Supporting Information:

A GPCR dimerization interface in human cone opsins

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Outline

This document contains supplemental figures with data and analyses to support the discussion and conclusions in the main document. Figures S1-S3 show raw PIE-FCCS data and fits for representative single cells expressing the wild-type cone opsins. Figure S4 illustrates the density dependence of the dimeric species. Figures S5 and S6 reveal FRET data. Figure S7 is a red/green opsin sequence alignment. Figures S8 and S9 present raw PIE-FCCS data and fits for representative single cells expressing the mutant opsins. Figure S10 compares the heterodimerization of red-TSV and wild-type red cone opsin to their homodimerization. Figure S11 summarizes PIE-FCCS for a green-IVM mutant. Figure S12 shows absorbance measurements of the GFP-opsin fusions.

Supporting Figures

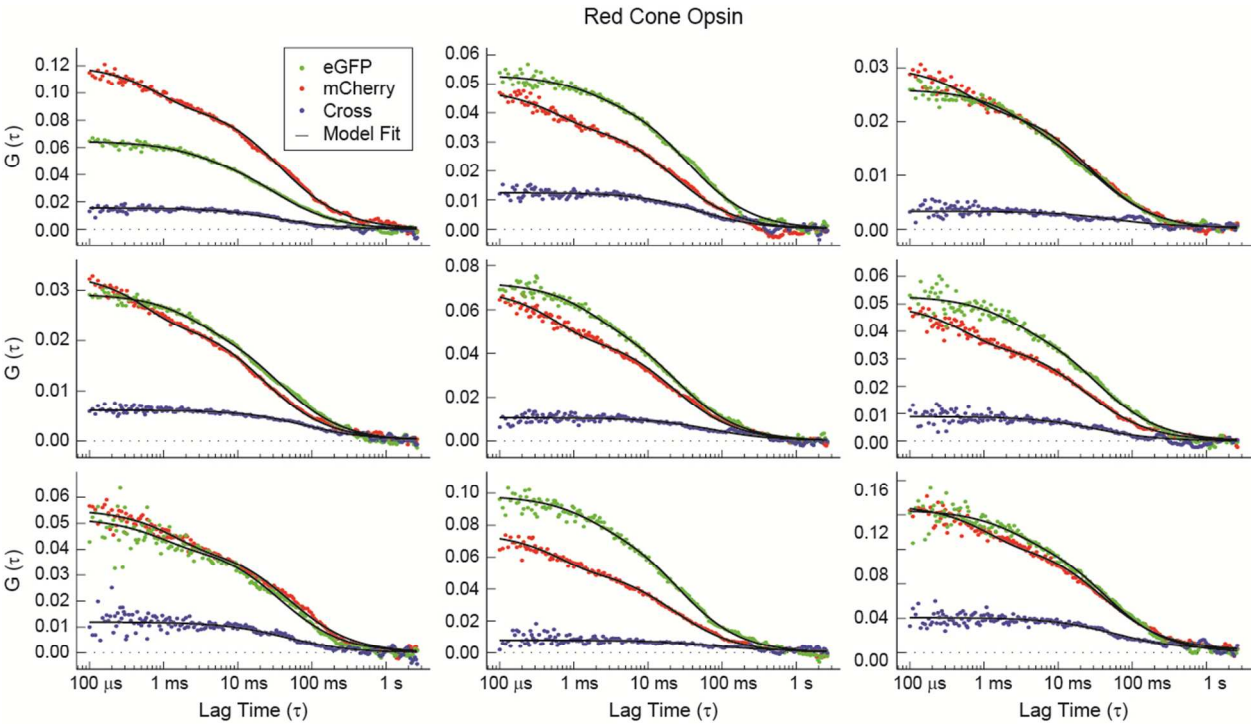


Figure S1. FCCS data are shown for nine representative single Cos-7 cells expressing human red cone opsin fusions to eGFP and mCherry. In each plot, the colored dots are the measured data points, whereas the solid black lines indicate the fitted functions (defined in the Material and Methods). Red dots are the FCS data for the mCherry fusion protein, $G_R(\tau)$; green dots are the FCS data for the eGFP fusion, $G_G(\tau)$; and blue dots are the FCCS data, $G_X(\tau)$. A horizontal dotted line marks the zero value in each plot. Amplitude data report directly on the concentration of diffusing species through the relationship: $G(0) = 1/\langle N \rangle = 1/A_{eff}\langle C \rangle$. The fraction correlated is calculated by comparing the relative amplitudes of the cross-correlation function and autocorrelation functions as described in the Materials and Methods.

