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

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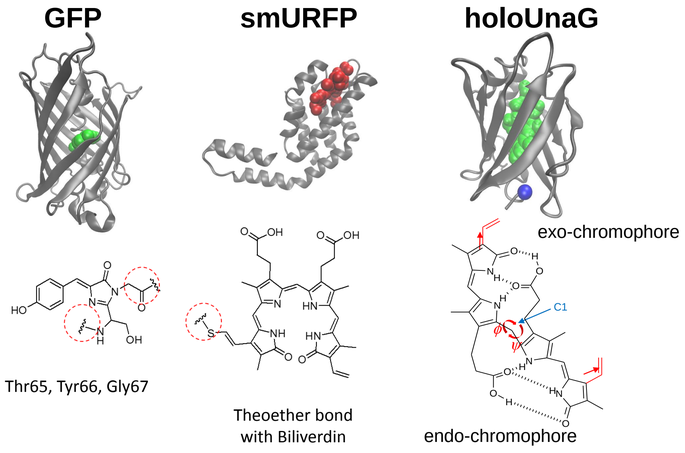
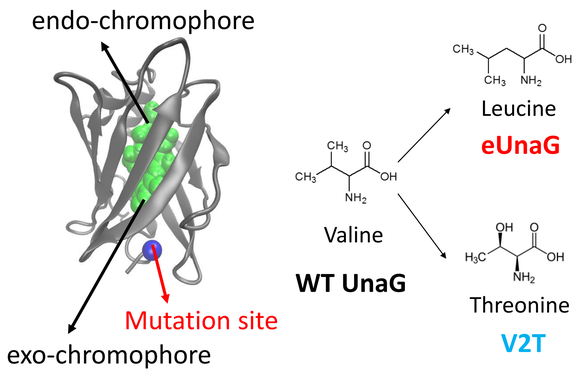


To monitor cellular processes in living system using microscope

Specific Mutant of UnaG : eUnaG (V2L), V2T

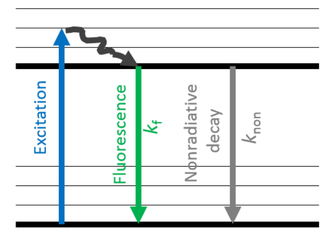


eUnaG ➡ **Enhanced fluorescence intensity (230 %)**

V2T ➡ **Reduced fluorescence intensity (69 %)**

Basic concept

Increased rigidity of chromophore
→ Decrease of non-radiative decay
→ Increase of quantum yield
→ Increase of fluorescence intensity



$$\emptyset = \frac{k_f}{k_f + k_{non}}$$

MD Simulation Methods

System1 : BR in water (55.14³ Å³)
System2 : BR-UnaG in water (57.20³ Å³)
System3 : BR-eUnaG in water (57.20³ Å³)
System4 : BR-V2T in water (57.20³ Å³)
Four systems in 5500 TIP3P water with SHAKE algorithm & Periodic condition using AMBER 16 program package

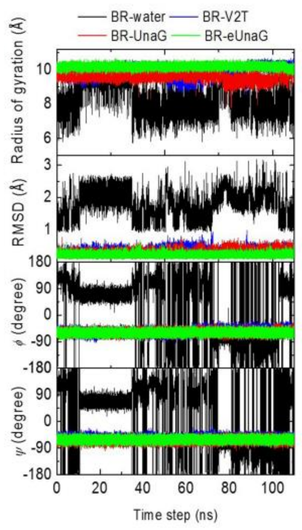
1st Minimization (6000 steps) with restraint for protein → 2nd Minimization (6000 steps) for whole system → NPT equilibration 4 ns → NVT equilibration 14 ns →
Production NVT MD simulation 110 ns / saved every 1 ps

