

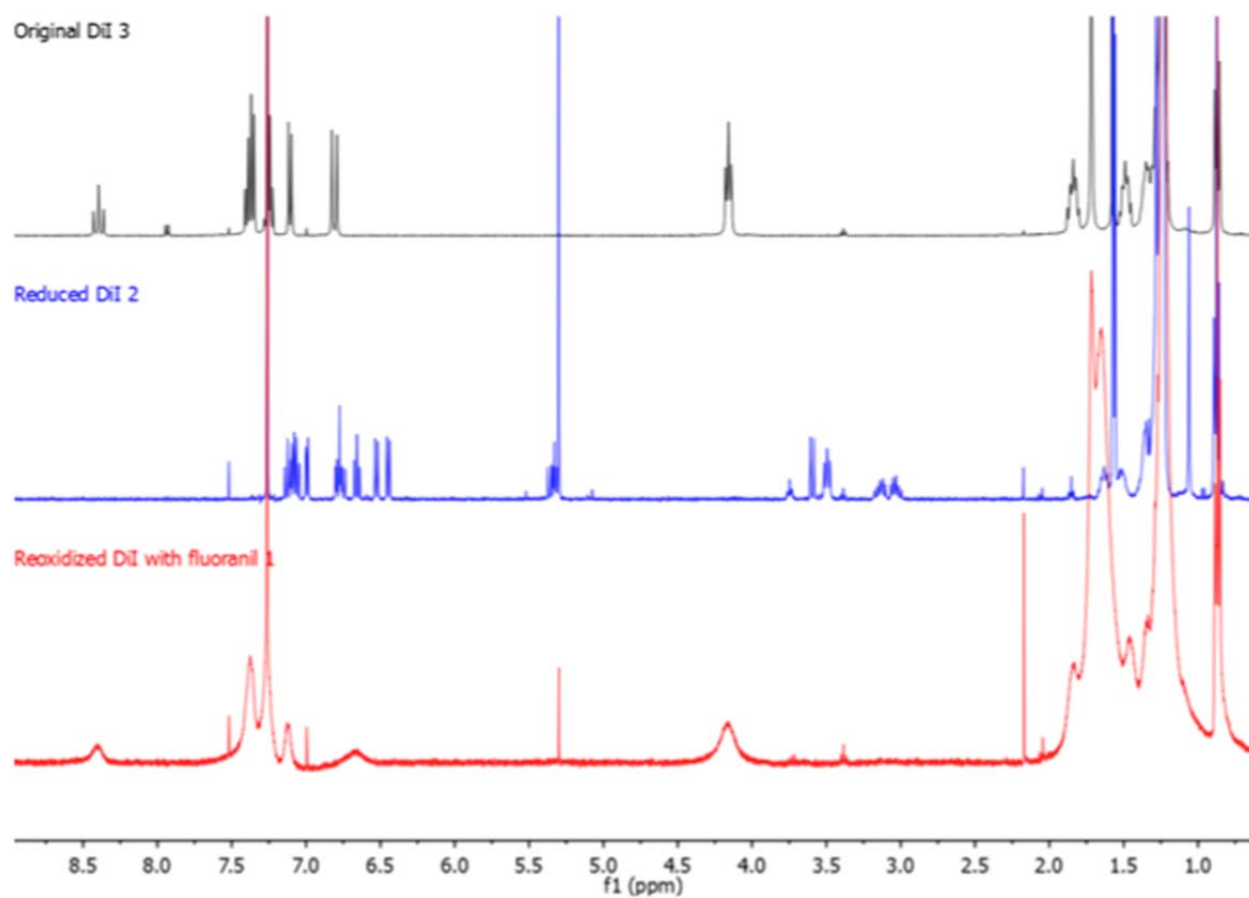
# Mutually-Reactive, Fluorogenic Hydrocyanine/Quinone Reporter Pairs for In- Solution Biosensing via Nanodroplet Association

*Rajarshi Chattaraj,<sup>§‡</sup> Praveena Mohan,<sup>‡‡</sup> Clare M. Livingston,<sup>‡</sup> Jeremy D. Besmer,<sup>‡</sup>*

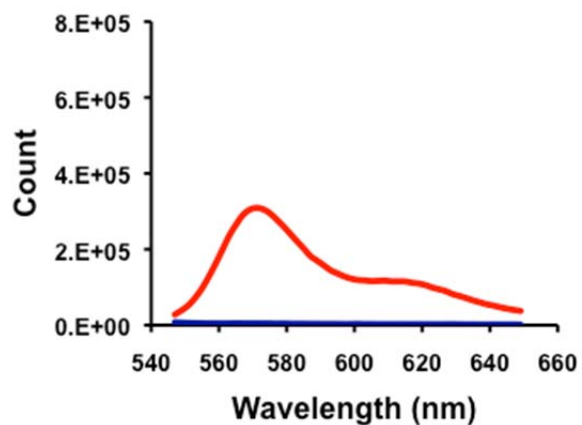
*Kaushlendra Kumar,<sup>‡</sup> and Andrew P. Goodwin<sup>‡\*</sup>*

<sup>§</sup> Department of Mechanical Engineering, University of Colorado Boulder. Boulder, CO 80309.

