Supplementary Text S1. **Plasmid construction for AtCRY-DASH production**

A double-stranded DNA fragment, containing 5’ and 3’ adapter sequences and a codon-optimized *Arabidopsis thaliana* cry3 gene (GenBank No. NP_568461 and Uniprot No. Q84KJ5) that encodes its 1–526\textsuperscript{th} amino acids (1610 bp, the supplementary Figure S6), was purchased from Genewiz. The fragment and the pET28a plasmid linearized by a treatment with *Nde*I and *Xho*I restriction enzymes were assembled by using the In-fusion HD cloning kit (Takara) according to the manufacture instruction. Stellar competent cells (Clontech) were transformed with the reaction mixture, and the obtained plasmid encoding the *Arabidopsis theriana* CRY-DASH (*AtCRYD*) gene (pET28a-*AtCRYD*) was sequenced and used for the following experiment.

The triple mutation of *AtCRYD*, where Arg487, Glu488, and Asp489 (see below) were replaced with Lys, Pro, and Leu, respectively, was introduced using QuikChange Site-Directed Mutagenesis kit (Agilent Technology). A set of primers, d(CTGGACCTACGTCGAGGTGTTGGAAATGACCCTAAGCCACTACGTTATTTCCTG ATCCCCAAAACAAGCA) and d(TGCTTGTTTTGGGATCGAGAAATAACGTAGTGGCTTAGGGTCATTTCCAACCT GCACCGTAGTGCAG), where the underlined sequences correspond to the triple mutation of R487K/E488P/D489L, were used for the polymerase chain reaction (PCR) using the pET28a-*AtCRYD* plasmid as a template. After the PCR, an aliquot of the mixture was treated with *Dpn*I, and the Stellar competent cells were transformed with the mixture. The obtained plasmid (pET28a-*AtCRYD*-RED-KPL) was sequenced and used for the following experiments.

Here, we need to make a caution about the numbering of the amino acid residues for *AtCRYD*. In the main text, the *AtCRYD* numbering was synchronized with that in the previous literatures for readability. However, we found that the *AtCRYD* numbering in the previous literatures are NOT identical to the current genetic data in GenBank and Uniprot. Although we describe the triple mutant as R443K/E444P/D445L in the main text, in fact it doesn’t match with the latest information, which is the R487K/E488P/D489L numbering as above-mentioned. To adapt the *AtCRYD* numbering with the latest information, all the numbers for *AtCRYD* in the main text should be shifted by adding 44.

Supplementary Text S2. **Protein purification**

The N-terminal His-tagged wild-type *AtCRYD* and its triple mutant were produced according to the previous report [1], with a modification. BL21 Star (DE3) competent cells (Invitrogen) were transformed with either pET28a-*AtCRYD* or pET28a-*AtCRYD*-RED-KPL, and the transformants were selected with Luria-Broth (LB) agar plates containing 20 µg/mL kanamycin. A single colony was inoculated into 40 mL of a LB medium containing the antibiotic, and the culture was shaken at 37°C for overnight. The overnight culture was inoculated into 2 L of a LB medium containing the antibiotic, and the culture was shaken at
37°C until turbidity detected at 600 nm came up to 0.7. Isopropyl β-D-1-thiogalactopyranoside was added at a final concentration of 10 µM, and the culture was further shaken at 25°C for 24 h. The cells were harvested by centrifugation (5000 × g) at 4°C for 15 min, and the supernatant was discarded. The cell pellet was suspended in a cold lysis buffer (30 mM, 50 mM NaH₂PO₄, 300 mM NaCl, 10 mM 2-mercaptoethanol, 5 mM imidazole, pH 7.5 adjusted by KOH, 0.5% Triton X-100, and 10% glycerol plus 50 mg lysozyme), and the cells were lysed by sonication. The mixture was centrifuged (20000 × g) at 4°C for 1 h, and the supernatant was passed through a column filled with 3 mL of Ni Sepharose 6 fast flow resin (GE Healthcare) equilibrated with the lysis buffer. Then, 3 column volumes (CV) of a wash buffer (50 mM NaH₂PO₄, 500 mM NaCl, 10 mM 2-mercaptoethanol, 10 mM imidazole, pH 7.5 adjusted by KOH, 10% glycerol) was applied to the column, followed by 3 CV of an elution buffer (50 mM NaH₂PO₄, 500 mM NaCl, 10 mM 2-mercaptoethanol, 500 mM imidazole, pH 7.5 adjusted by KOH, 10% glycerol). The green-colored fractions were pooled and concentrated with the Amicon Ultra-4 filter unit (MWCO 30000, Merck). The final purification of the protein was achieved by size exclusion chromatography using a Superdex 200 Increase 10/300 GL column (GE Healthcare) and a purification buffer (50 mM sodium phosphate, 100 mM NaCl, 10 mM 2-mercaptoethanol, 10% glycerol, pH 7.5). Small aliquots of fractions in the purification were sampled and analyzed by 10% SDS-PAGE, in which the band was visualized by Coomassie staining (the supplementary Figure S6). The protein concentration was quantified with the Bradford assay. Typically, ~1 mg of protein was obtained per a liter of culture.