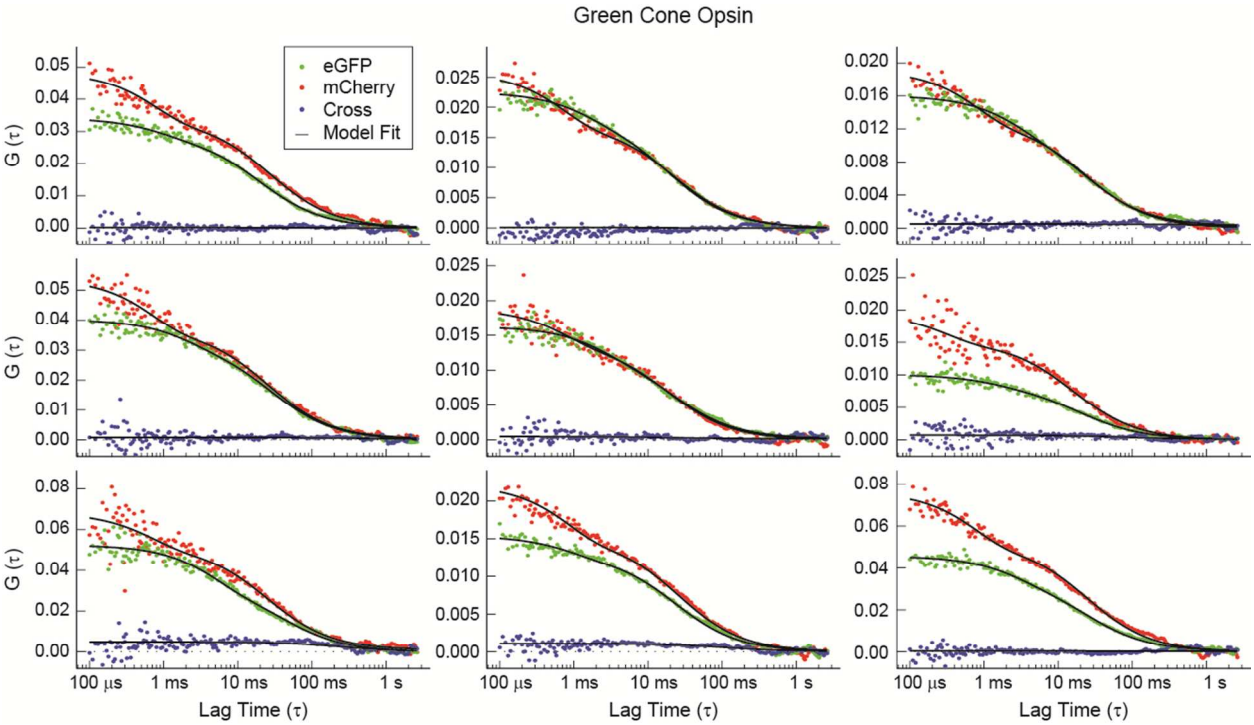


Figure S2. FCCS data are shown for nine representative single Cos-7 cells expressing human green cone opsin fusions to eGFP and mCherry. In each plot, the colored dots are the measured data points, whereas the solid black lines indicate the fitted functions (defined in the Material and Methods). Red dots are the FCS data for the mCherry fusion protein, G_R (τ); green dots are the FCS data for the eGFP fusion, G_G (τ); and blue dots are the FCCS data, G_X (τ). A horizontal dotted line marks the zero value in each plot. Amplitude data report directly on the concentration of diffusing species through the relationship: $G(0) = 1/\langle N \rangle = 1/A_{eff}\langle C \rangle$. The fraction correlated is calculated by comparing the relative amplitude of the cross-correlation function and autocorrelation functions as described in the Material and Methods.

