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Supporting Information

Investigation of dendrimers functionalized with eosin as macrophotoinitiators for polymerization-based signal amplification reactions

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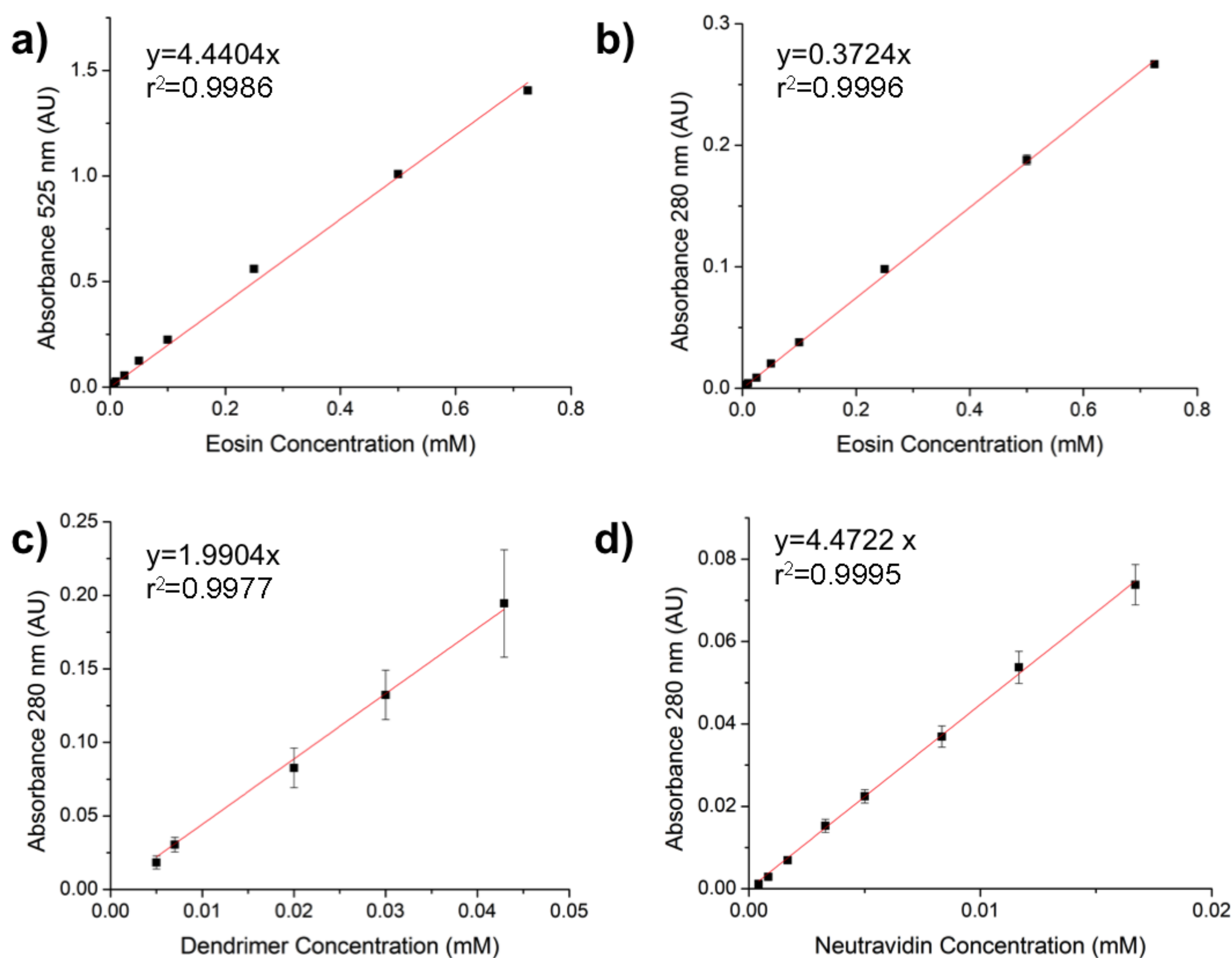


Figure S1. Absorbance standard curves. a) Absorbance standard curve for eosin at 525 nm (2 replicates). b) Absorbance standard curve for eosin at 280 nm (2 replicates). c) Absorbance standard curve for the poly (amidoamine) dendrimer at 280 nm (4 replicates). d) Absorbance standard curve for neutravidin at 280 nm (4 replicates). In all instances, the black squares represent the absorbances at the indicated wavelength for dilutions prepared in PBS.

