



STRUCTURAL
BIOLOGY

Volume 75 (2019)

Supporting information for article:

X-ray crystallographic studies on the hydrogen isotope effects of green fluorescent protein at sub-ångström resolutions

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Table S1 Bond lengths of the chromophore.

	d-GFP _{C3} at pD 8.5	d-GFP _{C3} at pD 7.0	h-GFP _{C3} at pD 8.5	h-GFP _{C3} at pH 8.5 (Takaba <i>et al.</i> , 2019)
C ^ε –O ⁿ	1.311(9) †	1.314(6)	1.310(8)	1.314(6)
C ^{ε2} –C ^ε	1.413(11)	1.415(8)	1.422(10)	1.414(8)
C ^{δ2} –C ^{ε2}	1.354(10)	1.361(8)	1.355(9)	1.364(8)
C ^{γ2} –C ^{δ2}	1.412(10)	1.419(8)	1.421(9)	1.417(8)
C ^{γ2} –C ^{δ1}	1.403(11)	1.415(7)	1.416(10)	1.407(8)
C ^{δ1} –C ^{ε1}	1.364(11)	1.372(8)	1.372(9)	1.372(8)
C ^{ε1} –C ^ε	1.422(11)	1.423(8)	1.405(9)	1.429(8)
C ^{β2} –C ^{γ2}	1.423(10)	1.422(7)	1.419(9)	1.430(8)
C ^{β2} –C ^{α2}	1.384(10)	1.384(7)	1.384(9)	1.369(7)
C ² –C ^{α2}	1.467(10)	1.456(7)	1.450(9)	1.448(7)
C ² –O ²	1.226(9)	1.238(6)	1.242(7)	1.245(6)
C ² –N ³	1.380(10)	1.383(7)	1.375(8)	1.385(7)
C ¹ –N ³	1.385(10)	1.393(7)	1.390(8)	1.399(7)
C ¹ –N ²	1.315(9)	1.304(6)	1.301(7)	1.305(6)
C ^{α2} –N ²	1.382(10)	1.395(7)	1.390(8)	1.417(7)

† Values in parentheses are the estimated standard deviations derived from the full-matrix least squares refinement.

Table S2 Summary of GFP structures determined at high resolutions.

PDB	Form	d_{\min} (Å)	Program	DPI (Å) [†]	Mutation
2awk	A	1.15	<i>SHELX</i>	0.078	F64L/S65T/R96M/F99S/M153T/V163A
2due	B	1.24	<i>SHELX</i>	0.078	S65T/H148N
2duh	B	1.2	<i>SHELX</i>	0.077	S65T/H148D
2fzu	ambiguous	1.25	<i>SHELX</i>	0.078	F64L/S65T/F99S/M153T/V163A
2hjo	B	1.25	<i>SHELX</i>	0.064	F64L/S65T/V224H
2hqz	B	1.2	<i>SHELX</i>	0.061	L42H/F64L/S65T
2qz0	A	1.2	<i>SHELX</i>	0.066	F64L/F99S/M153T/V163A/Q183E
2wur	ambiguous	0.9	<i>SHELX</i>	0.034	F64L/I167T/K238N
3dq7	A	1.23	<i>REFMAC</i>	0.126	S65G/V68L/Q69M/S72A/T203Y/H231L
3sry	A	1.16	<i>PHENIX</i>	0.057	S65G/S72A/Q183A/T203Y/H231L
3ssh	A	1.28	<i>PHENIX</i>	0.065	S65G/S72A/K79R/Q183A/T203Y/H231L
3st0	A	1.19	<i>PHENIX</i>	0.065	S65G/Q69T/S72A/K79R/V163A/T203Y/H231L
4ges	A	1.23	<i>REFMAC</i>	0.075	F99S/M153T/V163A
4gf6	A	1.1	<i>REFMAC</i>	0.055	F99S/M153T/V163A
6fww	ambiguous	1.13	<i>PHENIX</i>	0.067	V11K/Y39K/F64L/L221K
6ir7	A	1.28	<i>PHENIX</i>	0.128	F99S/M153T/V163A
6jgh	A	0.94	<i>SHELX</i>	0.032	F99S/M153T/V163A/T203I
6jgi	B	0.85	<i>SHELX</i>	0.023	S65T/F99S/M153T/V163A
6jgj	B	0.78	<i>MoPro</i>	0.018	F99S/M153T/V163A/E222Q
6ofk	B	1.15	<i>PHENIX</i>	0.061	S65T
6ofl	ambiguous	1.25	<i>PHENIX</i>	0.068	S65T/Y66(3-ClY)
6kkz	B	0.90	<i>SHELX</i>	0.027	S65T/F99S/M153T/V163A (d-GFP _{C3} at pD 8.5)
6kl0	B	0.80	<i>SHELX</i>	0.020	S65T/F99S/M153T/V163A (d-GFP _{C3} at pD 7.0)
6kl1	B	0.85	<i>SHELX</i>	0.023	S65T/F99S/M153T/V163A (h-GFP _{C3} at pD 8.5)