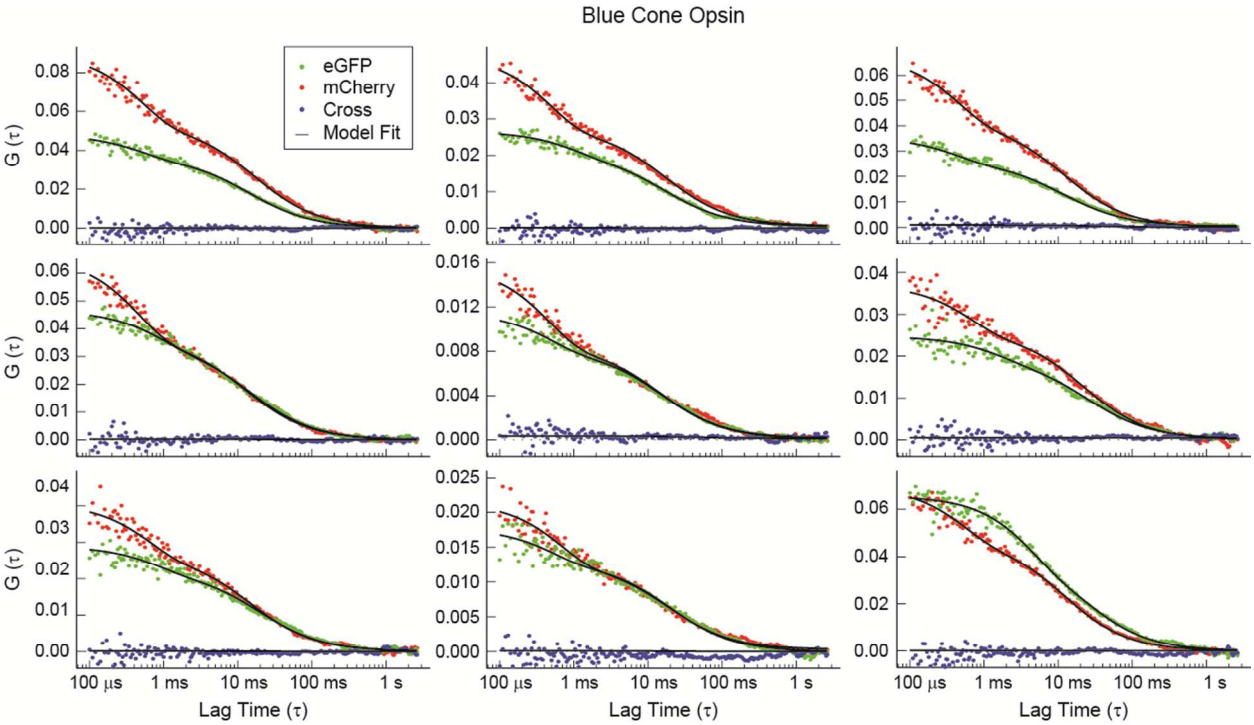


Figure S3. FCCS data are shown for nine representative single Cos-7 cells expressing human blue cone opsin fusions to eGFP and mCherry. In each plot, the colored dots are the measured data points, whereas the solid black lines indicate the fitted functions (defined in the Material and Methods). Red dots are the FCS data for the mCherry fusion protein, G_R (τ); green dots are the FCS data for the eGFP fusion, G_G (τ); and blue dots are the FCCS data, G_X (τ). A horizontal dotted line marks the zero value in each plot. Amplitude data report directly on the concentration of diffusing species through the relationship: $G(0) = 1/\langle N \rangle = 1/A_{eff}\langle C \rangle$. The fraction correlated is calculated by comparing the relative amplitudes of the cross-correlation function and autocorrelation functions as described in the main text.