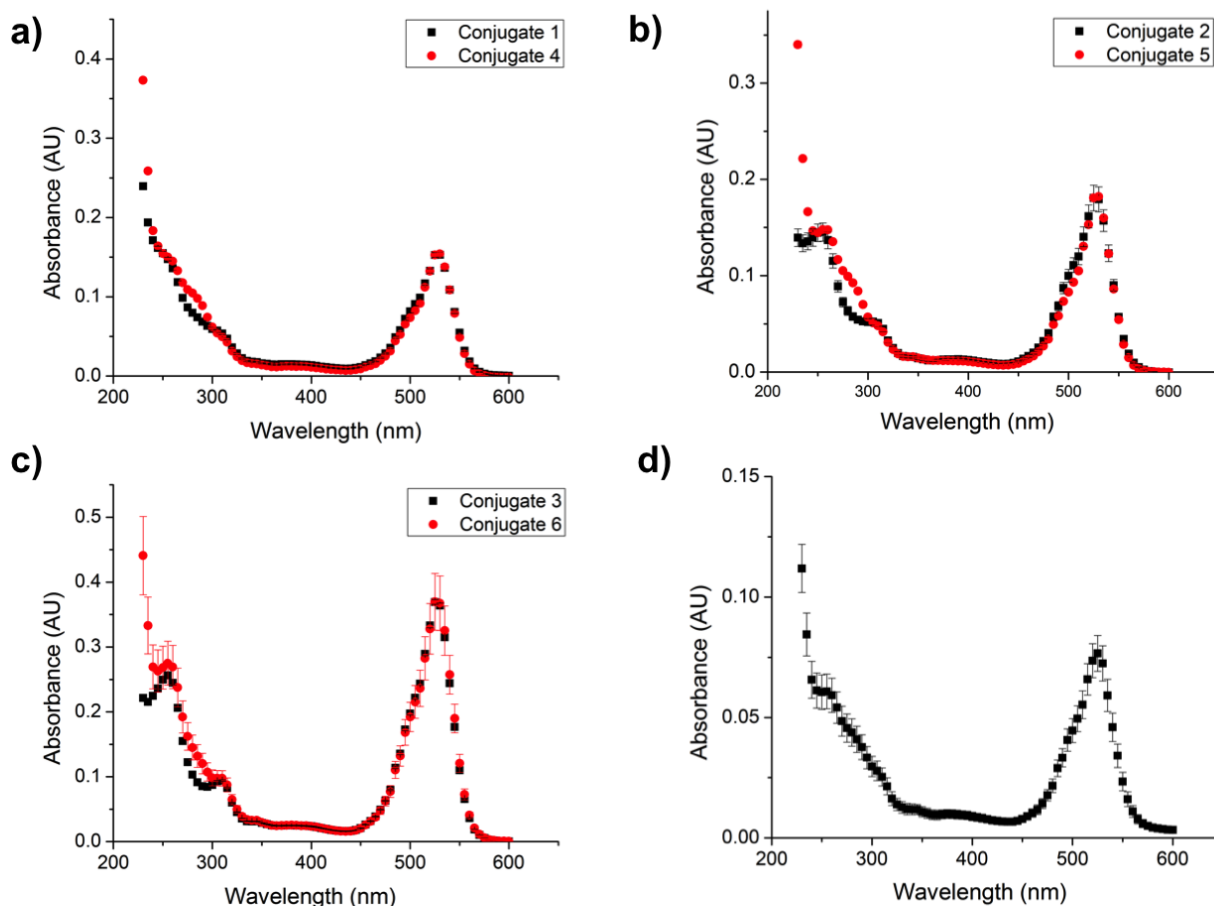


Figure S2. Conjugate characterization. Conjugates 1-3 were coupled to neutravidin to produce the corresponding conjugates 4-6. a) Absorbance spectra for conjugates 1 and 4. b) Absorbance spectra for conjugates 2 and 5. c) Absorbance spectra for conjugates 3 and 6. d) Absorbance spectrum for Neutravidin-eosin. Using the standard curves shown in supplementary figure 1, the number of eosin conjugated per dendrimer for conjugates 1-3 was determined. The characteristic absorbance of eosin at 525 nm allows for the subsequent determination of the number of neutravidin conjugated per dendrimer for conjugates 4-6 as the eosin densities for each dendrimer are known.

