Supplementary Information for "Fluorescence Correlation Spectroscopy at High Concentrations using Gold Bowtie Nanoantennas"

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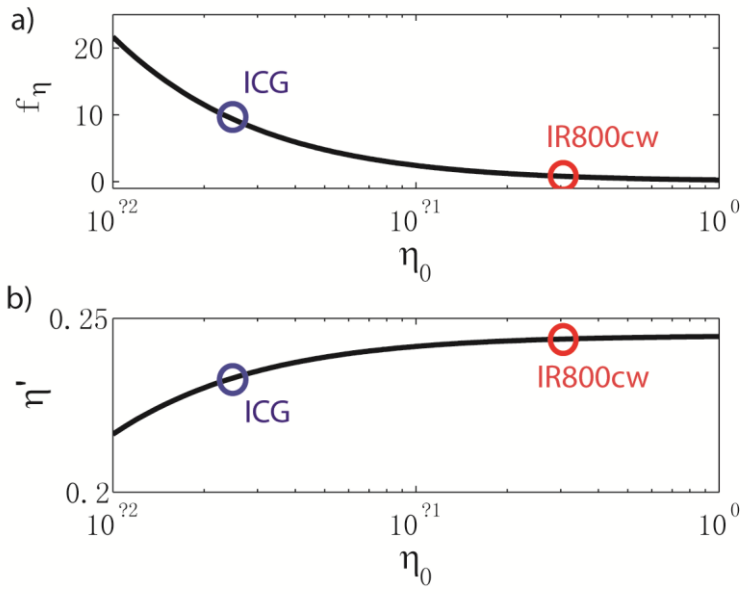
Finite-Difference Time-Domain (FDTD) Simulations

FDTD simulations of fluorescent molecules coupled to gold bowtie nanoantennas are described in Ref.[1]. In these simulations, it was confirmed that lower intrinsic fluorescence quantum efficiency molecules (η_0) have larger fluorescence enhancements. Supplementary Figure 1 plots the quantum efficiency (QE) enhancement (f_η) as a function of η_0 according to**:**

$$f_\eta = \frac{\eta'}{\eta_0} = \frac{\gamma_r' / \gamma_r}{1 - \eta_0 + \eta_0 \left(\gamma_r' / \gamma_r + \gamma_{nr}' / \gamma_r \right)}$$

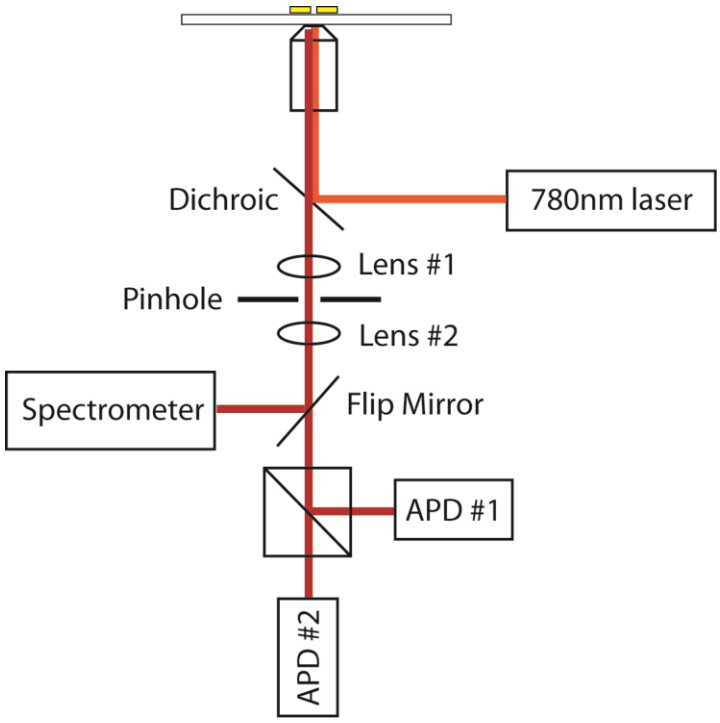
where η_0 is the molecule's intrinsic QE, η' is the molecule's QE when coupled to the bowtie, γ_r is the molecule's intrinsic radiative rate, and γ'_r and γ'_{nr} are the molecule's radiative and non-radiative rate when coupled to the bowtie. For ICG, the bowtie enhances the QE by a factor of 9.6, while for IR800cw, the QE is actually slightly quenched, by a factor of 0.8. The absorption enhancement (f_E = 180) for both molecules is independent of QE, so the total expected enhancement for ICG is f_F = 1700, while for IR800cw f_F = 144. These calculations, therefore, predict ICG to be a better molecule for bowtie-enhanced FCS as is demonstrated in Figure 3 of the main text.



