Supporting Information

Glas et al. 10.1073/pnas.0812665106

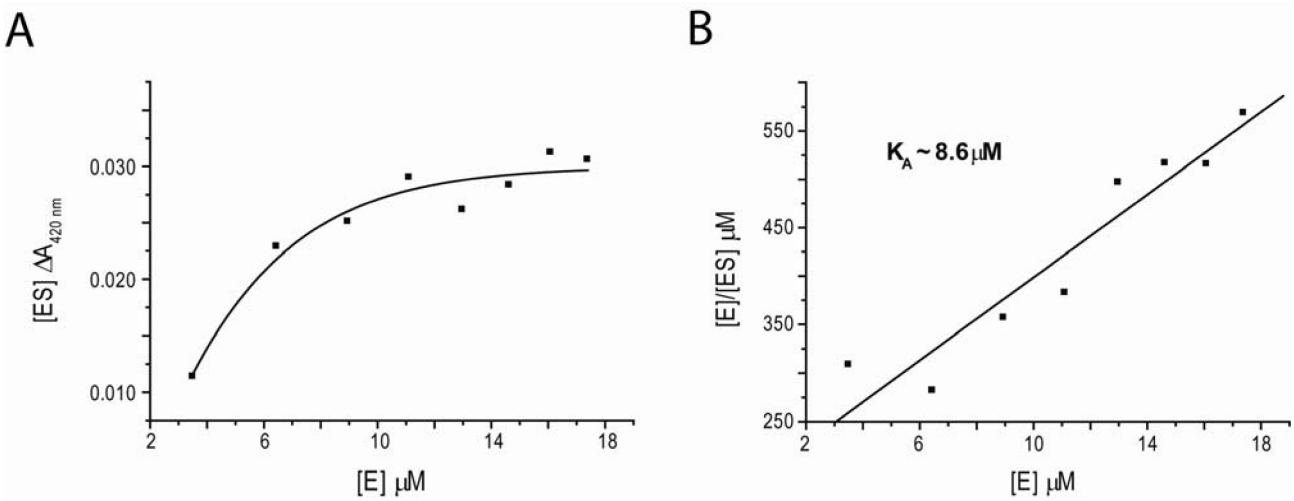

Fig. S1. Binding constant of F_0 to the (6-4) photolyase. (*A*) Graphical analysis of the binding data. (*B*) Hanes plot analysis of the binding event. K_a for F_0 binding to photolyase was estimated by titrating 25 μL of 40 μM F_0 with aliquots of 0.1 eq of (6-4) photolyase. The binding event was obtained by the total spectral difference at $\lambda = 420$ nm ($\Delta A_{420\ nm}$) after adding aliquots of (6-4) photolyase and waiting for 1 h for equilibration.