Table S1. Conjugate Concentrations from Characterization
Summary of concentrations for the components comprising each conjugate as calculated using the standard curves based on absorbance measurements obtained using the Tecan Infinite m200 microplate reader and a NanoQuant plate (path length=0.05 cm). The final row shows the concentration range of the standards used for generating the standard curves.

Conjugate	Eosin (mM)	G_7 PAMAM Dendrimer (mM)	Neutravidin (mM)
1	0.102	0.015	N/A
2	0.103	0.0076	N/A
3	0.287	0.012	N/A
4	0.077	0.011	0.0056
5	0.091	0.0067	0.0080
6	0.186	0.0077	0.0092
NAv-Eosin	0.039	N/A	0.0066
Linear Range	*0.005-0.725*	*0.005-0.0429*	*0.0004-0.017*

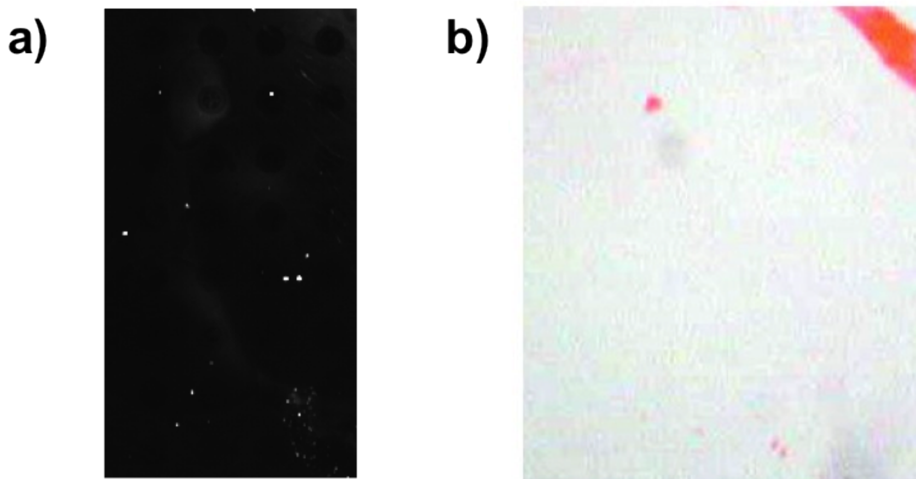
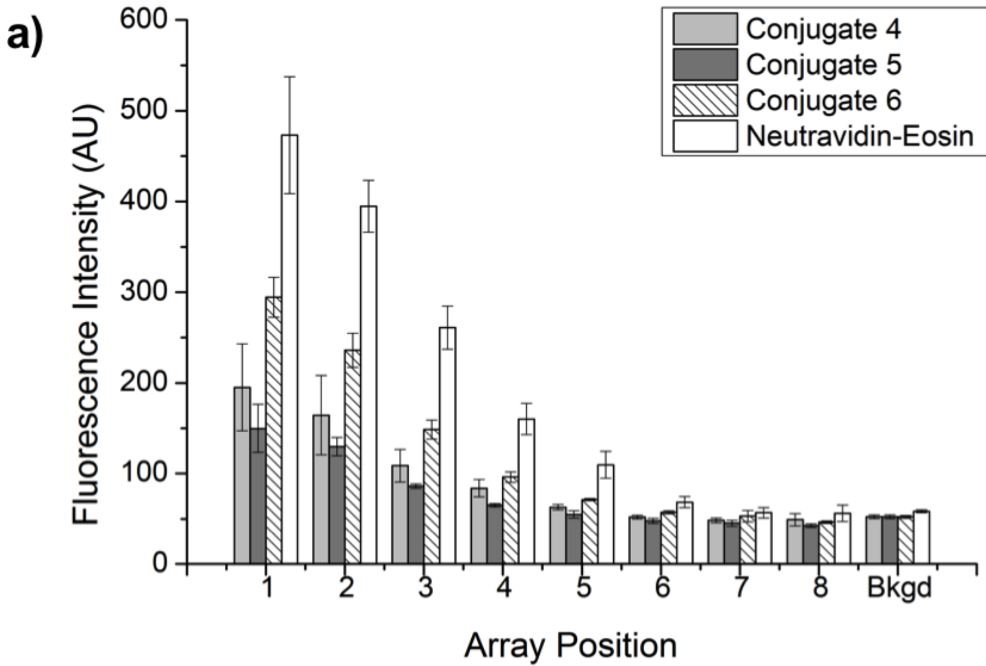


