

Supplementary Figures and Table

Figure S1. Gel filtration chromatography of VFP, mVFP, dVFP, and EGFP using Superdex S200 10/30 gel filtration column in FPLC at room temperature. Arrow indicated the injection time. D is dimer and M is monomer.

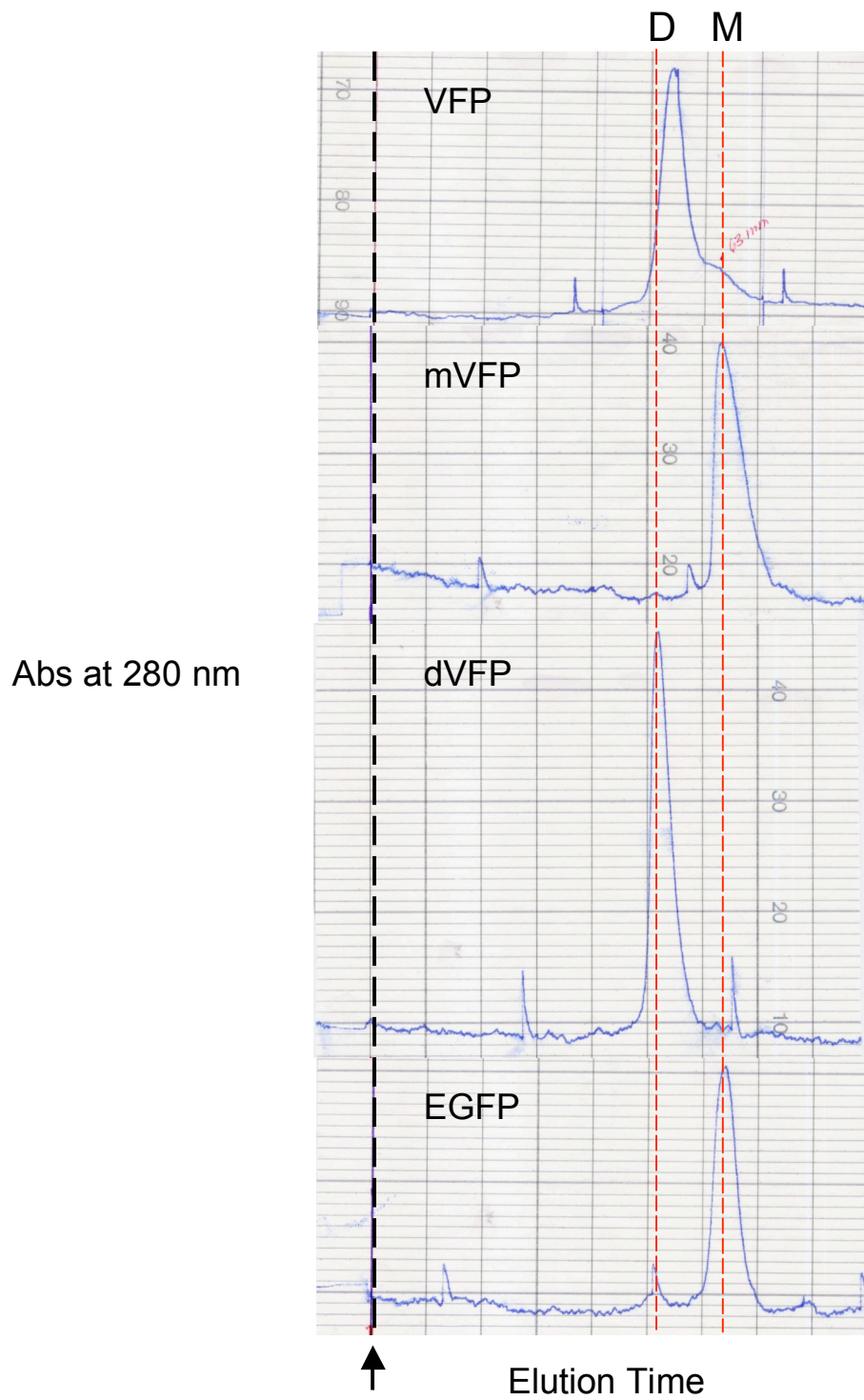


Figure S2. Absorption, fluorescence excitation and emission spectra of VFP and its variants. The samples were excited at 450 nm and emission spectra were measured from 465 nm to 650 nm. Fluorescence excitation spectra were obtained from 250 nm to 515 nm by monitoring the emission at 530 nm. The spectra were normalized at the maximum peak.

