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Electronic Supplementary Information (ESI) for

Fluorescence enhancement of a ligand-activated fluorescent protein induced by collective noncovalent interactions

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Figure S1. Calculated CD spectra of BR in UnaG, eUnaG, and V2T. The line shape of each peak is assumed to be a Lorentzian function, $\Delta\varepsilon(\omega) \propto \sum_n R_{0n}\Gamma / \{(\omega-\omega_n)^2+\Gamma^2\}$ where R_{0n}, Γ, and ω_n are the rotational strength of the *n*th electronic state, dephasing constant, and transition frequency, respectively. The dephasing constants for the low and high energy transitions are assumed to be 1300 and 2000 cm^{-1}, respectively.

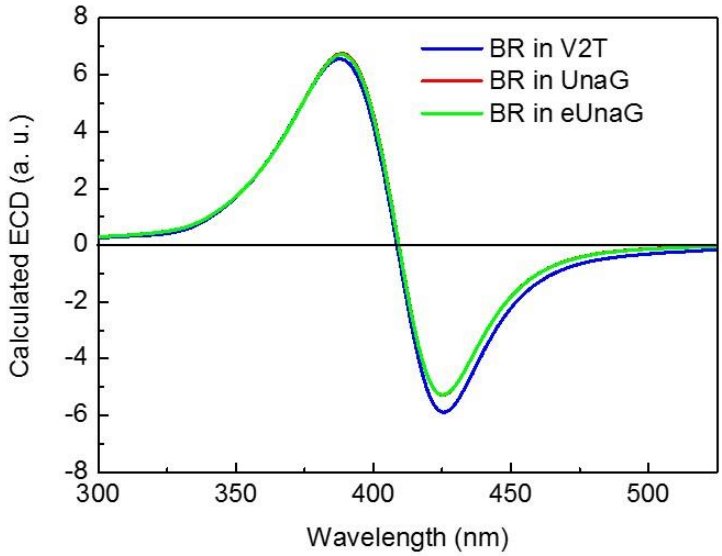

Figure S2. Distance distribution of protein atoms close to each heavy atom of BR (obtained from Eq. (3)) for the entire molecule in three protein systems.

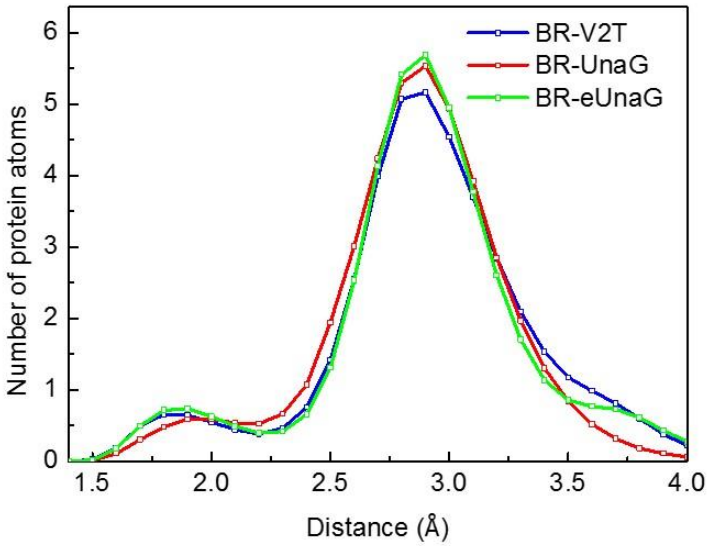

Figure S3. Open top and closed bottom structure of the protein (red surface-rendered structures) with the exposed BR (stick model circled in orange dashed line). BR is exposed to bulk water at the open top (a), but not at the closed bottom of the protein (b).

