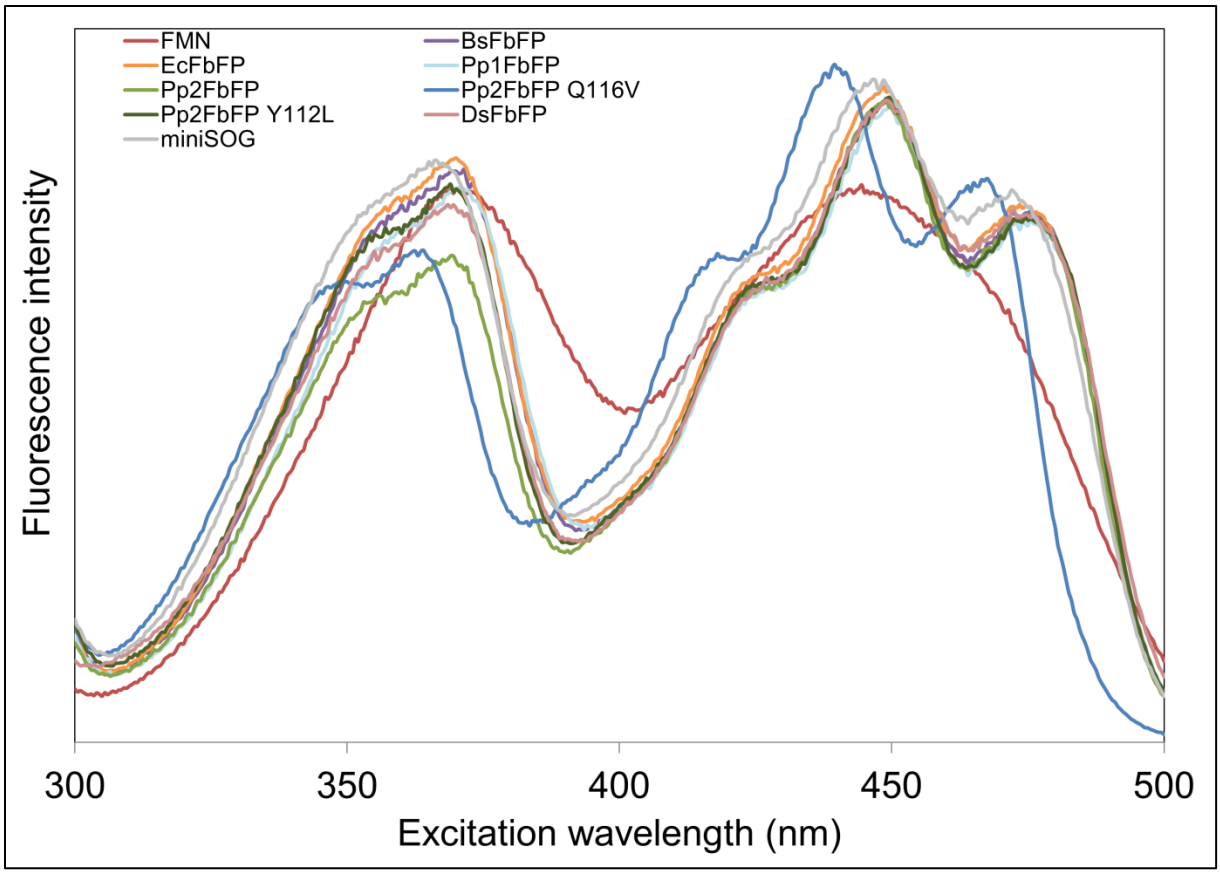
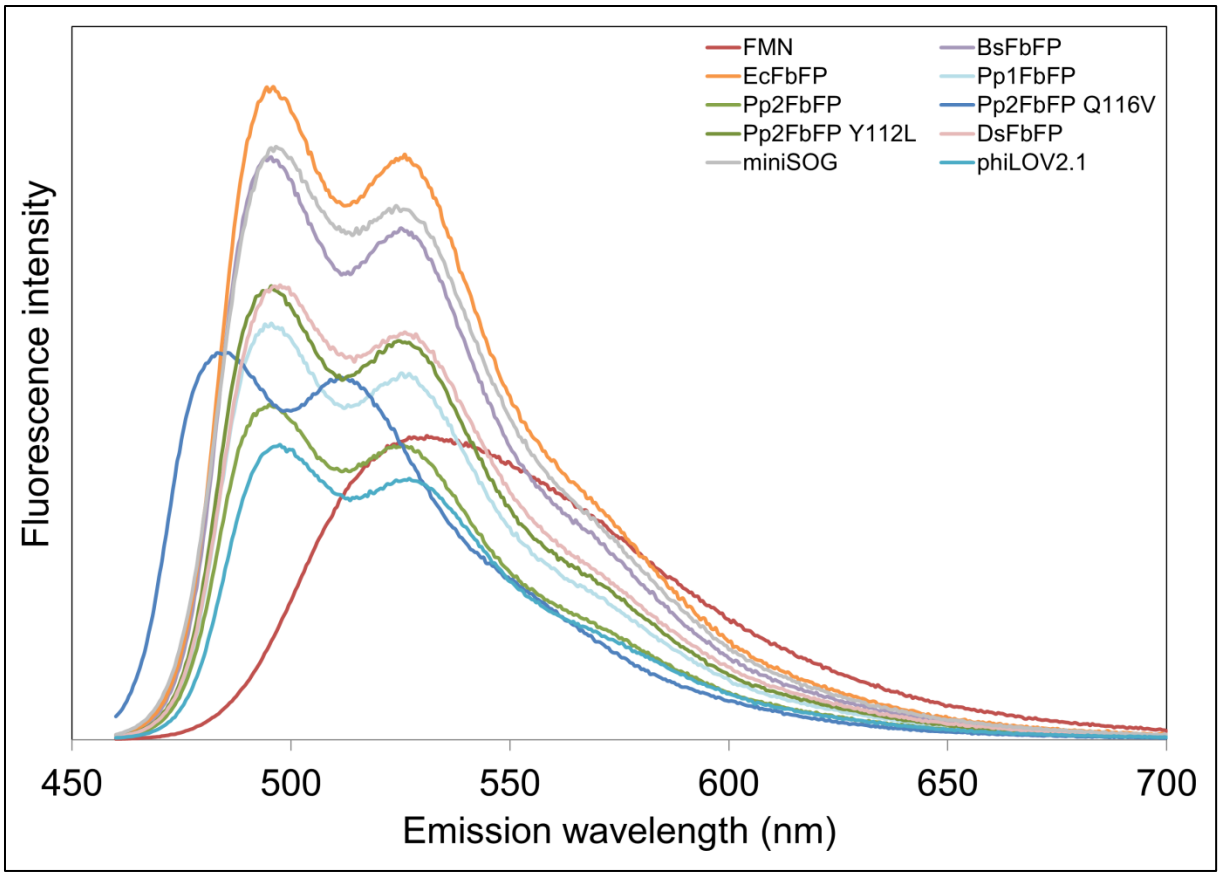
