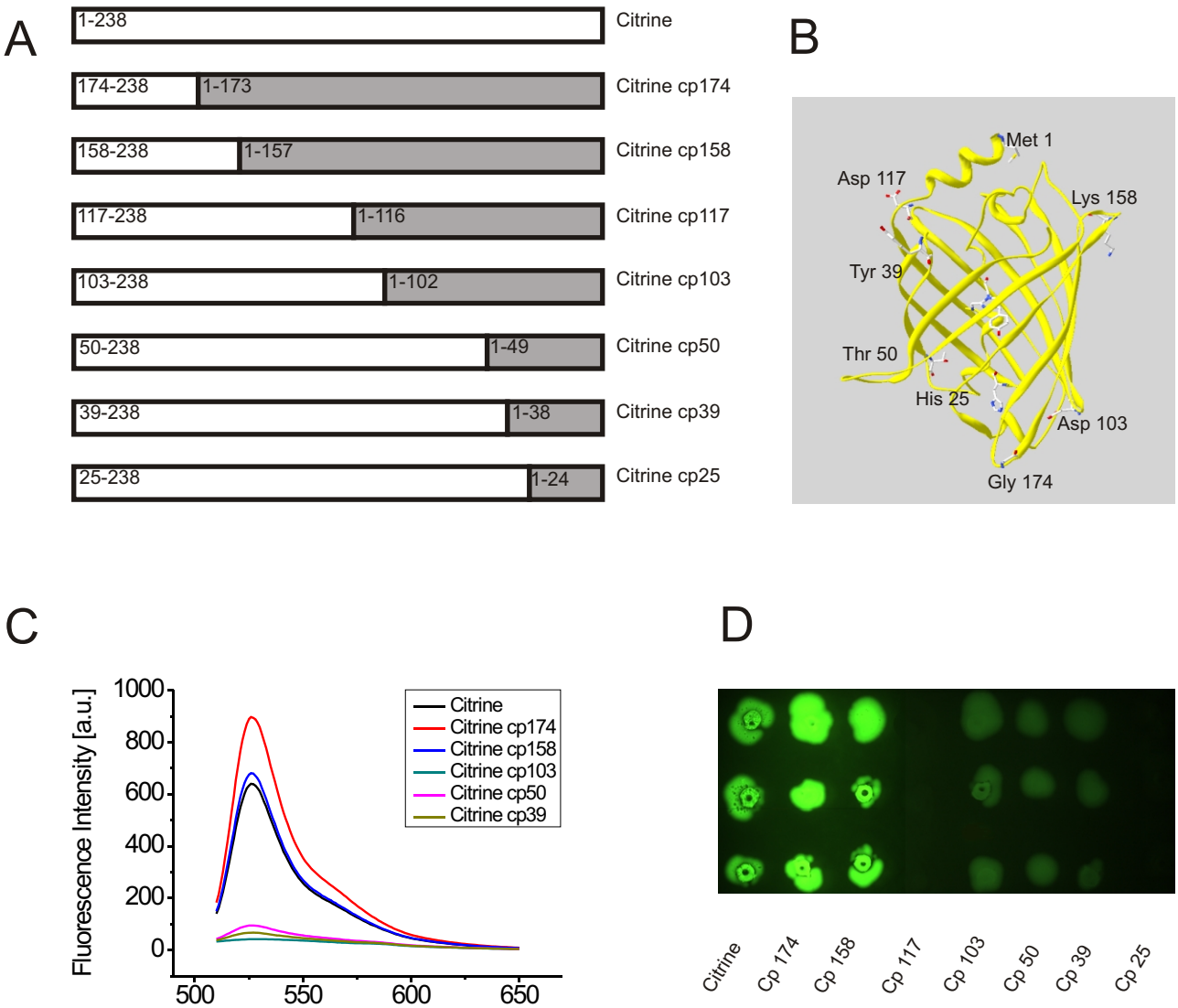
Supplementary Table 1: Spectroscopic properties of Citrine, Citrine cp175 and Citrine cp158.