Supplementary Figure 1. Finite-Difference Time-Domain simulation results for maximum fluorescence enhancement of a molecule located in the center of a 16 nm bowtie gap. Larger fluorescence enhancements are expected for lower intrinsic QE molecules.

Optical Setup

Supplementary Figure 2 is a schematic of the confocal microscope used to measure bowtie-enhanced FCS. In this microscope, a 785 nm diode laser is used in a typical confocal microscope setup with a 1.4 NA 60x Nikon objective. Fluorescence is collected through the same objective and, depending on the position of the flip mirror, can either be sent to a liquid nitrogen cooled CCD spectrometer for spectral measurement or to a 50/50 beam splitter and two Perkin Elmer APD's for FCS measurements.

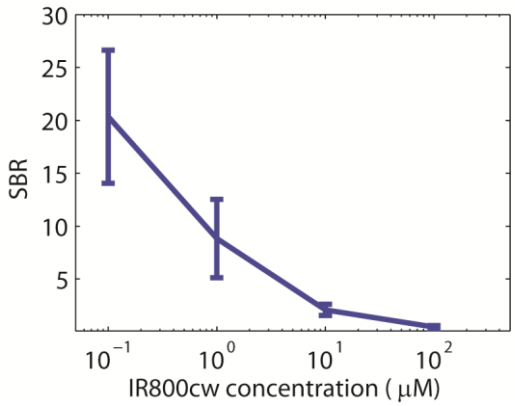


Supplementary Figure 2. Home-built confocal microscopy used for bowtie-enhanced FCS measurements.

IR800cw Bulk Enhanced Fluorescence

Bowtie nanoantennas were immersed in 4 different concentrations of IR800cw in ethanol and bulk fluorescence enhancement was measured with a confocal scan as in Figure 2b,c of the

main text. The enhanced fluorescence from 25 bowties in a single confocal scan were fit to 2D Gaussians in order to obtain the amplitude of the Gaussian focal spot. This amplitude was divided by the background level of unenhanced molecules in order to obtain the signal-to-background ratio (SBR) shown in Supplementary Figure 3. Error bars indicate one standard deviation in the amplitudes of the fits. Notice that the highest SBR is at 100nM and this steadily declines as the concentration increases. These data support the conclusion that the enhanced fluorescence is from molecules that are stuck to the substrate surface near the bowtie instead of molecules floating in solution, because the contrast ratio does not scale inversely with concentration.

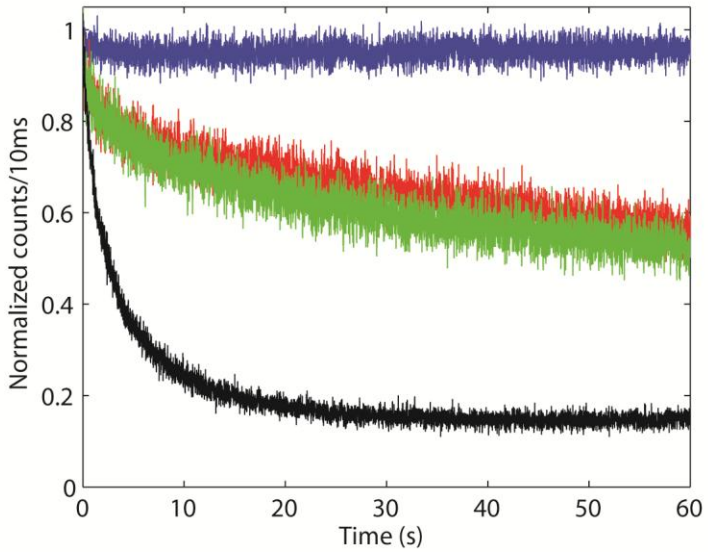


Supplementary Figure 3: SBR of IR800cw at different concentrations in ethanol.