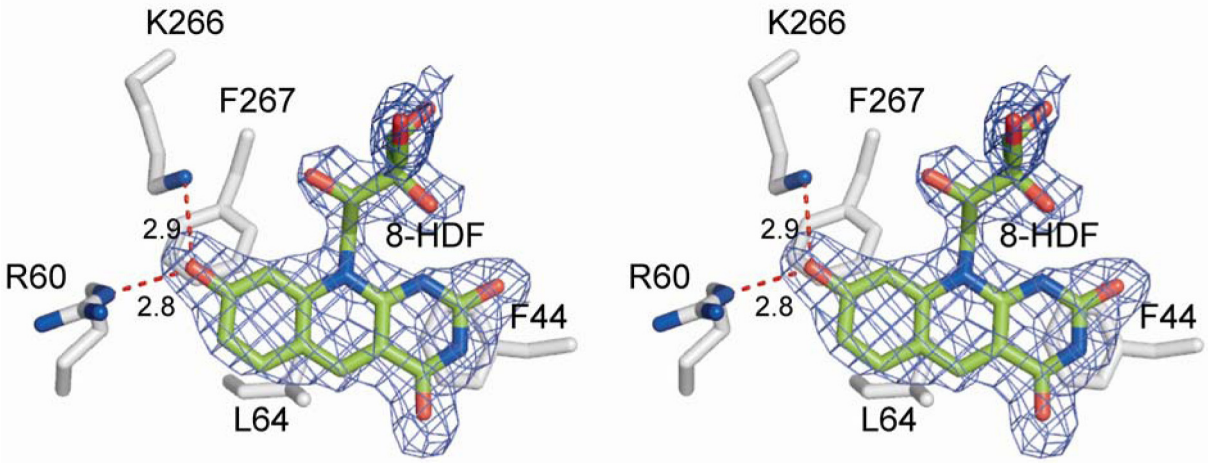

Fig. S2. Stereofigure of the F_0-binding site. $F_o - DF_c$ electron density [Emsley P, Cowtan K (2004) *Acta Crystallogr D Biol Crystallogr* 60:2126–2132; Read RJ (1986) *Acta Crystallogr A* 42:140–149] for the F_0 cofactor calculated before the incorporation of F_0 in the model, contoured at 2σ.

