

Spectral Distortions in Metal-Enhanced Fluorescence: Experimental Evidence for Ultra-Fast and Slow Transitions

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Supplemental Material

APPENDIX A:

ACRONYMS AND ABBREVIATIONS

MEF	- “metal-enhanced fluorescence”	<i>Refers to the phenomenon whereby an increase in fluorescence is observed for a fluorophore solution on a metal nanoparticle substrate as compared to a blank platform such as glass.</i>
CF	- “coupled fluorescence”	<i>Refers to the resulting spectrum when the intensities of the fluorophore excited in a blank (no MEF) well are subtracted from the intensities values measured for the fluorophore on the metal nanoparticle substrate</i>
EF	- “enhancement factor”	<i>Refers to the numerical value obtained when the fluorophore-metal intensity is divided by the control (or blank) intensity at the same emission wavelength.</i>
AgNPs	- Silver nanoparticle plasmonic platform	<i>Refers to silver nanoparticle substrates generated by the thermal deposition method. When used in legends, this indicates the platform on which the fluorophore was placed for emission detection.</i>
SiFs	- “silver island films”	<i>Refers to silver nanoparticle substrates generated by a wet deposition method. When used in legends, this indicates the platform on which the fluorophore was placed for emission detection.</i>
SD	- “slow dynamics” MEF	<i>Refers to those coupled fluorescent transitions that result in red-edge spectral distortion</i>
UFD	- “ultra-fast dynamics” MEF	<i>Refers to those coupled fluorescent transitions that result in blue-edge spectral distortion</i>
A	- “absorption”	<i>Refers to the maximum value of absorption detected for the metal nanoparticle substrate analyzed between 300-800 nm.</i>
-	- % area contribution	<i>Refers to the percentage of total integrated area of the fitted spectrum (comprised of several summed Gaussian distributions) that is due to a certain distribution.</i>
λ_{\max}	- peak wavelength	<i>Refers to emission wavelength at which the fluorophore achieves its intensity maximum for a given spectrum, detected on a control (no metal) substrate</i>
FWHM	- “full width half maximum”	<i>Full width half maximum of the emission spectrum</i>

APPENDIX B:

SAMPLE DATA EXTRACTION FROM IMAGE FILE

In order to extract the raw data from the original source image file, the image was first exported into Adobe® Photoshop® containing an activated grid as shown for one example plot in Figure A1. The grid was set to displays major intervals of 1 cm and minor subdivisions of 0.1 cm (130 px). The image was then transformed such that the axes aligned to a grid scale where 1 cm was equal to 10 nm in the x dimension and 50 arbitrary units (a.u.) for intensity in the y dimension.

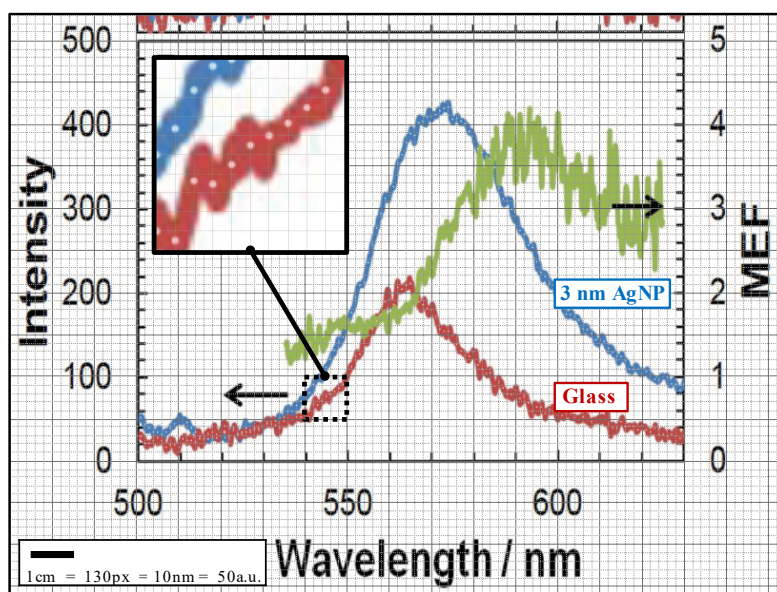


Figure A1. Example gridded fluorescence spectra for data extraction from image files. The spectra shown are for Rose Bengal ($\lambda_{\text{ex}} = 473 \text{ nm}$, 0.32-Wcm^{-2}) detected on glass or 3 nm silver nanoparticles (AgNP). **Inset** displays a magnified grid, showing measurements with 0.05 cm intensity tolerance at wavelength intervals with 0.1 cm tolerance.