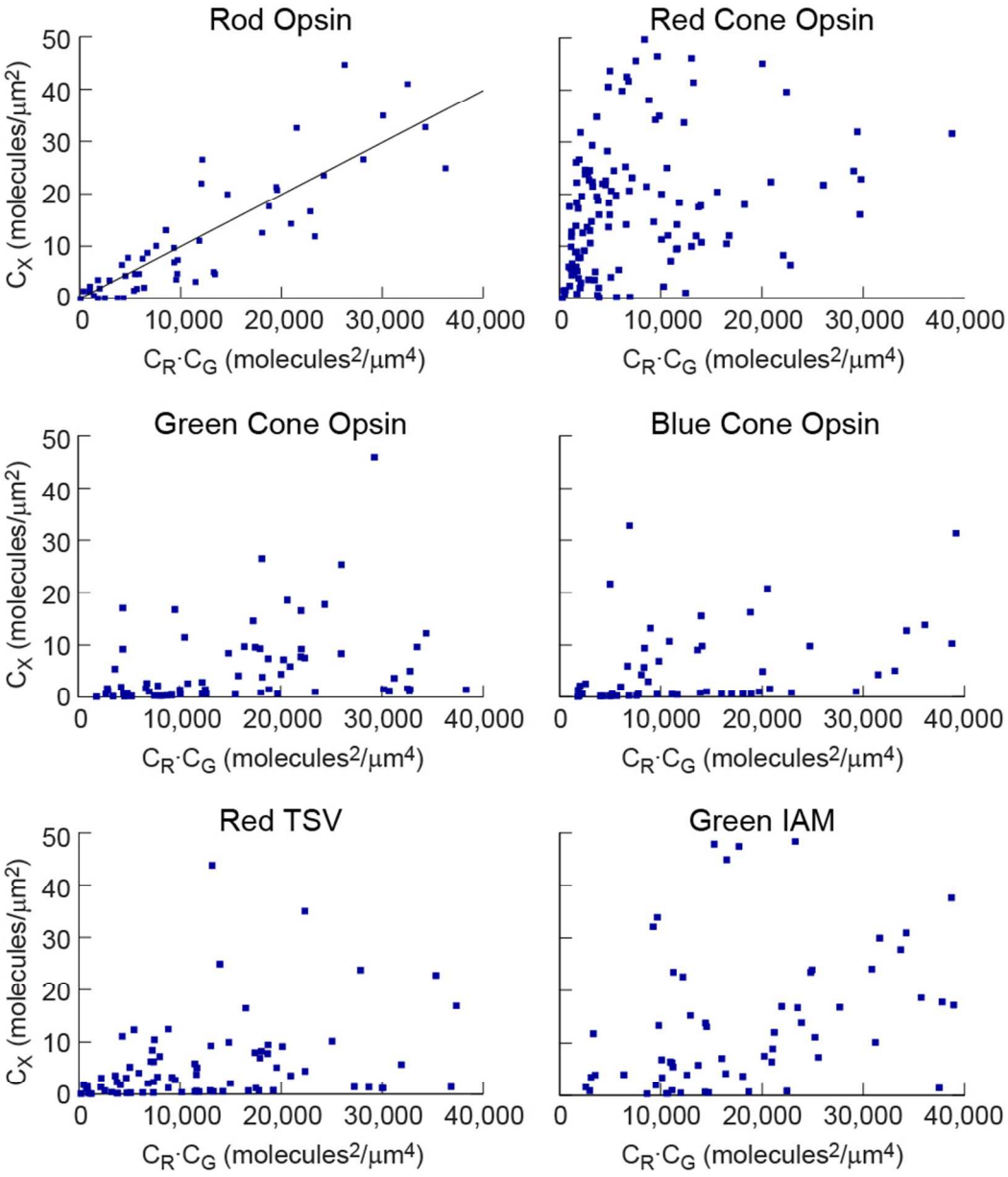


Figure S4. Density dependent dimerization. For each data set shown in Fig. 2D and Fig. 3B of the main text, we plotted the single cell values of the dimer concentration as a function of the product of the monomer concentrations. The rhodopsin data and their fits were published previously[1]. Plots for the other receptors indicate low correlations in a linear regression analysis: all R^2 values were less than 0.5 except for green-IAM.

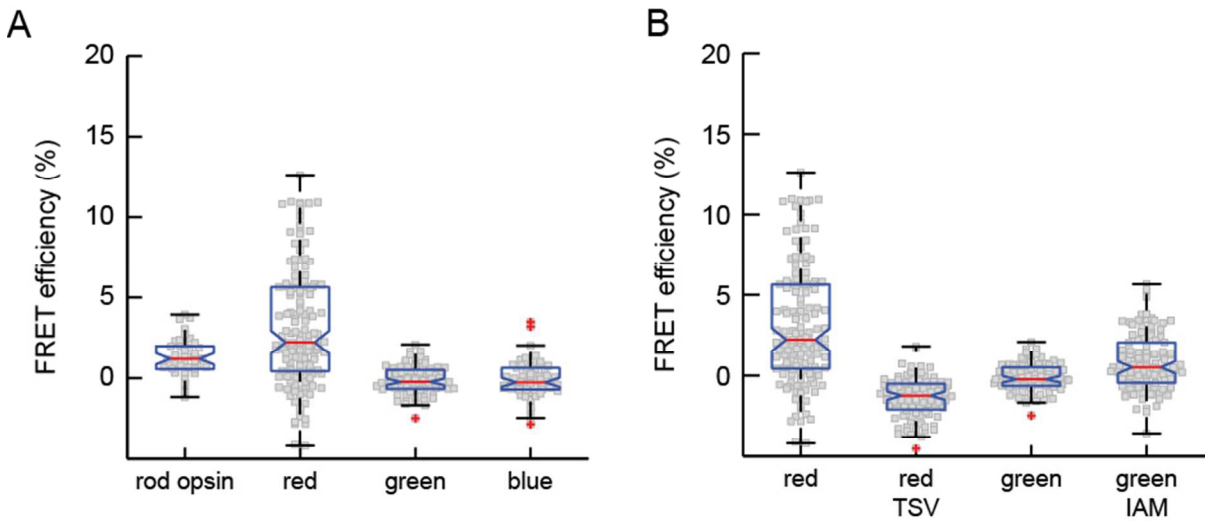


Figure S5. FRET analyses. The efficiency of FRET was determined by donor lifetime analysis, $FRET\ efficiency = \left(1 - \frac{\tau_{fl}}{\tau_0}\right)$, using the lifetime of the donor fluorophore in the presence (τ_{fl}) and absence (τ_0) of the acceptor. A higher FRET efficiency indicates an increase in dimerization. (**A**) Beeswarm plot of single Cos-7 cell FRET efficiency data is shown with box and whisker plots for rod opsin and the three human cone opsins. (**B**) FRET data for WT red and green cone opsins are compared with data from their mutants (described in the main text).

