Electronic Supplementary Information for

# ATP-binding Promotes Light-induced Structural Changes of Apoprotein of Arabidopsis Cryptochrome1

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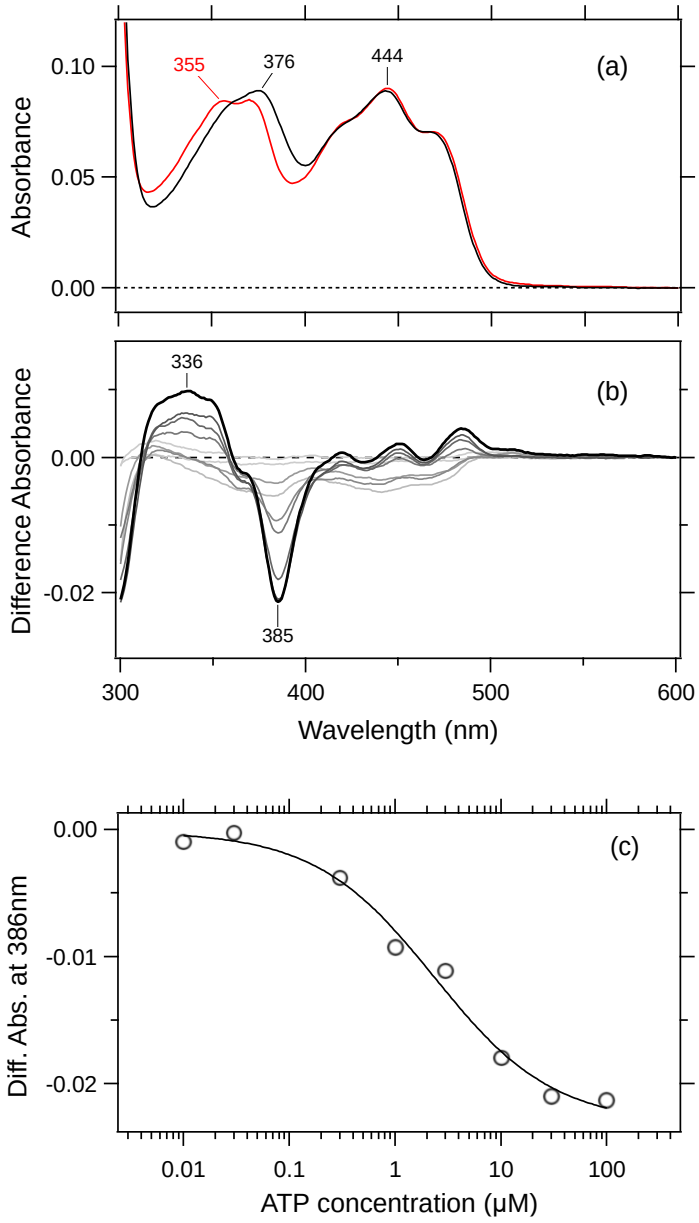


**Fig. S1.** Effects of ATP on the UV-vis absorption spectrum of $FAD_{ox}$ in AtCRY1-PHR. (a) UV-vis spectra of AtCRY1-PHR in the absence (black line) and presence (red line) of 100μM ATP. (b) The ATP minus no ATP difference spectrum. (c) Determination of the dissociation constant of the ATP/AtCRY1 complex. Absorbance differences at the wavelength of maximum change (385 nm) of AtCRY1-PHR. Fitting these points with a protein-ligand saturation isotherm for non-constant protein concentration (obtained with fitting Hill's formula) yields a dissociation constant Kd = 2.3 ± 0.8 μM.