Each spectrum was then manually marked at approximately the “center of mass” for each spectral line weight (see inset, Fig. A1), measured from the x axis in centimeters (or pixels), and were ultimately converted to arbitrary units of fluorescence intensity using the indicated scale. Tolerance for this process was 0.05 cm in the y dimension ($1.00 \pm 0.05 \text{ cm} = 50.0 \pm 2.5 \text{ a.u.}$) for the data set. Intensity values were collected at each minor interval in the x dimension, corresponding to an extracted

Appendix B: Sample Data Extraction from Image File

data point every nanometer ($1.0 \text{ cm} = 1.0 \text{ nm}$) or every second nanometer ($1.0 \text{ cm} = 2.0 \text{ nm}$) as indicated. These values were collected for all visible data points; in the case where the spectral line was obscured, approximations were made. In all cases, the metal-enhanced fluorescence (MEF) enhancement factor value (indicated in green, Fig. A1) was not analyzed. Rather, the enhancement factor (abbreviated “EF” in the main text) was calculated from the extracted points.

APPENDIX C:

PERCENT AREA CONTRIBUTION EXAMPLE CALCULATION

Following multi-Gaussian deconvolution analysis of the coupled fluorescence (CF) spectra contained in this report, percent area contribution (%AC) values were calculated based on the fit component integrated intensities, as described in Section 2.4 of the main article. For some data sets, these contributions were summed and reported according to a defined range relative to the peak wavelength detected on glass, or λ_{\max} . One example of this is shown in Figure 9 (refer also to plotted fits in Fig. 8) of the main text. In these cases, the percent area contributions are found according to equations B1 and B2,

$$A_X = \sum_{i_0}^i A_i \quad (\text{B1})$$

$$\%AC_X = A_X / A_T * 100 \quad (\text{B2})$$

where A is integrated area, i defines a peak component integer between 1 and n (with i_0 determining the lower bound) and X defines a range relative to λ_{\max} . Figure B1a shows two examples of these calculations.

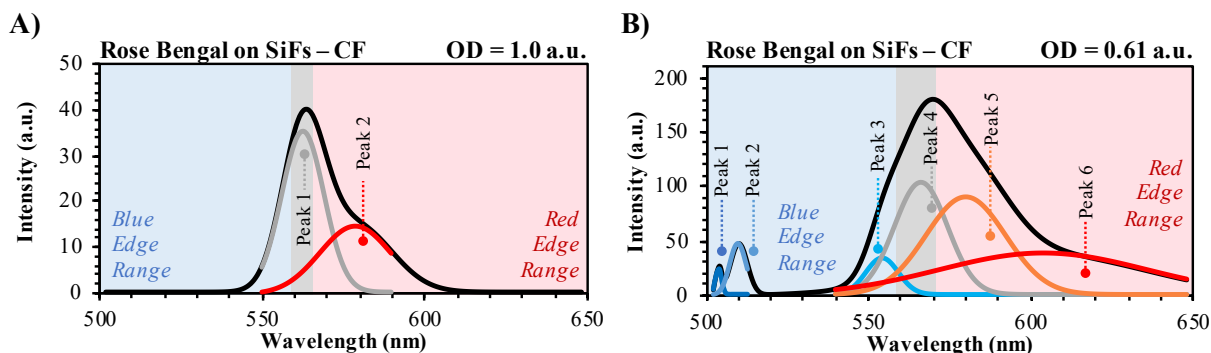


Figure B1. Example multi-Gaussian deconvolution analysis for coupled fluorescence (CF) spectra from Rose Bengal detected on silver island films (SiFs) with absorption values (O.D.) of A) 1.0 and B) 0.61 arbitrary units. Cumulative fits are indicated in black; component Gaussian distributions are indicated by colored spectra, with λ_{\max} indicated in grey. Peak component number (1 \rightarrow n) for each component is labeled. For original data and additional fit information, see Fig. 8 and supplemental Fig. S4.

In this example, Peak 1 was found to display a maximum wavelength approximately equal to that of the same fluorophore detected on glass. As such, this range ($i = i_0 = 1$) is designated “ λ_{\max} ” in Figure 9 and

Appendix C: Percent Area Contribution Example Calculation

has a percent area contribution of 62.4%. Peak 2, however, displayed a bathochromic peak wavelength relative to λ_{max} ; therefore, this range ($i = i_0 = 2$) is designated “Red Edge” in Figure 9, corresponding to a 37.6% area contribution. Figure B1b, however, has $n = 6$ single Gaussian component peaks. Peaks 1 through 3 comprise the “Blue Edge” range, where the peak maxima display a hypsochromic shift relative to λ_{max} . This range ($1 \leq i \leq 3$) had an associated area contribution of 9.1%. Peak 4 ($i = i_0 = 4$) is designated “ λ_{max} ” and constitutes 25.5% of the total integrated area; the “Red Edge” is defined by the sum of Peaks 5 and 6 ($5 \leq i \leq 6$), corresponding to the final 65.4%. Percent area contribution values were obtained in an equivalent manner for Rose Bengal detected on other SiFs of varied absorption values, not depicted in Figure B1. This procedure was also used for all other fluorophores and metal films for which summed percent area contributions were reported.