[†]Dispersion Precision Indicator as positional error calculated with the data deposited in PDB as follows: $\sigma(r, B_{\text{avg}}) = 3^{1/2}(N_i/p)^{1/2}C^{-1/3}R_{\text{free}}d_{\min}$ (Cruickshank, 1999).

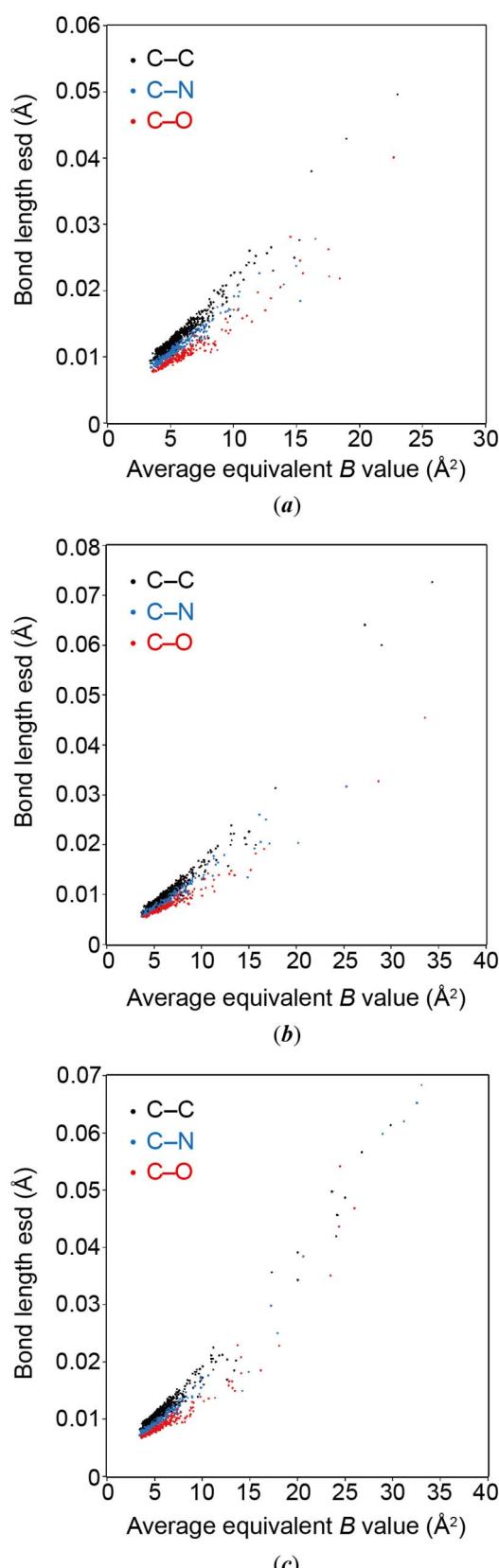


Figure S1 The plots of bond length estimated standard deviations versus average equivalent B values from unrestrained full matrix calculation for (a) d-GFP_{C3} (pD 8.5), (b) d-GFP_{C3} (pD 7.0) and (c) h-GFP_{C3} (pD 8.5).