Figure S3. Negative control experiment. To verify the specificity of conjugate binding as well as investigate the possibility of eosin conjugation increasing the hydrophobicity of the conjugates and thus contributing to nonspecific binding, a test surface was incubated with 0.27 µM dendrimer conjugate 3. Conjugate 3 was selected as it has the highest number of eosin per dendrimer, and is thus the most likely to exhibit nonspecific binding, and a concentration of 0.27 µM was used as it corresponds to the dendrimer concentration present for 20 µg/mL of conjugate 6 (the highest concentration assayed). a) Fluorescence image of test surface following incubation with conjugate 3 (intensity scale: 0-1500). The background intensity level is 83 $\pm$ 31. b) Polymerization result for test surface incubated with conjugate 3. No polymerization was observed.



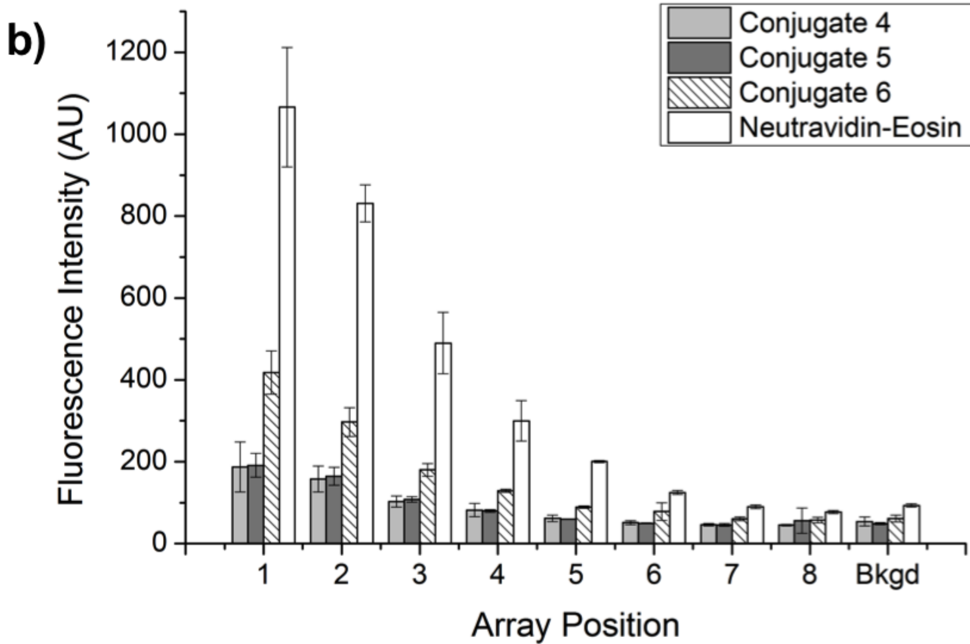


Figure S4. a) Summary of the average fluorescence intensities for each biotin dilution on test surfaces contacted with 5 µg/mL of each conjugate. b) Summary of the average fluorescence intensities for each biotin dilution on test surfaces contacted with 20 µg/mL of each conjugate. In both cases, the average comprises the surface features from two test surfaces.

