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Updated structure of *Drosophila* cryptochrome

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We report an improved model of the *Drosophila* cryptochrome structure that corrects errors in the original coordinates (3TVS.pdb). Further refinement of the structure with automated rebuilding algorithms in PHENIX¹ followed by manual building, indicated that a model of dCRY could be produced with excellent refinement statistics without taking into account the non-merohedral twinning originally reported (Table 1). The rebuilt structure has an RMSD on Ca positions of 2.4 Å compared to the deposited coordinates with most differences found in the conformation of surface loops (Fig. 1). However, the new analysis also indicates that the sequence register of the C-terminal tail helix (CTT) is displaced by two residues (Fig. 2). This change in sequence register offsets the invariant FFW motif along the helix axis such that Phe534, and not Trp536, approaches closest to the flavin ring (Fig. 3). In the new model, the three residues composing the FFW motif continue to make extensive interactions with the photolyase homology domain. This new position of the FFW motif is more consistent with the cellular data of Fig. S6, which shows that substitution of FFW to three alanine residues has a dramatic effect on dCRY stability, but that the W536A substitution alone, does not. The configuration of the flavin center is similar between the old and new models, with the largest difference in the angle of the ribityl-to-flavin (N10) bond (Fig. 4). Phosphorylation of Thr518 is not apparent in the new electron density maps despite identification of this modification by mass spectrometry. The errors in the original structure stemmed from model bias introduced during the detwinning procedure. Lower resolution data sets to which the original dCRY structure was built appear to suffer more from twinning than the 2.3 Å resolution data that the final model was refined against. Although the high-resolution data does contain indications of non-merohedral twinning, including intensity oscillations along the reciprocal space *l* axis and spurious Patterson peaks, a model that agrees well with the diffraction data as collected can be produced without compensation for these effects (Table 1). The new coordinates have been deposited in the PDB as 4GU5. B.R.C. apologizes for these errors.

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The new atomic coordinates have been deposited in the Protein Data Bank as 4GU5.

Author Contributions The New York authors (B.D.Z., A.T.V., D.T., J.W., M.W.Y. and B.R.C.) carried out the work presented in ref. 1; the Manchester authors (C.L., A.R.J., N.S.S. and D.L.) identified errors in the original coordinates; together, both sets of authors carried out the further refinement of the structure presented here. C.L. initially identified errors in the original coordinates, and C.L. and D.L. performed initial model rebuilding and crystallographic analysis, which was verified and discussed with A.R.J. and N.S.S. The New York authors B.D.Z. and B.R.C. completed and verified the model rebuilding and crystallographic analysis. The New York authors A.T.V., D.T., J.W. and M.W.Y. contributed to structural analysis and verified the new structure against existing biochemical and biological data. B.R.C. and B.D.Z. wrote the communication with input from all other authors.

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Figure 1. Superposition of Ca traces for 3TVS (yellow) and rebuilt dCry structures (cyan)
Both subunits within the asymmetric unit are depicted. Flavin cofactors are shown in orange.

Figure 2. Sequence shift in the CTT

Left: Superposition of C-terminal tail helix (CTT) from 3TVS (yellow) and rebuilt structure (pink). Sequence register in CTT of rebuilt structure is displaced two residues toward the C-terminus compared to 3TVS. Right: Electron density for the CTT using phases derived from the new model. The $F_{\text{obs}} - F_{\text{calc}}$ omit map electron density is contoured at 3 σ .

Figure 3. Flavin center of dCRY

Superposition of key residues in dCry flavin center between 3TVS (yellow) and rebuilt structure (pink). Position of Trp536 and linkage between flavin ring and ribityl chain differs in rebuilt structure compared to 3TVS.

Figure 4. dCRY Trp-triad photoreduction pathway

Arrangement of Cry Trp residues in 3TVS (yellow) and rebuilt structure (pink). Trp536 is replaced by Phe534 in the rebuilt structure. Trp536 is now separated from the flavin ring by the imidazole side chain of His378 (see Figure 3).

Table 1

Revised data collection and PHENIX refinement statistics

	Native	
Data collection	0.97918	
Space group	P2 ₁	
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	72.8, 112.3, 75.0	
<i>α</i> , <i>β</i> , <i>γ</i>	90.0, 114.9, 90.0	
Resolution (Å)	30 - 2.30 (2.38 - 2.30) *	
<i>R</i> _{sym}	0.126 (0.354)	
<i>I</i> / <i>σ</i> (<i>I</i>)	11.9 (1.8)	
Completeness (%)	90.1 (57.3)	
Redundancy	3.9	
Refinement	3TVS (Detwinned / Native)	Rebuilt (Detwinned / Native)
Resolution (Å)	30-2.3 Å (2.30-2.38) *	30-2.3 Å (2.30-2.38) *
No. reflections	47507	47507
<i>R</i> _{work} %	25.3 (30.0) / 34.2 (43.9)	22.2 (24.4) / 18.4 (24.2)
<i>R</i> _{free} %	29.8 (35.8) / 40.5 (49.3)	26.0 (34.2) / 24.7 (32.1)
Protein	8668	8653
Solvent	301	321
Ligand (FAD / Mg)	106 / 2	106 / 2
B-factors (Å ²)		
Protein	38.1	32.2
Ligand (FAD / Mg)	10.0	18.1
Water	28.6	30.7
R.m.s deviations		
Bond lengths (Å)	0.01	0.008
Bond angles (°)	1.8	1.1

* Highest resolution bin for compiling statistics.