Supporting Information

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SI Results and Discussion

pK_a Values of Glu46 and *p*-Coumaric Acid (*p*CA) in the R52A Photoactive Yellow Protein (PYP). Spectroscopic studies suggested that the two pK_a values approximately nine and approximately six, characteristic to the rate of recovery of the ground state in the PYP photocycle, were not significantly altered upon mutation of Arg52 to Ala (1). In agreement with the mutant study, the calculated pK_a(Glu46) and pK_a(*p*CA) in the R52A PYP model were considerably close to those in the wild-type PYP (Table 2), irrespective of the significant contribution of protonated Arg52 to pK_a(Glu46) (Table S2) and pK_a(*p*CA) (Table S3) in the wild-type PYP. Because Arg52 downshifted both pK_a(Glu46) and pK_a(*p*CA), removing Arg52 eliminates the influence on both pK_a(Glu46) and pK_a(*p*CA) simultaneously. In addition, the loss of the Arg52 volume that shielded the chromophore (2) enhances the availability of solvation of ionized *p*CA. Thus, the resulting pK_a(Glu46) and pK_a(*p*CA) are not significantly different upon R52A mutation.

The R52A PYP structure was modeled by removing the Arg side chain except for the C atom in the wild-type PYP crystal structure. We performed energy minimization only on the replaced Ala52 side chain.

1. Genick UK, et al. (1997) Active site mutants implicate key residues for control of color and light cycle kinetics of photoactive yellow protein. *Biochemistry* 36:8–14.
2. Borgstahl GE, Williams DR, Getzoff ED (1995) 1.4 Å structure of photoactive yellow protein, a cytosolic photoreceptor: Unusual fold, active site, and chromophore. *Biochemistry* 34:6278–6287.

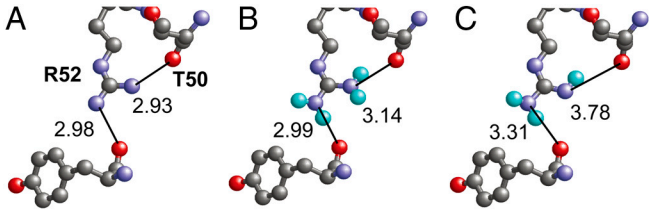
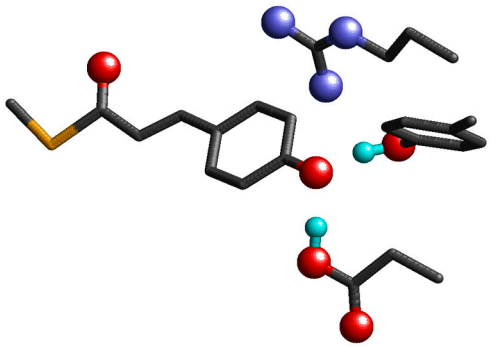


Fig. S1. Influence of the Arg52 protonation state on the H-bond geometry. H atoms are shown only for the quantum mechanical/molecular mechanical (QM/MM) optimized geometry (cyan spheres). (*A*) Crystal structure [Protein Data Bank (PDB) ID code 2ZOH]. QM/MM optimized geometry with (*B*) protonated Arg52 and (*C*) deprotonated Arg52.



Movie S1. Overlay of the QM/MM (H atoms; cyan spheres) and neutron diffraction (H atoms; green spheres) geometries (see Fig. 1 for details).

Movie S1 (GIF)

Table S1. Atomic coordinates and charges (ESP) of the QM/MM optimized geometry for *p*CA, Tyr42, Glu46, and Thr50