APPENDIX D:
SUPPLEMENTAL FIGURES

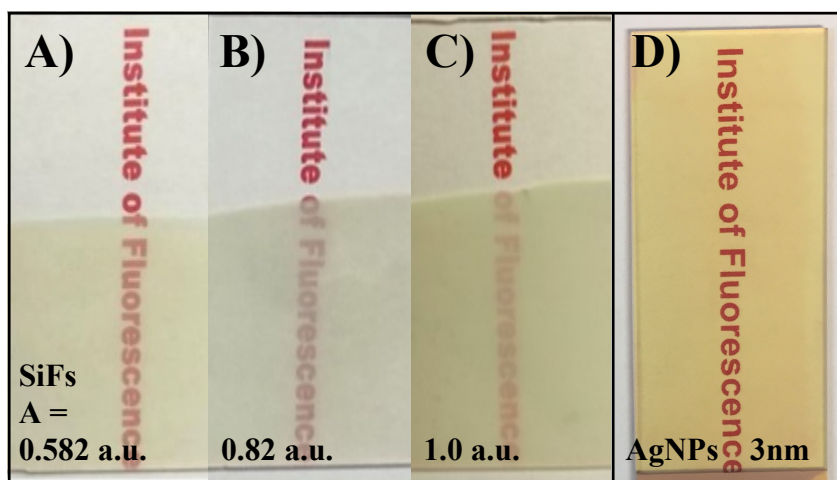


Figure S1. Real-color photographs of silver nanoparticle substrates. *A-C*) Silver island films (SiFs) deposited on glass by the wet deposition method, with absorption values (“A”) of *A*) 0.582, *B*) 0.82, and *C*) 1.0. *D*) Silver nanoparticles of 3 nm thickness deposited on glass by the vapor deposition method.

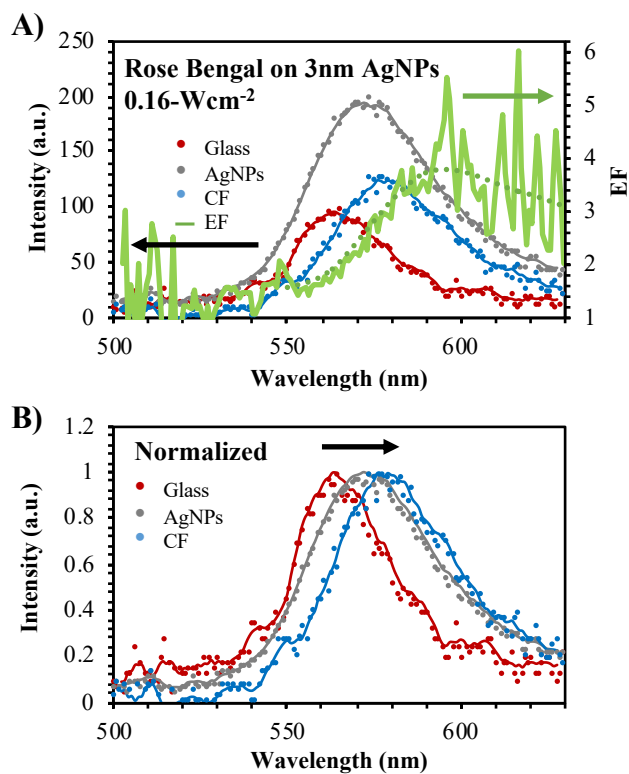


Figure S2. Fluorescence spectra for Rose Bengal ($\lambda_{\text{ex}} = 473 \text{ nm}$, 0.16-Wcm^{-2}) detected on glass or 3 nm silver nanoparticles (AgNPs). The resulting coupled fluorescence (CF) spectrum is also shown. *A)* Raw fluorescence intensity of each spectrum and resulting enhancement factor (EF) reported at each emission wavelength. *B)* Normalized fluorescence spectra displaying red-shifted maxima (solid arrow).