	Absorption (nm)	Emission (nm)	pKa	Quantum Yield*	ε (515nm)
wtCitrine	**515**	**527**	**5,9**	**0,76**	**82000**
Citrine cp174	**515**	**527**	**5,9**	**0,75**	**94000**
Citrine cp158	**515**	**527**	**5,9**	**0,76**	**93000**

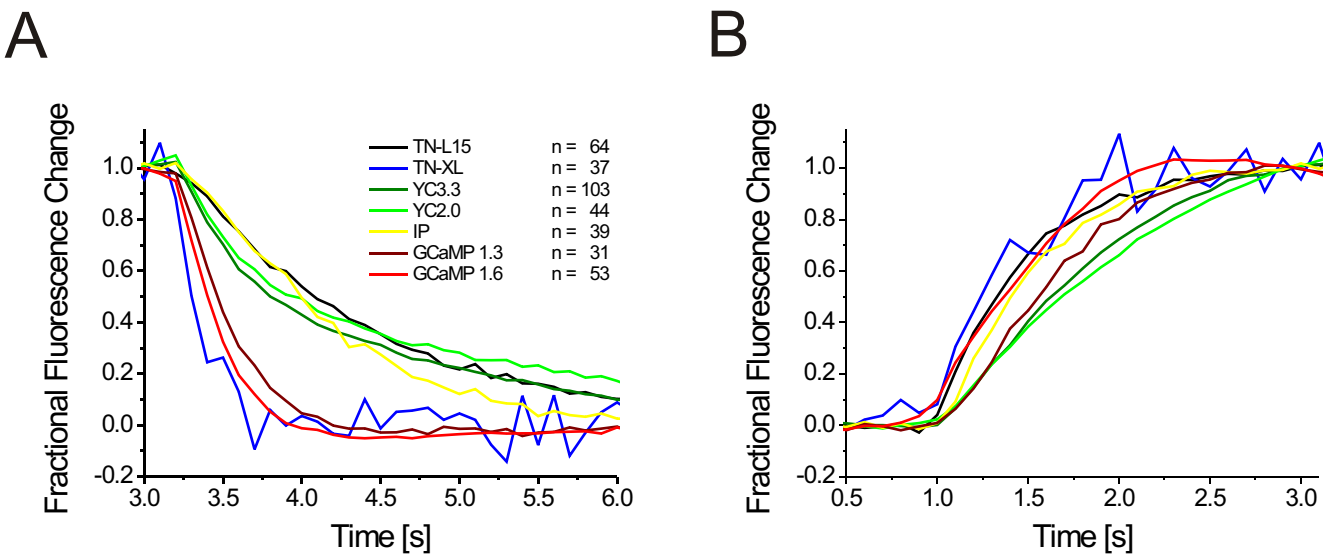
SUPPLEMENTARY TABLE 1. **Spectroscopic properties of Citrine, Citrine cp174 and Citrine cp158**. *as reference for determination of the Quantum Yields, a QY of 0.76 for Citrine was assumed (27). ε: Extinction coefficient in $M^{-1}cm^{-1}$.

SUPPLEMENTARY FIGURE 1. ***Scheme of circularly permuted (cp) variants of Citrine.*** **A**: Schematic presentation of the circularly permuted variants of Citrine. Indicated are the positions of the amino acids at which the cp variants were created. Note that the original Met[1] remained after the GGTGGS linker. **B**: Positions of the insertion of new termini in the permuted variants. **C**: Emission spectra obtained by the different variants when expressed in bacteria (BL21). Same amount of bacteria were measured by adjusting the solution to the same absorption at 600 nm. **D**: Bacteria colonies (BL21) expressing the variants. For each variant three different clones are shown.

SUPPLEMENTARY FIGURE 2. ***Decay (A) and rise (B) of the fluorescence signals of 4 ratiometric and 3 single chromophore indicators***. Single fluorophore sensor traces show fractional fluorescence changes and ratiometric indicator traces fractional ratio changes, all normalized to the maximum value. All close ups are taken from Fig. 4C and the corresponding time constants for the rise and decay are given in Table 2.



Supplementary Figure 1



Supplementary Figure 2