

Electronic Supplementary Information for

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

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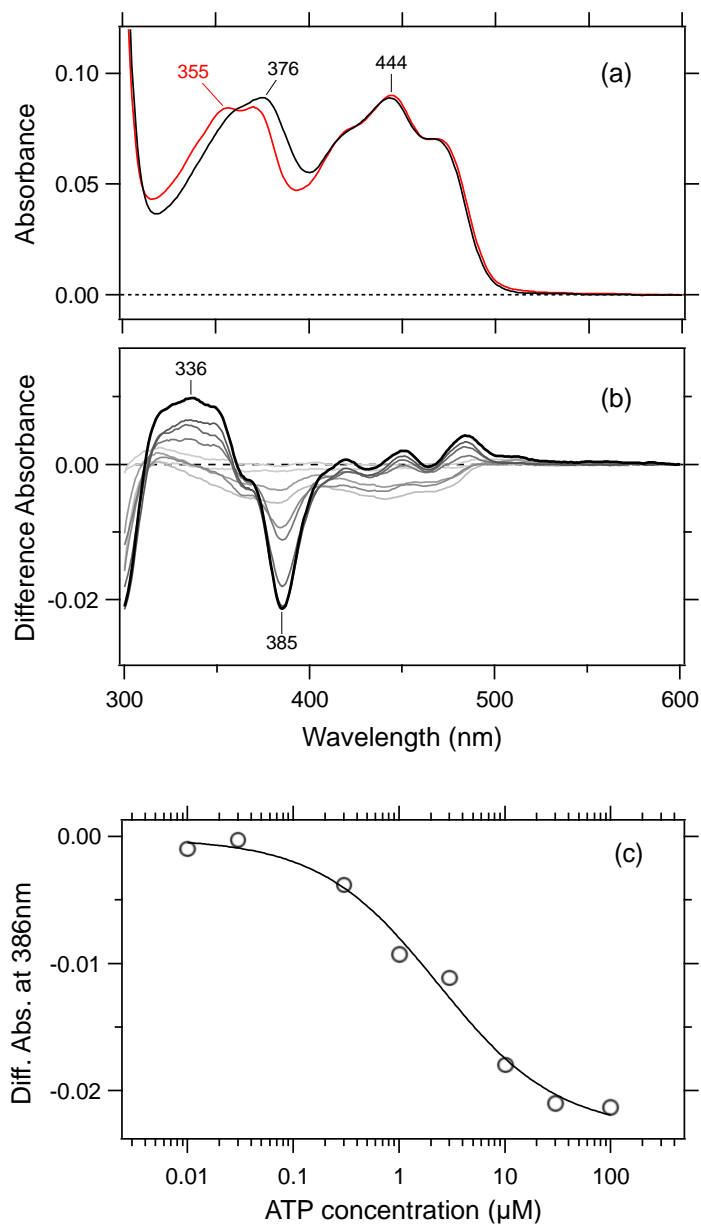


Fig. S1. Effects of ATP on the UV-vis absorption spectrum of FAD_{ox} in AtCRY1-PHR. (a) UV-vis spectra of AtCRY1-PHR in the absence (black line) and presence (red line) of 100 μ M ATP. (b) The ATP minus no ATP difference spectrum. (c) Determination of the dissociation constant of the ATP/AtCRY1 complex. 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 $K_d = 2.3 \pm 0.8 \mu$ M.