Bkgd: background (area surrounding surface features)

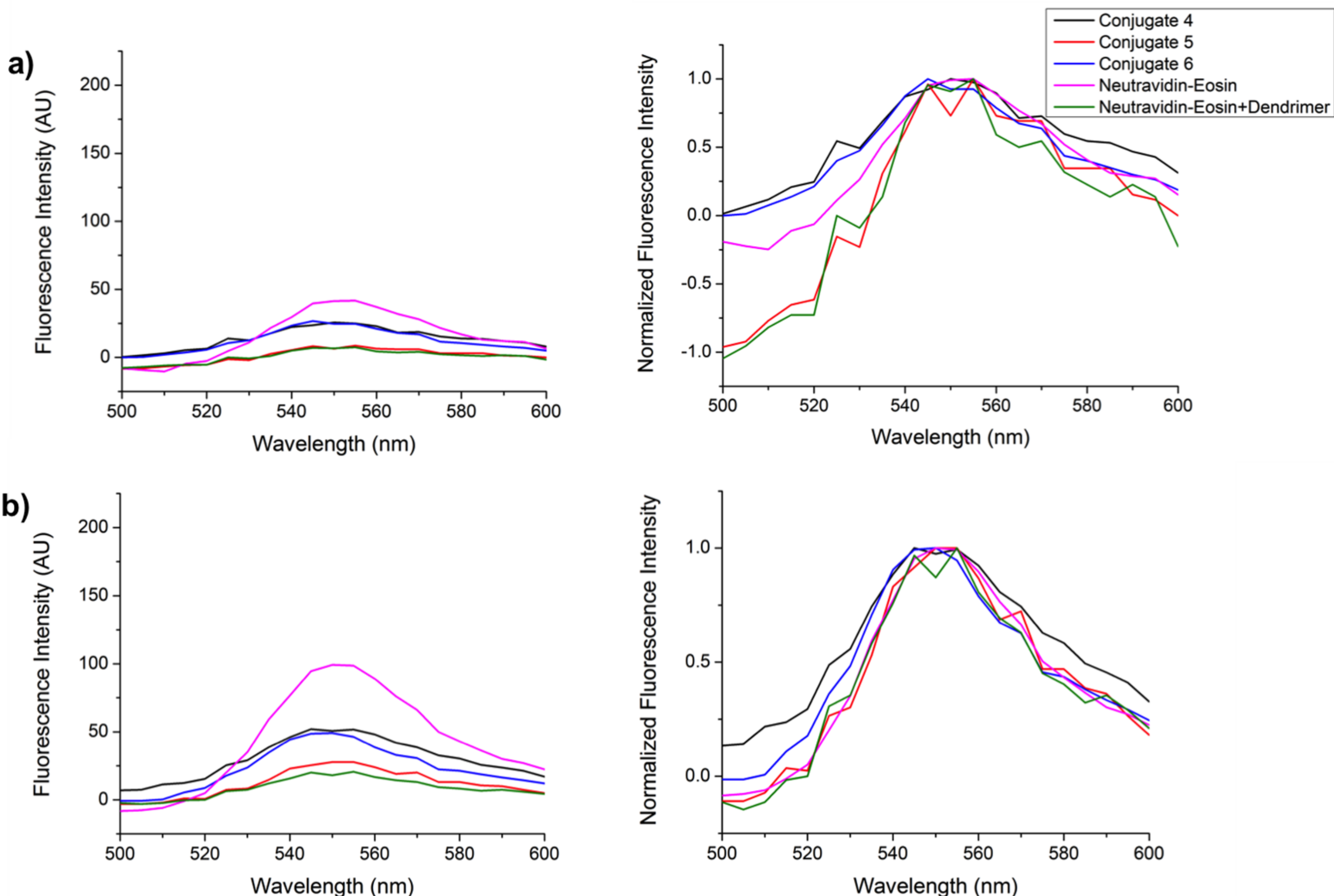


Figure S5. Emission spectra following excitation at 450 nm for 5 and 10 µg/mL of the conjugates as well as a dilution of neutravidin-eosin to which an equimolar amount of free dendrimer had been added. The spectra are corrected for the background fluorescence of the buffer solution (left column) and then normalized by peak height (right column). Note that these results are not normalized by the number of neutravidin conjugated per dendrimer, which is why the fluorescence intensities for conjugate 4 are higher than for conjugate 5. Conjugate 4 has approximately half the number of neutravidin conjugated compared with conjugate 5, so there are unconjugated dendrimers present and these contribute to an increase in the fluorescence intensity. a) Spectra for 5 µg/mL neutravidin. The normalized spectra (right) show that there is no shift in the maximum peak wavelength. b) Spectra for 10 µg/mL neutravidin. The spectra for 20 µg/mL neutravidin are shown in Figure 4. Progressively increasing the concentration of eosin more clearly demonstrates that there is no peak shift as the fluorescence intensity increases and the effect of noise is diminished. The higher concentrations are also more relevant from the perspective that aggregate formation is a concentration driven process and more likely to be observed at higher concentrations. The spectra are the average of three trials.