Residue		Atom	x	y	z	Charge
THR	50	CB	15.223	−4.62	−5.188	0.478
THR	50	HB	14.818	−3.922	−5.947	−0.123
THR	50	OG1	16.49	−5.1	−5.64	−0.873
THR	50	HG1	17.249	−4.554	−5.368	0.538
THR	50	CG2	15.271	−3.827	−3.878	−0.404
THR	50	HG21	15.757	−4.369	−3.063	0.139
THR	50	HG22	14.255	−3.555	−3.564	0.044
THR	50	HG23	15.816	−2.896	−4.026	0.137
GLU	46	CB	20.041	−4.89	−1.59	−0.346
GLU	46	HB1	20.883	−5.203	−2.214	0.188
GLU	46	HB2	20.46	−4.579	−0.63	0.09
GLU	46	CG	19.335	−3.701	−2.259	−0.09
GLU	46	HG1	18.936	−3.969	−3.242	0.13
GLU	46	HG2	20.072	−2.925	−2.475	0.09
GLU	46	CD	18.198	−3.062	−1.477	0.759
GLU	46	OE1	17.829	−3.443	−0.377	−0.648
GLU	46	OE2	17.57	−2.038	−2.069	−0.631
GLU	46	HE2	17.887	−1.857	−3.004	0.333
TYR	42	CB	24.614	−3.578	−4.898	−0.377
TYR	42	HB1	25.087	−4.291	−5.579	0.174
TYR	42	HB2	24.941	−2.585	−5.204	0.147
TYR	42	CG	23.103	−3.63	−5.03	0.164
TYR	42	CD1	22.382	−4.832	−5.096	−0.205
TYR	42	HD1	22.897	−5.772	−4.941	0.169
TYR	42	CD2	22.381	−2.438	−5.183	−0.135
TYR	42	HD2	22.909	−1.488	−5.146	0.139
TYR	42	CE1	21.009	−4.856	−5.327	−0.378
TYR	42	HE1	20.476	−5.796	−5.378	0.197
TYR	42	CE2	21.003	−2.438	−5.392	−0.419
TYR	42	HE2	20.461	−1.503	−5.48	0.17
TYR	42	CZ	20.314	−3.653	−5.463	0.545
TYR	42	OH	18.954	−3.67	−5.63	−0.748
TYR	42	HH	18.597	−2.784	−5.291	0.423
CYS	69	SG	10.997	3.802	−5.995	−0.043
PCA	169	C2	12.902	1.904	−6.213	−0.471
PCA	169	H2	12.905	2.037	−5.141	0.189
PCA	169	C3	13.734	1.024	−6.831	0.047
PCA	169	H3	13.637	0.934	−7.913	0.118
PCA	169	C1P	14.804	0.294	−6.225	−0.103
PCA	169	C6P	15.18	0.521	−4.881	0.051
PCA	169	H6P	14.612	1.217	−4.28	0.116
PCA	169	C2P	15.618	−0.559	−7	−0.048
PCA	169	H2P	15.382	−0.683	−8.054	0.078
PCA	169	C5P	16.276	−0.094	−4.327	−0.441
PCA	169	H5P	16.56	0.089	−3.297	0.179
PCA	169	C3P	16.724	−1.191	−6.462	−0.297
PCA	169	H3P	17.352	−1.838	−7.064	0.091
PCA	169	C4P	17.079	−0.985	−5.096	0.566
PCA	169	O4P	18.097	−1.585	−4.546	−0.639
PCA	169	C1	12.124	2.83	−7.005	0.502
PCA	169	O1	12.258	2.962	−8.219	−0.573

Table S2 Contribution of the PYP residues to pK_a; pK_a(Glu46) (left) upshifting and (right) downshifting residues

Residue	Side	b.b.	Total	Residue	Side	b.b.	Total
Asn43	1.0	0.5	1.5	Thr50	−1.3	−0.6	−1.9
*p*CA			1.3	Arg52	−1.6	−0.1	−1.8
Asp24	0.5	0.0	0.5	Tyr42	−0.9	−0.6	−1.5
				Gly29	0.0	−1.1	−1.1
				Ile49	-1.0	0.0	-1.0

Values may contain round errors. Residues that increase/decrease pK_a by 0.5 are listed. Side = side chain. b.b. = back bone.

Table S3 Contribution of the PYP residues to pK_a; pK_a(*p*CA) (left) upshifting and (right) downshifting residues

Residue	Side	b.b.	Total	Residue	Side	b.b.	Total
Glu46	0.7	0.1	0.8	Arg52	−2.7	−0.2	−2.9
Asn43	0.4	0.3	0.7	Tyr42	−2.0	−0.3	−2.3
Asp97	0.9	-0.4	0.5	Thr50	−1.1	−0.3	−1.5
				Cys69			−0.9
				Ile49	−0.5	0.0	−0.5

The entire Cys69 was treated as the backbone region of *p*CA.

Table S4. Contribution of the PYP residues to pK_a; pK_a(Arg52) (left) upshifting and (right) downshifting residues

Residue	Side	b.b.	Total	Residue	Side	b.b.	Total
*p*CA			2.2	Lys60	−0.4	0.0	−0.4
Tyr98	−0.2	2.0	1.8				
Thr50	−0.1	1.0	0.8				
Val66	0.0	0.7	0.7				
Met100	0.5	0.1	0.7				

Table S5. Atomic charges for *p*CA used for electrostatic titration

Atom	Protonated	Deprotonated
C1	0.471	0.423
O1	−0.496	−0.606
C2	−0.335	−0.498
H2	0.147	0.143
C3	−0.023	0.075
H3	0.116	0.083
C1′	0.071	−0.077
C2′	−0.127	−0.091
H2′	0.135	0.103
C3′	−0.268	−0.395
H3′	0.167	0.121
C4′	0.378	0.728
C5′	−0.268	−0.395
H5′	0.167	0.121
C6′	−0.127	−0.091
H6′	0.135	0.103
O4′	−0.506	−0.747
HO4	0.363	
total	0.000	-1.000