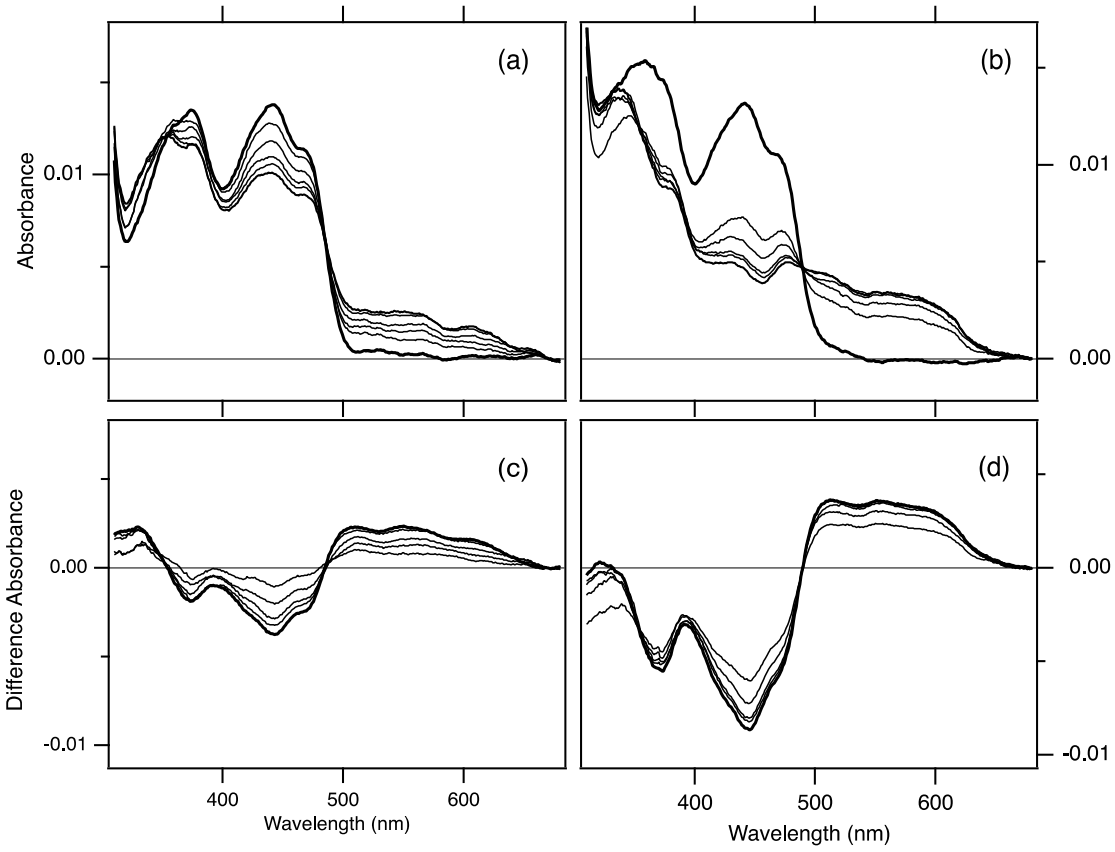


**Fig. S2.** (a, b) Typical absolute absorbance of concentrated AtCRY1-PHR solution without (a) and with ATP (b). Specrta were measured before (bold) and after (thin) 450 nm light illumination every 1 min. Total illumination was carried out for 5 min. (c, d) Light-induced difference spectra of AtCRY1-PHR without (c) and with ATP (d).

