

Supplementary Materials for

Photoactivation of *Drosophila melanogaster* cryptochrome through sequential conformational transitions

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Supplementary materials

Table S1. TRXSS kinetic modeling.

	n-comp	k_1	k_2	k_3	k_4	k_5
WT pH 7	4	0.4±0.0 μs^{-1}	2.9±0.4 ms^{-1}	0.3±0.0 ms^{-1}	-	-
WT pH 7	5	10.1±0.6 μs^{-1}	1.3±0.1 μs^{-1}	3.9±0.3 ms^{-1}	0.4±0.30 ms^{-1}	-
WT pH 7	6	6.3±25.8 μs^{-1} *	6.5±26.3 μs^{-1} *	0.9±0.1 μs^{-1}	3.3±0.2 ms^{-1}	0.3±0.0 ms^{-1}
H378A pH 7	3	2.3±0.2 ms^{-1}	0.3±0.0 ms^{-1}	-	-	-
H378A pH 7	4	0.2±0.0 μs^{-1}	1.2±0.1 ms^{-1}	0.3±0.0 ms^{-1}	-	-
H378A pH 7	5	0.4±0.1 μs^{-1}	24.2±2.0 ms^{-1}	0.8±0.1 ms^{-1}	0.4±0.1 ms^{-1}	-
WT pH 9	3	0.2±0.0 μs^{-1}	3.5±0.2 ms^{-1}	-	-	-
WT pH 9	4	0.2±0.0 μs^{-1}	3.9±0.3 ms^{-1}	0.3±0.0 ms^{-1}	-	-
WT pH 9	5	0.6±1.3 μs^{-1} *	0.6±1.3 μs^{-1} *	4.4±0.2 ms^{-1}	0.3±0.0 ms^{-1}	-
H378A pH 9	2	2.9±0.1 ms^{-1}	-	-	-	-
H378A pH 9	3	4.1±0.4 μs^{-1}	1.3±0.2 ms^{-1}	-	-	-
H378A pH 9	4	3.0±163.8 μs^{-1} *	2.3±0.2 ms^{-1}	-0.5±0.0 ms^{-1} *	-	-

*Non-sensible values

Table S2. Changes in R_g .

	$DmCry_\alpha$ (Å)	$DmCry_\beta$ (Å)	$DmCry_\gamma$ (Å)	$DmCry_\delta$ (Å)	$DmCry_\epsilon^*$ (Å)
WT pH 7	-0.02±0.005**	0.15±0.004	-0.04±0.004	-0.10±0.004	-0.09±0.004
H378A pH 7	n/a	0.09±0.004	-0.17±0.005	-0.09±0.004	-0.07±0.004
WT pH 9	n/a	0.03±0.004	-0.17±0.004	-0.16±0.004	-0.00±0.004
H378A pH 9	n/a	n/a	0.03±0.004	-0.09±0.004	0.00±0.004

*For the pH 9 samples this is instead the final state, ** The error ranges represent the statistical uncertainty based on the noise level of the data (see Materials and Methods) and does not include experimental uncertainty.

Table S3. SAXS parameters.

	$DmCry$ (dark)	$DmCry$ (light)
R_g (Guinier analysis)	30.3±0.3 Å	30.8±0.3 Å
$I(q=0)$ (Guinier analysis)	21.9±0.12	22.8±0.13
MW (Q_r)	61 kDa	61 kDa

Table S4. Rate constants for TRXSS measurements of $DmCry$ at pH 9.

	k_1	k_2	k_3
WT pH 9	0.2±0.0 μs^{-1}	3.9±0.3 ms^{-1}	0.3±0.0 ms^{-1}
H378A pH 7	n/d	4.1±0.4 ms^{-1}	1.3±0.2 ms^{-1}

Table S5. Rate constants for TA measurements of *DmCry*.

