Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supplementary Information

Emission Shaping in Fluorescent Proteins: Role of Electrostatics and π-Stacking

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Text S1. Details of diabatic state responses.

In this supplementary text, we describe the responses of individual diabatic states in a detailed way. The bare chromophore diabatic Hamiltonian **D** is perturbed by $\Delta\mathbf{D}_{res}$ with electrostatic interactions with an amino acid residue *res* as

$$\Delta\mathbf{D}_{res} = \sum_{A}^{\text{int}} \sum_{N}^{res} \frac{q_N}{|\mathbf{X}_N - \mathbf{X}_A|} \mathbf{q}_A \,.$$

The diabatic electronic population matrix $\mathbf{q}_A$ for chromophore atoms A at X_A is used for characterizing the charge distributions in the chromophore, and the partial charges q_N for the atom N at $\mathbf{X}_N$ in *res* is used for describing the electrostatic interactions involving *res*. This equation is equivalent to eq. (6) in the "Computational details" section in the main text. This quantity displays the degrees of perturbation to the diabatic states due to the presence of the residue. When $\Delta\mathbf{D}_{res}$ and the diabatic Hamiltonian in vacuo **D** are available, we can also estimate the gap energy modulations by comparing the eigenvalues of the matrices **D** and $\mathbf{D}+\Delta\mathbf{D}_{res}$. The eigenvectors obtained with this diagonalization process will of course represent how the diabatic state contributions toward forming the adiabatic states change by the electrostatic perturbation.

The elements in the matrices **D** and $\Delta\mathbf{D}_{res}$, and corresponding eigenvalues and eigenvectors for all the residues considered in this work (namely, *res* = Thr62, Phe64, Val68, Arg96, Tyr145, His148, and Thr203) are presented in Table S1. The quantities in this table were calculated by averaging **D** and $\Delta\mathbf{D}$ over 20,000 trajectory snapshots. The eigenvalues and eigenvectors were obtained by diagonalizing averaged **D** and $\mathbf{D}+\Delta\mathbf{D}$. Average $\Delta\mathbf{D}$ from each residue shows how the residue perturbs the chromophore and change the S_0–S_1 gap energies. For example, including Tyr145 stabilize the *P*-state more than the *B*- and the *I*-

states. This makes the *P*-state and the *I*-state more contributing in S_0 and S_1 adiabatic states respectively, leading to the increased (blue-shifted) $S_0 - S_1$ gap energies.

Table S1. The elements in the matrices **D** and $\Delta\mathbf{D}_{res}$ for each residue and their corresponding eigenvalues (i.e. adiabatic energies) and eigenvectors. The definitions of the elements are

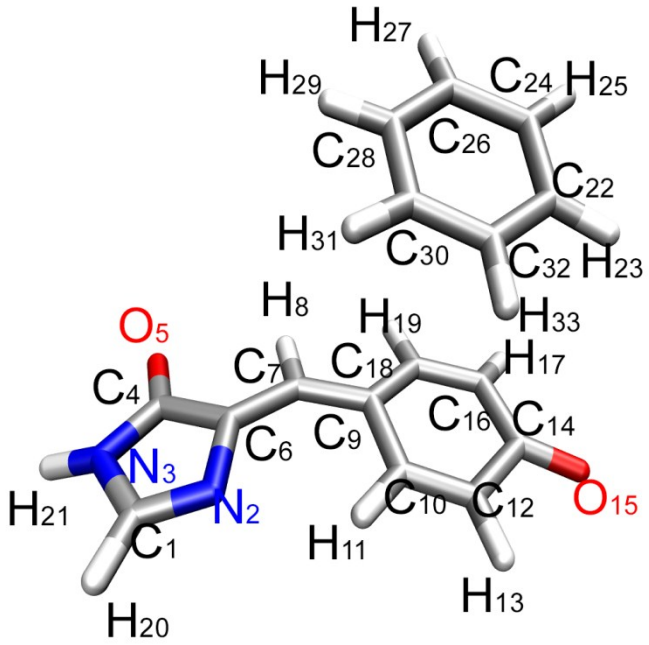
$$\mathbf{D} = \begin{pmatrix} D_{PP} & D_{PI} & D_{PB} \\ D_{PI} & D_{II} & D_{IB} \\ D_{PB} & D_{IB} & D_{BB} \end{pmatrix} \text{ and } \Delta\mathbf{D} = \begin{pmatrix} \Delta D_{PP} & \Delta D_{PI} & \Delta D_{PB} \\ \Delta D_{PI} & \Delta D_{II} & \Delta D_{IB} \\ \Delta D_{PB} & \Delta D_{IB} & \Delta D_{BB} \end{pmatrix}.$$