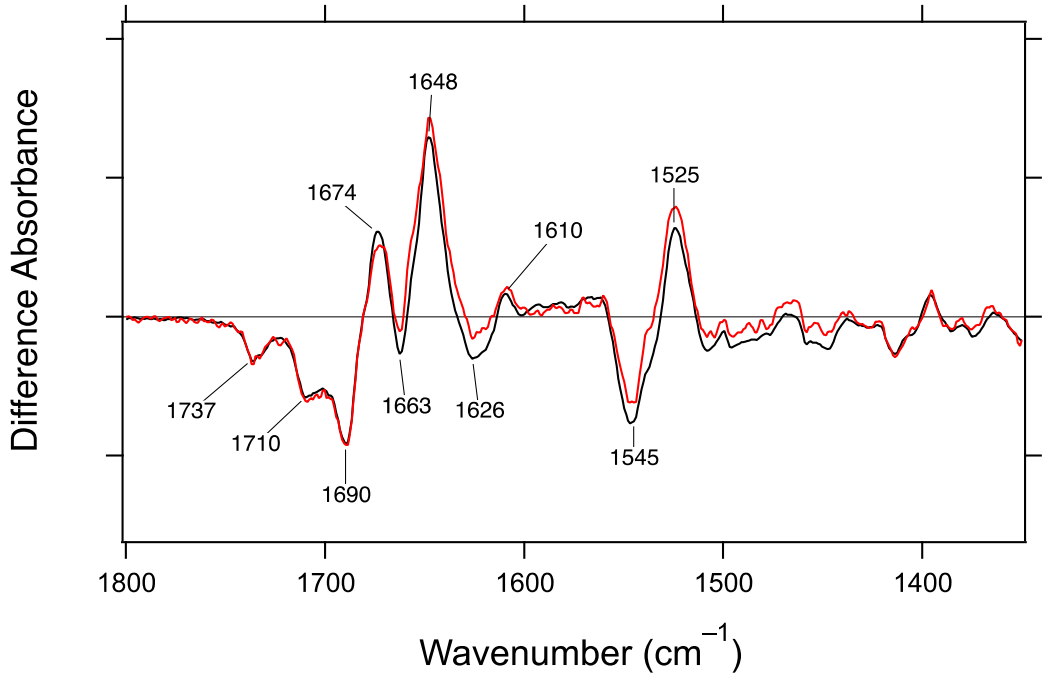


**Fig. S3.** Comparison of difference FTIR spectra of WT AtCRY1 in the presence of ATP (black line) and ADP (red line). One division of the y-axis corresponds to 0.001 absorbance units.

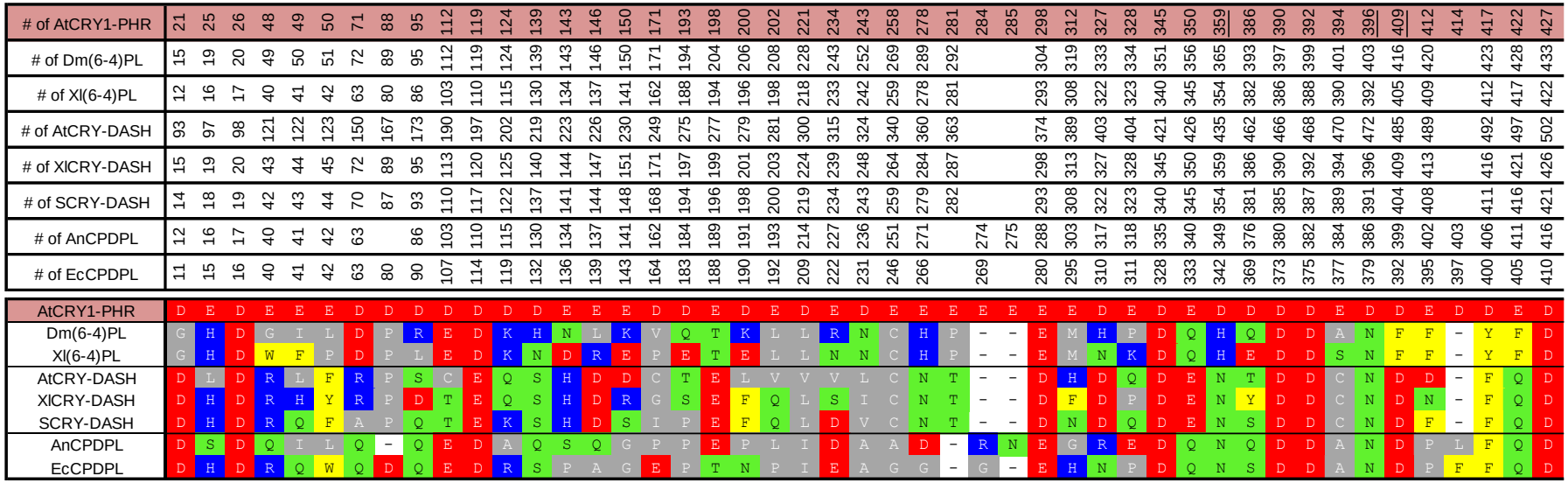


**Fig. S4.** Aamino acid alignment of CRY/PLs. Carboxylic acid residues in AtCRY1-PHR are aligned. Abbreviation: At, *Arabidopsis thaliana*; Dm, *Drosophila melanogaster*; Xl, *Xenopus laevis*; S, *Synechocystis sp.*; An, *Anacystis nidulans*; Ec, *Escherichia coli*.

