Supplementary Information for

Optofluidic FRET Lasers Using Aqueous Quantum Dots as Donors

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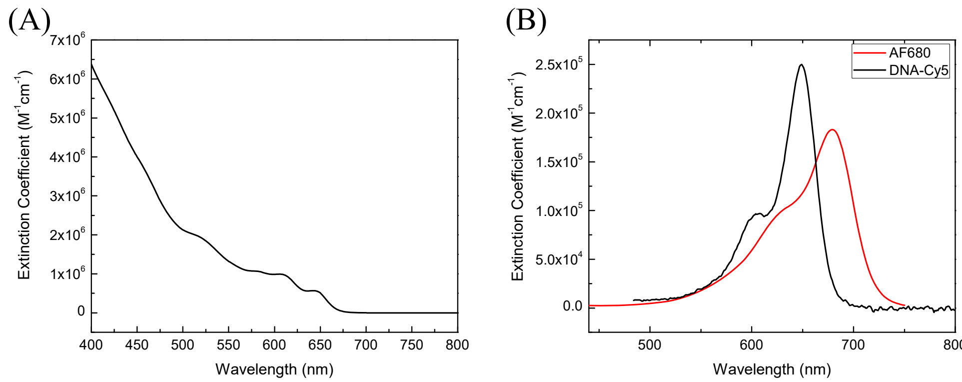

Figure S1. (A) Extinction coefficient of QDot 655 derived from absorption spectrum exported from Life Technologies® Fluorescence SpectraViewer. The extinction coefficient at 450 nm is 4e6 $M^{-1}cm^{-1}$. (B) Extinction coefficient of AF680 and Cy5. The extinction coefficient for AF680/Cy5 is 1.83e5 $M^{-1}cm^{-1}$/2.5e5 $M^{-1}cm^{-1}$ at 680 nm/648 nm, respectively. The absorption spectrum of AF680 is from Chroma Technology Corp® Chroma Spectra Viewer. The absorption spectrum of Cy5 is recorded by Nanodrop 2000c UV-Vis spectrometer with our DNA-Cy5 sample. The absorption cross section can be calculated from the extinction coefficient ε using the following equation: $\sigma_a = \ln(10)\frac{1000}{NA}\varepsilon = 3.82e-21\varepsilon\ cm^2$, where NA is the Avogadro's constant.

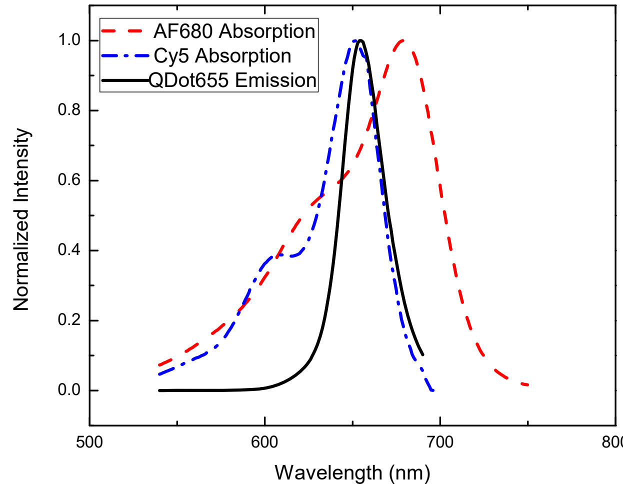

Figure S2. Emission or Absorption spectrum for QDot 655, Cy5, and AF680.

The Forster distance R_0 of a FRET pair is given by $R_0^6 = \frac{9\ln(10)\kappa^2\Phi_D J}{128\pi^5 n^4 N_A}$, where κ^2 is the dipole-dipole orientation factor ($\kappa^2 = 2/3$ for isotropically oriented dipoles), ϕ_D is the quantum yield of donor in the absence of the acceptor, n is the refractive index of the medium, N_A is Avogadro's number. J is the spectral overlap integral and $J = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda$, where F_D is the normalized donor emission spectrum and ε_A is the extinction coefficient of the acceptor. The emission spectrum of QDot 655 and absorption spectrum of Cy5 and AF 680 are shown in Fig. S2. The extinction coefficient is 250,000 $M^{-1}cm^{-1}$/183,000 $M^{-1}cm^{-1}$ for Cy5/AF680 at the absorption maximum. With a nominal quantum yield of 50% for the quantum dots, $\kappa^2 = 2/3$ and n=1.33, the Förster distance of QDot 655-Cy5/QDot 655-AF680 pair can be calculated as 8.2 nm/7.9 nm, which means QD-Cy5 has higher intrinsic FRET transfer rate than QD-A F680.

*QDot 655 emission spectrum is from Life Technologies® Fluorescence SpectraViewer.

*Cy5 and AF680 absorption spectra are from Chroma Technology Corp® Chroma Spectra Viewer.

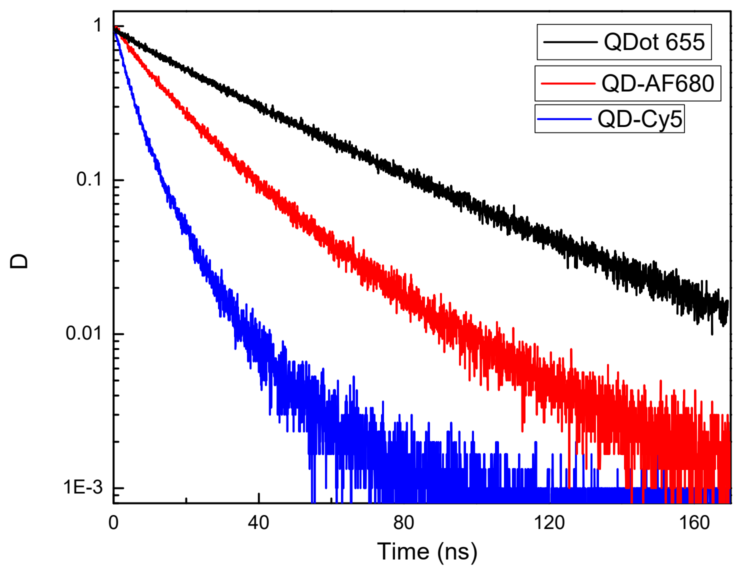

Figure S3. QD Fluorescence lifetime measurement of pure QDot 655 (black), QD-AF680 conjugate (red) and QD-Cy5 conjugate. Emission was recorded at 620 nm to avoid contribution from dye molecules for the conjugate sample.