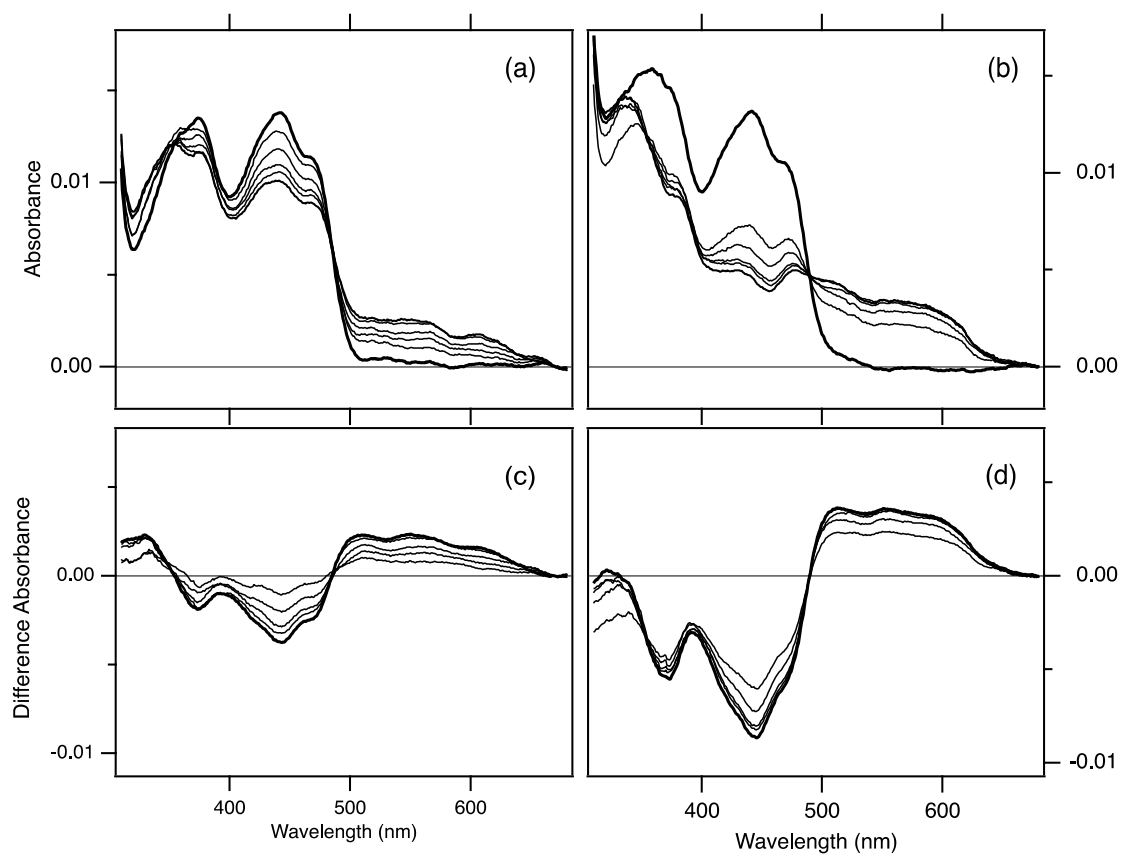


Fig. S2. (a, b) Typical absolute absorbance of concentrated AtCRY1-PHR solution without (a) and with ATP (b). Spectra were measured before (bold) and after (thin) 450 nm light illumination every 1 min. Total illumination was carried out for 5 min. (c, d) Light-induced difference spectra of AtCRY1-PHR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