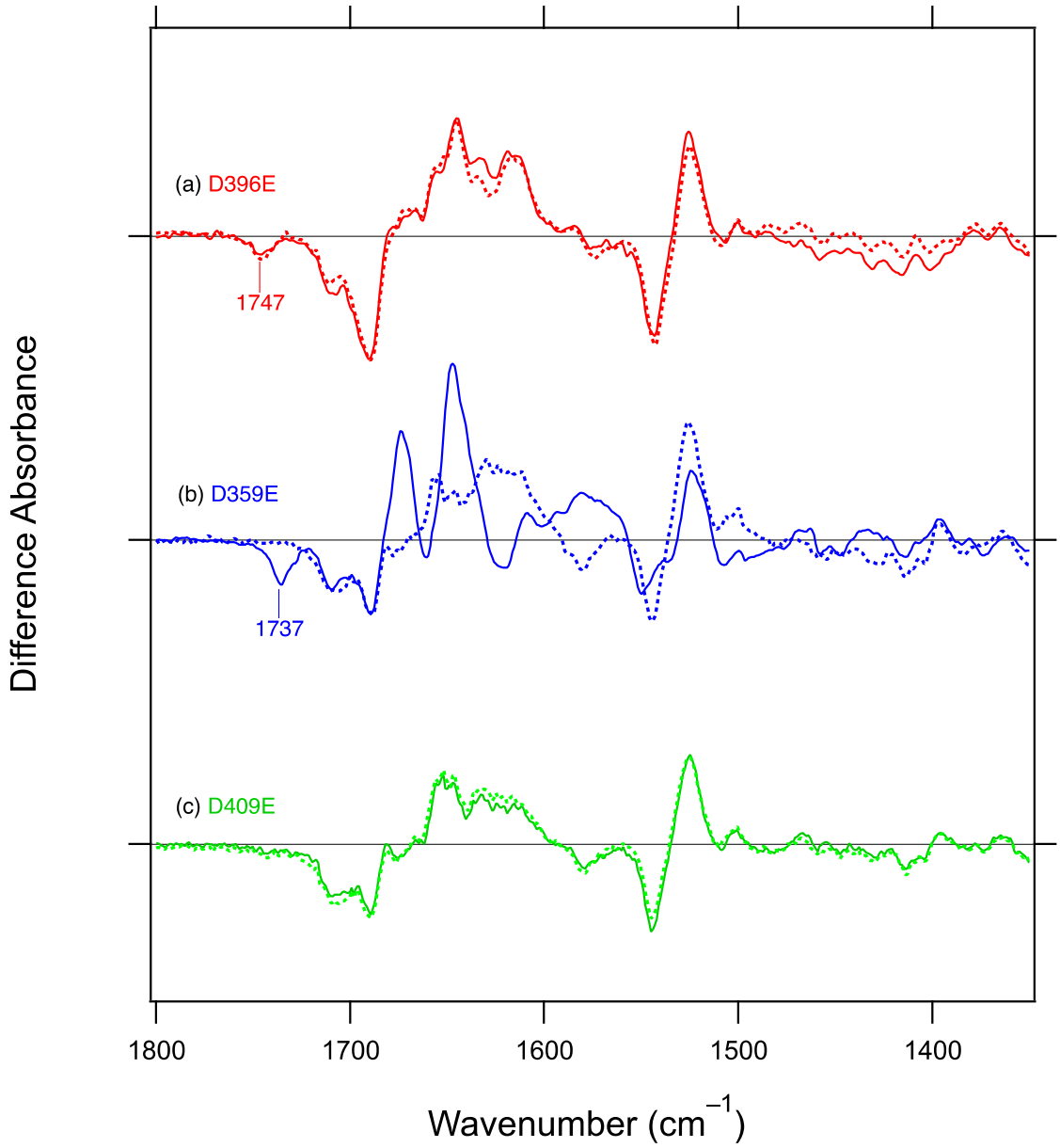


**Fig. S5.** Comparison of difference FTIR spectra of D396E (a), D359E (b), and D409E (c) mutants of AtCRY1 in the absence (dotted lines) and presence (solid lines) of ATP. One division of the y-axis corresponds to 0.003 absorbance units.