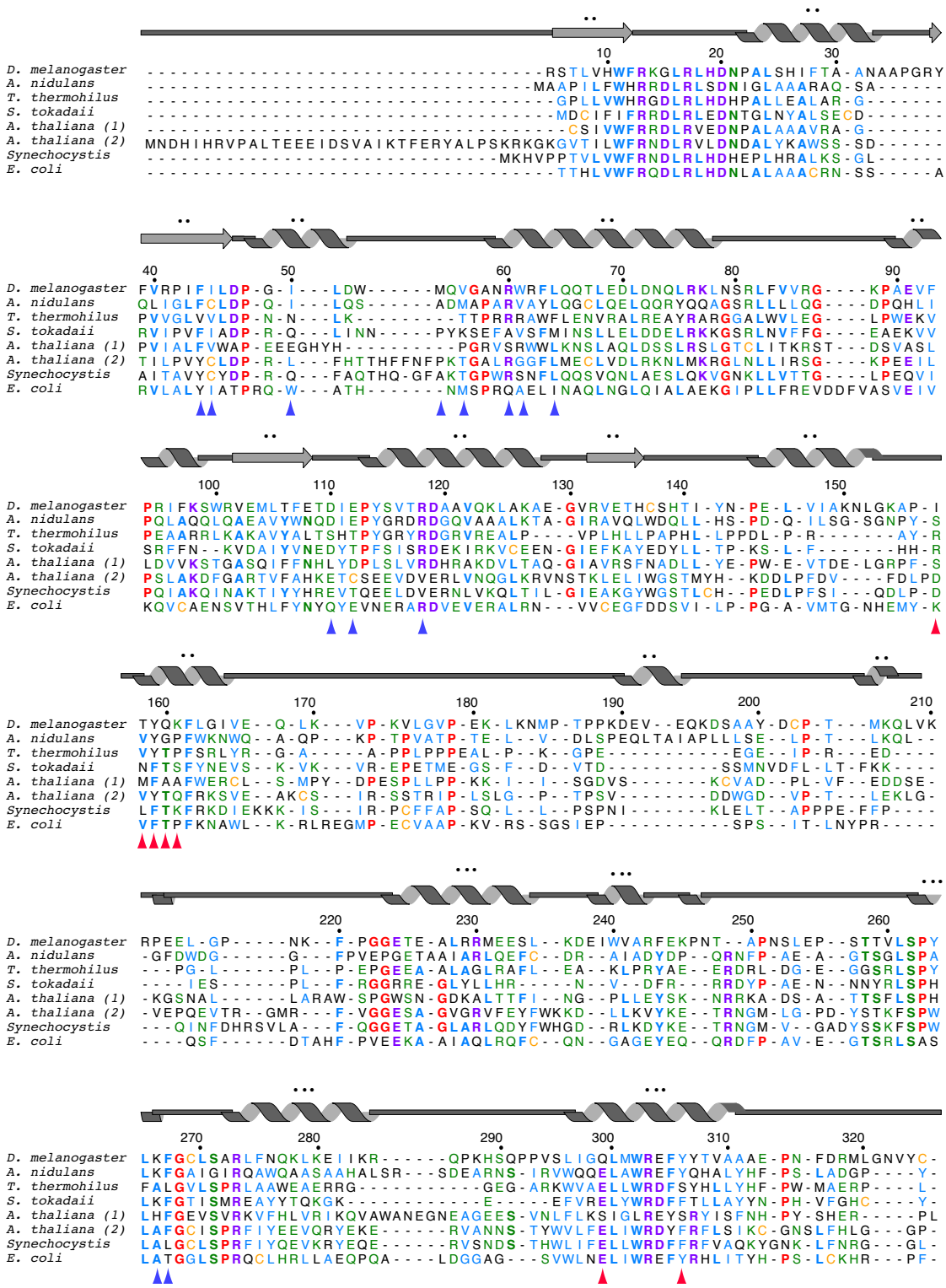

Fig. S3. Structure-based sequence alignment of the (6-4) photolyase of *D. melanogaster* with homologous structures. Refer to Table S2 for the PDB ID codes, values of sequence identity, and rmsd {with numbers of aligned residues obtained from secondary structure matching [Krissinel E, Henrick K (2004) *Acta Crystallogr D Biol Crystallogr* 60:2256–2268]} carried out with COOT [Emsley P, Cowtan K (2004) *Acta Crystallogr D Biol Crystallogr* 60:2126–2132]. Numbering and secondary structure on top of the alignment correspond to the *D. melanogaster* (6-4) photolyase. The alignment was annotated by using a program by T. Stevens [Stevens TJ, Paoli M (2008) *Proteins* 70:378–387]. Residues involved in DNA binding and/or important for enzymatic activity are highlighted with red arrowheads. Residues of the cofactor-binding pocket are marked with blue arrowheads. No. 1: *A. thaliana* cryptochrome PDB ID code 1U3C [Brautigam CA, et al. (2004) *Proc Natl Acad Sci USA* 101:12142–12147], no. 2: *A. thaliana* cryptochrome PDB ID code 2IJG [Brudler R, et al. (2003) *Mol Cell* 11:59–67].