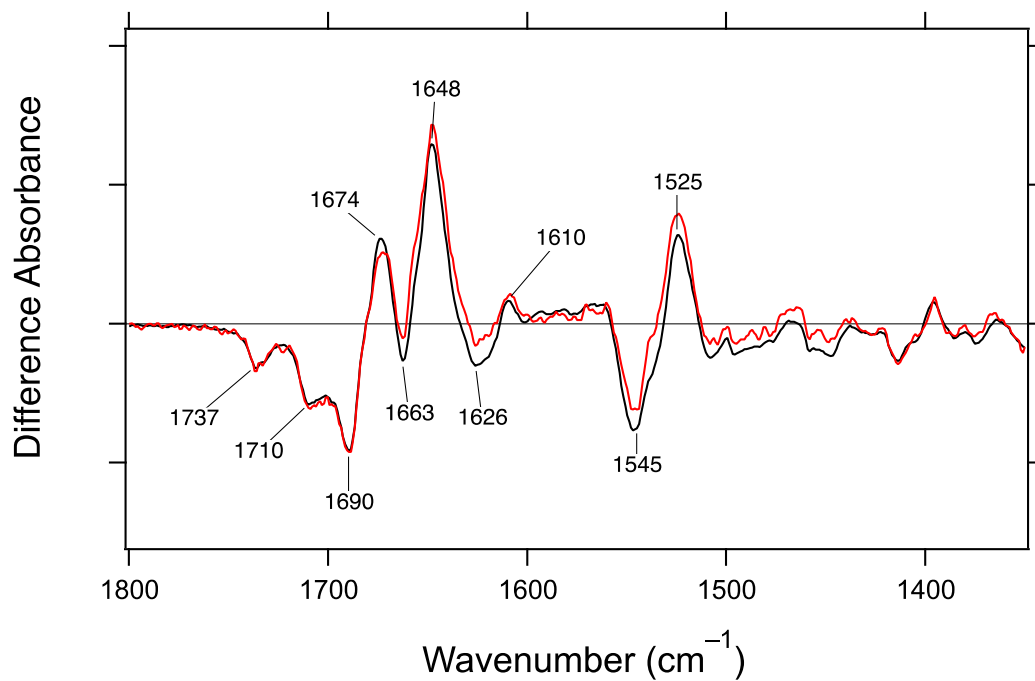


Fig. S3. Comparison of difference FTIR spectra of WT AtCRY1 in the presence of ATP (black line) and ADP (red line). One division of the y-axis corresponds to 0.001 absorbance units.

# of AtCRY1-PHR	21	25	26	48	49	50	51	71	88	95	112	119	124	139	143	146	150	171	193	198	200	202	221	234	243	258	278	281	284	285	298	312	327	334	345	350	359	386	390	392	394	396	409	412	414	417	422	427		
# of Dm(6-4)PL	15	19	20	49	50	51	72	89	95	103	112	119	124	139	143	146	150	171	193	198	200	202	221	234	243	258	278	281	284	285	298	312	327	334	345	350	359	386	390	392	394	396	409	412	414	417	422	427		
# of Xl(6-4)PL	12	16	17	40	41	42	63	80	86	95	103	112	119	124	139	143	146	150	171	193	198	200	202	221	234	243	258	278	281	284	285	298	312	327	334	345	350	359	386	390	392	394	396	409	412	414	417	422	427	
# of AtCRY-DASH	93	97	98	121	122	123	150	167	173	180	190	197	202	219	223	226	230	249	275	277	279	281	282	288	298	304	312	327	334	345	350	359	386	390	392	394	396	409	412	414	417	422	427							
# of XICRY-DASH	15	19	20	43	44	45	72	89	95	103	112	119	124	139	143	146	150	171	193	198	200	202	221	234	243	258	278	281	284	285	298	312	327	334	345	350	359	386	390	392	394	396	409	412	414	417	422	427		
# of SCRY-DASH	14	18	19	42	43	44	70	87	93	103	110	117	122	137	141	144	147	168	194	199	201	203	209	227	234	243	258	278	281	