Supporting Information for

Hidden Electronic Excited State of Enhanced Green Fluorescent Protein

Haruko Hosoi[†], *Shoichi Yamaguchi*[†], *Hideaki Mizuno*[‡], *Atsushi Miyawaki*[‡], *and Tahei Tahara**[†]

1. TPA Spectra

The TPA measurements of the proteins were carried out with three different ω_1 wavelengths of 1300, 1490, and 1820 nm as shown in Figure S1. The spectra measured with the three ω_1 were smoothly connected to provide TPA spectrum in the wide wavelength range for each protein.

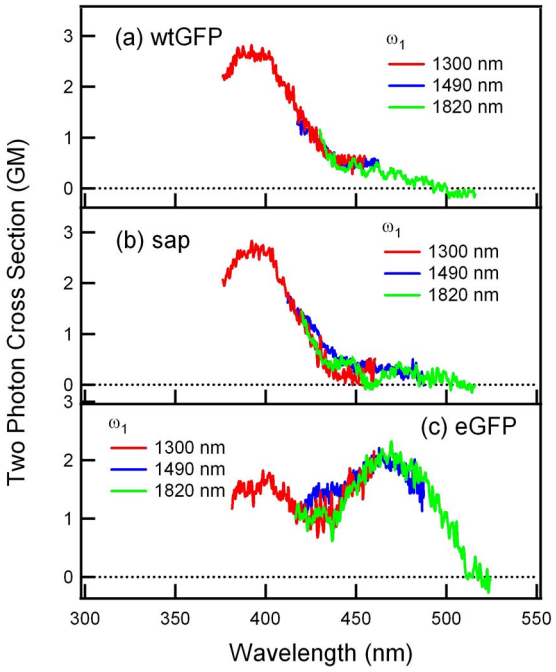
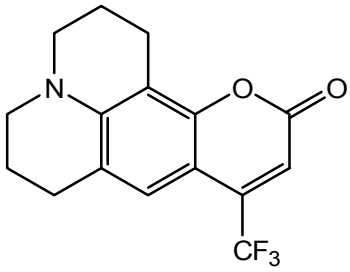


Figure S1. Two-photon absorption spectra of wtGFP (a), sap (b), and eGFP (c) measured with the ω_1 wavelengths of 1300, 1490, and 1820 nm.

2. One-photon and two-photon absorption spectra of a coumarin dye, coumarin 153

(a)



(b)

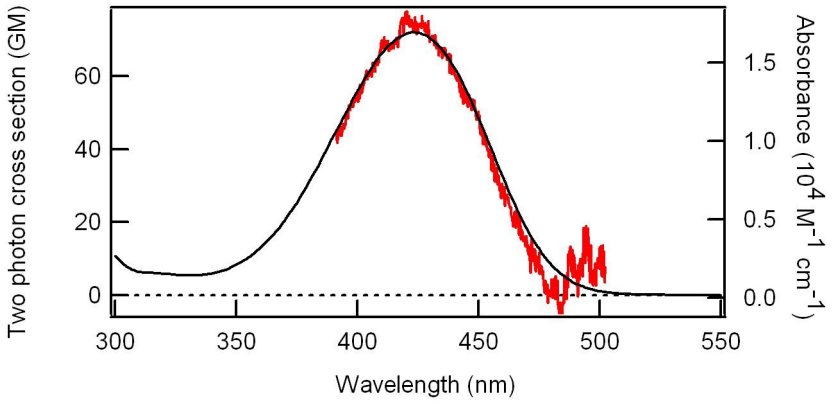


Figure S2. (a) Structure of coumarin 153. (b) One-photon (black) and two-photon (red) absorption spectra of coumarin 153 in methanol solution (9.3 mM, ω_1 : 1440 nm).