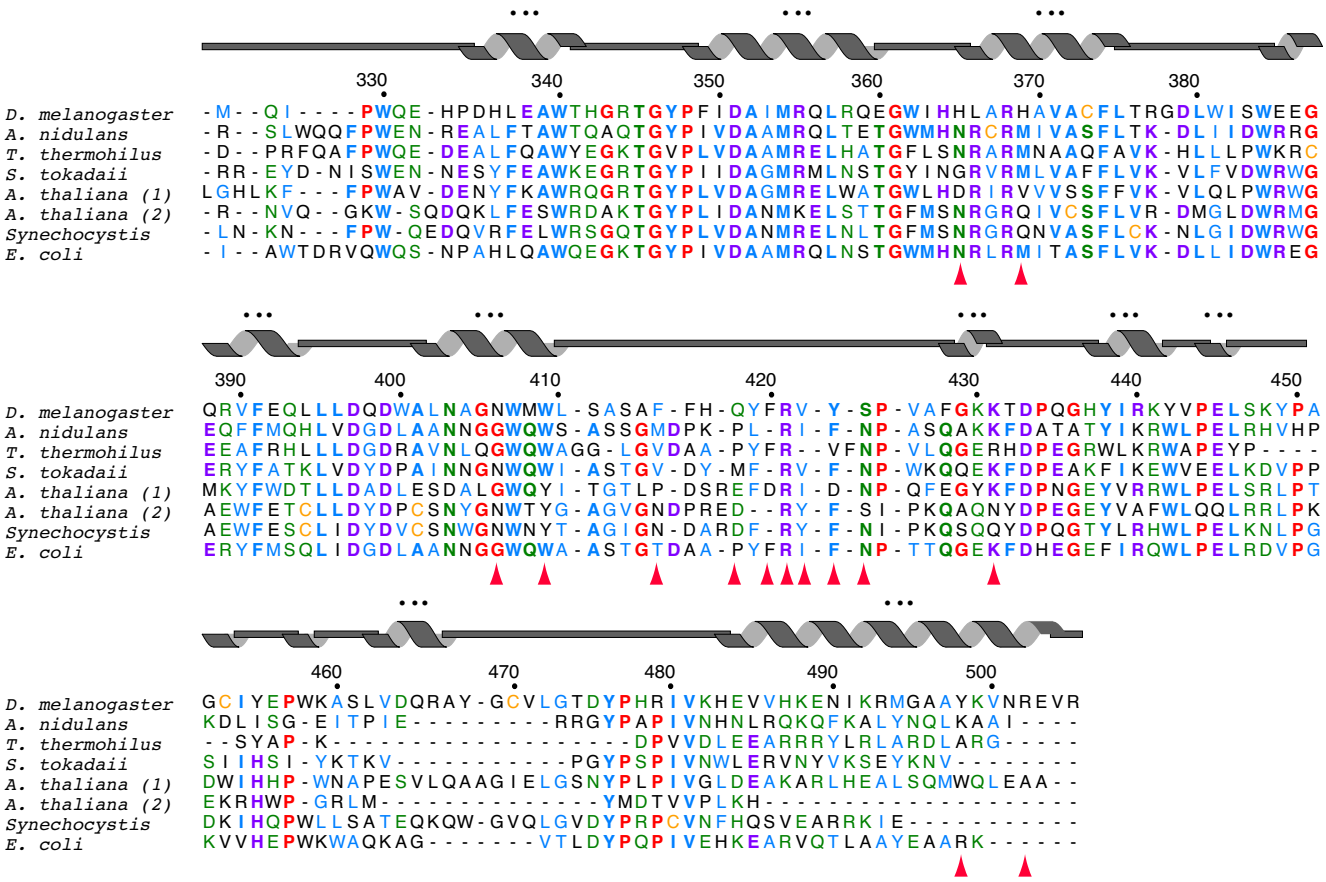

Fig. S3. Continued.

Fig. S4. Multiple-sequence alignment of F_0 synthases. CofG and CofH homologues share ≈25% sequence identity, including conserved residues within the radical SAM motif and the F_0 synthase subunit. CofG and CofH are homologous to the N-terminal and C-terminal halves of the *Mycobacterium bovis* FbiC, respectively. *M. bovis* FbiC and the *O. tauri* putative F_0 synthase (CAL57234.1) share ≈51.6% consensus and 39.4% identity positions. Highly conserved residues are shown in green, and similarly conserved residues are shown in yellow. Sequences were aligned by using ClustalX [Thompson JD, Higgins DG, Gibson TJ (1994) *Nucleic Acids Res* 22:4673–4680] and annotated with VectorNTI (Invitrogen).

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            M. bovis FbiC  (779) GR-ISHIQTSWVKLGVRRTQVMLEGGANDLGGTLMEETISRMAGSEHGSAKTVAELVAIAEGIGRPARQRTTTYA
O. tauri putative protein  (698) PIGITNIQASWVKMGPEFAAHLLHAGCNDMGGTLNNESITRAAGAQFGQEIDAAAMRRIIENAGRISRQRTTLYA
        S. elongatus CofG  (316) ----------------------------------------------------------------------------
        S. elongatus CofH  (314) QW-IVNHQPSWVKLGLRGATMALNWGCNDLGGTLMEEHITSVAGAQGGTGVSPEDLVAAIHSLGRTPQQRTTLYN
          S. sviceus FbiC  (772) PY-IPNIQTSWVKLGTEGAAEMLRSGANDLGGTLMEETISRMAGSSYGGSYKSVKDLIAVAEAAGRPARPRTTLYG
          S. tropica FbiC  (783) GR-VHNIQCSWVKLGDEGSAELLRGGCNDLGGTLMEETISRMAGSGNGGSARTEEQLIAIAAAAGRPANKRTTAYG
                Consensus  (826)    I NIQ SWVKLG EGAA LL GGCNDLGGTLMEETISRMAGS  GSAKS  DLIAIAEAAGRPARQRTTLYA
                           901                   925
            M. bovis FbiC  (853) LLAA--------------------
O. tauri putative protein  (773) DAPSDRVPISR--------------
        S. elongatus CofG  (316) -------------------------
        S. elongatus CofH  (388) PVGERQ-------------------
          S. sviceus FbiC  (846) EVPQERQKAARVSDGHLPELLPVLD
          S. tropica FbiC  (857) HRVG--------------------
                Consensus  (901)  V
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Fig. S4. Continued.

