

Supporting information for Rothwell *et al.* (2003) *Proc. Natl. Acad. Sci. USA*,  
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**Fig. 5.** (A) Unmodified intensity signal measurements for Fig. 2A. (B) Ratio of the green to red signal intensities vs. the fitted lifetimes. In this graph, the distinction between species Ia and Ib is already visible. The lifetimes have been left as fluorescence-weighted lifetimes. (C) The ratio of fluorescence intensities is plotted vs. the species-weighted lifetimes. The fluorescence intensities were calculated by correcting the signal intensities for background, relative detection efficiencies, and crosstalk ( $B_G = 1.9$  kHz,  $B_R = 0.59$  kHz,  $g_G/g_R = 0.52$ ,  $\alpha = 0.011$ ). The equation  $F_D/F_A = a_{\text{trans}}(\Phi_D/\Phi_A) \bar{\tau}_{D(A)}/(\bar{\tau}_{D(0)} - \bar{\tau}_{D(A)})$  is plotted over the data, with  $\Phi_D = 0.63$ ,  $\Phi_A = 0.40$ ,  $a_{\text{trans}} = 0.80$ , and  $\bar{\tau}_{D(0)} = 3.1$  ns. The correction from fluorescence-weighted to species-weighted was calculated by using the polynomial  $\bar{\tau}_{D(A)} = -0.533\tau_{D(A)}^2 + 0.9583\tau_{D(A)} + 0.0139$ .