284	285	298	312	327	334	345	350	359	386	390	392	394	396	409	412	414	417	422	427	
# of AnCPDPL	12	16	17	40	41	42	63	80	86	93	103	110	117	122	137	140	144	147	168	194	199	201	203	209	227	234	243	258	278	281	284	285	298	312	327	334	345	350	359	386	390	392	394	396	409	412	414	417	422	427
# of EcCPDPL	11	15	16	40	41	42	63	80	86	93	103	110	117	122	137	140	144	147	168	194	199	201	203	209	227	234	243	258	278	281	284	285	298	312	327	334	345	350	359	386	390	392	394	396	409	412	414	417	422	427
AtCRY1-PHR	D	E	D	E	E	E	D	D	D	D	D	D	D	D	E	E	E	D	D	E	D	E	D	E	D	E	E	E	E	E	E	D	E	D	E	D	D	D	D	D	E	D	D	E	D	D	E	D		
Dm(6-4)PL	G	H	D	G	I	L	D	P	R	E	D	K	H	N	L	K	V	Q	T	K	L	L	R	N	C	H	P	-	-	E	M	H	P	D	Q	H	Q	D	D	A	N	F	F	-	Y	F	D			
Xl(6-4)PL	G	H	D	W	F	F	D	P	L	E	D	K	N	D	R	E	P	E	F	E	L	L	N	N	C	H	P	-	-	E	M	N	K	D	Q	H	E	D	D	S	N	F	F	-	Y	F	D			
AtCRY-DASH	D	L	D	R	L	F	R	P	S	C	E	Q	S	H	D	D	C	T	E	L	V	V	V	L	C	N	T	-	-	D	H	D	Q	D	E	N	T	D	D	C	N	D	D	-	F	Q	D			
XICRY-DASH	D	H	D	R	H	Y	R	P	D	T	E	Q	S	H	D	R	G	S	E	F	Q	L	S	I	C	N	T	-	-	D	F	D	P	D	E	N	Y	D	D	C	N	D	N	-	F	Q	D			
SCRY-DASH	D	H	D	R	Q	F	A	P	Q	T	E	K	S	H	D	S	I	P	E	F	Q	L	D	V	C	N	T	-	-	D	N	D	Q	D	E	N	S	D	D	C	N	D	F	-	F	Q	D			
AnCPDPL	D	S	D	Q	I	L	Q	-	Q	E	D	A	Q	S	Q	G	P	P	E	P	L	I	D	A	A	D	-	R	N	E	G	R	E	D	Q	N	Q	D	D	A	N	D	P	L	F	Q	D			
EcCPDPL	D	H	D	R	Q	W	Q	D	Q	E	D	R	S	F	A	G	E	P	T	N	P	I	E	A	G	G	-	G	-	E	H	N	P	D	Q	N	S	D	D	A	N	D	P	F	F	Q	D			