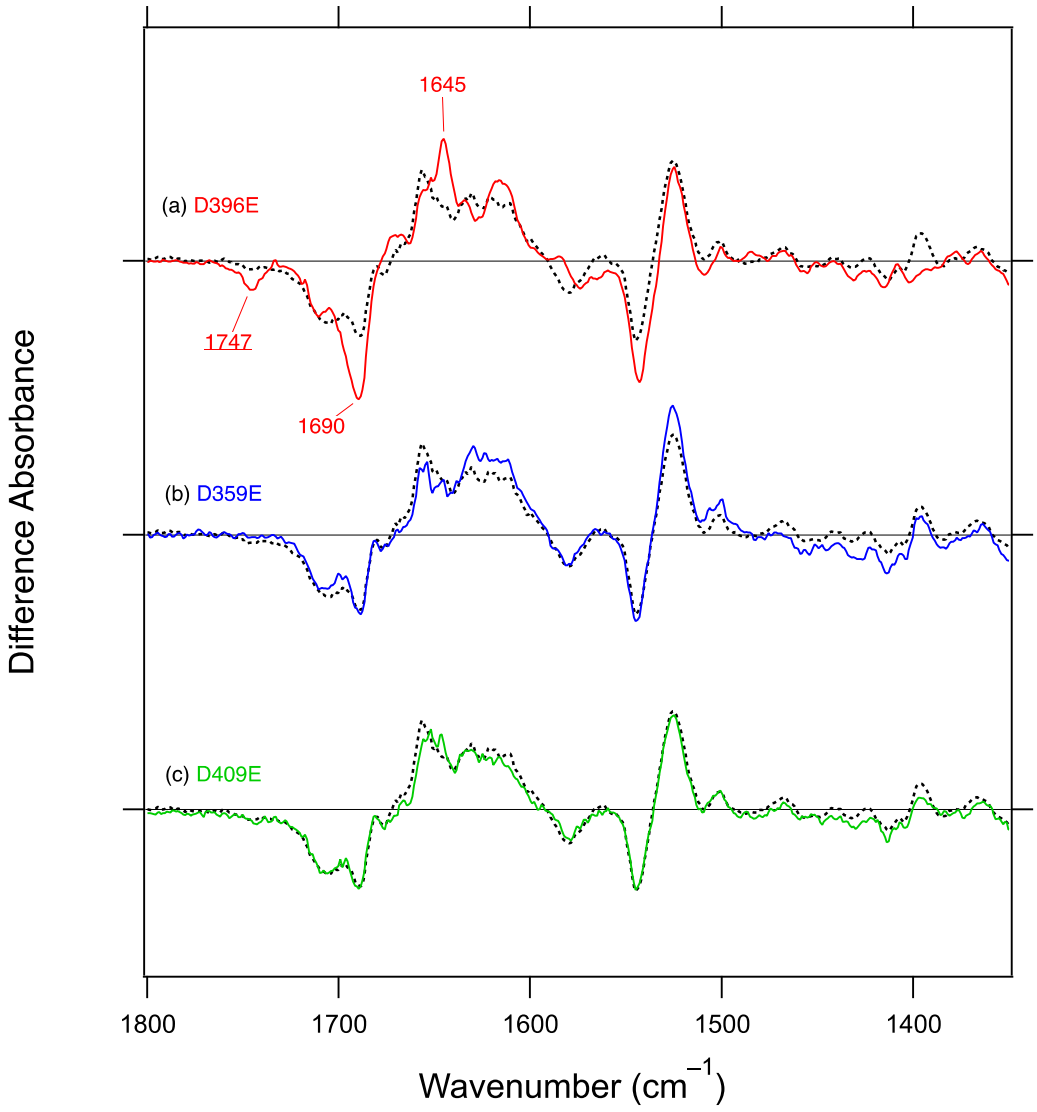


**Fig. S6.** Light-induced difference FTIR spectra of WT (dotted lines) and Asp to Glu mutants (solid lines) of AtCRY1 in the absence of ATP. D396E (a), D359E (b), and D409E (c) mutants were measured. One division of the y-axis corresponds to 0.0025 absorbance units.