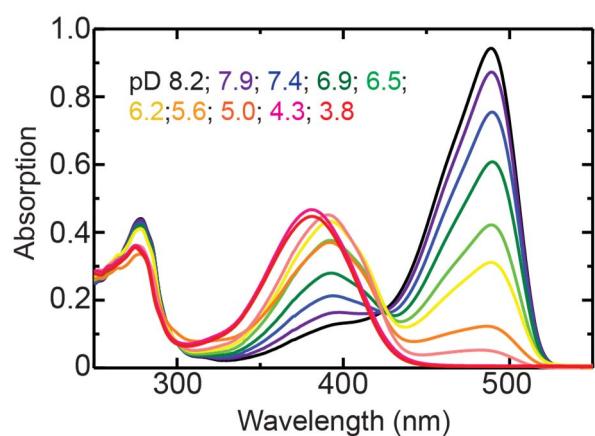


Figure S2 UV-vis absorption spectra of d-GFP_{C3} in D₂O at various pD values are shown in the wavelength range from 250 to 550 nm.

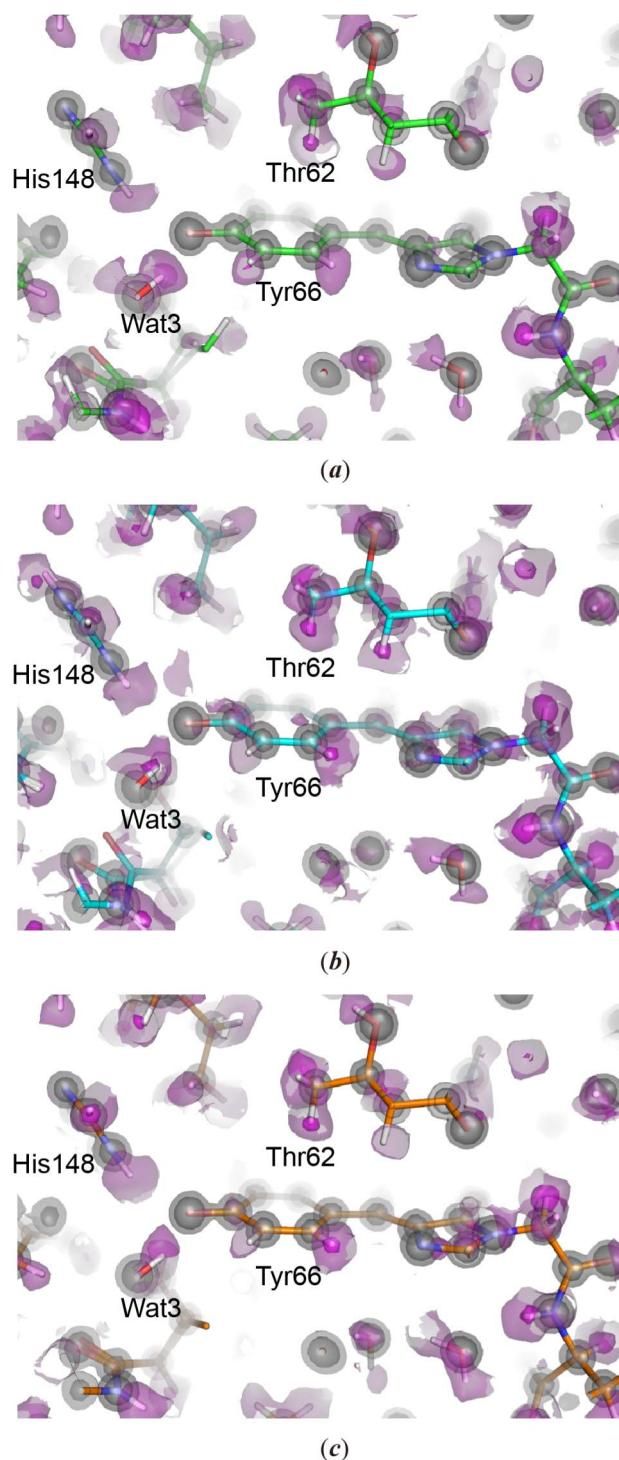


Figure S3 Electron density maps. The $2F_{\text{obs}} - F_{\text{calc}}$ map (3σ and 6σ levels) is shown in grey. In addition, the $F_{\text{obs}} - F_{\text{calc}}$ hydrogen omit map (1.5σ and 4σ levels) is shown in pink. (a) d-GFP_{C3} (pD 8.5). (b) d-GFP_{C3} (pD 7.0). (c) h-GFP_{C3} (pD 8.5).