Table S6. Atomic coordinates of the QM/MM optimized geometries for Arg52, Met100 and carbonyl backbone groups of Thr50 and Tyr98. Arg52 protonated

Residue		Atom	x	y	z
ARG	52	CB	18.522	−5.213	−8.767
ARG	52	HB1	19.585	−5.095	−9.003
ARG	52	HB2	18.45	−5.118	−7.685
ARG	52	CG	17.763	−4.068	−9.472
ARG	52	HG1	17.908	−4.125	−10.562
ARG	52	HG2	18.207	−3.123	−9.138
ARG	52	CD	16.267	−4.03	−9.169
ARG	52	HD1	16.125	−3.971	−8.088
ARG	52	HD2	15.776	−4.939	−9.537
ARG	52	NE	15.604	−2.875	−9.787
ARG	52	HE	16.114	−2.297	−10.441
ARG	52	CZ	14.318	−2.58	−9.553
ARG	52	NH1	13.549	−3.425	−8.864
ARG	52	HH11	13.87	−4.342	−8.574
ARG	52	HH12	12.711	−3.096	−8.377
ARG	52	NH2	13.791	−1.438	−10.035
ARG	52	HH21	12.783	−1.306	−10.008
ARG	52	HH22	14.387	−0.649	−10.257
MET	100	CB	9.658	−4.41	−8.391
MET	100	HB1	10.565	−4.507	−8.992
MET	100	HB2	9.672	−5.229	−7.662
MET	100	CG	9.629	−3.046	−7.659
MET	100	HG1	8.827	−2.982	−6.919
MET	100	HG2	9.488	−2.231	−8.362
MET	100	SD	11.154	−2.558	−6.782
MET	100	CE	11.049	−3.625	−5.307
MET	100	HE1	11.109	−4.681	−5.569
MET	100	HE2	10.109	−3.428	−4.786
MET	100	HE3	11.886	−3.36	−4.657
THR	50	C	14.162	−6.607	−6.405
THR	50	O	13.493	−6.153	−7.317
TYR	98	C	9.908	−0.784	−10.794
TYR	98	O	10.809	−1.45	−10.32

Table S7. Atomic coordinates of the QM/MM optimized geometries for Arg52, Met100, and carbonyl backbone groups of Thr50 and Tyr98; Arg52 deprotonated

Residue			Atom	x	y	z
ARG	A	52	CB	18.544	−5.232	−8.722
ARG	A	52	HB1	19.623	−5.135	−8.896
ARG	A	52	HB2	18.418	−5.159	−7.643
ARG	A	52	CG	17.851	−4.058	−9.435
ARG	A	52	HG1	18.072	−4.087	−10.516
ARG	A	52	HG2	18.294	−3.131	−9.052
ARG	A	52	CD	16.339	−3.967	−9.241
ARG	A	52	HD1	16.116	−3.952	−8.171
ARG	A	52	HD2	15.838	−4.851	−9.667
ARG	A	52	NE	15.82	−2.746	−9.836
ARG	A	52	HE	16.258	−2.456	−10.7
ARG	A	52	CZ	14.455	−2.472	−9.783
ARG	A	52	NH1	13.543	−3.119	−9.151
ARG	A	52	HH11	13.88	−3.977	−8.72
ARG	A	52	NH2	14.113	−1.35	−10.546
ARG	A	52	HH21	13.125	−1.149	−10.436
ARG	A	52	HH22	14.711	−0.535	−10.415
MET	A	100	CB	9.693	−4.522	−8.417
MET	A	100	HB1	10.568	−4.81	−9.006
MET	A	100	HB2	9.587	−5.28	−7.628
MET	A	100	CG	9.949	−3.137	−7.789
MET	A	100	HG1	9.059	−2.718	−7.307
MET	A	100	HG2	10.292	−2.444	−8.552
MET	A	100	SD	11.305	−3.082	−6.552
MET	A	100	CE	10.484	−3.866	−5.126
MET	A	100	HE1	10.267	−4.922	−5.3
MET	A	100	HE2	9.563	−3.337	−4.869
MET	A	100	HE3	11.181	−3.806	−4.285
THR	A	50	C	14.1	−6.653	−6.376
THR	A	50	O	13.28	−6.362	−7.222
TYR	A	98	C	9.912	−0.781	−10.798
TYR	A	98	O	10.815	−1.44	−10.339