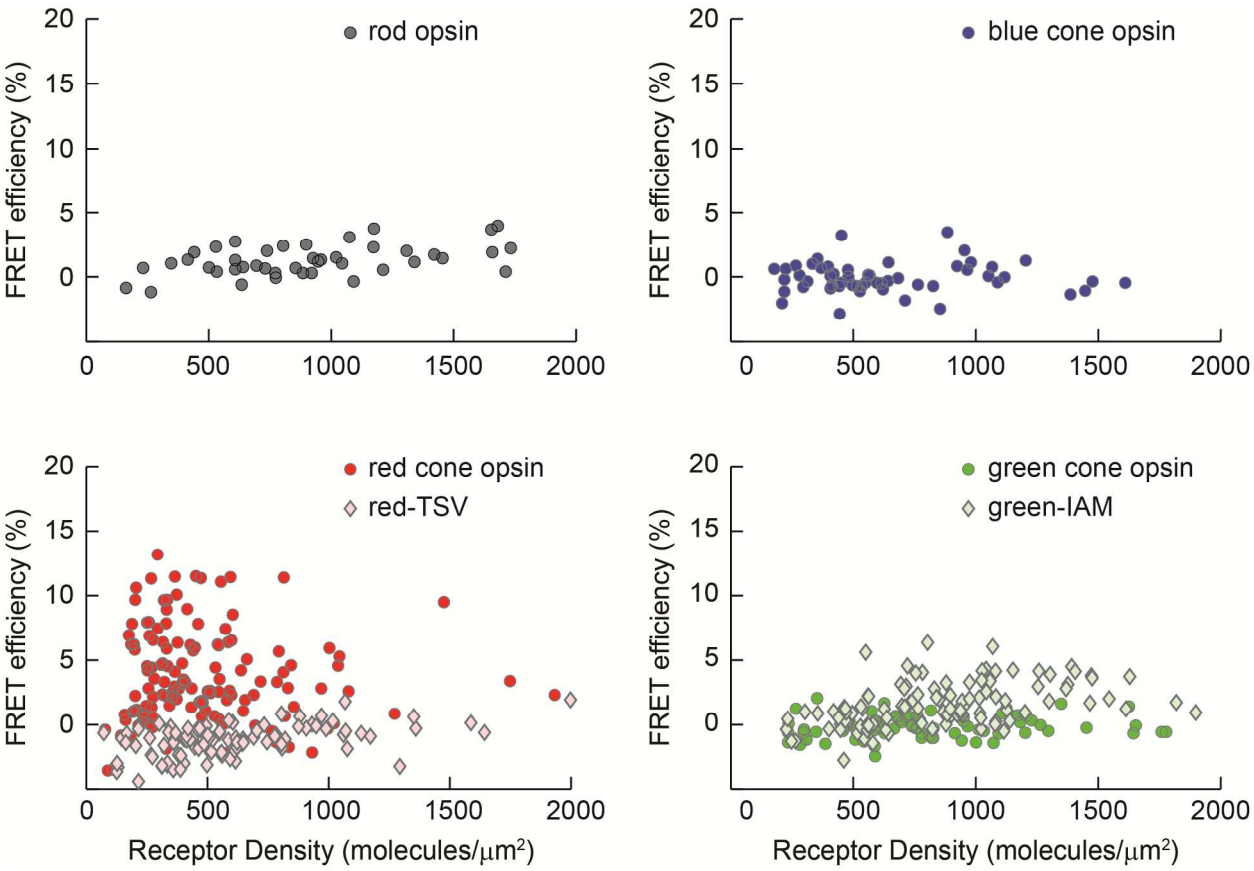


Figure S6. Density dependence of FRET efficiency. FRET values from Fig. S5 are plotted against the receptor density calculated from the FCS data.

Figure S7. A sequence alignment of red and green cone opsins produced with Clustal2.1. Conserved residues are highlighted in grey. The symbols below the sequences indicate: conserved sequences (*), conserved mutations (:) and semi-conserved mutations (.).