Energies are shown in the eV unit.

	D	$\Delta\mathbf{D}_{Thr62}$	$\Delta\mathbf{D}_{Phe64}$	$\Delta\mathbf{D}_{Val68}$	$\Delta\mathbf{D}_{Arg96}$	$\Delta\mathbf{D}_{Tyr145}$	$\Delta\mathbf{D}_{His148}$	$\Delta\mathbf{D}_{Thr203}$
$(\Delta)D_{PP}$[a]	1.96	–0.12	–0.57	–0.35	–0.59	–0.55	–0.82	–0.52
$(\Delta)D_{II}$[a]	2.35	0.23	–0.31	–0.59	–1.27	–0.27	–0.48	–0.24
$(\Delta)D_{BB}$[a]	3.81	–0.02	–0.51	–0.42	–0.97	–0.33	–0.53	–0.31
$(\Delta)D_{PI}$[a]	–0.96	0.00	–0.01	0.01	0.02	–0.01	–0.02	–0.01
$(\Delta)D_{PB}$[a]	–0.63	–0.03	–0.02	0.02	0.01	0.02	0.02	0.01
$(\Delta)D_{BI}$[a]	–0.90	–0.02	–0.06	0.05	–0.02	0.02	0.03	0.02
$E(S_0)$[b]	0.80	0.80	0.29	0.38	–0.13	0.39	0.14	0.41
$E(S_1)$[b]	3.07	3.12	2.61	2.61	2.17	2.68	2.45	2.71
$E(S_2)$[b]	4.25	4.29	3.82	3.77	3.25	3.91	3.70	3.93
$E(S_1)–E(S_0)$	2.27	2.32	2.32	2.22	2.30	2.29	2.31	2.29
Shift[c]	0.00	0.06	0.06	–0.04	0.03	0.03	0.04	0.03
$\langle P\|S_0\rangle$[d]	0.70	0.75	0.73	0.68	0.60	0.75	0.76	0.75
$\langle I\|S_0\rangle$[d]	0.63	0.57	0.59	0.66	0.72	0.59	0.58	0.59
$\langle B\|S_0\rangle$[d]	0.34	0.34	0.35	0.33	0.35	0.31	0.31	0.32
$\langle P\|S_1\rangle$[d]	0.71	0.66	0.68	0.73	0.79	0.66	0.65	0.66
$\langle I\|S_1\rangle$[d]	–0.68	–0.69	–0.67	–0.67	–0.61	–0.70	–0.71	–0.70
$\langle B\|S_1\rangle$[d]	–0.22	–0.29	–0.29	–0.15	–0.09	–0.26	–0.26	–0.26
$\langle P\|S_2\rangle$[d]	–0.09	–0.07	–0.06	–0.12	–0.15	–0.07	–0.06	–0.07
$\langle I\|S_2\rangle$[d]	–0.39	–0.44	–0.45	–0.34	–0.33	–0.40	–0.40	–0.40
$\langle B\|S_2\rangle$[d]	0.92	0.90	0.89	0.93	0.93	0.91	0.91	0.91

[a] Bare chromophore Hamiltonian **D** or its residue perturbation $\Delta\mathbf{D}$. The distinction is apparent from the top row.
[b] Eigenvalues of **D** or $(\mathbf{D}+\Delta\mathbf{D}_{res})$ matrix. These are equivalent to the adiabatic energies.
[c] Gap energy ($E(S_1)–E(S_0)$) shift from the bare chromophore.
[d] Eigenvectors of **D** or $(\mathbf{D}+\Delta\mathbf{D}_{res})$ matrix. These values represent the diabatic compositions of a given adiabatic state denoted with the ket vector.