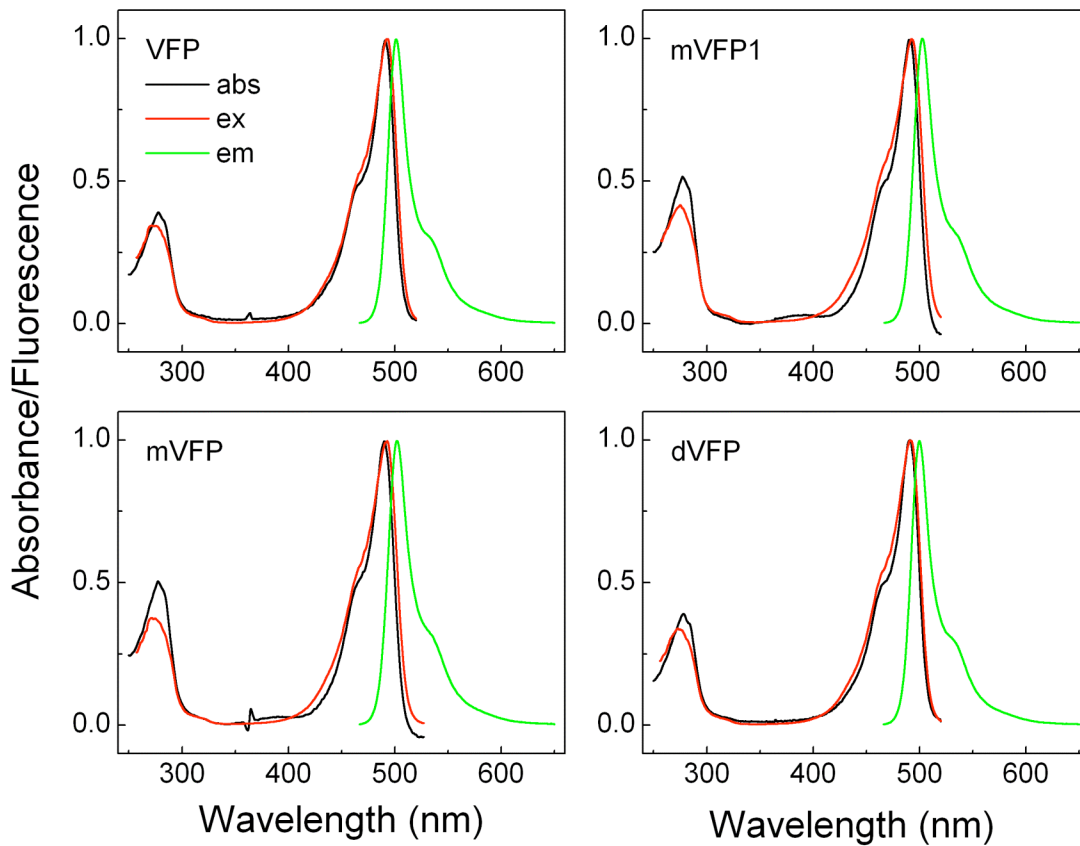


Figure S3. pH dependence of the fluorescence of VFP and its variants in comparison with EGFP and Venus. VFP and its variants have similar pH stability profiles to EGFP, between pH 6 to 10.