Table S2. Conjugate Concentrations for Solution Spectroscopy

Summary of concentration ranges for the components comprising each conjugate for the solution spectroscopy experiments, which were performed in 96 well plates (path length=0.23 cm).

Conjugate	Eosin (mM)	G_7 PAMAM Dendrimer (mM)	Neutravidin (mM)
4	$5.83x10^{-4}$-$2.33x10^{-3}$	$1.67x10^{-4}$-$6.67x10^{-4}$	$8.33x10^{-5}$-$3.33x10^{-4}$
5	$1.17x10^{-3}$-$4.67x10^{-3}$	$8.33x10^{-5}$-$3.33x10^{-4}$	$8.33x10^{-5}$-$3.33x10^{-4}$
6	$2x10^{-3}$-$8x10^{-3}$	$8.33x10^{-5}$-$3.33x10^{-4}$	$8.33x10^{-5}$-$3.33x10^{-4}$
NAv-Eosin	$5x10^{-4}$-$2x10^{-3}$	N/A	$8.33x10^{-5}$-$3.33x10^{-4}$

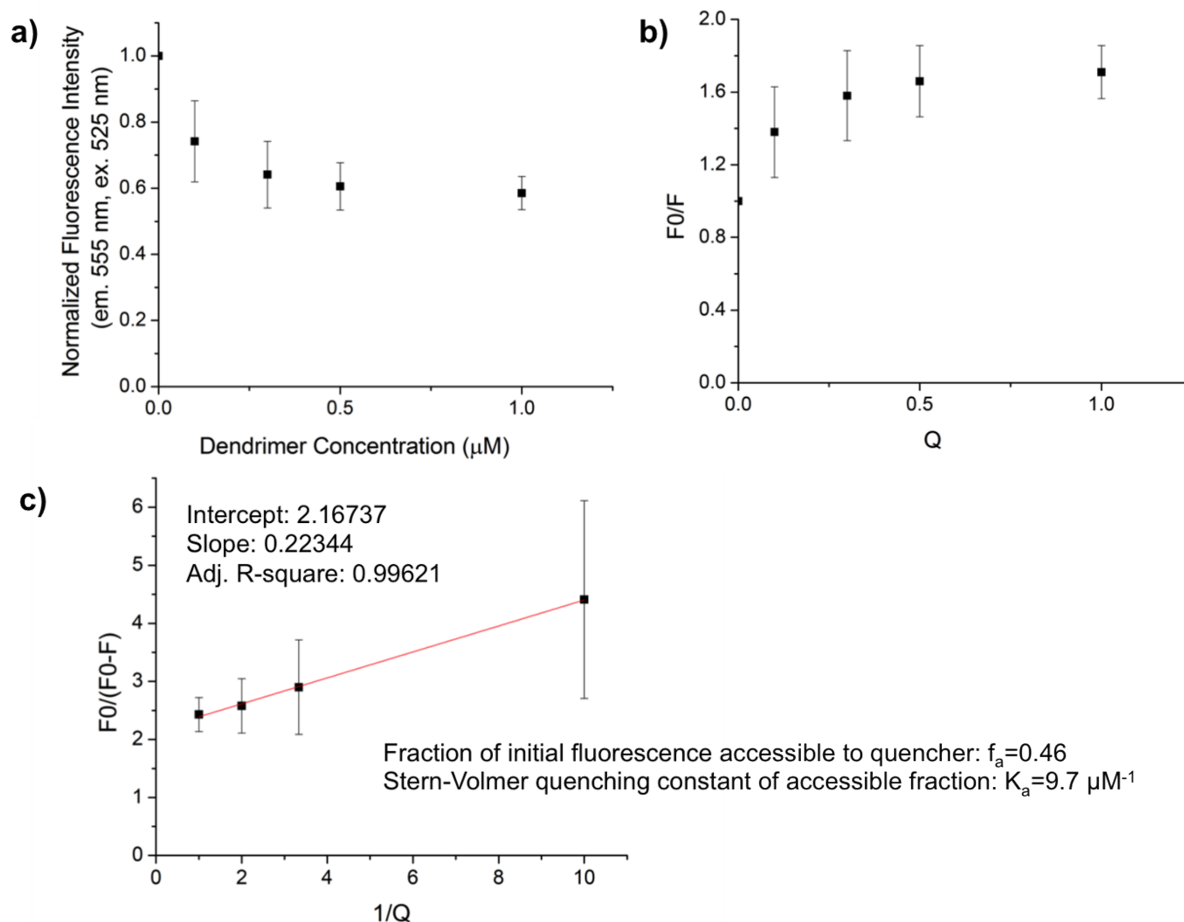


Figure S6. Stern-Volmer Plots. a) The concentration of free dendrimer in a 1 μM eosin solution was varied between 0.1 and 1 μM (three trials). b) The ratio of the fluorescence intensity in the absence of quencher, F_0, to the fluorescence intensity, F, was plotted as a function of quencher concentration, $|Q|$, in accordance with the Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + k_q \tau_0 |Q|_1$$