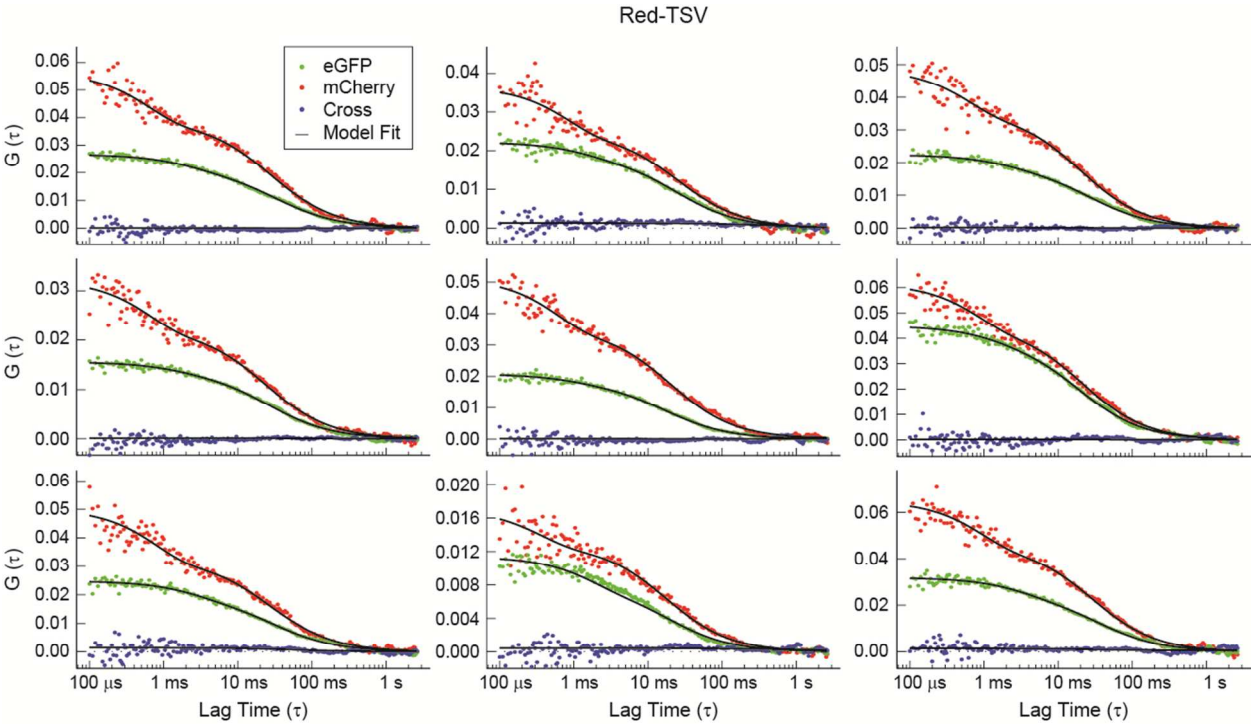


Figure S8. FCCS data are shown for nine representative single Cos-7 cells expressing red-TSV fusions to eGFP and mCherry. In each plot, colored dots are the measured data points, whereas the solid black lines indicate the fitted functions (defined in the Material and Methods). Red dots are the FCS data for the mCherry fusion protein, $G_R(\tau)$; green dots are the FCS data for the eGFP fusion, $G_G(\tau)$; and blue dots are the FCCS data, $G_X(\tau)$. A horizontal dotted line marks the zero value in each plot. Amplitude data report directly on the concentration of diffusing species through the relationship: $G(0) = 1/\langle N \rangle = 1/A_{eff}\langle C \rangle$. The fraction correlated is calculated by comparing the relative amplitudes of the cross-correlation function and autocorrelation functions as described in the Material and Methods.

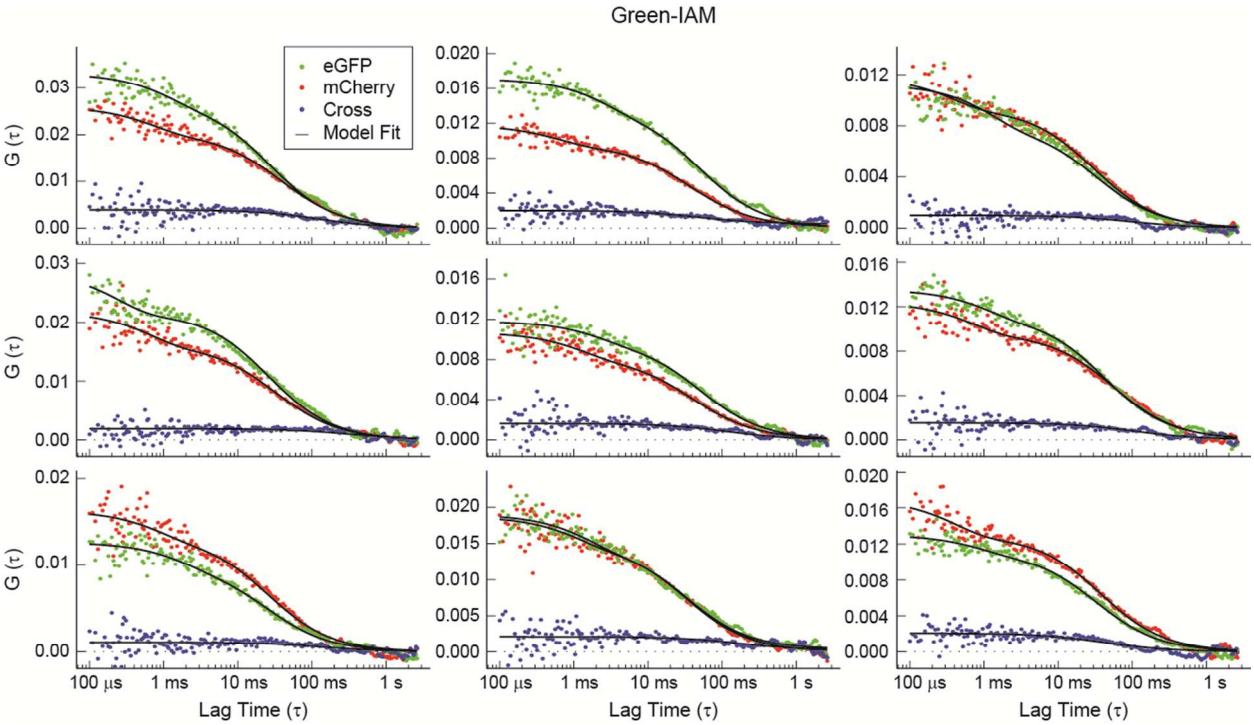
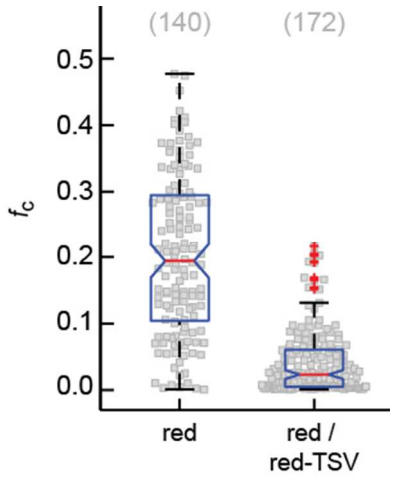