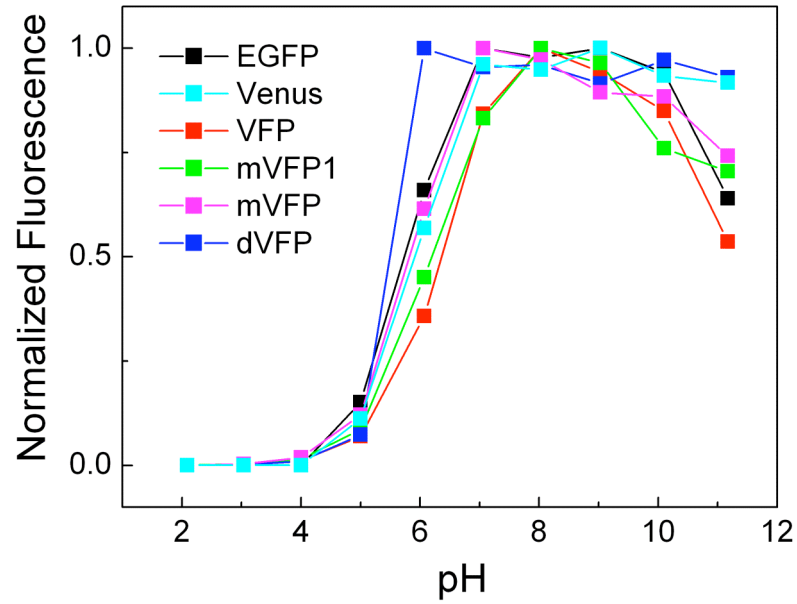


Figure S4. Representative FCS autocorrelation curves of Venus, VFP, mVFP1 and dVFP taken at increasing laser power intensities from 0.25 to 5 mW. The curves are normalized to the number of molecules obtained from the fitting for comparison.

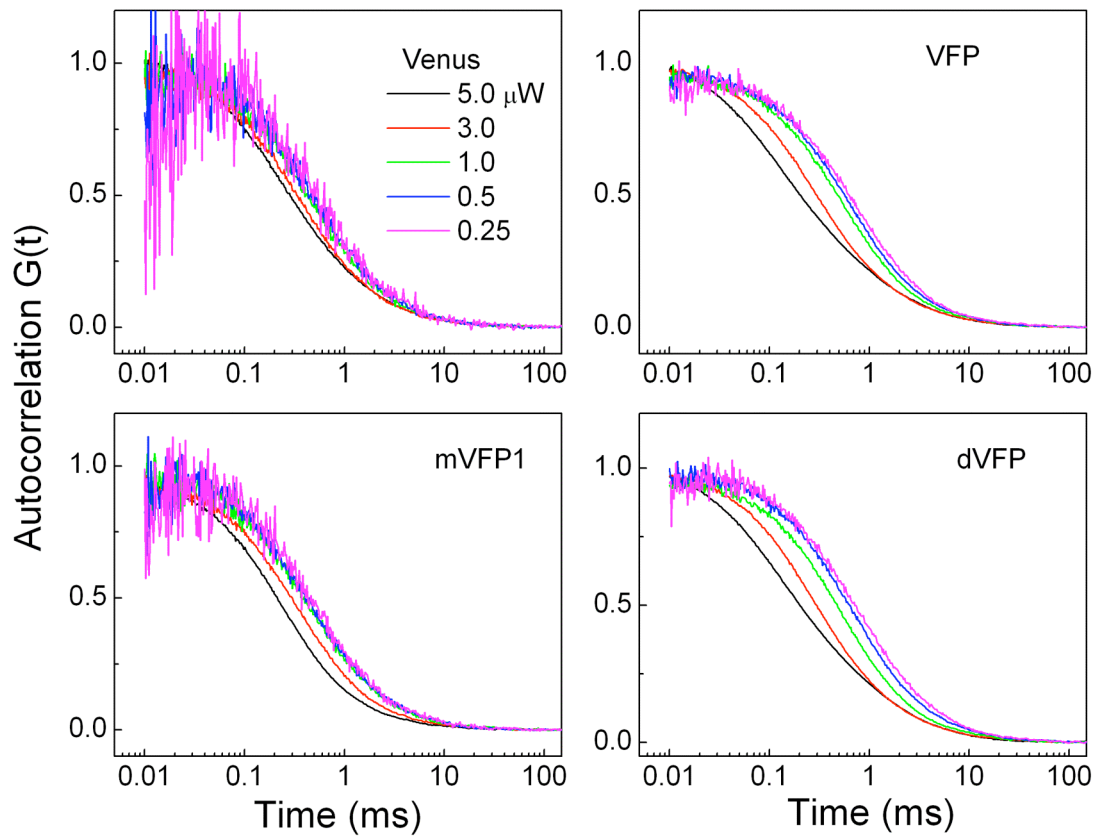


Figure S5. Photobleaching curves for EGFP, Venus, VFP and its variants under mercury arc lamp illumination. The relative photostability of the FPs is reported in Table 1.