3. OPA and TPA spectra of eGFP and the anion of HBDI plotted with a wavenumber axis.

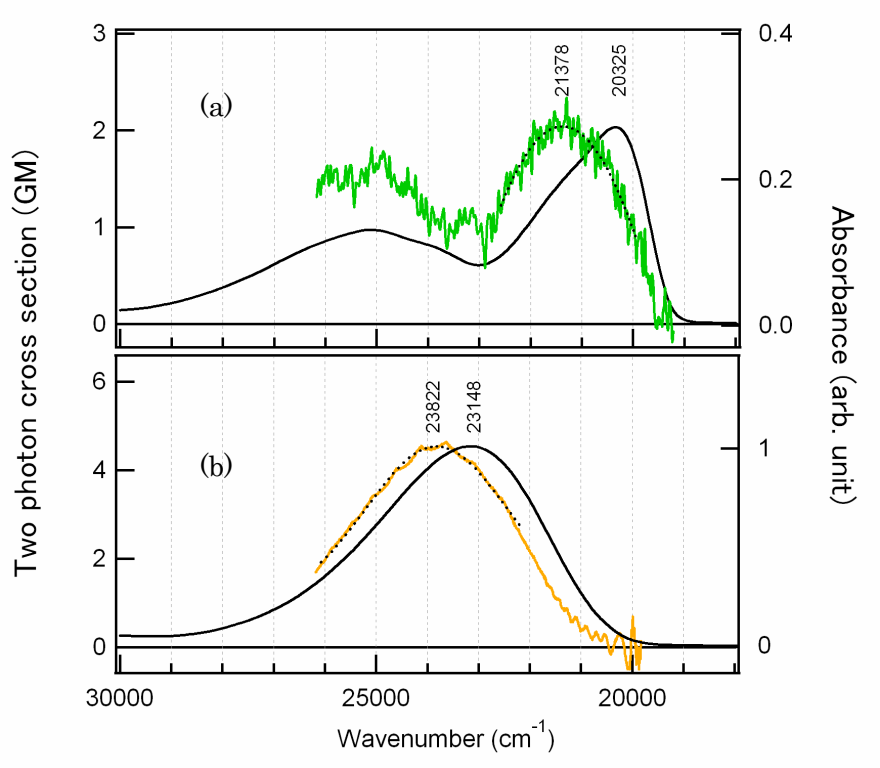


Figure S3. One-photon (black) and two-photon (color) absorption spectra of eGFP (a) and the anion of HBDI in methanol (b). Dotted lines indicate the fitting results using a Gaussian function.

4. Steady state fluorescence spectra of eGFP

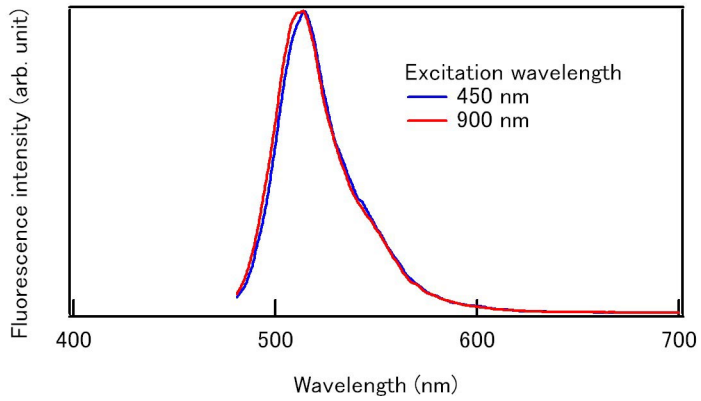


Figure S4. Fluorescence spectra of eGFP obtained with one-photon excitation (450 nm, blue) and two-photon excitation (900 nm, red).

5. Time-resolved fluorescence signals of eGFP

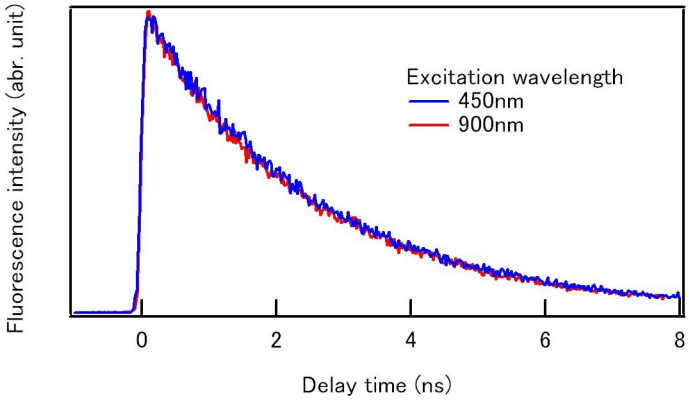


Figure S5. Time-resolved fluorescence signals (observed in the range of 500-550 nm) of eGFP observed with one-photon excitation (450 nm, blue) and two-photon excitation (900 nm, red).