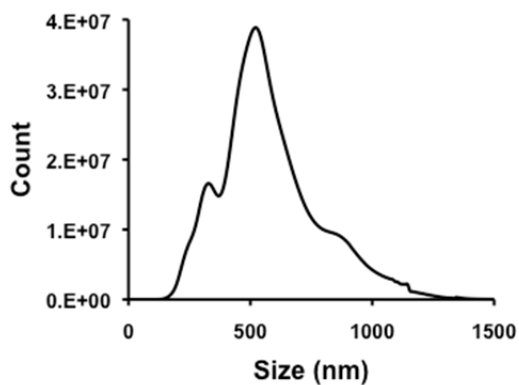
<sup>‡</sup>Department of Chemical and Biological Engineering. University of Colorado Boulder. Boulder, CO 80303.



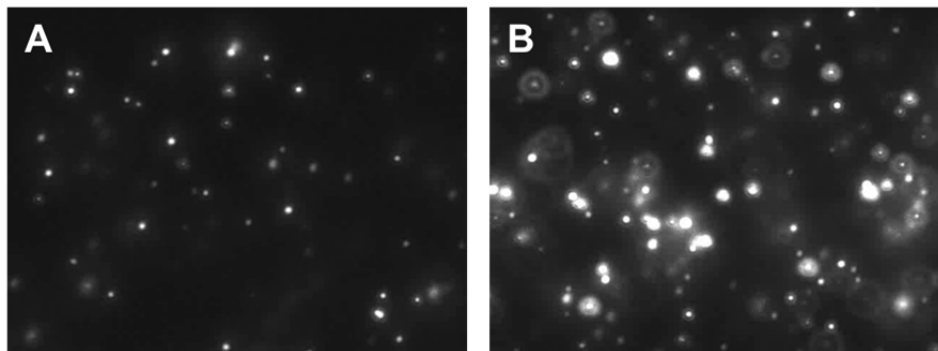
**Figure S1.** <sup>1</sup>H-NMR spectra (CDCl<sub>3</sub>) of DiI (*black*), HDiI(*blue*), and HDiI mixed with 4 molar equivalents of p-fluoraniI (*red*).



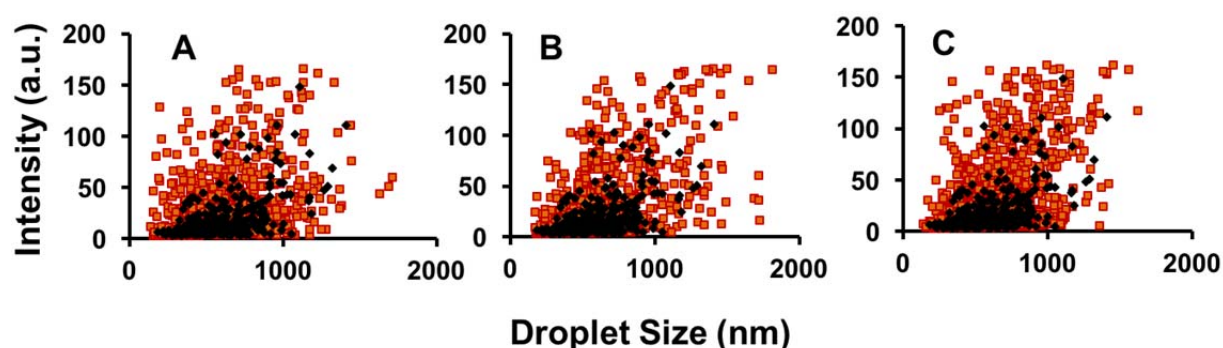
**Figure S2.** Fluorescence emission spectra ( $\lambda_{\text{exc}} = 532 \text{ nm}$ ) of HDiI mixed with 1 molar equivalent of p-fluoranol (*red*) in soybean oil, and HDiI only (*blue*) in soybean oil.



**Figure S3.** Size distribution of droplets containing NEOBEE oil as measured by NTA. Mean droplet diameter: 575.5 nm; SD = 203.5 nm.



**Figure S4.** Representative frames of videos captured through a fluorescence filter under a 532 nm excitation laser, by the Malvern NanoSight LM10, for subsequent analysis by the NTA 3.0 software. Figures show fluorescence for biotinylated mixed HDiI droplets and p-Fluoranol droplets incubated without (**A**) and with (**B**) 1nM streptavidin.



**Figure S5.** Representative NTA fluorescence scatter plots of biotinylated HDiI droplets and p-fluoranol droplets mixed without (black diamonds) or with (orange squares) a respective concentration of streptavidin (**A**: 1 pM; **B**: 100 pM; **C**: 1 nM)

T-Test values							
ttest values between each STV conc and Control				ttest values between each VEGF conc and Control			
<i>Figure 4C</i>				<i>Figure 6C</i>			
Conc of stv	p-value			VEGF conc	p-value		
1.0E-13	0.00126337			1E-13	0.0106526		
1.0E-12	0.00177514			1E-12	0.02369324		
1.0E-10	0.0007076			1E-10	0.00210828		
1.0E-09	0.00013669			1E-09	0.00037667		
1.0E-08	0.00865701						

**Figure S6.** P-values calculated from one-tailed t-test for data in Figures 4C and 6C.