2010. **Defining the Limits of Single-Molecule FRET Resolution in TIRF Microscopy**. Holden SJ, Uphoff S, Hohlbein J, Yadin D, Le Reste L, Britton OJ, and Kapanidis AN. *Biophys J*. 99:3102–3111.

We have identified an error in our manuscript Holden et. al, 2010, Biophys. J. Eq. 6 (also referred to as Eq. S15) was given as:

$$\sigma(E) \; = \; \sqrt{\frac{f_G^2 E_0 (1-E_0)}{N} + \frac{4\pi}{\alpha^2 N^4} \left(D^2 s_D^2 b_D^2 + A^2 s_A^2 b_A^2 \right)}.$$

In this equation, the donor and acceptor photon count terms, *D* and *A* respectively, were erroneously switched. Eq. 6 should read:

$$\sigma(E) = \sqrt{\frac{f_G^2 E_0 (1 - E_0)}{N} + \frac{4\pi}{\alpha^2 N^4} \left(A^2 s_D^2 b_D^2 + D^2 s_A^2 b_A^2 \right)}.$$

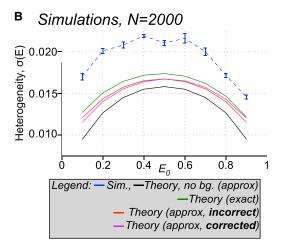
The same error affects Eq. S22, which should read:

$$\sigma_{ap}(E) = \sqrt{\frac{f_G^2 E_0 (1 - E_0)}{N} + \frac{1}{N^4} (A^2 \alpha_D b_D^2 + D^2 \alpha_A b_A^2)}.$$

All results apart from those presented in Fig. 2A and 2B used the numerically in-tegrated version of the theoretical predictions, Eq. S14, and are therefore unaffected. However, the red lines on Fig. 2A and 2B do use Eq. 6.

Symmetry at FRET efficiency of 0.5 means that Fig. 2A requires no correction. A corrected Fig. 2B, showing both the incorrect and corrected theoretical predictions (red line and magenta line respectively), is presented below. For the parameters used in the calculations for Fig. 2B, the theoretical prediction of Eq. 6 is changed by less than 2.5% in the range $0.2 \le E0 \le 0.8$ and less than 5% over the entire calculated range, $0.1 \le E0 \le 0.9$. Therefore none of the conclusions of the manuscript are affected by this error.

We note also a typographical error, which does not affect any of the calculations or conclusions of the manuscript: the negative exponent of the Gaussian function in Equations 4, 5, 7, and S7 was erroneously omitted.



Corrected Figure 2B: Red line, incorrect theoretical prediction; magenta line, corrected theoretical prediction



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