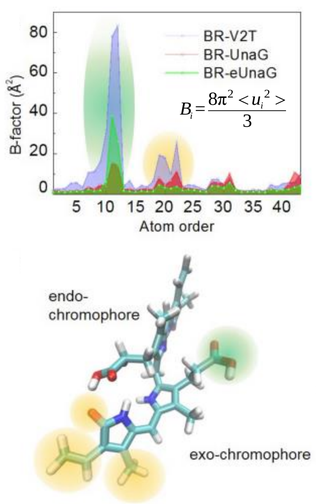
Quantum Chemistry Calculation Methods

1.Partial Geometry Optimization with fixed dihedral angles of BR
2.Time-dependent DFT calculation to obtain rotational strengths with B3LYP/6-31+G(d) in both of two calculations
3.Lorentzian function of line shape to ECD spectra

$$\Delta\varepsilon(\omega) \propto \sum_n R_{0n}\Gamma / \{(\omega-\omega_n)^2 + \Gamma^2\}$$

Conformational rigidity of BR





2D Potential of Mean Force

$$\Delta A(\phi,\psi) = -RT\ln\left(P(\phi,\psi)\right)$$

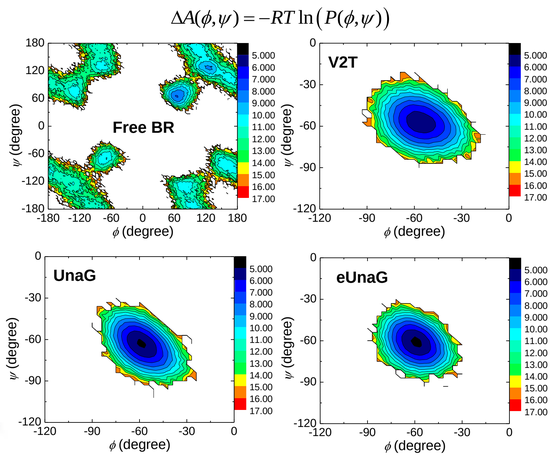
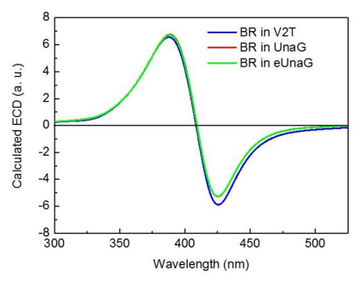


Table. Average values and standard deviations for 110 ns

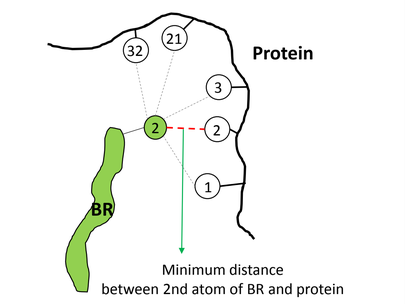
	UnaG	eUnaG	V2T
Radius of gyration	9.58 ± 0.20 Å	10.19 ± 0.12 Å	9.96 ± 0.28 Å
RMSD	0.29 Å	< 0.22 Å	0.35 Å
ϕ (exo)	-58.37 ± 7.11°	-59.31 ± 7.55°	-55.68 ± 9.12°
ψ (endo)	-63.69 ± 7.12°	-61.41 ± 7.65°	-57.18 ± 7.90°

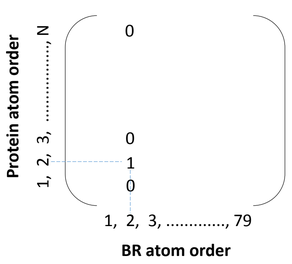
ECD spectra obtained from Time-dependent DFT calculation



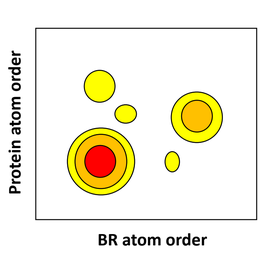
∴ Rigidity of BR
eUnaG > UnaG > V2T

BR-protein encounter map





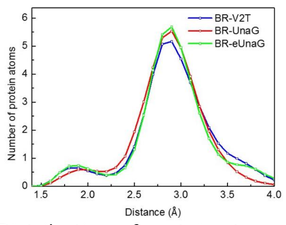
Count & Stack for 110 ns → divide by whole time steps → **"Encounter probability"**