Figure S9. FCCS data are shown for nine representative single Cos-7 cells expressing green-IAM fusions to eGFP and mCherry. In each plot, colored dots are the measured data points, whereas the solid black lines indicate the fitted functions (defined in the Material and Methods). Red dots are the FCS data for the mCherry fusion protein, $G_R(\tau)$; green dots are the FCS data for the eGFP fusion, $G_G(\tau)$; and blue dots are the FCCS data, $G_X(\tau)$. The horizontal dotted line marks the zero value in each plot. Amplitude data report directly on the concentration of diffusing species through the relationship: $G(0) = 1/\langle N \rangle = 1/A_{eff}\langle C \rangle$. The fraction correlated is calculated by comparing the relative amplitudes of the cross-correlation function and autocorrelation functions as described in the Material and Methods.



FigureS10. Comparison of red cone opsin homodimerization with heterodimerization of WT red cone opsin and red-TSV. The PIE-FCCS data show that coexpression of WT red-mCherry and red-TSV-eGFP results in a significant decrease in dimerization ($P < 0.001$). This result is consistent with the conclusion that TM5 is part of the dimerization interface, and that other interfaces likely play only minor roles.

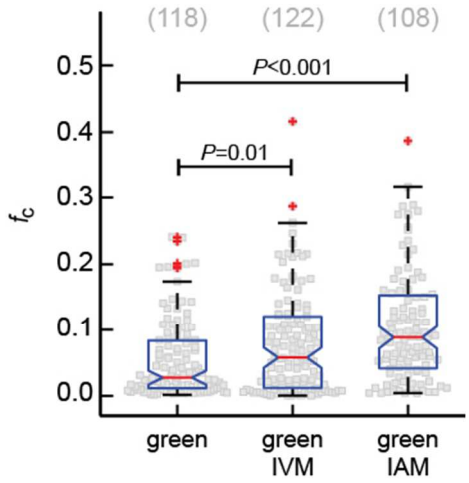


Figure S11. Comparison of dimerization for green cone opsin, green-IAM and green-IVM. The plot contains a summary of cross-correlation values, f_c, for green cone opsin, green-IAM, (T230I, S233A, V236M) and green-IVM (T230I, S233V, V236M). The median of the f_c distribution for green-IVM is between those of WT green and green-IAM. This indicates that the Val substitution at position 233 weakens the dimerization affinity relative to the Ala residue at the same position.