Table S2. Natural atomic charges on a π-stacked geometry.



	$q(S_0)$	$q(S_1)$	$q(S_1) - q(S_0)$	$\Sigma(q(S_1) - q(S_0))$
C1	0.26573	0.21329	−0.05244	
N2	−0.53114	−0.58328	−0.05213	
N3	−0.75340	−0.75339	0.00001	
C4	0.76688	0.69607	−0.07081	
O5	−0.79086	−0.76083	0.03003	
C6	−0.16412	0.10870	0.27281	
C7	−0.00269	−0.38813	−0.38544	
H8	0.23487	0.24390	0.00903	
C9	−0.29370	0.03535	0.32905	
C10	−0.12799	−0.25048	−0.12249	Chromophore:
H11	0.24600	0.24790	0.00190	
C12	−0.39186	−0.32403	0.06783	0.00625
H13	0.22099	0.21809	−0.00290	
C14	0.56993	0.50697	−0.06296	
O15	−0.79415	−0.76215	0.03200	
C16	−0.40840	−0.29743	0.11097	
H17	0.22272	0.21843	−0.00429	
C18	−0.10478	−0.19913	−0.09435	
H19	0.21321	0.21428	0.00107	
H20	0.19965	0.19977	0.00012	
H21	0.42775	0.42698	−0.00076	

C_{22}	0.23670	0.23665	−0.00004	
H_{23}	−0.22573	−0.22650	−0.00076	
C_{24}	−0.24768	−0.24702	0.00065	
H_{25}	0.22543	0.22539	−0.00004	
C_{26}	−0.24803	−0.24860	−0.00057	
H_{27}	0.22346	0.22345	−0.00002	
C_{28}	−0.24294	−0.24482	−0.00188	Tyr203 Ring:
H_{29}	0.23011	0.22940	−0.00071	−0.00625
C_{30}	−0.22009	−0.22171	−0.00161	
H_{31}	0.24297	0.24266	−0.00031	
C_{32}	−0.22257	−0.22338	−0.00081	
H_{33}	0.24375	0.24360	−0.00014	

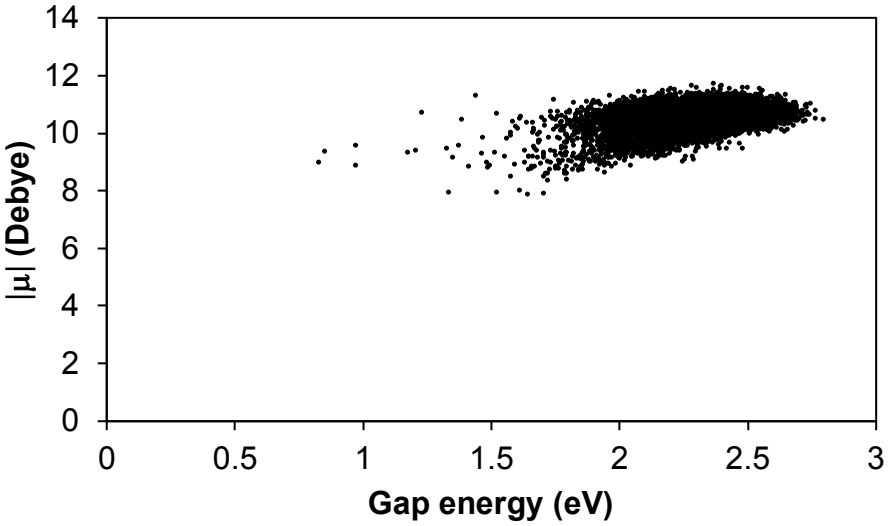


Figure S1. Constancy of the transition dipole sizes. Here, the same conformations adopted for the wild-type GFP fluorescence simulation were adopted, after reducing the total number by a factor of 1/100 for computational economy. Transition dipoles were computed at the SA3-CAS(4,3)/6-31G* level of theory.