Supplementary Text S3. DNA repair assay

A qualitative DNA repair assay was performed according to the previous literature [2]. Briefly, a protein solution (95 µL, 2 µM protein, 5 mM fresh dithiothreitol, 50 mM phosphate, 10 mM 2-mercaptoethanol, 10% glycerol, pH 7.5) prepared in a 1.5 mL plastic tube was put on ice holding the lid open, and the mixture was illuminated with fluorescent lamps (15W × 4) at a distance of 20 cm for 30 min, to photoreduce the enzymes. A solution (5 µL) of either a single-stranded 14-mer oligonucleotide (1 nmol), d(ATCGGCTTCGCGCA), where the underlined TT represents the CPD, or a double-stranded form of the 14-mer oligonucleotide hybridized with its complementary strand, were added to the photoreduced solution. The DNA-protein mixture was illuminated again with the same setup for 10 min. Then, protein was removed by phenol/chloroform extraction, and the oligonucleotides were collected by ethanol precipitation. The recovered oligonucleotides were analyzed by a Gilson gradient-type analytical HPLC system equipped with a Waters 2998 photodiode array detector. Separation was achieved on a µBondapak C18 5 µm 300 Å column heated at 50°C, with a linear gradient of 5–13% acetonitrile in 0.1 M triethylammonium acetate buffer (pH 7). For the double-stranded DNA, the same experiment with increasing protein concentration (20 µM) and prolonged illumination for DNA repair up to 60 min was also performed. The results are shown in the supplementary Figure S8.
To estimate the electronic coupling matrix element TDA for evaluation of ET...

...by the GROMACS version 5.0.4 program package [63].

We used these model structures as the initial structures for PL was replaced with An...

...each optimized structure of simulations, depending on the purpose. We used either i) (QM/MM) calculation or the equilibrated structures of MD [21]) by quantum mechanics/molecular mechanics.

For the observation of the dynamical behavior of...

...aligning (PDB ID: 2VTB) were aligned by TM-align [47], and the internucleoside moiety (PDB ID: 1TEZ [46]) and containing a CPD-analog with a formacetal linkage in its complex. The structures of...

...converted to the CPD with the natural phosphate by applying a force field upon the tLeap procedure described below. These model structures were used as the initial...

...simulation of the artificially CPD-analog in 2VTB was applied during temperature heating and production run (gradually heated for nine steps under the...

...the tLeap module of the AMBER program package by...

...molecules [55] and constructed square simulation boxes with a margin of at least 30 Å from proteins to the box boundaries. Finally, to neutralize each system, counterions (Na+) were added. In order to obtain the force fields of CPD, FADH–, 5,10-methenyltetrahydrofolate (MTHF), and 8-hydroxy-7,8-pyrimidine dimer calculated using the restrained electrostatic potential (RESP) method...

...methionine (CRYDQ395M) by QM/MM calculation, or ii) many...

...coordinates after geometry optimization and electron structure calculation for each molecule with a B3LYP/6-31G(d) and MP2/6-31+G(d,p) level of theory using Gaussian 16 [48]. Here, the geometric coordinate of CPD was obtained from Figure S1. Sequence alignment between AnPL (PDB ID: 1TEZ) and AnCRYD (PDB ID: 2VTB). Red highlight is the relevant amino acid residues to the electron transfer, whereas blue highlight is the relevant amino acid residues to the DNA binding. Here BLAST tool used the sequence alignment (https://blast.ncbi.nlm.nih.gov/Blast.cgi).
Supplementary Figure S2. Aligned structure of *An*PL and *At*CRYD. (a) The structures are displayed by Newcartoon using VMD visualization, the blue and red colors are *An*PL and *At*CRYD, respectively. (b) Some amino acid residues and cofactor that exist active site of *An*PL (*At*CRYD), namely Glu283 (Glu325), Trp286 (Trp328), Asn349 (Asn391), Met353 (Gln395), Trp392 (Tyr434), and FADH−, are displayed.
Supplementary Figure S3. Setting of QM region in QM/MM simulation. (a) is AtCRYD, and FADH\textsuperscript{−}, CPD, Glu325, Asn391, and Gln395 are included in QM region. (b) is AtCRYD\textsuperscript{Q395M}, and FADH\textsuperscript{−}, CPD, Glu325, Asn391, and Met395 are included in QM region. Notably, water molecules and counter ions were taken into account as point charge, although they are not shown in this figure.
Supplementary Figure S4. Edit each molecule in CIS calculation. For FADH\(^{-}\), the ribitol part is replaced by CH\(_3\), and the ribose part of adenine moiety add H atom. For CPD, the phosphate group and CH\(_2\) of 5\(^{\prime}\) side is replaced by H atom, and oxygen atom of 3\(^{\prime}\) side is replaced by H atom. For Glu, Asn, Gln, and Met, the main chain is replaced by CH\(_3\).
Supplementary Figure S5. The root-mean-square deviation (RMSD) for MD simulations. (a) and (b) are the RMSD for AtCRYD and AtCRYDQ395M. Here the RMSD is calculated for all atom of protein and DNA.
Supplementary Figure S6. Sequence of the DNA fragment purchased from Genewiz.
Supplementary Figure S7. SDS-PAGE analysis of Ni-affinity column and size exclusion chromatography of wild-type and mutant of AtCRYD.
Supplementary Figure S8. HPLC analysis of repair of either the single- or double-stranded substrates by wild-type (WT) or triple mutant of AtCRYD. In the case of the single-stranded substrate, the original substrate containing CPD at the retention time (Rt) of 15.4 min was converted to the repaired substrate at Rt = 16.7 min, indicating that the repair reaction successfully proceeded. In the case of the double-stranded substrate, however, no production of the repaired substrate was observed, even in the presence of increasing concentration of proteins in addition to the prolonged white light illumination up to 60 min. Note that the peaks emerged at Rt = 6.6 min and 14.7 min correspond to residual phenol and the complementary strand, d(TGCGCGAAGCCGAT), respectively.
Supplementary Figure S9. Distance and structure for triple mutant *AtCRYD* (Arg443Lys/Glu444Pro/Asp445Leu). (a) is the distances among the nitrogen atom NZ of Arg443Lys and the two oxygen atoms O1 or O2 of the phosphate of CPD and (b) is the distances among the nitrogen atom NZ of Arg443Lys and the two oxygen atoms O11 or O12 of the internucleoside phosphate of CPD. (c) is the distances among the nitrogen atom NH1 of Arg446 and the two oxygen atoms O11 or O1 of the internucleoside phosphate of CPD and (d) is the distances among the nitrogen atom NH2 of Arg446 and the two oxygen atoms O11 or O12 of the internucleoside phosphate of CPD. Here, the MD simulations were performed during 1000 ns.
**Supplementary Table S1.** Comparison of the distance between N6 of adenine moiety and O4 of 5’-side of CPD or N6 of adenine moiety and O4 of 3’-side of CPD of the QM/MM optimized geometry