Bulk Photobleaching to Measure Sticking

Additional support for the conclusion that enhanced fluorescence comes primarily from molecules that stick to the surface is found by the following bulk fluorescence experiment. In order to ascertain if molecules do indeed stick to the surface of the ITO coated quartz coverslip, the coverslip is immersed in 1μM concentrations of IR800cw and ICG in both ethanol and water.

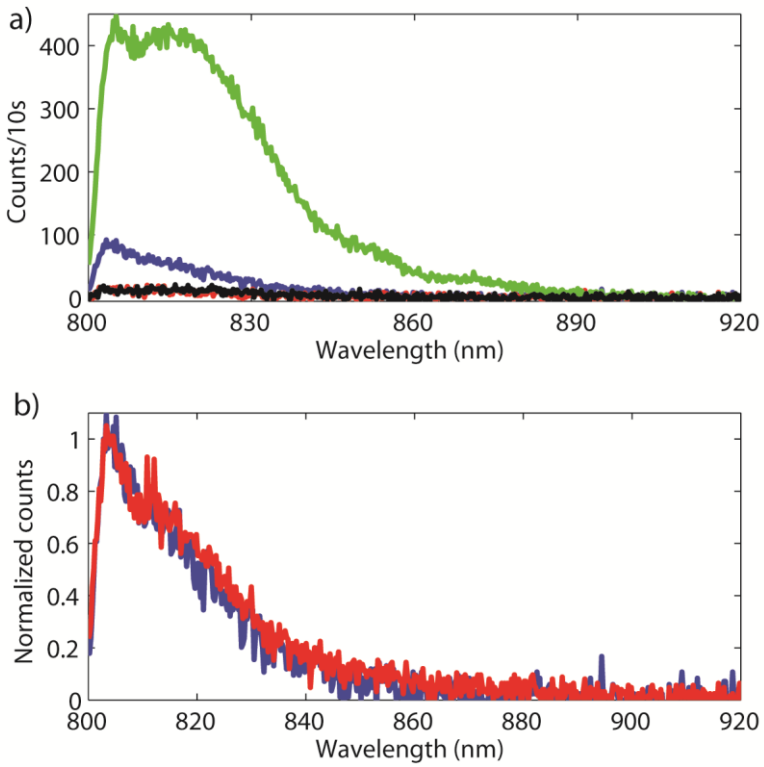
If a substantial portion of the molecules are sticking to the surface, then photobleaching should be observable. Otherwise, molecules will diffuse out of the focal volume on the millisecond time scale, so new unphotobleached molecules will always be present in the focal volume and no photobleaching would be measured beyond 1 ms of measurement. As seen in Supplementary Figure 4, all of the dye/solvent combinations exhibit photobleaching except for ICG in ethanol, which was shown in the Figure 2f of the main text to not show significant enhanced bulk fluorescence.



Supplementary Figure 4. Photobleaching of IR800cw and ICG in water and ethanol. Blue: 1μM ICG in ethanol. Red: 1μM ICG in water. Black: 1μM IR800cw in ethanol. Green: 1μM IR800cw in water. Only ICG in ethanol fails to show photobleaching, so it is the only combination that does not tend to stick to the ITO surface.