Fig. S4. Amino 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*.

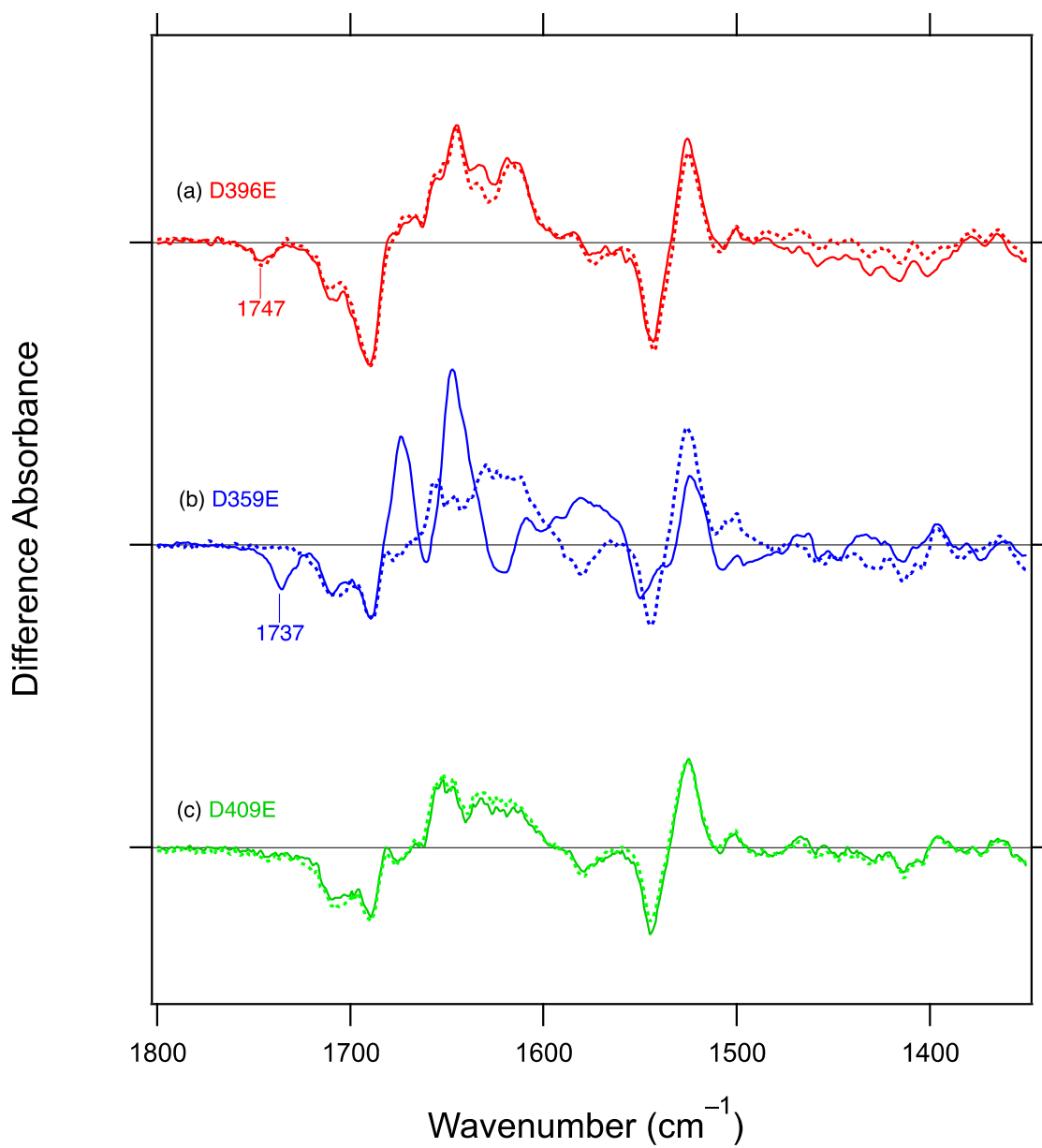


Fig. S5. Comparison of difference FTIR spectra of D396E (a), D359E (b), and D409E (c) mutants of AtCRY1 in the absence (dotted lines) and presence (solid lines) of ATP. One division of the y-axis corresponds to 0.003 absorbance units.