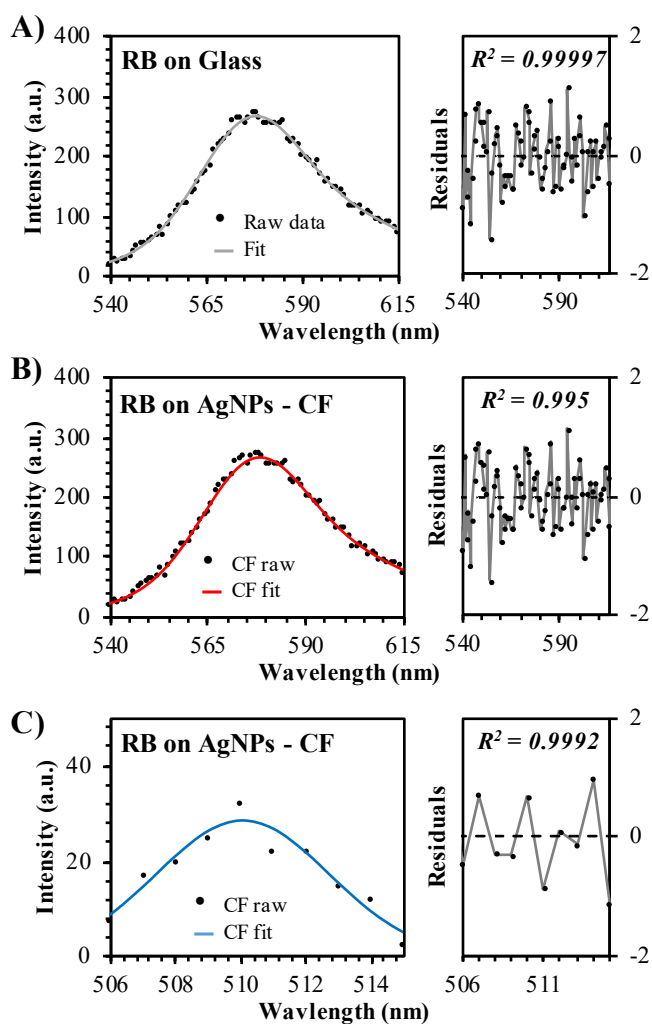


Figure S3. Multi-Gaussian fitting parameters for Rose Bengal ($\lambda_{\text{ex}} = 473 \text{ nm}$) detected on glass or silver nanoparticles (AgNPs). Fits included are *A*) the raw spectrum from Rose Bengal on glass, *B*) the red region of the coupled fluorescence (CF) spectrum, and *C*) the blue region of the CF spectrum. For all panels, the *left* – displays the fit and the *right* – displays the corresponding residuals.