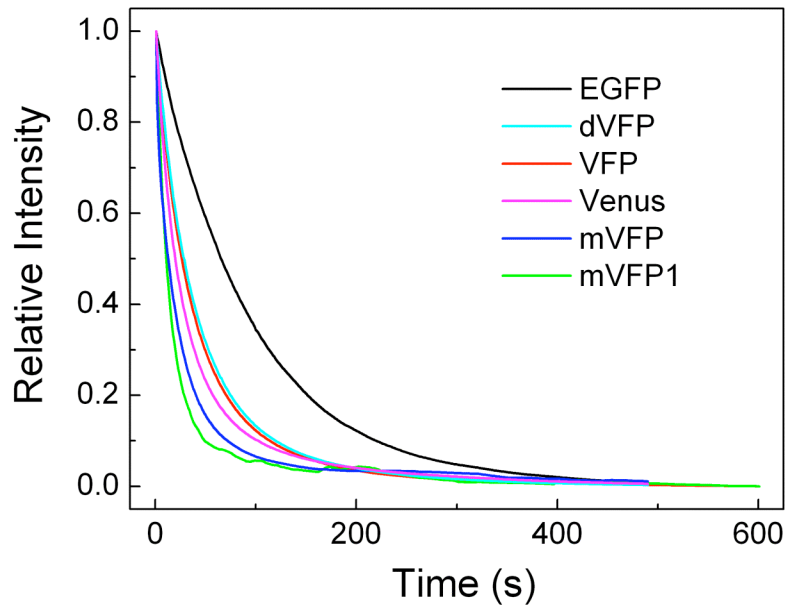


Table S1: Summary of Fluorescence Correlation Spectroscopy (FCS) Analysis. The autocorrelation curves were fitted using single-diffusion component equation. The brightness expressed as counts per molecules was calculated by dividing the intensity by number of molecules.

FPs	Power Intensity (μW)	Diffusion time (ms)	Intensity (Hz) 1×10^4	Counts per molecules (kHz/molecule)
EGFP	1	0.500 ± 0.013	8.43 ± 0.05	0.914 ± 0.005
	0.5	0.487 ± 0.012	5.01 ± 0.04	0.536 ± 0.004
	0.25	0.486 ± 0.012	2.49 ± 0.05	0.262 ± 0.005
VFP	1	0.475 ± 0.003	6.67 ± 0.29	5.50 ± 0.25
	0.5	0.584 ± 0.003	4.48 ± 0.34	3.38 ± 0.25
	0.25	0.646 ± 0.004	4.07 ± 0.14	1.76 ± 0.06
mVFP1	1	0.413 ± 0.006	8.92 ± 0.11	1.64 ± 0.02
	0.5	0.443 ± 0.006	5.32 ± 0.13	0.947 ± 0.023
	0.25	0.460 ± 0.007	3.40 ± 0.19	0.485 ± 0.028
mVFP	1	0.384 ± 0.006	11.4 ± 0.20	1.72 ± 0.03
	0.5	0.433 ± 0.007	7.12 ± 0.04	0.970 ± 0.006
	0.25	0.472 ± 0.007	3.70 ± 0.14	0.476 ± 0.018
dVFP	1	0.547 ± 0.003	6.52 ± 0.19	4.90 ± 0.14
	0.5	0.668 ± 0.003	4.39 ± 0.21	3.00 ± 0.14
	0.25	0.763 ± 0.004	3.00 ± 0.11	1.55 ± 0.06
Venus	1	0.459 ± 0.016	10.8 ± 0.25	0.897 ± 0.02
	0.5	0.511 ± 0.017	6.50 ± 0.03	0.509 ± 0.002
	0.25	0.543 ± 0.019	3.25 ± 0.02	0.251 ± 0.002