| distance [Å] | AtCRYD | AtCRYD\(^{Q395M}\) |
|--------------|--------|-----------------|
| \(d_1\) (N6-O4) 5’ | 2.79   | 3.27            |
| \(d_2\) (N6-O4) 3’ | 3.45   | 3.27            |

**Supplementary Table S2.** Example of excitation energies [eV] and oscillator strengths (in bracket) for optional MD snapshots, which CT state to CPD reverses among S_4, S_3 and S_6. I, A and C note the abbreviation of isoalloxazine ring, adenine moiety and CPD, respectively. Here the calculation level is CIS/6-31G(d)

**Example 1**

| Excited states | AtCRYD | Excited states | AtCRYD\(^{Q395M}\) |
|----------------|--------|----------------|-----------------|
| S_1 (LE state (I)) | 4.0724 (0.02) | S_1 (LE state (I)) | 4.2706 (0.02) |
| S_2 (LE state (I)) | 4.6779 (0.23) | S_2 (LE state (I)) | 4.5714 (0.18) |
| S_3 (CT state (A)) | 4.8882 (0.00) | S_3 (CT state (A)) | 4.6100 (0.01) |
| S_4 (CT state (A)) | 5.4586 (0.02) | S_4 (CT state (A)) | 5.4590 (0.39) |
| S_5 (CT state (C)) | 5.4826 (0.00) | S_5 (CT state (C)) | 5.5808 (0.00) |
| S_6 (LE state (A)) | 5.5215 (0.32) | S_6 (CT state (A)) | 5.6555 (0.02) |

**Example 2**

| Excited states | AtCRYD | Excited states | AtCRYD\(^{Q395M}\) |
|----------------|--------|----------------|-----------------|
| S_1 (LE state (I)) | 4.1097 (0.04) | S_1 (LE state (I)) | 4.1613 (0.02) |
| S_2 (LE state (I)) | 4.7213 (0.20) | S_2 (LE state (I)) | 4.6017 (0.19) |
| S_3 (CT state (A)) | 5.3923 (0.01) | S_3 (CT state (A)) | 5.4721 (0.00) |
| S_4 (LE state (A)) | 5.5574 (0.31) | S_4 (LE state (A)) | 5.7901 (0.51) |
| S_5 (LE state (I)) | 5.7320 (0.33) | S_5 (LE state (I)) | 5.7971 (0.29) |
| S_6 (CT state (C)) | 5.7626 (0.00) | S_6 (CT state (C)) | 5.9367 (0.00) |
Supplementary References

[1]. Pokorny, R., Klar, T., Essen, L-O. & Batschauer, A. Crystallization and preliminary X-ray analysis of cryptochrome 3 from Arabidopsis thaliana. *Acta. Cryst.* **F61**, 935-938 (2005). DOI: 10.1107/S1744309105028897

[2]. Yamamoto, J., Hitomi, K., Todo, T. & Iwai, S. Chemical synthesis of oligodeoxyribonucleotides containing the Dewar valence isomer of the (6-4) photoproduct and their use in (6-4) photolyase studies. *Nucleic. Acids. Res.* **34**,4406-4415 (2006). DOI: 10.1093/nar/gkl572