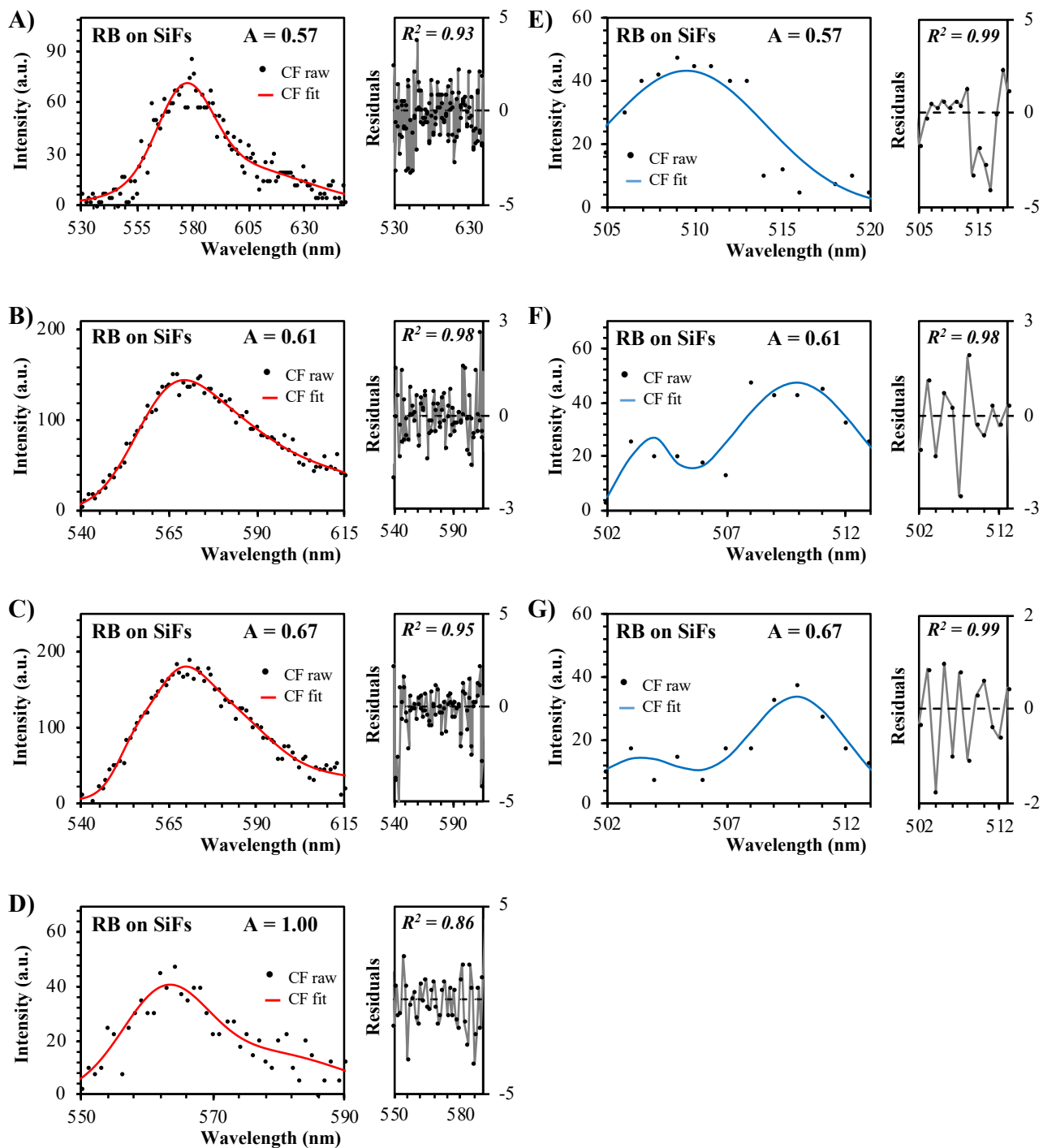


Figure S4. Fitting parameters for the multi-Gaussian fits of the coupled fluorescence (CF) spectra for Rose Bengal (RB, $\lambda_{\text{ex}} = 473$ nm) detected on glass versus silver island films with absorption values ("A") of A/E) 0.57, B/F) 0.61, C/G) 0.67 and D) 1.0 arbitrary units. A-D) Fitting for the red-shifted region. E-G) Fitting for the blue-shifted region. For each panel, *left* – displays the fit and the *right* – displays corresponding residuals.

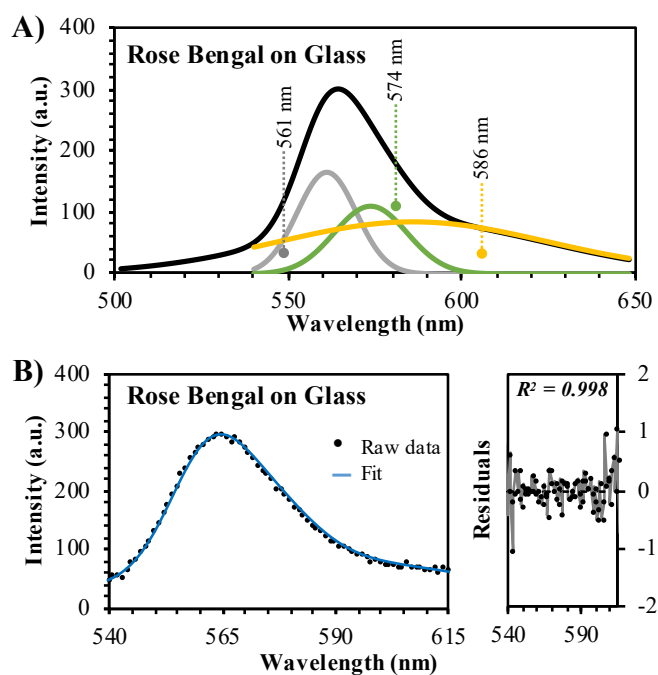


Figure S5. Multi-Gaussian fit of the raw spectrum from Rose Bengal ($\lambda_{\text{ex}} = 473$ nm) detected on glass. *A*) Spectral fit (black spectrum) with corresponding Gaussian distributions (colored spectra). Peak wavelengths for each component are labeled. *B*) Fitting parameters for (A) showing *left* – the fit and *right* – the corresponding residuals.