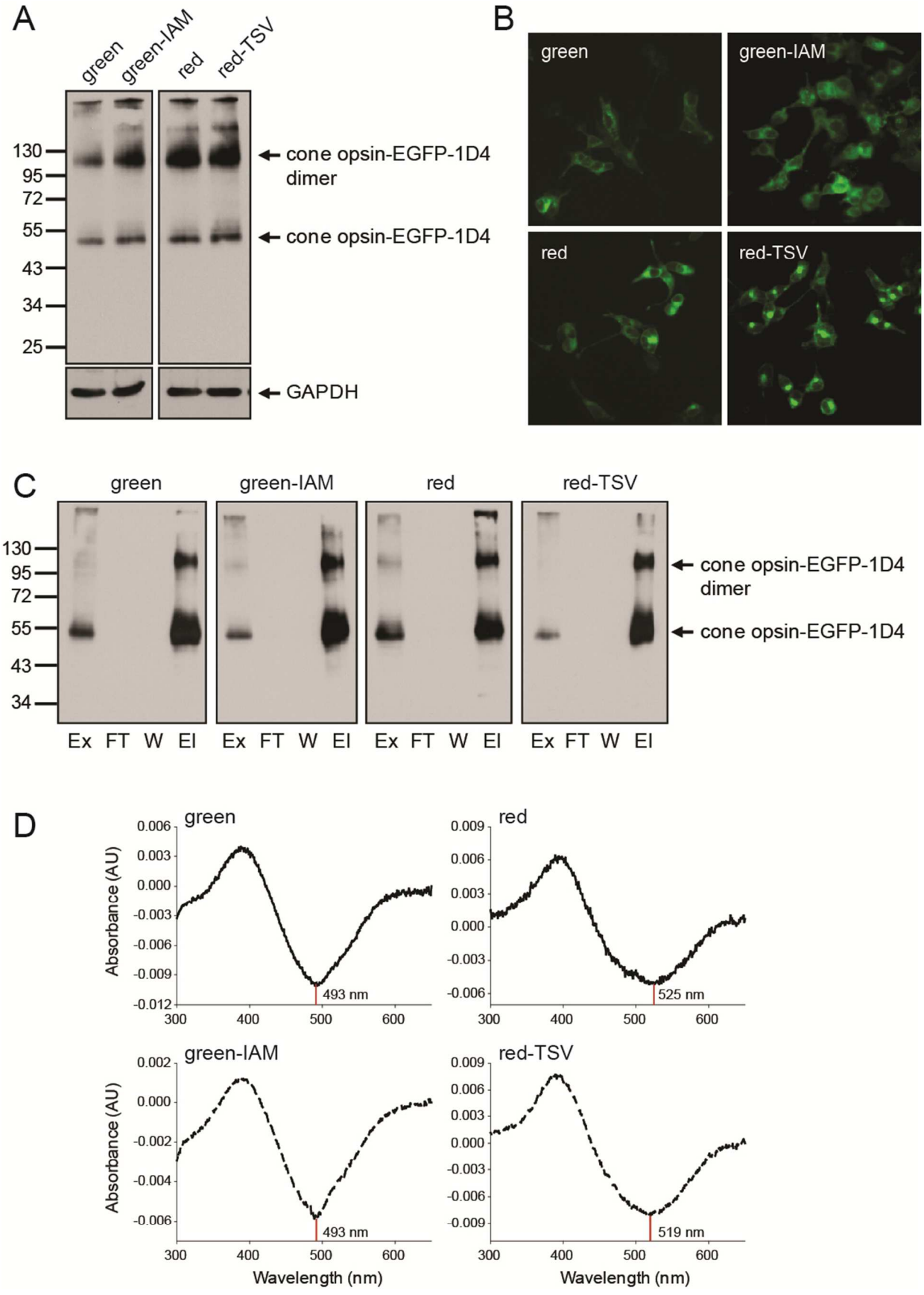

A
green
green-IAM
red
red-TSV
130
95
72
55
43
34
25
cone opsin-EGFP-1D4 dimer
cone opsin-EGFP-1D4
GAPDH
B
green
green-IAM
red
red-TSV
C
green
green-IAM
red
red-TSV
130
95
72
55
43
34
cone opsin-EGFP-1D4 dimer
cone opsin-EGFP-1D4
Ex FT W El Ex FT W El Ex FT W El Ex FT W El
D
green
red
green-IAM
red-TSV
Absorbance (AU)
493 nm
525 nm
493 nm
519 nm
Wavelength (nm)

Figure S12. Biochemical characterization and spectral tuning of eGFP constructs. (**A**) Immunoblots indicating expression levels of green cone opsin, green-IAM, red cone opsin and red-TSV, each with an eGFP-1D4 fusion tag and transiently expressed in HEK-293 cells. Measurements were made with an anti-rhodopsin C-terminal 1D4 tagged antibody on 50 µg of total protein cell lysate 48 h after transfection. GAPDH was the protein loading control. (**B**) Membrane localization of opsins was determined by detecting eGFP fluorescence in live cells. (**C**) Immunoblots of green cone opsin, green-IAM, red cone opsin and red-TSV regenerated with 9-*cis*-retinal and purified by immunoaffinity chromatography and detected with the anti-rhodopsin C-terminal 1D4 tagged antibody. Ex – extract (total lysate), FT – flow through, W – last wash, El – elution. (**D**), Difference absorption spectra of green cone opsin, green-IAM, red cone opsin and red-TSV regenerated with 9-*cis*-retinal and purified by immunoaffinity chromatography. The spectrum of red-TSV shows a 6 nm spectral shift, whereas the green-IAM mutant opsin does not.

Reference

1. Comar, W. D.; Schubert, S. M.; Jastrzebska, B.; Palczewski, K.; Smith, A. W., *J. Am. Chem. Soc.* **2014,** *136,* 8342-8349.