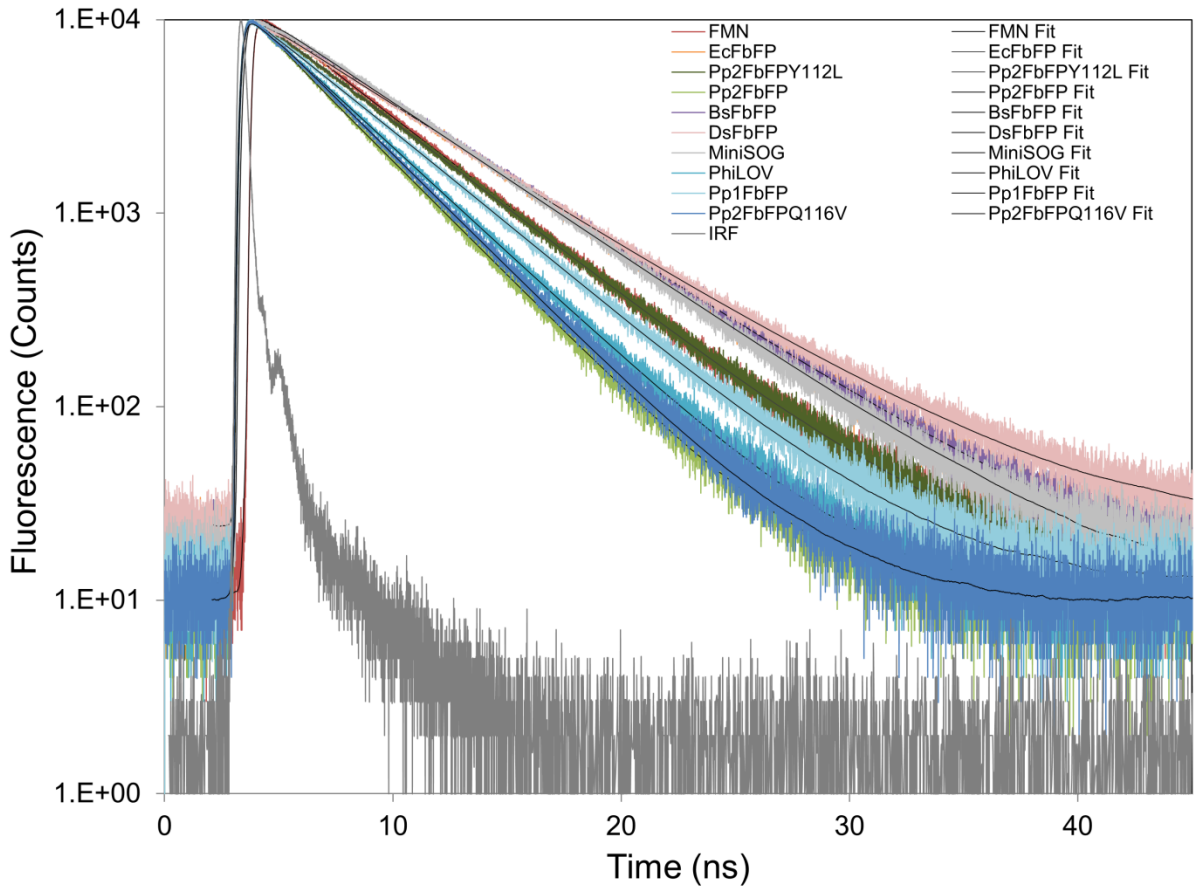