	$k_1(\mu\text{s}^{-1})^*$	$k_2(\mu\text{s}^{-1})^*$	$k_3(\mu\text{s}^{-1})^{**}$	$k_4(\mu\text{s}^{-1})^*$	$k_5(\text{ms}^{-1})$	$k_6(\text{ms}^{-1})$	$k_{recom.}^{***}(\text{ms}^{-1})$
WT pH 7	$3.1 \cdot 10^3 \pm 9.97 \cdot 10^7$	8.68 ± 0.39	$0.37 \pm 4.3 \cdot 10^{-3}$	$0.7 \cdot 10^{-3} \pm 1.2 \cdot 10^{-3}$	$0.5 \pm 1.97 \cdot 10^{-2}$	$0.069 \pm 2.54 \cdot 10^{-3}$	0.0998
H378A pH 7	217.7 ± 22	218 ± 22	$0.4 \pm 5.173 \cdot 10^{-3}$	$9.48 \cdot 10^{-3} \pm 7.2 \cdot 10^{-4}$	2.85 ± 0.7	0.14 ± 0.06	0.213
WT pH 9	146.1 ± 2.55	4.44 ± 0.16	$0.80 \pm 6.0 \cdot 10^{-3}$	$2 \cdot 10^{-3} \pm 0.3 \cdot 10^{-3}$	$0.63 \pm 1.65 \cdot 10^{-2}$	$0.098 \pm 1.0 \cdot 10^{-3}$	0.099
H378A pH 9	139 ± 22	3.19 ± 0.19	$0.73 \pm 21 \cdot 10^{-3}$	$8.0 \cdot 10^{-3} \pm 8.5 \cdot 10^{-4}$	12.35 ± 2.87	0.12 ± 0.01	0.1648

* k_1 , k_2 and k_4 remain unassigned in this paper. We follow Ref. (19) for assignments

** k_3 is the time constant assigned to deprotonation of W394

***The time constant for radical pair recombination is calculated as $k_{recom.} = (k_5 + w_5 + k_6 \cdot w_6) / (w_5 + w_6)$