Enhanced Emission Spectra

Since the enhanced emission is due to molecules sticking to the substrate surface, it is also possible that the emission may not be metal-enhanced fluorescence (MEF), but is instead due to Surface Enhanced Raman Scattering (SERS). In SERS, as opposed to MEF, the highest enhancement position is for the molecule to be attached to the gold because large chemical enhancement effects are needed to see the extremely weak Raman signal. SERS has a very different spectral signature as well, and the spectra in Supplementary Figure 5 do not show any sharp Raman peaks characteristic of SERS. Therefore, the emission is indeed MEF and not SERS. In Supplementary Figure 5b, the bowtie and no bowtie spectra for IR800cw are normalized in order to show that the shape of the fluorescence spectrum does not markedly change with the presence of the bowtie nanoantenna. In principle, plasmonic antennas can change the fluorescence emission of molecules coupled to them[2-7], but the bowtie's resonance is relatively broad and well matched to the molecules' emission spectra (Figure 1b), so this does not occur.



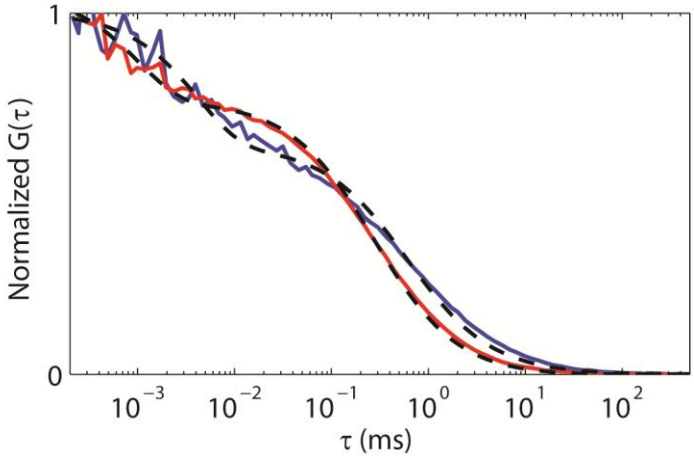
Supplementary Figure 5. a) Spectra integrated over 10s from a 100nM concentration solution of IR800cw in ethanol with (blue) and without (red) a bowtie present, as well as spectra from a 1μM concentration solution of ICG in water with (green) and without (black) a bowtie present. Notice that none of the spectra contain Raman peaks. b) Normalized spectra from 100nM IR800cw with (blue) and without (red) a bowtie present. Notice that the shape of the spectrum does not change depending on the bowtie's presence or absence. For both figures, the laser filter cuts off emission 800nm and shorter, causing aberrations near the cutoff, particularly at ~810 nm.

Low Concentration FCS

Supplementary Figure 6 plots the measured FCS curve for a 10pM concentration of ICG in ethanol (blue) and for a 10pM concentration of IR800cw in ethanol (red) both in the absence of bowtie nanoantennas. The autocorrelation function in each case was computed from the photon arrival times using a commercial package (SymPhoTime, Picoquant). These curves have been normalized to the value of $G(100\text{ns})$. They can be fit with the following standard equation[8], which includes contributions from diffusion through the focal volume as well as a short-lived dark state and are plotted as black dashed lines:

$$G(\tau) = \frac{1}{N}\frac{1}{\left(1+\frac{\tau}{\tau_D}\right)\left(1+\frac{\tau}{\kappa^2\tau_D}\right)}\left[1-D+De^{-\tau/\tau_{dark}}\right]$$

where N relates to the average number of molecules in the focal volume, τ_D is the diffusion time, κ is a shape factor that describes the asymmetry of the ellipsoidal Gaussian focus, D is the amplitude of the dark state contribution, and τ_{dark} is the dark state lifetime. For ICG, τ_D is 608 μs, which corresponds to the amount of time the molecule tends to spend in a diffraction-limited volume of water and τ_{dark} is 4 μs. For IR800cw, τ_D is 274 μs and τ_{dark} is 1 μs. The difference in the two diffusion times is likely due to an inadvertent difference in focal spot size.



Supplementary Figure 6. FCS from low concentration solutions of ICG and IR800cw

Wide-field Bowtie Enhanced Fluorescence Movie

Also included in this supplement is a wide-field fluorescence movie[9] with 50ms frames of a3x3 array of bowtie nanoantennas immersed in a 1μM solution of ICG in water. Each of the 9 bowties in this movie flash brightly when new ICG molecules stick near the bowtie nanoantenna and emit enhanced fluorescence until photobleaching.

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