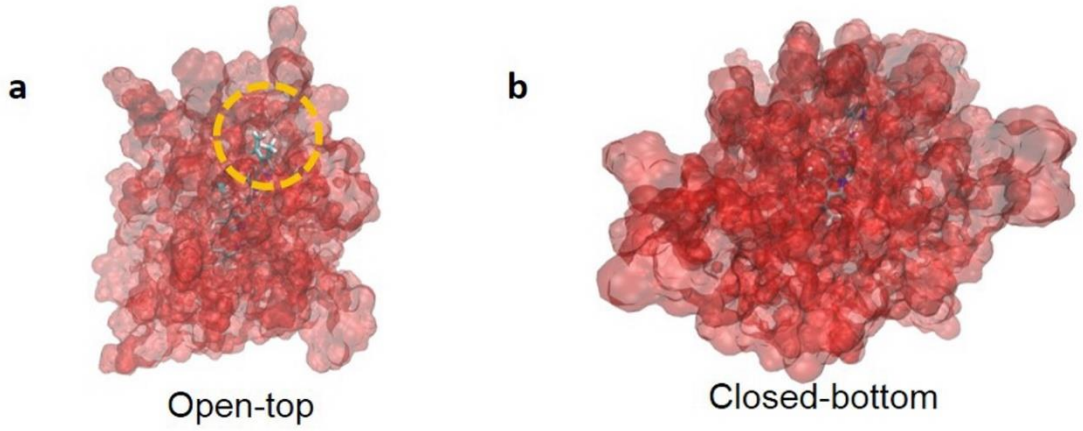

Figure S4. Water distribution near BR in the water-binding cavity. (a) Bound water 1 (blue dot) and 2 (white dot) distributions confined near BR (stick model) for 1 ns MD trajectories in eUnaG. (b) Escape and return of a bound water molecule from the binding pocket in UnaG. The example period trapped in the binding cavity corresponds to MD simulation steps between 100 and 101 ns. Before 20 ns from the trapped period, the water exists outside of the binding pocket, and after 20ns, it gradually moves out of the binding pocket. (c-e) Counts of water molecules within 3 Å from the water-binding atoms of BR in V2T (d), in UnaG (e), and in eUnaG (f). Red arrows represent the bound water 1 and 2 obtained from the B-W map.

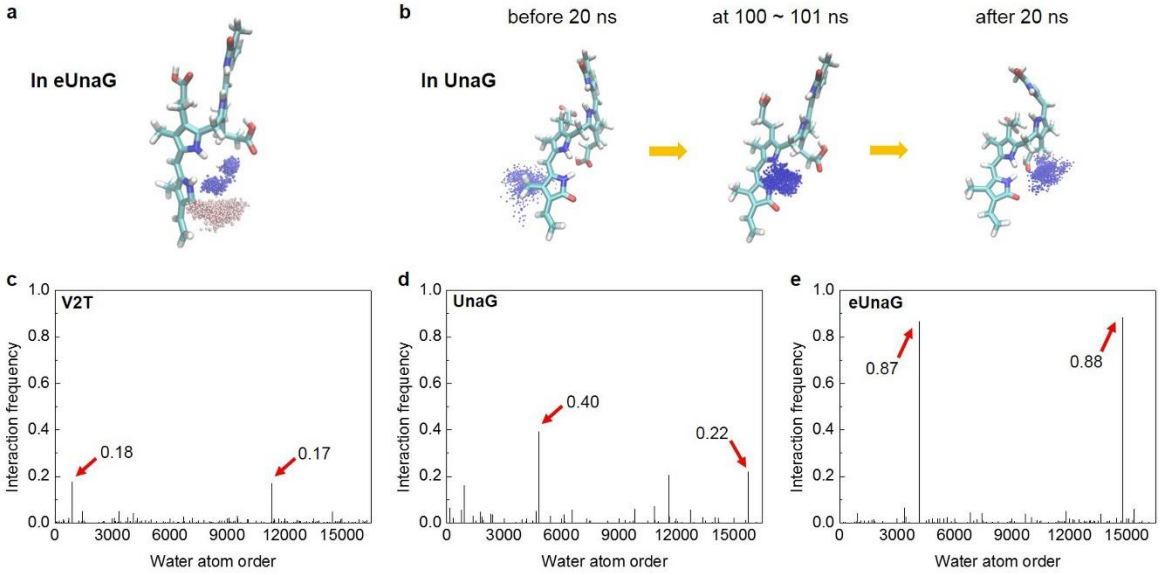

Figure S5. Molecular orbitals involved in the dominant transition, with large oscillator strength, from the ground state to the second excited state **2** (see Table S3) of BR with no H-bonded water molecule. The relative weighting factors of these three transition configurations can be found in Table S3.