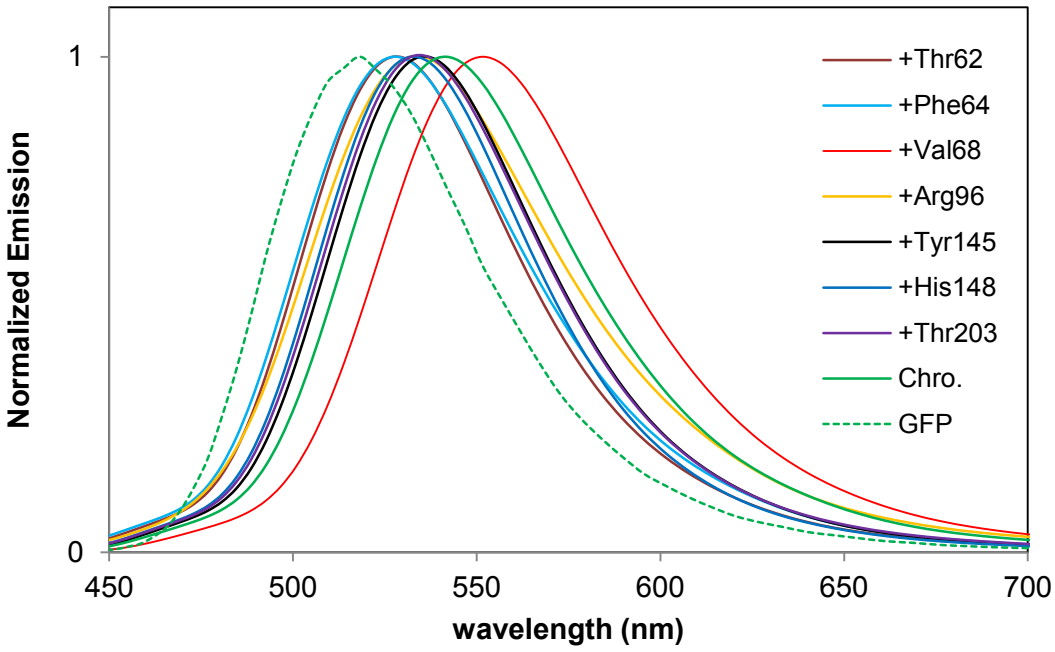


Figure S2. Full version of Figure 4 in the main text.