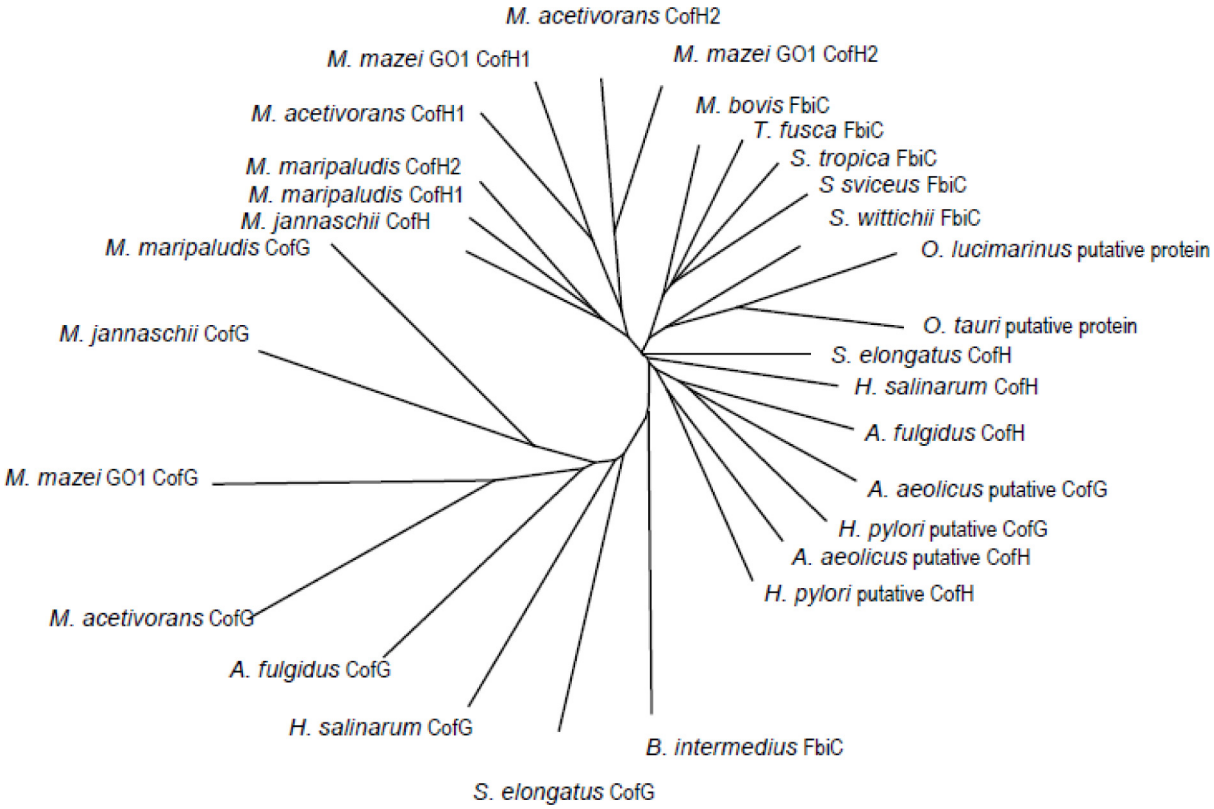

Fig. S5. Phylogeny of CofG/CofH and their bifunctional FbiC homologues. CofG and CofH are widely distributed among organisms known to produce F_{420} and/or F_0. The *cofG* and *cofH* genes appear to have been descended from a cyanobacterial F_0 synthase and vertically inherited in euryarchaeal, cyanobacterial, and actinomycetal lineages. Several bacteria and archaea have 2 *cofH* homologues that most likely evolved by gene duplication. Both *cofG* and *cofH* must be fused in the actinomycete lineage [Graham DE, Xu H, White RH (2003) *Arch Microbiol* 180:455–464]. Compared F_0 synthases are from *Aquifex aeolicus* (gb|AAC06943 and gb|AAC06853), *Archaeoglobus fulgidus* (sp|O29461.1 and sp|O29460.2), *Bacillus intermedius* (emb|CAA66710.1), *Halobacterium salinarum* (sp|Q9HNU9.1 and sp|Q9HNU8.2), *Helicobacter pylori* (gb|AAD06182.1 and gb|AAD06180.1), *Methanocaldococcus jannaschii* (sp|Q57888 and sp|Q58826), *Methanococcus maripaludis* (sp|Q6LYV9.1, sp|Q6M161.1 and sp|Q6M160.1), *Methanosarcina acetivorans* (sp|Q8TQP9.1, sp|Q8TQQ0.1 and sp|Q8TQQ1.2), *Methanosarcina mazei* (sp|Q8PU55.1, sp|Q8PU54.1 and sp|Q8PU53.2), *Mycobacterium bovis* (sp|Q7U0G9.1), *Ostreococcus lucimarinus* (gb|ABO99077.1), *O. tauri* (emb|CAL57234.1), *Salinaspora tropica* (ref|YP_001157801.1), *Sphingomonas wittichii* (ref|YP_001261222.1), *Streptomyces sviceus* (ref|YP_002209454.1), *Synechococcus elongatus* (sp|Q8DIR6.1 and sp|Q8DII8.1), and *Thermobifida fusca* (ref|YP_290575.1). Phylogenetic tree was calculated with ClustalX [Thompson JD, Higgins DG, Gibson TJ (1994) *Nucleic Acids Res* 22:4673–4680] and annotated by using TreeView [Page RD (2002) *Curr Protoc Bioinformatics* Chapter 6:Unit 6.2].

Table S1. Data collection, processing, and structure refinement statistics