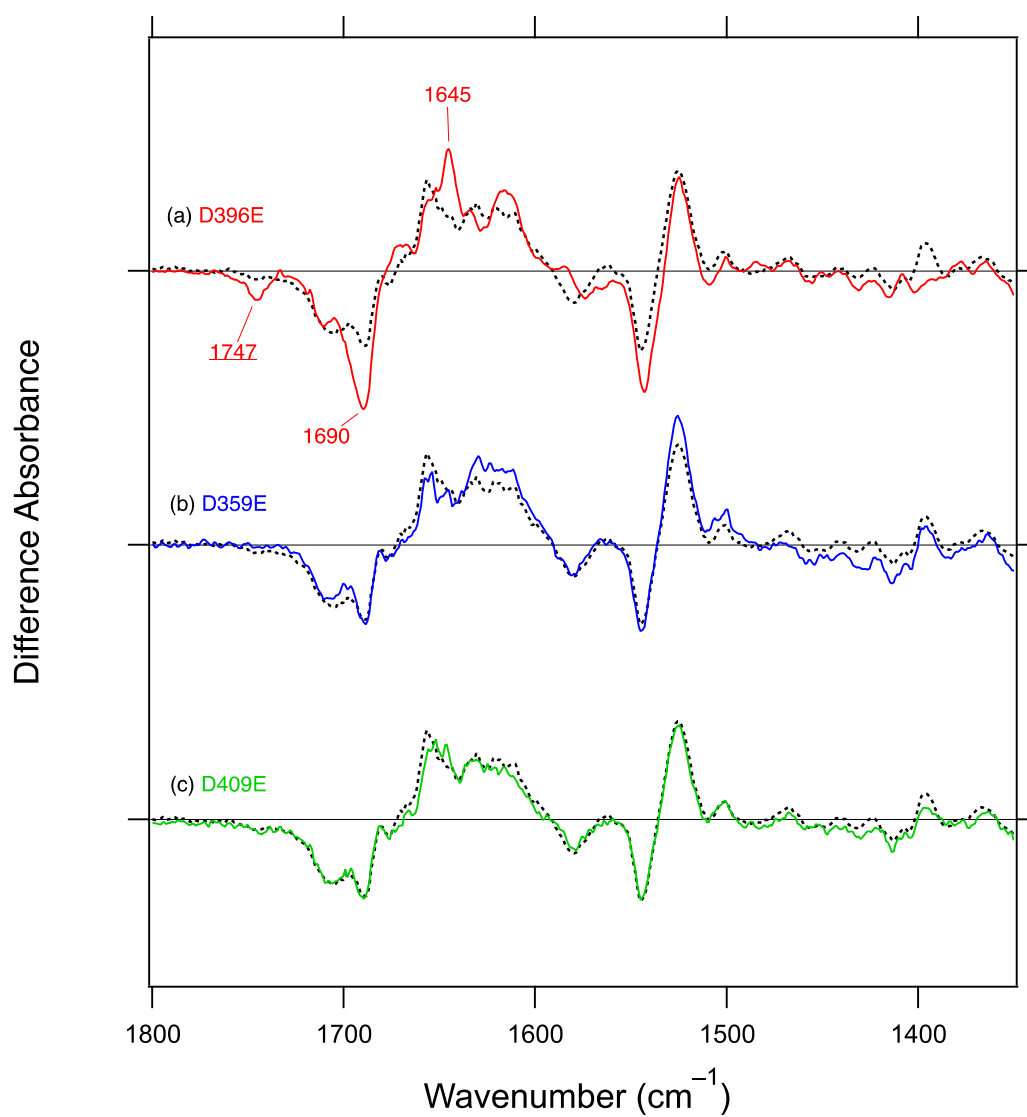


Fig. S6. Light-induced difference FTIR spectra of WT (dotted lines) and Asp to Glu mutants (solid lines) of AtCRY1 in the absence of ATP. D396E (a), D359E (b), and D409E (c) mutants were measured. One division of the y-axis corresponds to 0.0025 absorbance units.

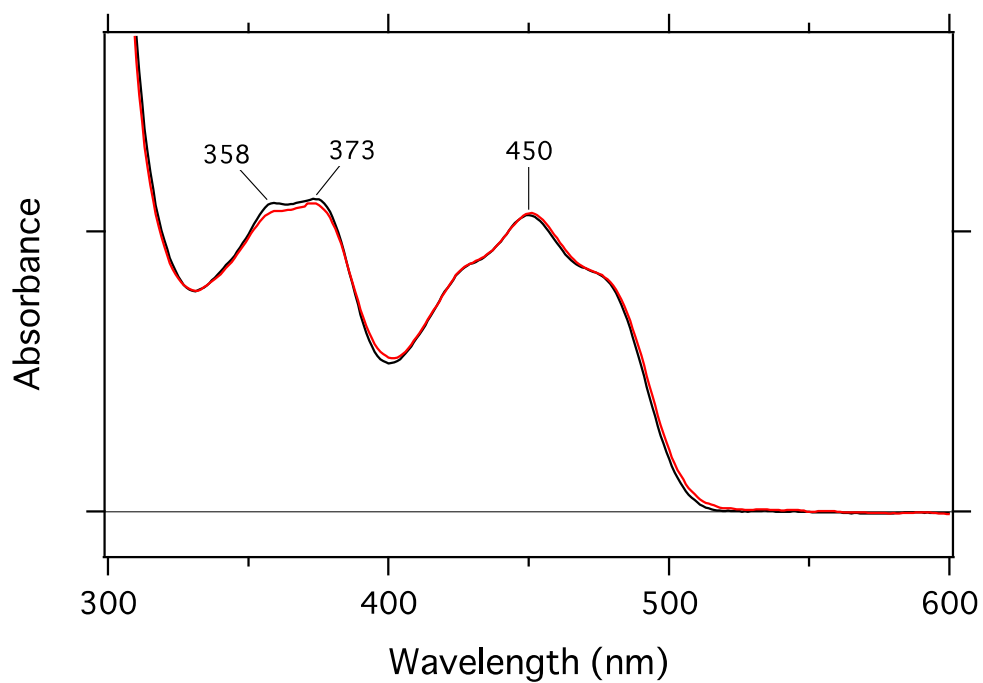


Fig. S7. UV-vis spectra of D396E mutant of AtCRY1 in the absence (black line) and presence (blue line) of 100 μ M ATP. One division of the y-axis corresponds to 0.05 absorbance units.