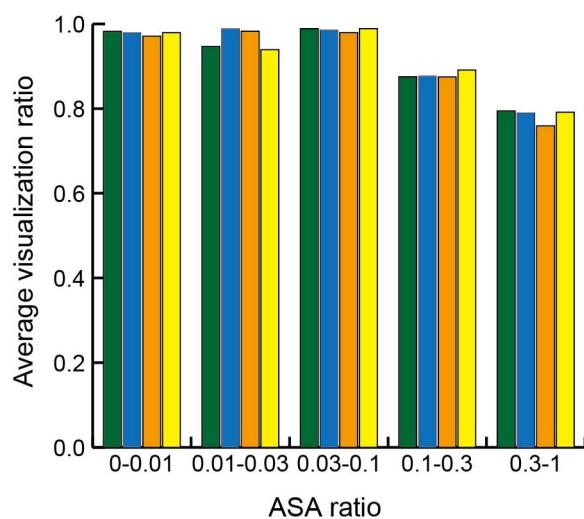


Figure S4 The histogram of average hydrogen visualization ratio for residues in each bin of certain ASA ratio ranges for d-GFP_{C3} (pD 8.5), d-GFP_{C3} (pD 7.0), h-GFP_{C3} (pD 8.5) and h-GFP_{C3} (pH 8.5) which are shown in green, blue, orange and yellow, respectively.

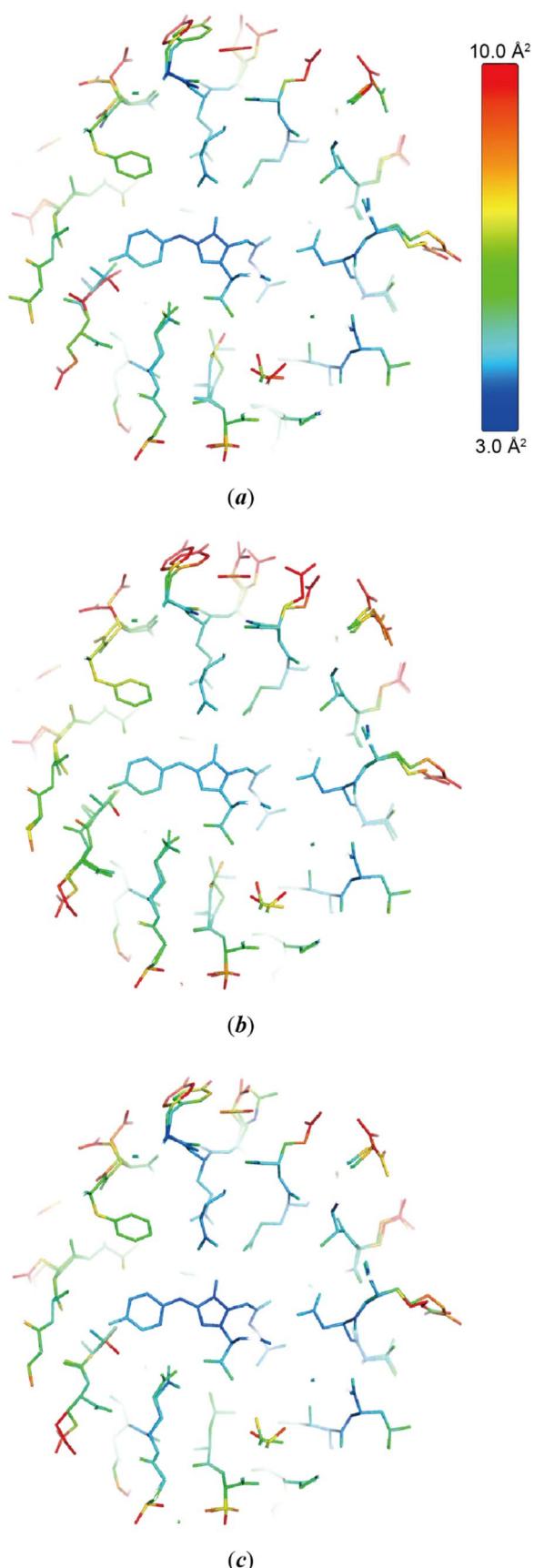


Figure S5 The distribution of *B* factor of the residues around the chromophore for (a) d-GFP_{C3} (pD 8.5), (b) d-GFP_{C3} (pD 7.0) and (c) h-GFP_{C3} (pD 8.5).