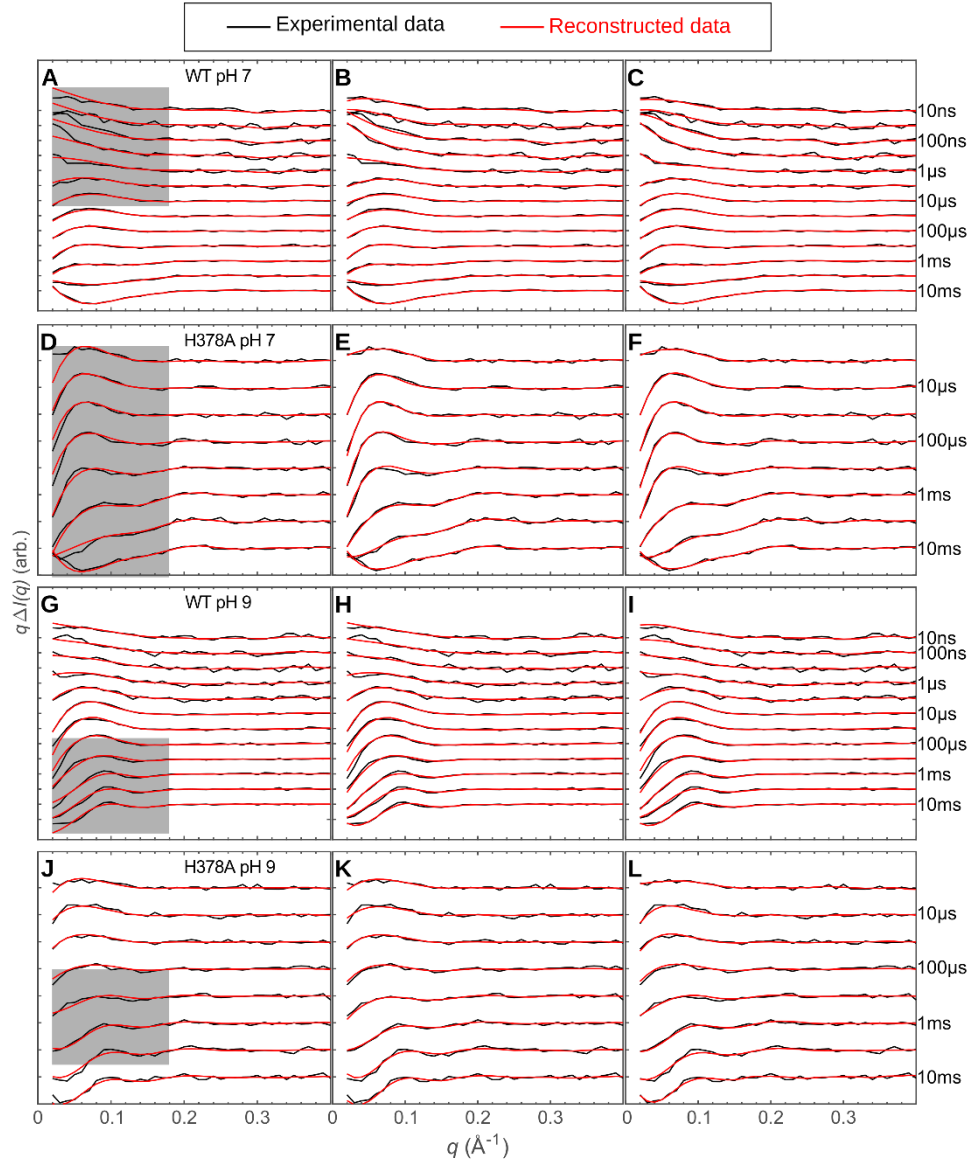


Fig. S1. TRXSS data and the reconstructed data using a kinetic model with different numbers of components. Wild type *DmCry* at pH 7 with 4 (A), 5 (B) or 6 (C) components. H378A *DmCry* at pH~7 with 3 (D), 4 (E) or 5 (F) components. Wild type *DmCry* at pH 9 with 3 (G), 4 (H) or 5 (I) components. H378A *DmCry* at pH 9 with 2 (J), 3 (K) or 4 (L) components. Shaded areas highlight the regions where too few species result in systematic deviations.

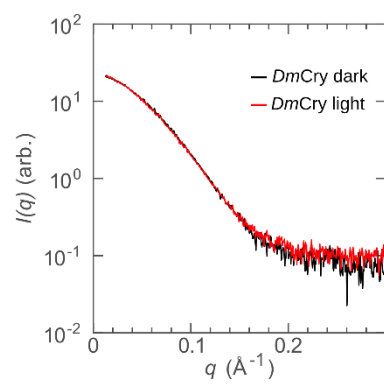


Fig. S2. SAXS scattering profiles for *DmCry* in the dark and under blue-light illumination.