**Supplementary Fig. 1** Excitation spectra of LOV-based FPs and FMN. Fluorescence emission at 520nm was recorded and all spectra were normalized to the extinction coefficient of the proteins and FMN, respectively, at the blue absorption maximum (see Tab. 1). The excitation spectrum of phiLOV2.1 is not shown because the protein aggregated at 95°C and no extinction coefficient could be determined (the overall shape of its excitation spectrum does not differ from that of the other LOV-based FPs).




**Supplementary Fig. 2** Fluorescence emission spectra of LOV-based FPs and FMN.
All spectra were normalized to the absorption of the samples at the excitation wavelength (450 nm) so that the integrals of the spectra are proportional to the quantum yield of the respective FP and FMN, respectively.




**Supplementary Fig. 3** Fluorescence decay curves of all characterized LOV-based FPs and FMN, respectively. Experimental and fitted fluorescence decay curves (excitation: 440 nm, detection: 495 nm with the exception of FMN (530 nm)) and the instrument response function (IRF; excitation and detection: 440 nm) are shown.

| | $\alpha_1$ (%) | $\tau_1$ (ns) | $\alpha_2$ (%) | $\tau_2$ (ns) | $\tau_{Fl\ ave}$ | $\chi^2$ |
|---|---|---|---|---|---|---|
| BsFbFP | 100 | 5.68 | | | 5.68 | 1.24 |
| EcFbFP | 100 | 5.70 | | | 5.70 | 1.3 |
| Pp1FbFP | 81 | 4.60 | 19 | 1.12 | 3.90 | 1.05 |
| Pp2FbFP | 85 | 3.60 | 15 | 0.93 | 3.17 | 1 |
| Pp2FbFP Y112L | 95 | 4.78 | 5 | 0.51 | 4.56 | 1 |
| Pp2FbFP Q116V | 94 | 3.70 | 6 | 1.02 | 3.53 | 1 |
| DsFbFP | 57 | 6.60 | 43 | 4.41 | 5.66 | 1.12 |
| miniSOG | 100 | 5.46 | | | 5.46 | 1.8 |
| phiLOV2.1 | 85 | 3.92 | 15 | 0.33 | 3.36 | 1 |
| FMN | 89 | 4.80 | 11 | 0.68 | 4.36 | 1.1 |

**Supplementary Tab. 1** Fluorescence lifetime analysis of all characterized LOV-based FPs. Experimental data are fitted with a mono-exponential functions in case of BsFbFP, EcFbFP, miniSOG and bi-exponential functions in case of Pp1FbFP, Pp2FbFP, Pp2FbFP Y112L, Pp2FbFP Q116V, DsFbFP, phiLOV2.1 and FMN. $\alpha$ (amplitude), $\tau$ (fluorescence lifetime) $\tau_{Fl\ ave}$ (average fluorescence lifetime).