numerical values reflect the integral of the electron density beyond ± 3.0 σ and are corrected for variations in photolysis yield. They are normalized and averaged as in Fig. 2. Representative comparison of negative and positive difference electron densities are shown for the heme and Gln-64 features. For the vast majority of features, positive density significantly exceeds negative density, which implies that the photo-excited structures are more ordered than the resting, dark structures. The evolution of positive and negative densities over time displays comparable trends, which is expected in the absence of laser-induced crystal disorder.

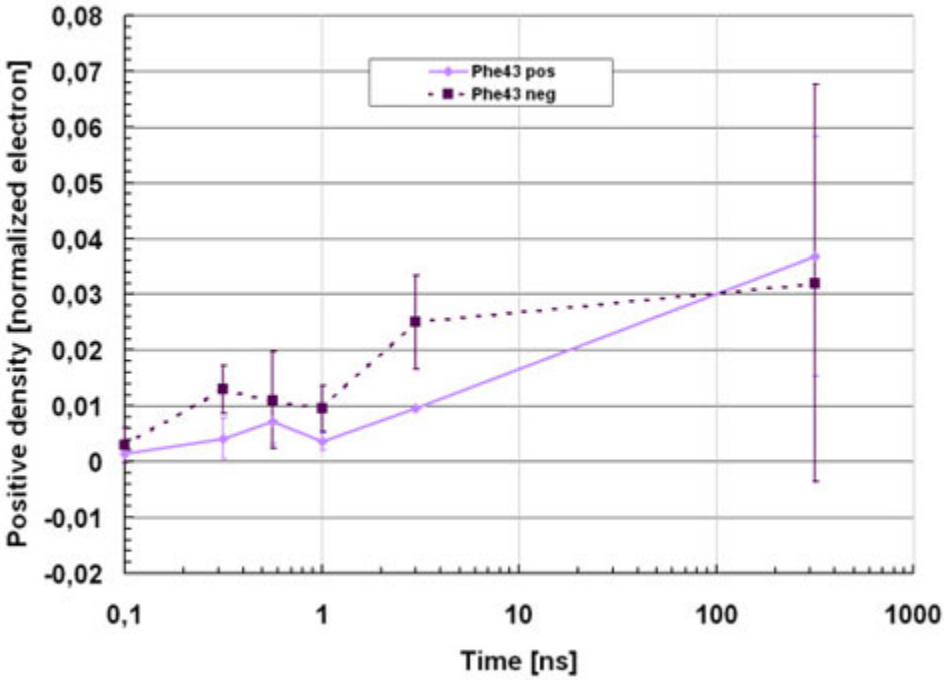

Fig. 5. Time dependence of the difference electron density for Phe-43(CD1). The numerical values were obtained as in Figs. 2 and 4. In contrast with the rest of the protein matrix, the Phe-43(CD1) negative density exceeds positive density, which implies that the photo-excited structure is locally less ordered than the resting structure.

Table 2. Deoxy Mb structure and conformation of Phe-46(CD4) in 11 variants

Deoxy-Mb variant	C_{α}E7-Fe distance, Å	F46 conformation	Protein Data Bank code
YQR	8.0	YQR-Mb	1F63
F46V *	8.22	wt-Mb	1MTJ
H64Q	8.26	YQR-Mb	2MGG
H64T	8.28	YQR-Mb	1MOD
V68A	8.38	wt-Mb	1MLG
H64G	8.41	wt-Mb	1MOB
H64L	8.41	wt-Mb	2MGD
V68F	8.42	wt-Mb	1MLK
V68L	8.50	wt-Mb	1MLR
wt	8.51	wt-Mb	2MGL
L29F	8.55	wt-Mb	1MOA

Shown is how the distance between the Cα of residue at position E7 and the heme iron can be used as diagnostic of the conformation of Phe-46, which can be like either wt-Mb or YQR-Mb (see text).

*In the case of F46V the diagnostic distance is <8.3 Å, which is attributed to the absence of a bulky residue in position CD4.