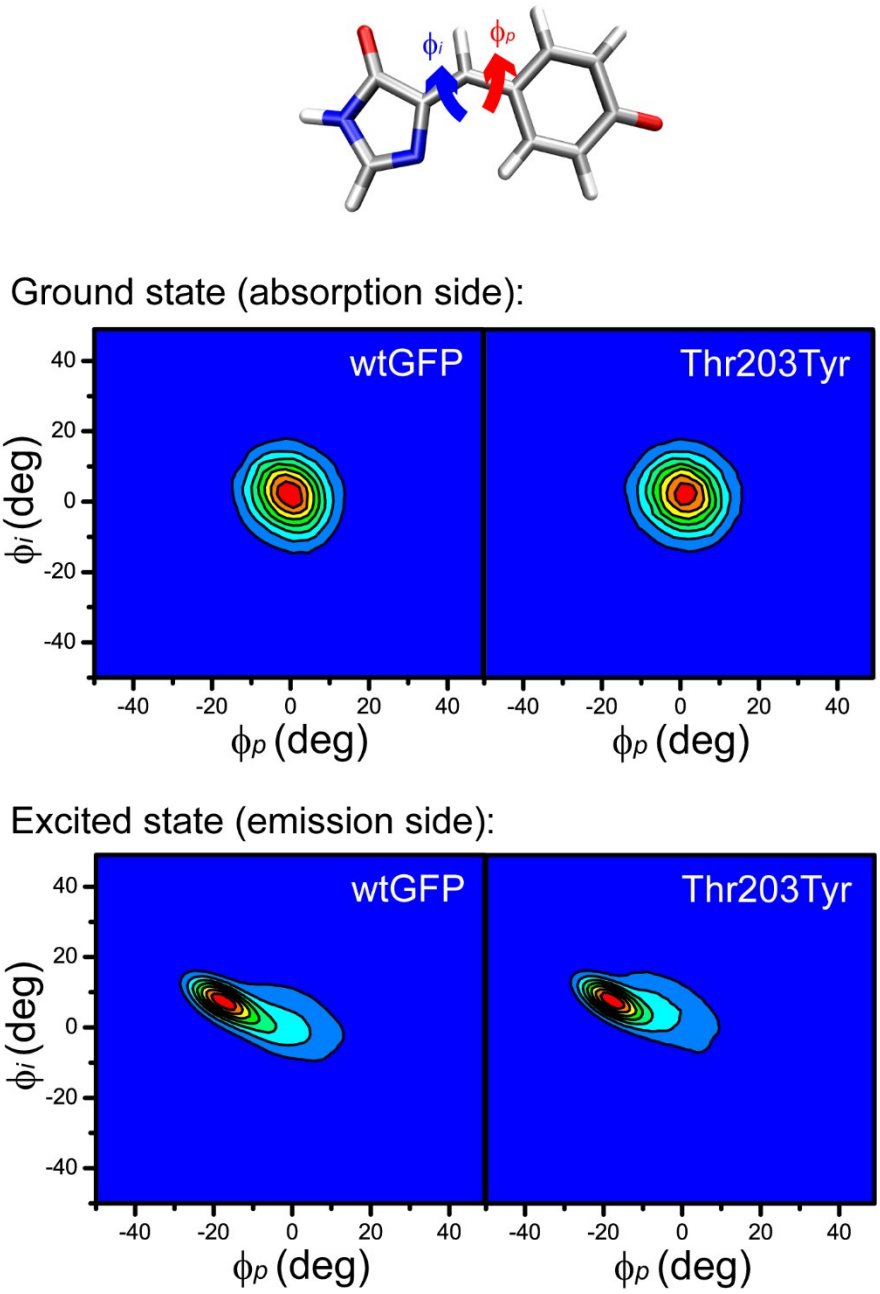


Figure S3. Distributions of the bridge dihedral angles of the chromophores in wtGFP and Thr203Tyr mutant. Red is for more frequent appearances and blue is for zero appearance.