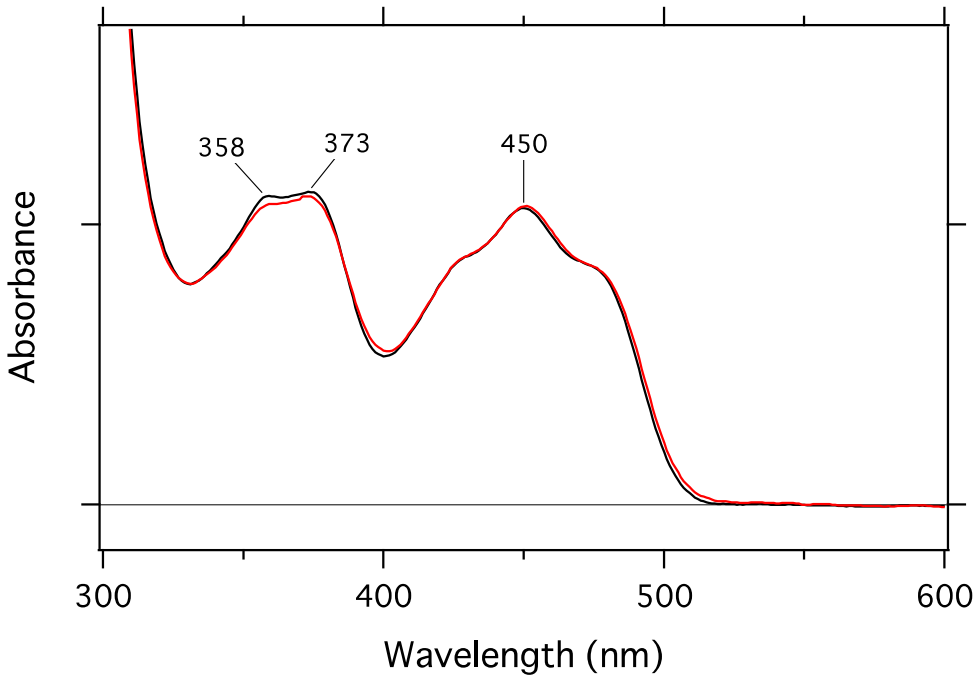


**Fig. S7.** UV-vis spectra of D396E mutant of AtCRY1 in the absence (black line) and presence (blue line) of 100 μM ATP. One division of the y-axis corresponds to 0.05 absorbance units.

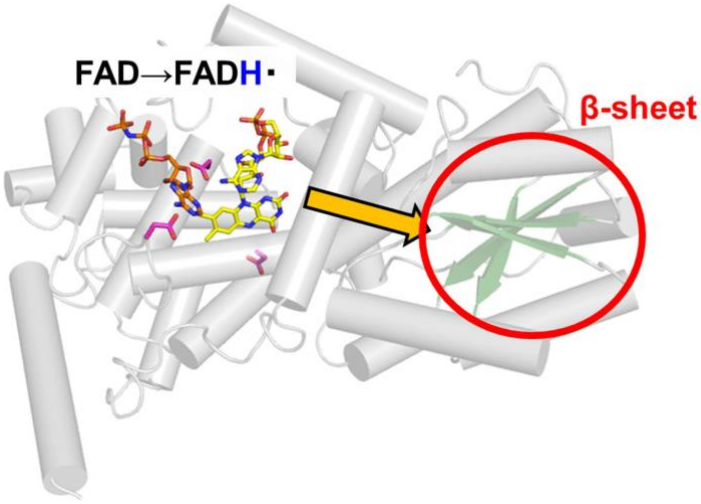


**Fig. S8.** Overall structure on the AtCRY-PHR (PDB code: 1U3C). Upon the photoreduction of FAD, structural changes are transmitted from FAD binding domain to the region including β-sheets (yellow arrow). α/β subdomain are highlighted in red.

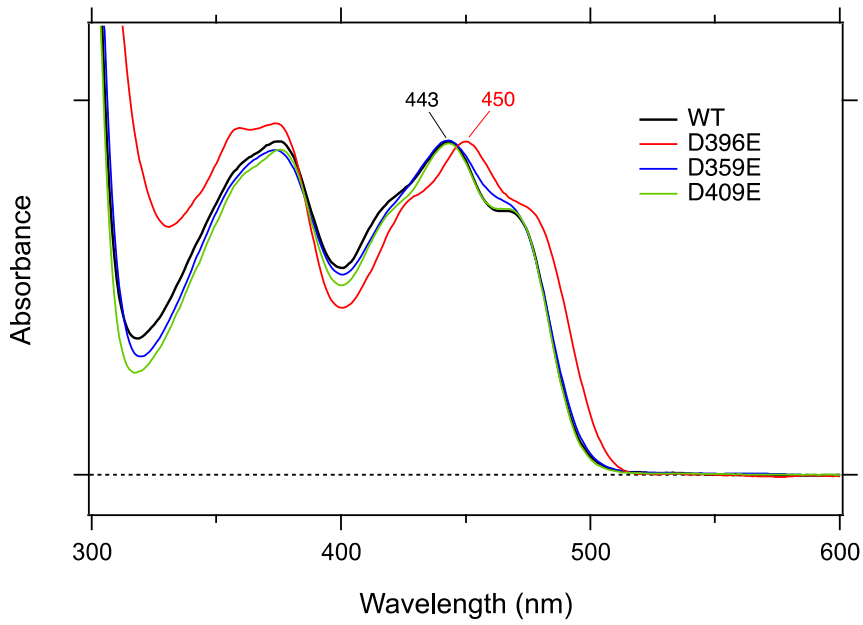


**Fig. S9.** UV-vis spectra of WT AtCRY1 (black line), D396E (red line), D359E (blue line), and D409E (green line) mutants in the absence of ATP.