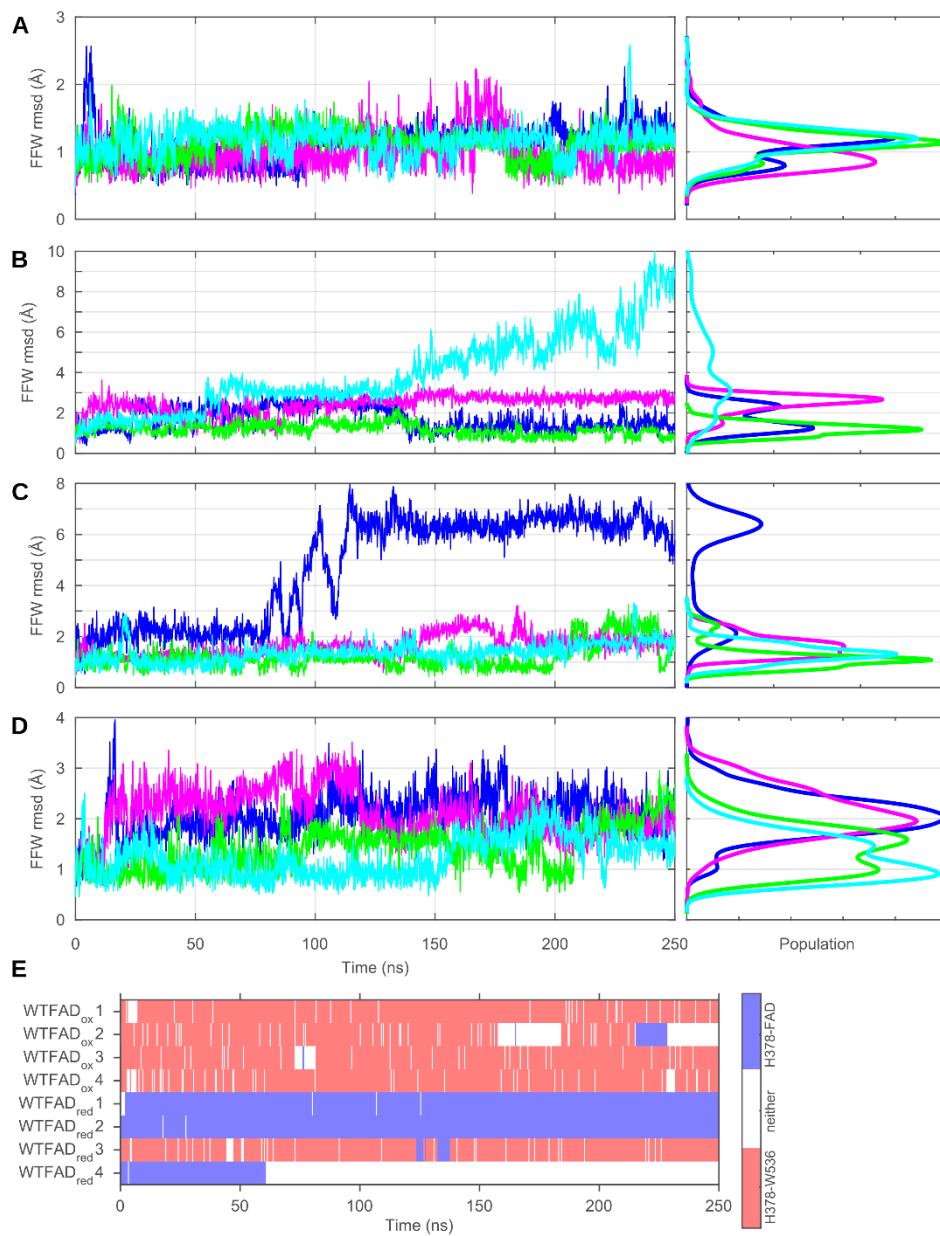


Fig. S3. The RMSD of the FFW motif at each simulation frame. (A) Four simulations with oxidized FAD, (B) four simulations with reduced FAD, (C) four simulations with oxidized FAD and H378A mutation, (D) four simulations with reduced FAD and H378A mutation. (E) Existence of a hydrogen bond between H378 and W536 or H378 and FAD for wild type trajectories using oxidized (ox) or reduced (red) chromophore parameters

