Supporting Information.

FDTD simulations- The incident wave was a unit amplitude x-polarized ramped sinusoidal electric field tuned to 470nm (excitation wavelength for the Alexa Fluor 488 FCS experiment), and the viewing slice was taken at 10 nm away from the aperture-substrate interface, where the fields are expected to be mostly confined. The buffer solution used in the experiment was modeled as a static dielectric inside the apertures with an index of refraction close to the water value of 1.33. The aluminum film was modeled as a Drude metal with parameters (ω_p=1.67e16 s^{-1}, τ_c=1.14e-15 s, ε_∞=2.10) obtained by fitting tabulated complex permittivity values from the *Handbook of Optical Constants of Solids* by Palik[1].

E and S Calculation and 2-D E-S histogram analysis - In order to determine the fluorescence intensities used in Equations 1 and 2 for calculating *E* and *S*, it is necessary to identify intensity bursts from the raw photon data. This is done, by first generating multi-channel time traces using the PicoQuant SymphoTime 4.2 (SPT) software. Photon counts are binned into 1 ms time bins by using just the photon arrival time. One-dimensional *E* and *S* histograms can be directly generated from the SPT software using the FRET analysis feature. Here, the intensity vs. time traces for the donor and acceptor fluorescence intensities as needed in the expressions for *E* and *S* are generated by selecting the appropriate time gate and detector channel information. Photon bursts for each component, $F_{D\,ex}^{D\,em}$, $F_{D\,ex}^{A\,em}$, and $F_{A\,ex}^{A\,em}$ for *E* calculation with PIE filtering, and $F_{D\,ex}$ and $F_{A\,ex}$ for *S* calculation, are detected when the bin count for each respective is above a selected threshold. Photon bursts must be above the threshold value for all components required for *E* or *S* calculation. Furthermore, *E* calculation can selectively be carried out only on intact FRET pairs by selecting PIE filtering. That is, only events with bursts above the threshold set for the $F_{A\,ex}^{A\,em}$ component will be used in the determination of the *E* histogram. Thus, independent one-dimensional *E* and *S* histograms will be generated using the following expressions.

$$E = \frac{n_A}{n_A + n_D} \tag{3.}$$

$$S = \frac{n_{D\,ex}}{n_{D\,ex} + n_{A\,ex}} \tag{4.}$$

n_A is the number of acceptor photon counts with donor excitation, n_D is the number of donor photon counts with donor excitation. $n_{D\,ex}$ is the total number of photon counts in both detection channels with donor excitation, and $n_{A\,ex}$ is the total number with acceptor excitation. Here we have again assumed that γ~1 for this particular FRET pair and that the donor and acceptor emission leakage into the opposite channels and direct excitation of the acceptor with donor excitation are also negligible. Due to these assumptions, it is possible to generate *S* histograms using the FRET analysis of the SPT software, as the expressions for *E* and *S* are similar and only require different time gate and detection channel values. However, when taking into account leakage and direct excitation cross-talk contributions as in Lee et. al. [2], one must calculate *S* offline, because in this case these expressions do differ significantly so that a simple swapping of terms in the ratio expressions cannot be performed. Similarly, *E* and *S* for each burst cannot be calculated simultaneously within the SPT software. Therefore, the temporal time traces for each of the emission components were exported into IGOR Pro (v. 5.02) (Wavemetrics, Lake Oswego, OR), and a burst identification using thresholds and *E* and *S* calculation was employed similar to the SPT software, using the same expressions for *E* and *S*. However, *E* and *S* values for each detected burst were now coupled and binned to a 2D *E-S* histogram. PIE filtering can also be implemented in the 2-D analysis by filtering out bursts in which the $F_{A\,ex}^{A\,em}$ component is absent.

A donor-only complex should have an *E* value of ~ 0 and an *S* value of ~1. Likewise, an acceptor-only complex should yield *E* and *S* values of ~0. Donor-Acceptor complexes will have different *E* and *S* values depending on their relative distance and stoichiometry. As *S* is a parameter that depends on the relative excitation intensities of the donor and acceptor lasers, *S* values will depend on the ratio between the two intensities, and hence must initially be adjusted and calibrated for each individual pair of FRET dyes. It was shown[3] that adjusting the excitation powers of the two lasers to yield a value of *S* = 0.5 for a 1:1 donor-acceptor stoichiometry results in the best resolution of *S* values for different stoichiometries. As will be seen below from our results, a 1:1 donor to acceptor ratio corresponds to S = 0.4.

References

1. Palik, E., *Handbook of optical contstants of solids*. Academic Press: Orlando, 1985.

2. Lee, N. K.; Kapanidis, A. N.; Wang, Y.; Michalet, X.; Mukhopadhyay, J.; Ebright, R. H.; Weiss, S. *Biophysical Journal* **2005,** 88, (4), 2939-2953.

3. Kapanidis, A. N.; Lee, N. K.; Laurence, T. A.; Doose, S.; Margeat, E.; Weiss, S. *Proceedings of the National Academy of Sciences of the United States of America* **2004,** 101, (24), 8936-8941.