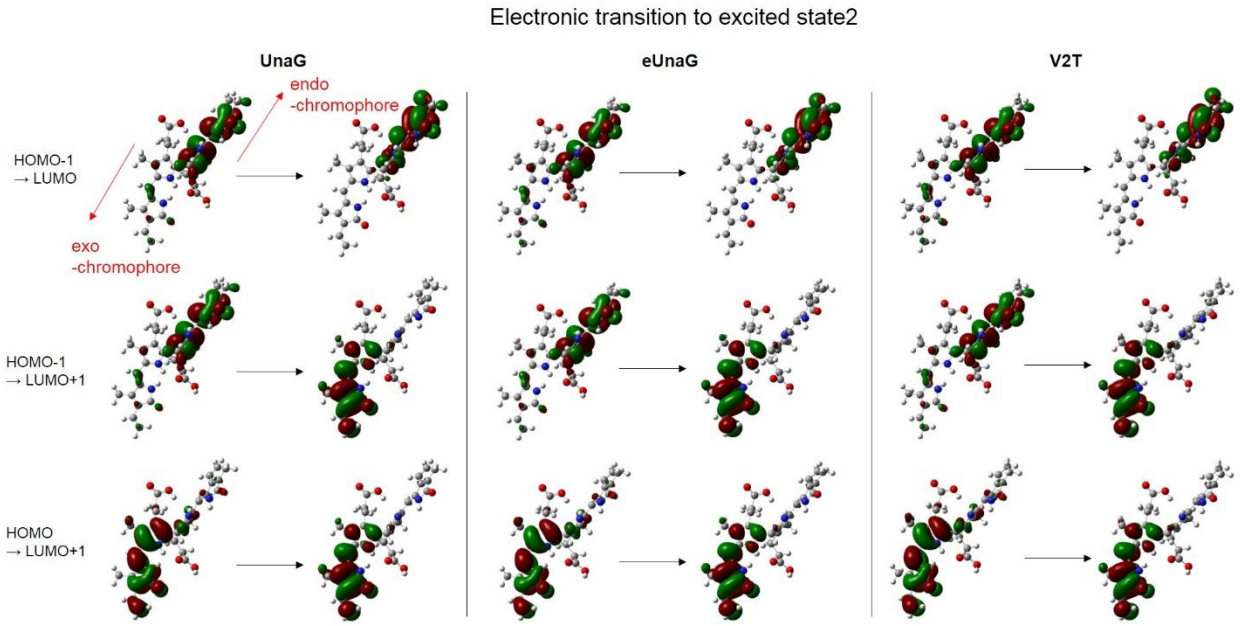

Figure S6. Molecular orbitals involved in the electronic transition to the second excited state **2** (see Table S4) of BR with two H-bonded water molecules for the three protein systems. The water H-bonding sites on each BR were chosen by analyzing our MD trajectories of the eUnaG.

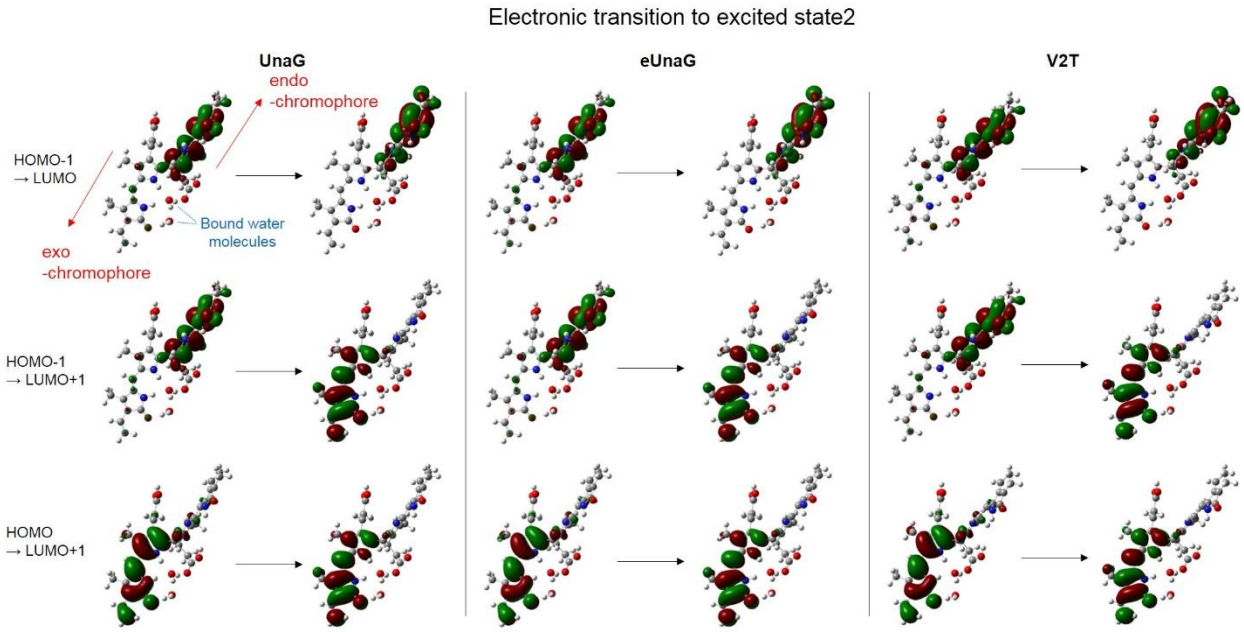

Table S1. Averages and standard deviations of radius gyration, RMSD and dihedral angles from MD traces in Fig. 2a-d.

