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Reply to 'Contradictions in X-ray structures of intermediates in the photocycle of photoactive yellow protein'

Y. O. Jung[1,2], **J. H. Lee**[2], **J. Kim**[2], **M. Schmidt**[3], **K. Moffat**[4,5], **V. Srajer**[5], and **H. Ihee**[1,2,*]

[1]Center for Nanomaterials and Chemical Reactions, Institute for Basic Science, Daejeon, 305-701, Republic of Korea

[2]Center for Time-Resolved Diffraction, Department of Chemistry, KAIST, Daejeon, 305-701, Republic of Korea

[3]Physics Department, University of Wisconsin–Milwaukee, Milwaukee, Wisconsin 53201, USA

[4]Department of Biochemistry and Molecular Biology, and Institute for Biophysical Dynamics, The University of Chicago, 929 East 57th Street, Chicago, Illinois 60637, USA

[5]Center for Advanced Radiation Sources, The University of Chicago, 5620 South Ellis Avenue, Chicago, Illinois 60637, USA

We recently reported picosecond time-resolved crystallographic investigations on photoactive yellow protein[1]. Now Schotte and colleagues[2] challenge the structural interpretation of our results based on their work[3]. In particular, they disagree with structural details of our earliest intermediate I_T and the next intermediate I_{CT} based on their density functional theory (DFT) calculations. We stand by our results because, for both intermediates, the time-resolved X-ray data and the experimental electron densities favor the structures that we reported over the structures derived from DFT.

Our study[1] and that by Schotte *et al.* [2] used the same experimental technique but differ in that (1) in our work we also studied the E46Q mutant as well as the wild-type (WT) protein, (2) the crystals were grown under quite different conditions, and (3) the X-ray data quality and crystallographic completeness differ.

Figure 1 shows the dependence of the R factor on three dihedral angles. Whereas I_T is located at the minimum R factor, both the DFT structure (I_T^{DFT}) and their earliest structure of Schotte *et al.* [2] (pR_0), which supposedly corresponds to I_T, are far from the minimum. Notably, in `Structure Refinement' section of the Supporting Information of their paper, they report that pR_0 also tends to adopt a C_1'–C_3'–C_2'–C_1 dihedral angle close to 90° as found in I_T when the structure was not restrained to resemble I_T^{DFT}. Thus, the refined dihedral angle varies depending on whether the structure is restrained to mimic I_T^{DFT} (their approach) or is allowed to follow the experimental electron density (our approach). Our approach was to compare the qualitative features of I_T^{DFT} and I_T instead of using I_T^{DFT} as the structural restraints. Although I_T obtained without such restraints has a different dihedral angle than that of I_T^{DFT}, we were content with the fact that I_T^{DFT} also supports a non-planar structure

*Corresponding author. hyotcherl.ihee@kaist.ac.kr.

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consistent with I_T. We believed that forcing the structural refinement of the experimental density to meet the restraints from the DFT structure removes the possibility that the experimental data could provide any new information other than the boundary given by DFT. Strictly speaking, no new information was obtained in their study, and such an approach would always yield only those structures compatible with DFT even when new experimental data with better temporal and spatial resolution become available from experiments at X-ray free electron lasers. The possibility of interactions that stabilize such a highly strained dihedral angle but are not fully accounted for by DFT cannot be ruled out. In addition, because DFT is a single-reference based method, DFT may not well describe such a highly distorted structure of the chromophore whose multiconfigurational character might be strong. It should also be noted that I_T is observed in both E46Q and WT with a dihedral angle $C_1'–C_3'–C_2'–C_1$ that is smaller in E46Q by ~15°.

Regarding the choice between I_{CT} and I_{CP}: our maps in the present study are consistent with those Ihee et al. reported earlier[4]. In that earlier work, due to the limited time resolution, Ihee et al. used only a single structure, I_{CP}, to fit the maps at the time delays on nanosecond time scales. This fit was only partly satisfactory and left some residual density. Further, the single I_{CP} structure does not have the minimum R-factor. Our work in ref. 1 explains these observations: the maps in both the present and the earlier studies are structurally heterogeneous and contain I_{CT} and pR_1. Schotte et al.[2] explain this residual, non-planar density differently: they assume an equilibrium between the first and second intermediates, but this kinetic scenario gives a worse fit to our experimental densities for both WT and E46Q. Moreover, such an equilibrium is highly unlikely because, at early times, the chromophore is highly strained and the reactions is likely to proceed strongly downhill.

Although Schotte et al. [2] direct their major attention to the detailed structural features of the early intermediates, a more serious discrepancy between us is found even on the well-established microsecond time range. They identify[2] only one structural species (pR_2) whereas our study[11] and others[4,5] reported that two species (pR_1 and pR_2) co-exist. It is not clear yet whether this discrepancy arises from the experimental conditions, or from data analysis and interpretation. Our conditions of lower salt (50 mM NaCl) and neutral pH (pH 7.0) have been extensively used for earlier time-resolved X-ray crystallographic investigations of PYP. In contrast, Schotte et al. used crystals grown in high salt (1.1 M NaCl) and D_2O (pD 9.0), although their ammonium sulfate concentration (~2.5 M) was close to ours (~2.6 M). Because salt and pH may well affect the structure and dynamics[6–8], the exact origin of these discrepancies remains to be studied.

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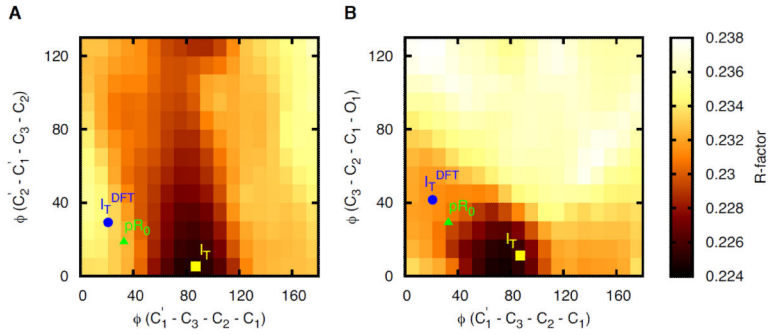

Figure 1.

The dependence of the R factor on the three dihedral angles (A: C_2'–C_1'–C_3'–C_2 vs C_1'–C_3'–C_2'–C_1 and B: C_3'–C_2'–C_1'–O_1 vs C_1'–C_3'–C_2'–C_1). Whereas I_T is located at the minimum R factor, I_T^{DFT} and pR_0 are far from the minimum. The same situation is found also for I_{CT} vs I_{CP} (data not shown).

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