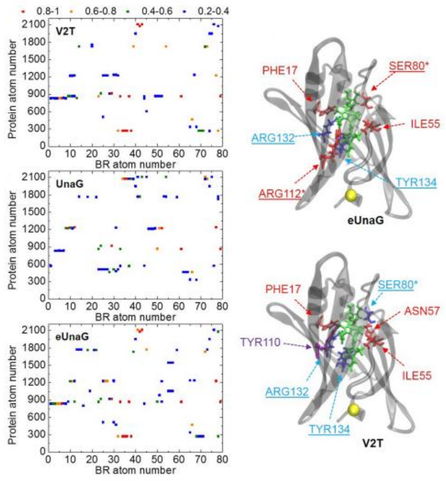
Encounter probability = 1
→ Interaction between two atoms is always keeping

High encounter probability = strong interaction
Weak encounter probability = weak or no interaction

Local protein structure around BR



▶ Distribution of protein atoms from BR.
There is no any difference, indicating that the steric effect is not reason of enhanced rigidity.



▶ Left : **The B-P map**
▶ Right :changed interacting degree of binding residues
Increased(red) or decreased(blue) encounter probability

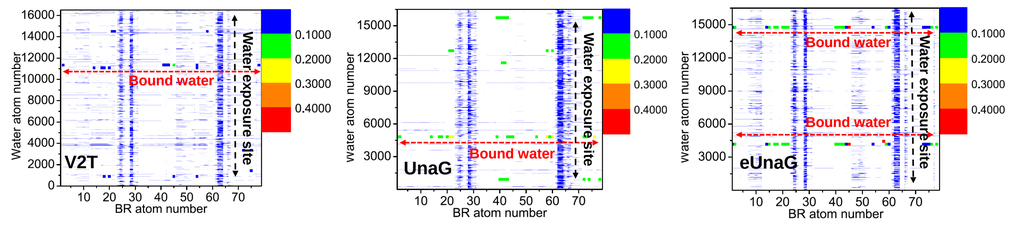
Table. The average number of H-bonding with BR (cpptraj)

	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

H-bonding interaction between protein and BR
eUnaG > UnaG > V2T

H-bonding interaction between bound water molecules and BR
eUnaG > UnaG > V2T

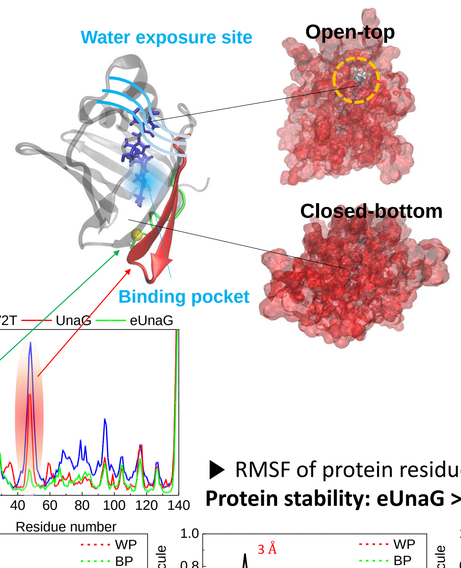
Captured water molecules around BR



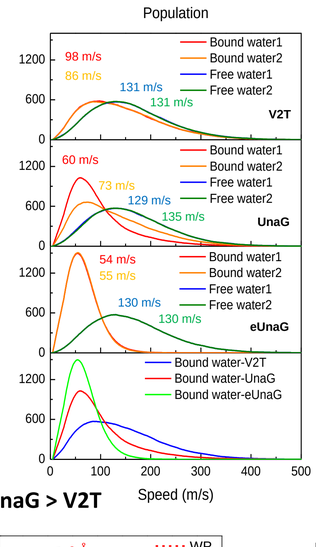
▶ **The B-W map**

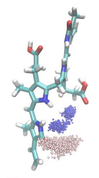
Vertical lines
with low encounter probability
→ **Water exposure site**