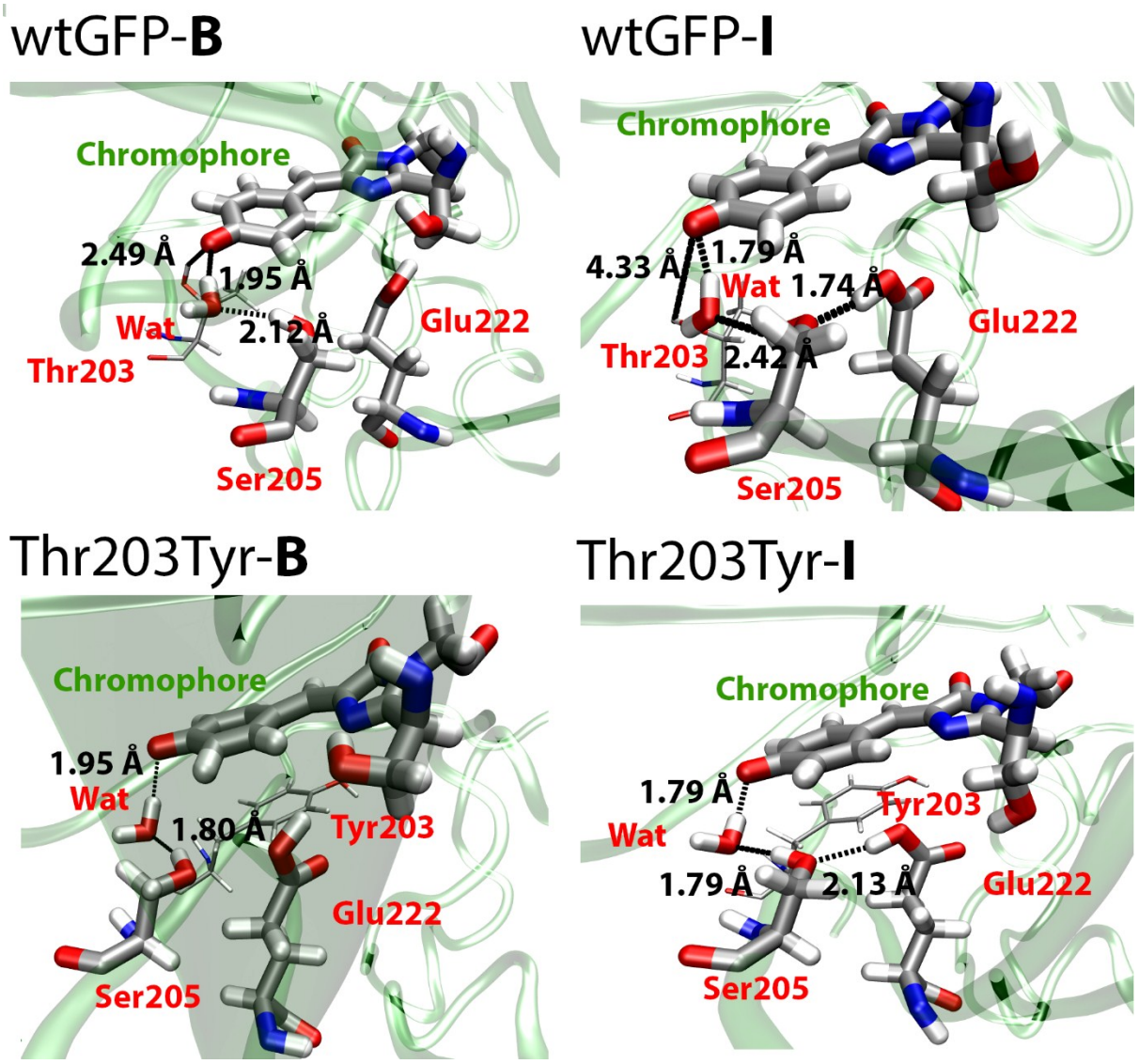


Figure S4. Representative proton wire configurations of **B** and **I** states in the wtGFP and Thr203Tyr mutant.

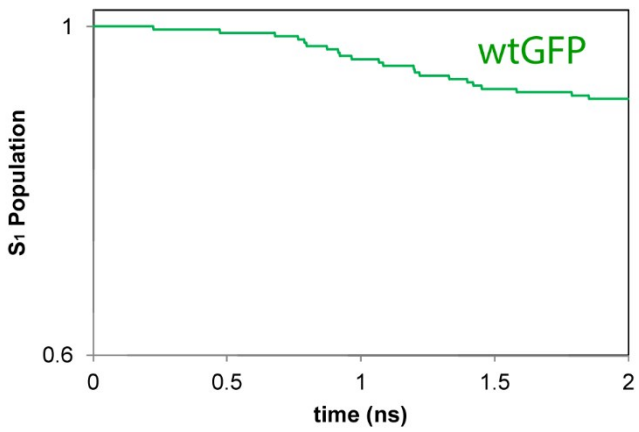


Figure S5. Population decay pattern of wtGFP obtained with Landau-Zener surface hopping simulations.