In the above expression, k_q is the bimolecular quenching constant, and τ_0 is the lifetime of the fluorophore in the absence of quencher. The nonlinearity of the Stern-Volmer plot indicates that more than one type of quenching may have occurred. The downward curvature of this plot suggests that the quenching observed here might be better described by a modified Stern-Volmer equation that allows for two fluorophore populations, one accessible to the quencher and the other inaccessible. In this expression (below), f_a is the fraction of initial fluorescence that is accessible to the quencher and K_a is the Stern-Volmer quenching constant of the accessible fraction.

$$\frac{F_0}{F_0 - F} = \frac{1}{f_a K_a |Q|} + \frac{1}{f_{a1}}$$

c) The modified Stern-Volmer plot. While quenching was consistently observed, the linear fit for the modified plot is not convincing. For each plot, the error bars represent the standard deviation of the dependent variable across three trials.

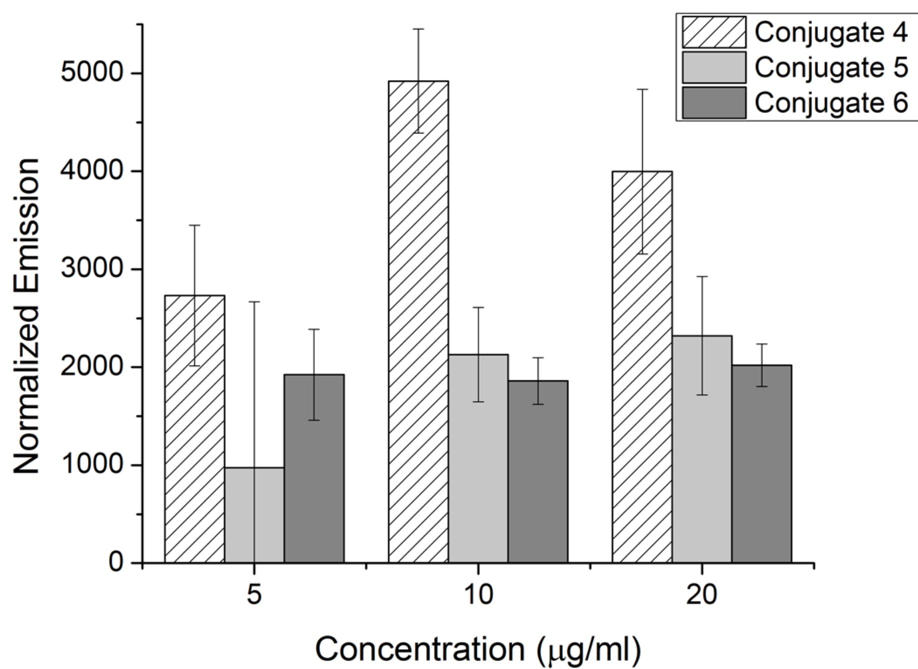


Figure S7. Emission normalized by absorbance. The emission at 545 nm (ex. 450 nm) was normalized by the peak absorbance at 525 nm for each dendrimer conjugate. As the number of eosin substituents per dendrimer increases, the normalized emission decreases.

References

(1) Lakowicz, J. R. (2006) Principles of Fluorescence Spectroscopy Third Edit. Springer, New York, NY.