	6-4 photolyase, DNA lesion, F_0
Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c*, Å	86.4, 88.7, 90.4
a, b, g, °	90, 90, 90
Wavelength, nm	0.97925
Resolution, Å	40.0–2.1 (2.15–2.1)
R_{merge}	0.082 (0.362)
Mean $I/\sigma I$	15.2 (5.0)
Completeness, %	97.0 (93.0)
Redundancy	5.2
Refinement	
Resolution, Å	40.0–2.1
No. of reflections (unique)	39,271 (4,836)
R_{work}/R_{free}	0.166/0.213
No. of atoms	5,435
Protein	4,259
DNA/FAD/F_0	53/609/26
Waters	468/16
B-factors	
Protein	34.4
DNA/FAD/F_0	27.3/46.2/28.2
Waters	44.6/52.9
rmsd	
Bond lengths, Å	0.009
Bond angles, °	0.1393

Table S2. Comparison of the (6-4) photolyase of *D. melanogaster* with homologous structures

PDB ID code	Species	Function	rmsd, Å	Sequence ID, %	Residues used in alignment/total no. of residues	Cofactor
1QNF	*A. nidulans*	CPD photolyase	2.1	29.5	430/475	FAD/F_0
1U3C	*A. thaliana*	Cryptochrome	1.7	26.1	414/485	FAD/−
2IJG	*A. thaliana*	CPD photolyase	1.98	25.4	394/492	FAD/MTHF
1DNP	*E. coli*	CPD photolyase	1.97	28.8	416/470	FAD/MTHF
2EI0	*S. tokadaii*	CPD photolyase	1.9	29.1	399/428	FAD/FAD
1NP7	*Synechocystis*	Cryptochrome	3.4	28.7	421/483	FAD/MTHF
2J07	*T. thermophilis*	CPD photolyase	1.8	27.0	381/419	FAD/F_0

Comparison of the (6-4) photolyase of *D. melanogaster* with homologous structures, as identified by Blast search [Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) *J Mol Biol* 215:403–410] of the protein database. The table lists the PDB ID codes, percentage of sequence identity, rmsd, and the respective numbers of aligned residues obtained from secondary structure matching [Krissinel E, Henrick K (2004) *Acta Crystallogr D Biol Crystallogr* 60:2256–2268] carried out with COOT [Emsley P, Cowtan K (2004) *Acta Crystallogr D Biol Crystallogr* 60:2126–2132].