Horizontal lines
with high encounter probability
→ **Bound water**

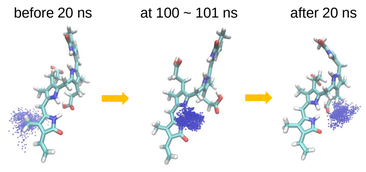


▶ RMSF of protein residues
Protein stability: eUnaG > UnaG > V2T

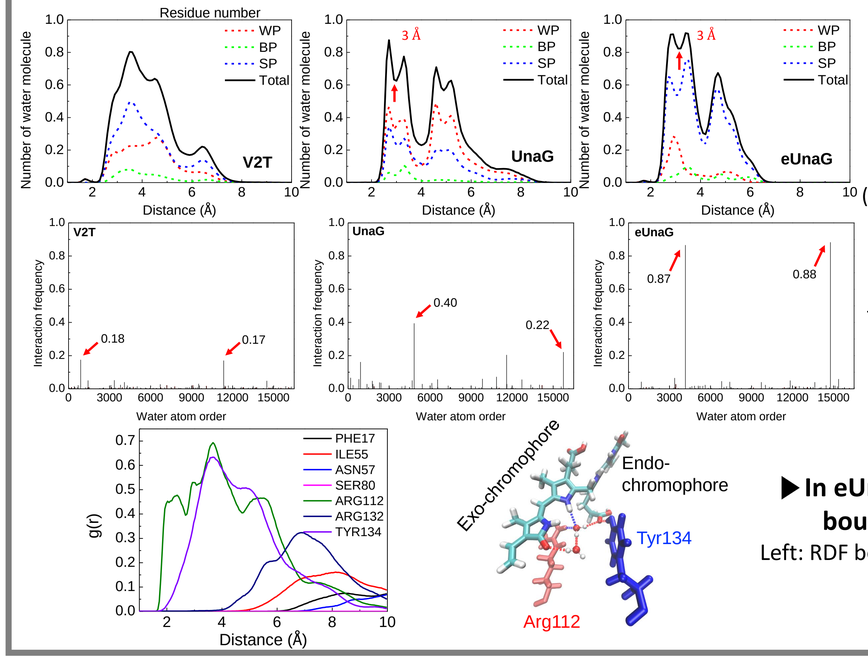




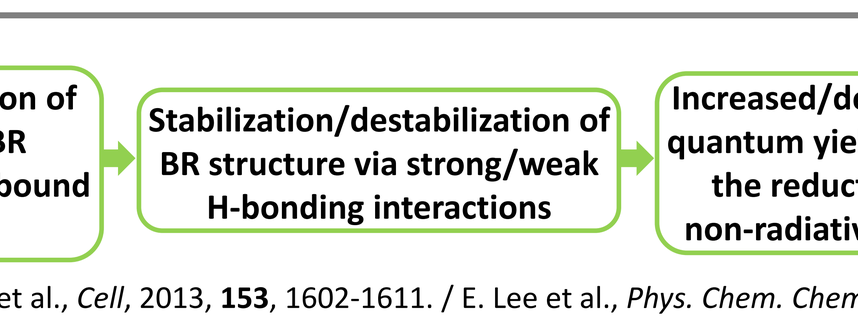
▶ **In eUnaG** : Tightly binding & exchange their position in binding pocket



▶ **In UnaG and V2T** : Moving around BR entering & escaping binding pocket
(The bound water molecules in V2T are faster than those in UnaG)



▶ **Classification of bound water molecules**
WP : Water-pointing water
(Two H atoms of water point water phase)
BP : BR-pointing water
(Two H atoms of water point BR surface)
SP : Surface-parallel water
(Two H atoms of water straddle BR surface and water phase)



▶ **Interaction frequency between BR and water molecules (within 3 Å)**
The water molecules pointed by red arrows correspond to the bound water revealed from the B-W map

▶ **In eUnaG, Strong interaction network between BR, bound water molecules, and protein residues**
Left: RDF between the bound water molecule and binding residues
Right: Interaction network snapshot

Summary

Mutation (eUnaG/V2T) → **Protein stabilization/ destabilization** → **Stabilization/destabilization of local environment of BR (Binding residues and the bound water molecules)** → **Stabilization/destabilization of BR structure via strong/weak H-bonding interactions** → **Increased/decreased quantum yield due to the reduction of non-radiative decay** → **Enhanced/reduced fluorescence intensity**

Reference: J. T. H. Yeh et al., *Sci. Rep.*, 2017, **7**, 41619. / A. Kumagai et al., *Cell*, 2013, **153**, 1602-1611. / E. Lee et al., *Phys. Chem. Chem. Phys.*, 2017, **19**, 20008-20015.