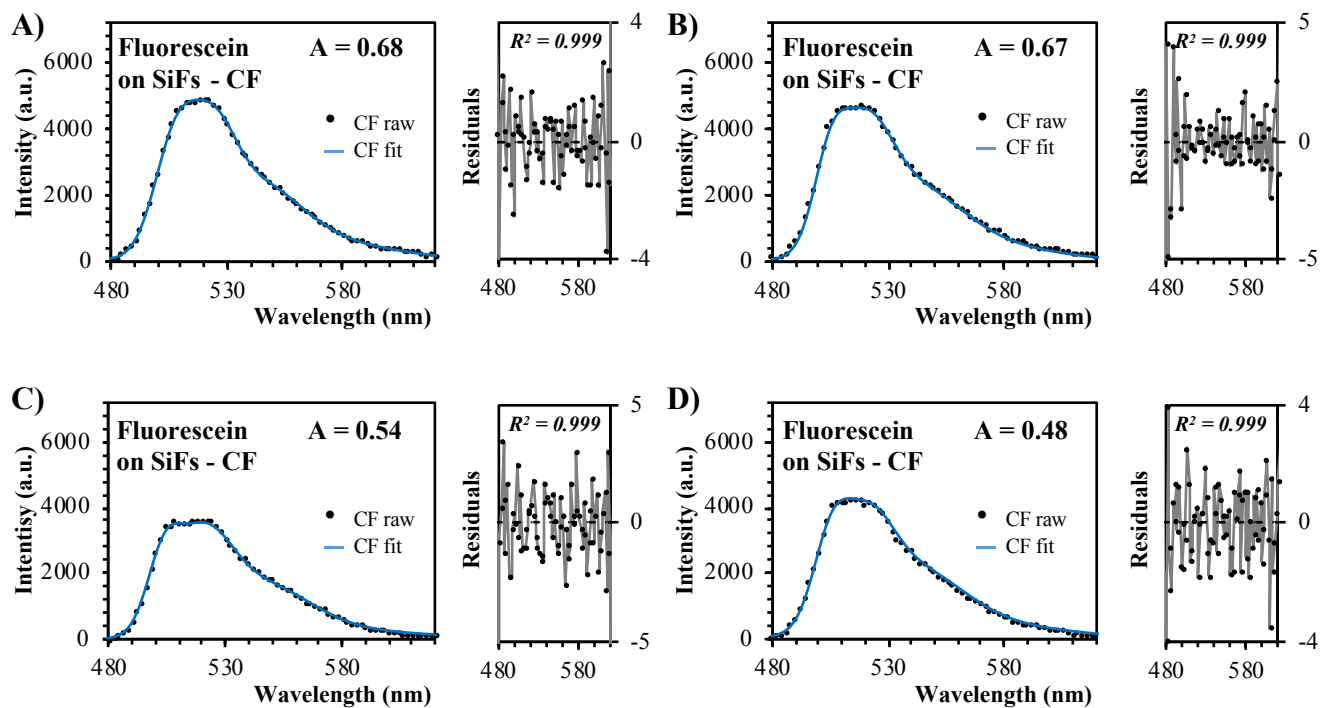


Figure S6. Fitting parameters for the multi-Gaussian fits of the coupled fluorescence (CF) spectra for Fluorescein ($\lambda_{\text{ex}} = 473$ nm) detected on glass versus silver island films with absorption values (“A”) of A) 0.68, B) 0.67, C) 0.54 and D) 0.48 arbitrary units. For each panel, *left* – displays the fit and the *right* – displays corresponding residuals.

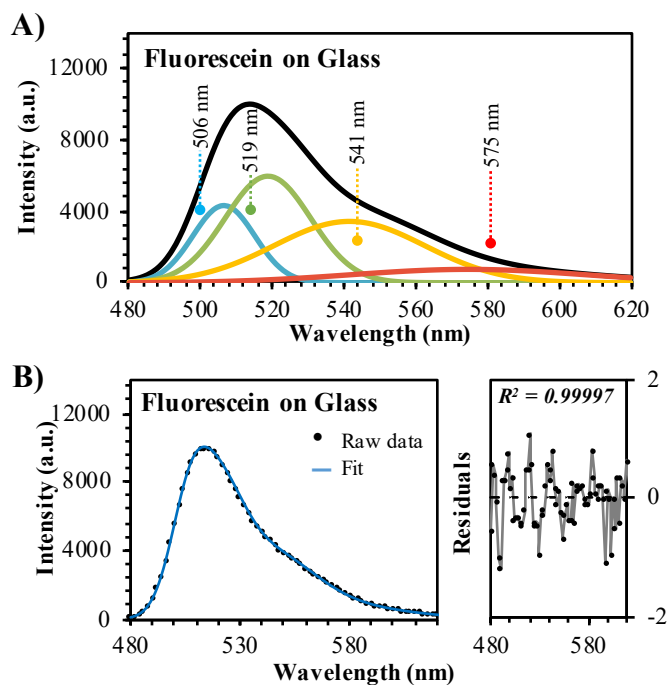


Figure S7. Multi-Gaussian deconvolution analysis of the raw spectrum from Fluorescein ($\lambda_{\text{ex}} = 473$ nm) detected on glass. *A)* Spectral fit (black spectrum) with corresponding Gaussian distributions (colored spectra). Peak wavelengths for each component are labeled. *B)* Fitting parameters for (A) showing *left* – the fit and *right* – the corresponding residuals.

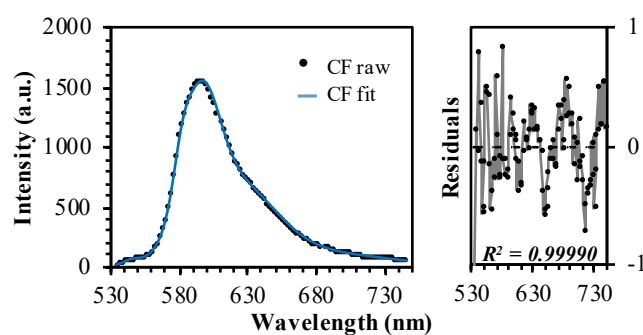


Figure S8. Multi-Gaussian fit of the coupled fluorescence (CF) spectrum from Rose Bengal ($\lambda_{\text{ex}} = 532$ nm) detected on glass or silver island films (SiFs) reported in Figure 15 of the main article. *Left* – fit for CF spectrum and *right* – corresponding residuals. Data was analyzed from reference [S1].