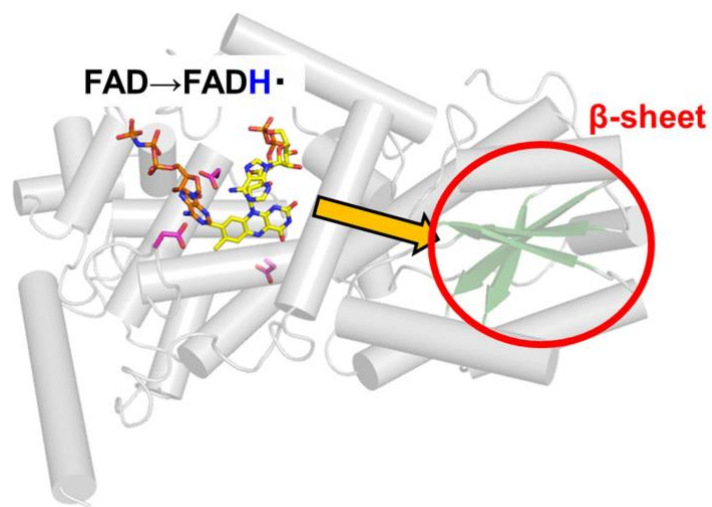


Fig. S8. Overall structure on the AtCRY-PHR (PDB code: 1U3C). Upon the photoreduction of FAD, structural changes are transmitted from FAD binding domain to the region including β -sheets (yellow arrow). α/β subdomain are highlighted in red.

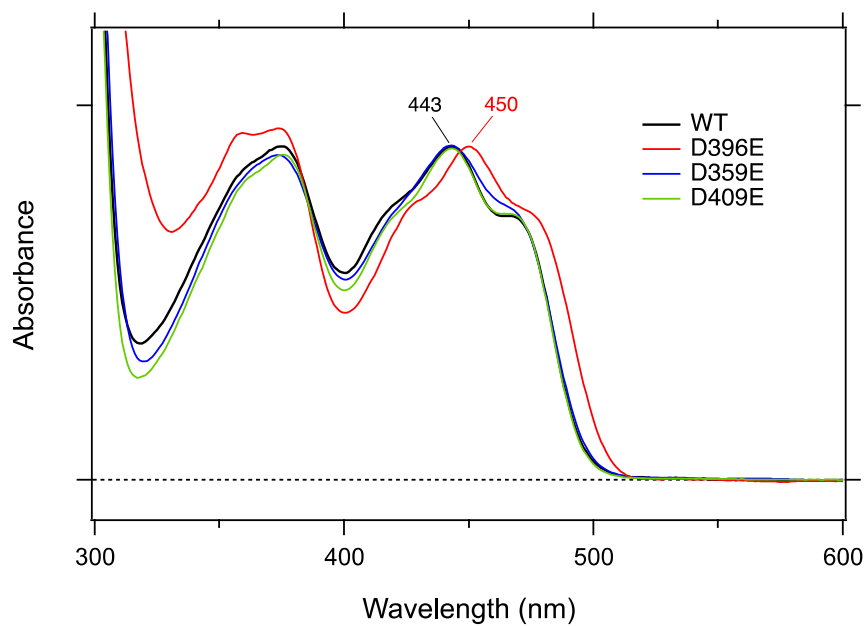


Fig. S9. UV-vis spectra of WT AtCRY1 (black line), D396E (red line), D359E (blue line), and D409E (green line) mutants in the absence of ATP.