	UnaG	**eUnaG**	**V2T**
Radius of gyration	9.58 ± 0.20 Å	10.19 ± 0.12 Å	9.96 ± 0.28 Å
RMSD	0.29 Å	< 0.22 Å	0.35 Å
ϕ (exo)	-58.37 ± 7.55°	-59.31 ± 7.11°	-55.68 ± 9.12°
ψ (endo)	-63.69 ± 7.65°	-61.41 ± 7.12°	-57.18 ± 7.90°

Table S2. Average Hydrogen-bond (H-bond) number between all atoms of BR and protein, and between all atoms of BR and each changed binding residue determined from the B-P map in Fig. 4, for the 110 ns MD trajectories. The cutoff of distance (acceptor to donor heavy atom) is 3.5 Å and of angle is 120°, which are widely used.

	UnaG	**eUnaG**	**V2T**
All atoms of protein	34.09	35.18	32.01
Phe17	0.71	2.07	2.04
Ile55	1.67	2.95	2.65
Asn57	2.85	3.87	3.90
Ser80	1.76	3.79	2.28
Arg112	1.83	1.46	0.99
Arg132	3.57	1.49	1.36
Tyr134	1.98	1.36	1.48

Table S3. Time-dependent DFT calculation results on BR in UnaG (a), eUnaG (b), and V2T (c). Configurations involved in each electronic transition and their weighting factors are given in the second column of this table. Then, the transition energy (eV), transition wavelength (nm), and oscillator strength associated with each transition are summarized in this table. In all cases (a-c), the electronic transition with the highest oscillator strength corresponds to the excited state **2** (gray shaded).

a. BR in UnaG

Excited state	Transition	Transition Energy (eV)	Transition Wavelength (nm)	Oscillator strength
1	HOMO→LUMO (0.70472)	2.4280	510.64	0.0944
2	HOMO-1→LUMO (-0.38251) HOMO-1→LUMO+1 (0.13572) HOMO→LUMO+1 (0.57179)	2.9460	420.85	1.2007
3	HOMO-1→LUMO (0.57023) HOMO-1→LUMO+1 (0.18329) HOMO→LUMO+1 (0.34962)	3.1324	395.81	0.1648
4	HOMO-1→LUMO+1 (0.66664) HOMO→LUMO+1 (-0.20961)	3.2537	381.06	0.1967
5	HOMO-5→LUMO+1 (0.15596) HOMO-2→LUMO (0.59041) HOMO-2→LUMO+1 (-0.28251) HOMO→LUMO+2 (0.17115)	3.7038	334.75	0.0284
6	HOMO-2→LUMO (-0.16916) HOMO→LUMO+2 (0.67498)	3.7093	334.26	0.0012

b. BR in eUnaG

Excited state	Transition	Transition Energy (eV)	Transition Frequency (nm)	Oscillator strength
1	HOMO→LUMO (0.70471)	2.4310	510.01	0.0951
2	HOMO-1→LUMO (-0.38035) HOMO-1→LUMO+1 (0.13683) HOMO→LUMO+1 (0.57302)	2.9460	420.86	1.1951
3	HOMO-1→LUMO (0.57125) HOMO-1→LUMO+1 (0.18600) HOMO→LUMO+1 (0.34638)	3.1331	395.73	0.1691
4	HOMO-1→LUMO+1 (0.66565) HOMO→LUMO+1 (-0.21165)	3.2528	381.16	0.1986
5	HOMO-5→LUMO+1 (0.14777) HOMO-2→LUMO (0.52151) HOMO-2→LUMO+1 (-0.27004) HOMO→LUMO+2 (0.34011)	3.7048	334.66	0.0251
6	HOMO-2→LUMO (-0.31332) HOMO-2→LUMO+1 (0.11277) HOMO→LUMO+2 (0.60788)	3.7082	334.35	0.0054