[S1] Zhang, Y.; Aslan, K.; Previte, M.J.R.; Malyn, S.N.; Geddes C.D. “Metal-Enhanced Phosphorescence: Interpretation in Terms of Triplet-Coupled Radiating Plasmons.” *J. Phys. Chem. B*, **2006**, 110, 25108-25114.

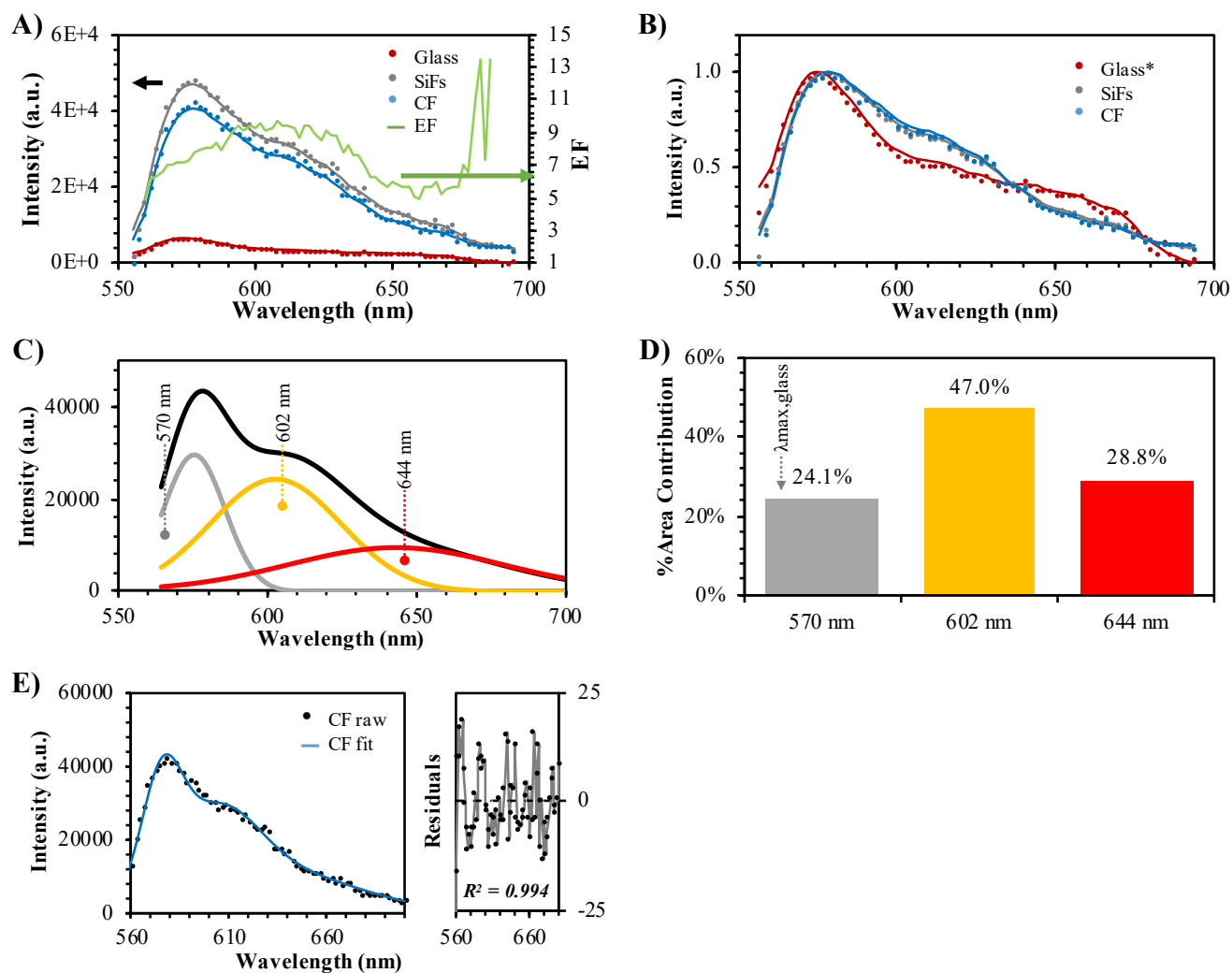


Figure S9. Fluorescence spectra for trimethine cyanine dye ($\lambda_{\text{ex}} = 530$ nm) detected on glass or silver island films (SiFs, $A \sim 0.85$ a.u.) and the resulting coupled fluorescence (CF) spectrum. *A*) Extracted raw intensity values for each spectrum and corresponding enhancement factors (EF) at each emission wavelength. *B*) Normalized spectra from (A). Note that the *Glass** spectrum had baseline noise subtracted for clarity of spectral shape. *C*) Multi-Gaussian deconvolution analysis of the CF spectrum (black spectrum) with corresponding Gaussian distributions (colored spectra). Peak wavelengths for each component are labeled. *D*) Percent area contribution of each Gaussian distribution to the total integrated area of the resulting fit from (C). *E*) *Left* – fit for CF spectrum and *right* – corresponding residuals. Data was analyzed from reference [S2].