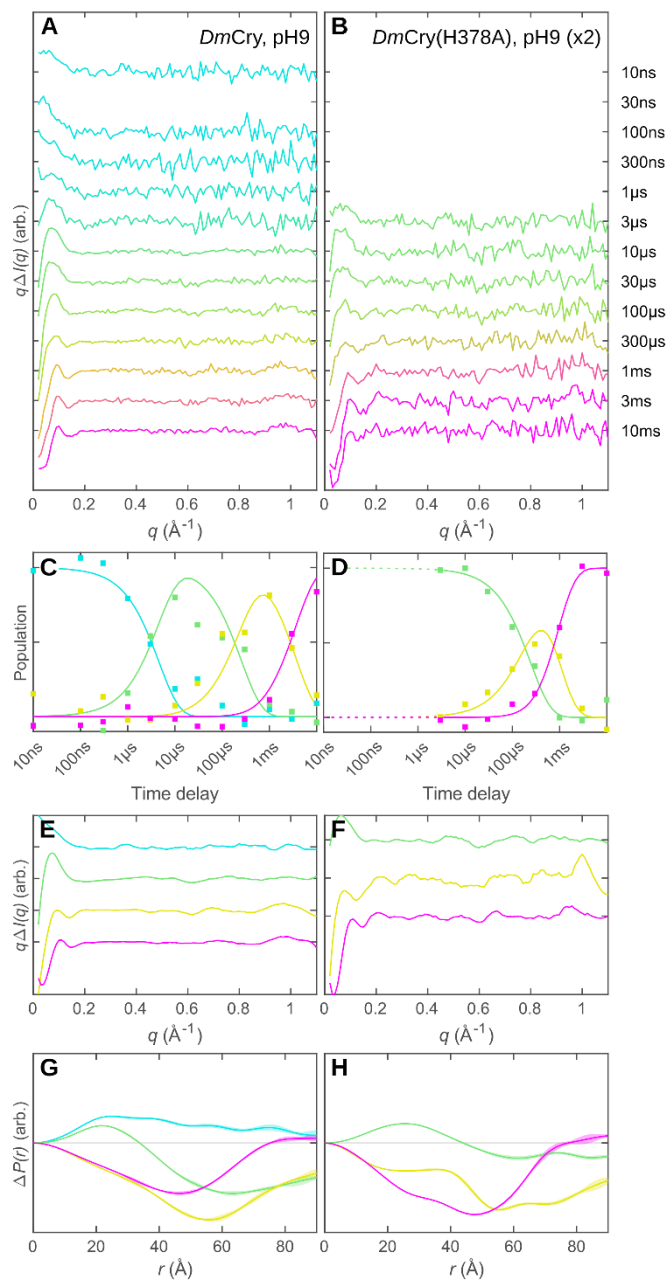


Fig. S4. TRXSS data and kinetic modeling for wild-type *DmCry* and H378A at pH 9. Scattering data (A,B), transient populations (C,D), species associated spectra (E,F) and $\Delta P(r)$ (G,H). The coloring is according to the similar spectra that are found in Fig. 3, with the exception of the *DmCry*_s state (magenta), which is significantly different at pH 9.

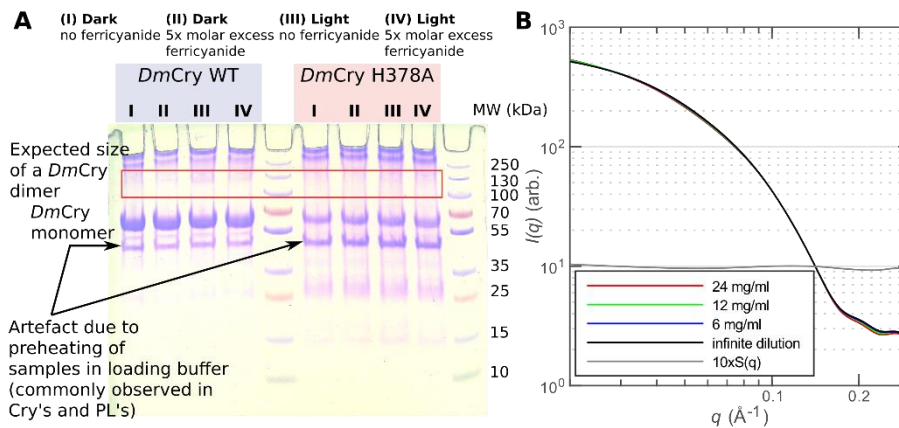


Fig. S5. Stability analysis of the used samples via SDS-PAGE and SAXS. (A) SDS-PAGE analysis of *DmCry* WT and H378A performed under non reducing conditions (without beta-mercaptoethanol), with and without ferricyanide, in the dark and directly after 5 minutes of blue-light. No presence of *DmCry* dimer can be observed. (B) Concentration dependence of scattering profile, measured during TRXSS experiment. $S(q)$ is the ratio between the 24 mg/ml and the infinite dilution scattering curves.