c. BR in V2T

Excited state	Transition	Transition Energy (eV)	Transition Frequency (nm)	Oscillator strength
1	HOMO→LUMO (0.70461)	2.4578	504.46	0.0955
2	HOMO-1→LUMO (-0.35862) HOMO-1→LUMO+1 (0.14549) HOMO→LUMO+1 (0.58523)	2.9413	421.53	1.1432
3	HOMO-4→LUMO (-0.10188) HOMO-1→LUMO (0.58050) HOMO-1→LUMO+1 (0.21143) HOMO→LUMO+1 (0.31422)	3.1356	395.41	0.2138
4	HOMO-1→LUMO (-0.11289) HOMO-1→LUMO+1 (0.65626) HOMO→LUMO+1 (-0.22821)	3.2338	383.40	0.2086
5	HOMO-5→LUMO+1 (0.24908) HOMO-2→LUMO (0.50509) HOMO-2→LUMO+1 (-0.38598)	3.7188	333.40	0.0396
6	HOMO-8→LUMO (-0.13203) HOMO-4→LUMO (-0.12385) HOMO→LUMO+2 (0.66533)	3.7360	331.86	0.0028

Table S4. Time-dependent DFT calculation results on BR with two H-bonded water molecules for UnaG (a), eUnaG (b), and V2T (c). Configurations involved in each electronic transition and their weighting factors are given in the second column of this table. Then, the transition energy (eV), transition wavelength (nm), and oscillator strength associated with each transition are summarized in this table. In all cases (a-c), the electronic transition with the highest oscillator strength corresponds to the excited state **2** (gray shaded).

a. BR with the bound water molecules in UnaG

Excited state	Transition	Transition Energy (eV)	Transition Frequency (nm)	Oscillator strength
1	HOMO→LUMO (0.70522)	2.3218	534.00	0.0794
2	HOMO-1→LUMO (0.40959) HOMO-1→LUMO+1 (-0.11581) HOMO→LUMO+1 (0.55861)	2.9176	424.95	1.3691
3	HOMO-1→LUMO (0.55583) HOMO-1→LUMO+1 (0.15094) HOMO→LUMO+1 (-0.39001)	3.1005	399.88	0.1404
4	HOMO-1→LUMO+1 (0.67884) HOMO→LUMO+1 (0.18089)	3.2459	381.97	0.1588
5	HOMO-2→LUMO (0.66226) HOMO-2→LUMO+1 (-0.23108)	3.5635	347.93	0.0117
6	HOMO-2→LUMO (0.24375) HOMO-2→LUMO+1 (0.64060)	3.6766	337.22	0.0214

b. BR with the bound water molecules in eUnaG

Excited state	Transition	Transition Energy (eV)	Transition Frequency (nm)	Oscillator strength
1	HOMO→LUMO (0.70527)	2.3243	533.44	0.0756
2	HOMO-1→LUMO (0.40762) HOMO-1→LUMO+1 (-0.11456) HOMO→LUMO+1 (0.56043)	2.9175	424.96	1.3641
3	HOMO-1→LUMO (0.55718) HOMO-1→LUMO+1 (0.15073) HOMO→LUMO+1 (-0.38811)	3.1004	399.90	0.1518
4	HOMO-1→LUMO+1 (0.67914) HOMO→LUMO+1 (0.17959)	3.2432	382.29	0.1539
5	HOMO-2→LUMO (0.66059) HOMO-2→LUMO+1 (-0.23563)	3.5646	347.82	0.0115
6	HOMO-2→LUMO (0.24833) HOMO-2→LUMO+1 (0.63909)	3.6736	337.50	0.0217

c. BR with the bound water molecules in V2T

Excited state	Transition	Transition Energy (eV)	Transition Frequency (nm)	Oscillator strength
1	HOMO→LUMO (0.70537)	2.3233	533.66	0.0711
2	HOMO-1→LUMO (-0.43200) HOMO-1→LUMO+1 (-0.10901) HOMO→LUMO+1 (0.54248)	2.9250	423.88	1.3547
3	HOMO-1→LUMO (0.53820) HOMO-1→LUMO+1 (-0.16214) HOMO→LUMO+1 (0.41068)	3.1021	399.68	0.1771
4	HOMO-1→LUMO+1 (0.67751) HOMO→LUMO+1 (0.18440)	3.2545	380.96	0.1560
5	HOMO-2→LUMO (0.67395) HOMO-2→LUMO+1 (0.19848)	3.5606	348.22	0.0092
6	HOMO-2→LUMO (-0.20960) HOMO-2→LUMO+1 (0.65156)	3.6767	337.22	0.0236