According to fluorescence lifetime measure as shown in Fig. S3, we can derive the fluorescence lifetime of QDot 655 in each sample. For pure QDot 655, single exponential fitting gives a QD lifetime of $\tau_0 = 34.6$ ns. For the conjugation samples, the data fit to second-order exponential decay. The dominant decay constant gives a total life time of 4.4 ns for QD-Cy5 sample and 12 ns for QD-AF680 sample.

The FRET efficiency in the conjugation can be calculated by $E = 1 - \frac{\tau}{\tau_0}$, where τ is the lifetime of the QD in the presence of FRET. In the QD-Cy5 sample, $\tau = 4.4$ ns, E=87%. In the QD-AF680 sample, $\tau = 8.7$ ns, E=65%. The energy transfer rate can be calculated by $k_f = \tau_f^{-1} = \tau^{-1} - \tau_0^{-1}$. For QD-Cy5, $k_f = (5 \text{ ns})^{-1}$.

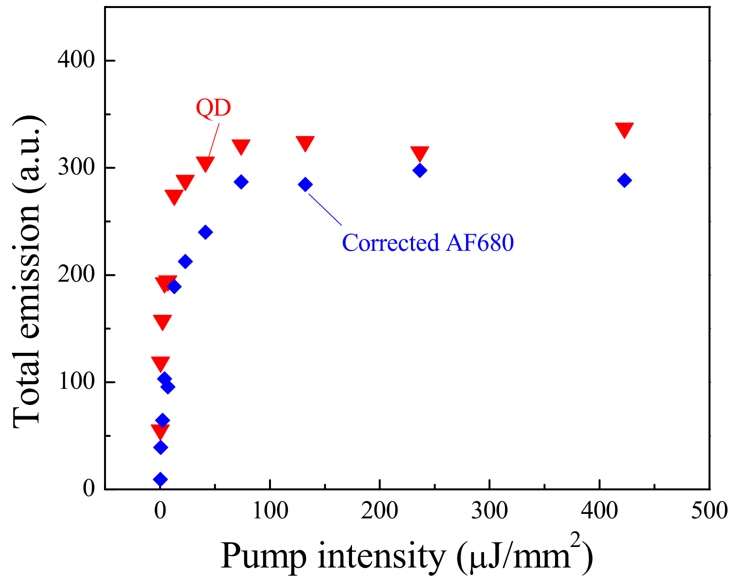

Figure S4. Fluorescence intensity of AF680 in QD-AF680 sample corrected for direct excitation in QD-AF680 sample.

AF680 emission resulting from direct excitation can be written as $I_{direct} = AN_{AF680}\sigma_{AF680}(450nm)q_{AF680}$, where A is the system collection coefficient. N_{AF680} is the total concentration of AF680. σ_{AF680} and q_{AF680} are the absorption cross section and quantum yield, respectively. A can be derived from the slope of the QD emission. Fig. S4 shows the AF680 emission intensity after removing direct excitation contribution. We can see that the AF680 emission follows the same saturation behavior as the QD emission. The saturation in the AF680 emission is attributed to the saturation in the QD excitation (*i.e.*, single excitons). It cannot be explained by the saturation caused by direction excitation of AF680 at 450 nm, which requires a pump intensity of 1940 μJ/mm^2 (*i.e.*, $P_s = 1/\sigma_a(\lambda_P)\tau_F = 8.8\text{e}16\ \text{photons}/(cm^2 \cdot ns)$), much higher than 100 μJ/mm^2 shown in Fig. S4. Thus, we can confirm that the emission of AF680 shown in Fig. S4 is due solely to FRET through QDs.

τ_0	34.5 ns	Life time of pure QDs
τ_{QD-Cy5}	4.4 ns	Life time of QDs in QD-Cy5
$\tau_{QD-AF680}$	12 ns	Life time of QDs in QD-AF680
τ_{Cy5}	1 ns	Life time of pure Cy5 [1]
τ_{AF680}	1.2 ns	Life time of pure AF680 [2]
$\tau_{FRET,QD-Cy5}$ ($1/k_F$)	5 ns	FRET life time of QD-Cy5
$\sigma_{e,Cy5}(730nm)$	2.5e-16 cm^2	Emission cross-section of Cy5
$\sigma_{a,Cy5}(500nm)$	3.1e-17 cm^2	Absorption cross-section of Cy5 at 500 nm
$\sigma_{a,AF680}(450nm)$	0.95e-17 cm^2	Absorption cross-section of AF680 at 450 nm.
q_{AF680}	0.3	Quantum yield of AF680
q_{Cy5}	0.27	Quantum yield of Cy5
N_{AF680}	38 μM	Concentration of AF680 in QD-AF680 sample

Table S1. Summary of the parameters used in the main text and the Supplementary Information.

[1]http://www.iss.com/resources/reference/data_tables/LifetimeDataFluorophores.html
[2]https://www.thermofisher.com/us/en/home/references/molecular-probes-the-handbook/tables/fluorescence-quantum-yields-and-lifetimes-for-alexa-fluor-dyes.html