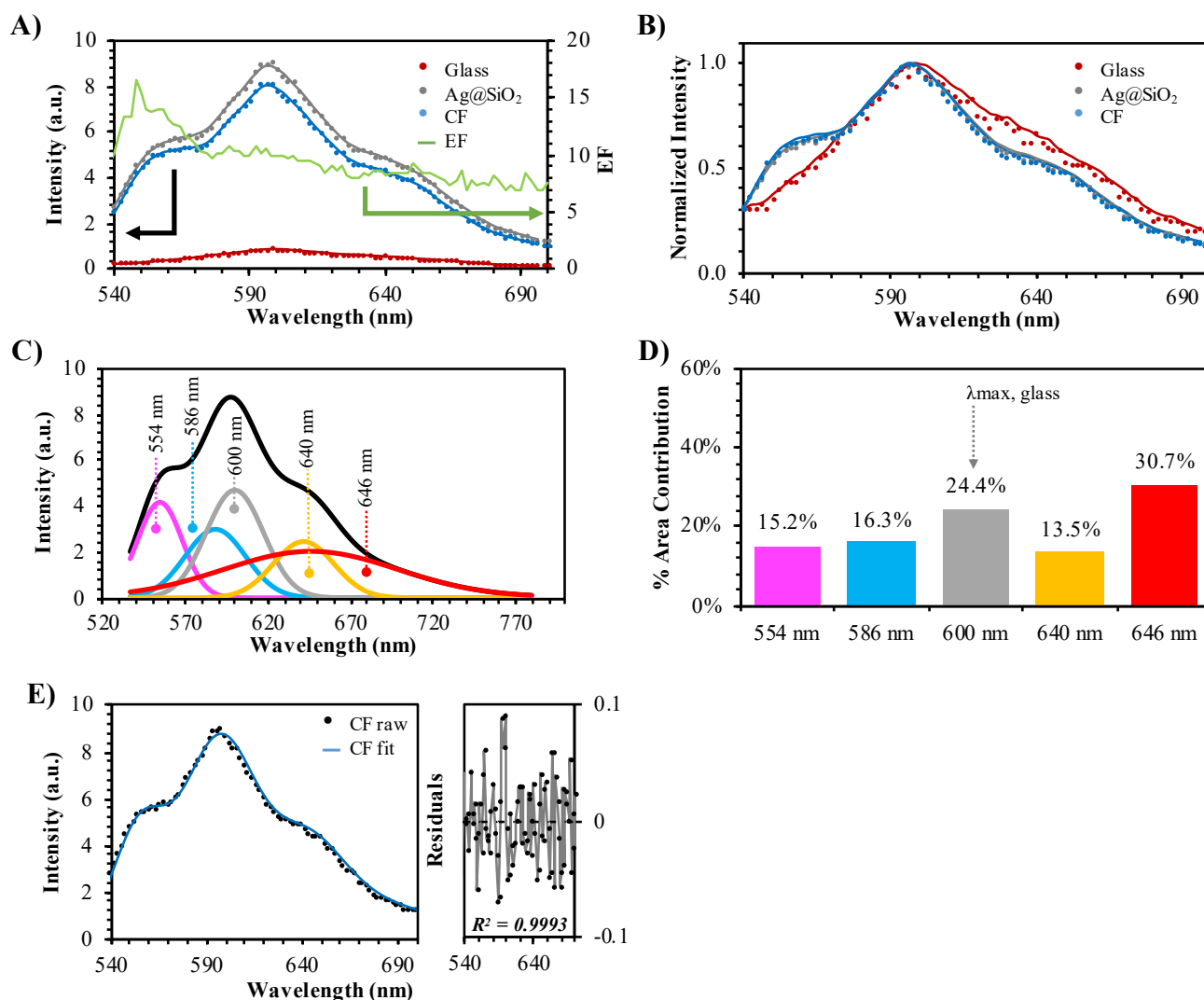


Figure S10. Fluorescence spectra for Valrubicin ($\lambda_{\text{ex}} = 470$ nm) detected on glass or silver core-shell nanostructure coated glass (Ag@SiO₂) and the resulting coupled fluorescence (CF) spectrum. *A)* Extracted raw intensity values for each spectrum and corresponding enhancement factors (EF) at each emission wavelength. *B)* Normalized spectra from (A). *C)* Multi-Gaussian deconvolution analysis of the CF spectrum (black spectrum) with corresponding Gaussian distributions (colored spectra). Peak wavelengths for each component are labeled. *D)* Percent area contribution of each Gaussian distribution to the total integrated area of the resulting fit from (C). *E)* *Left* – fit for CF spectrum and *right* – corresponding residuals. Data was analyzed from reference [S3].

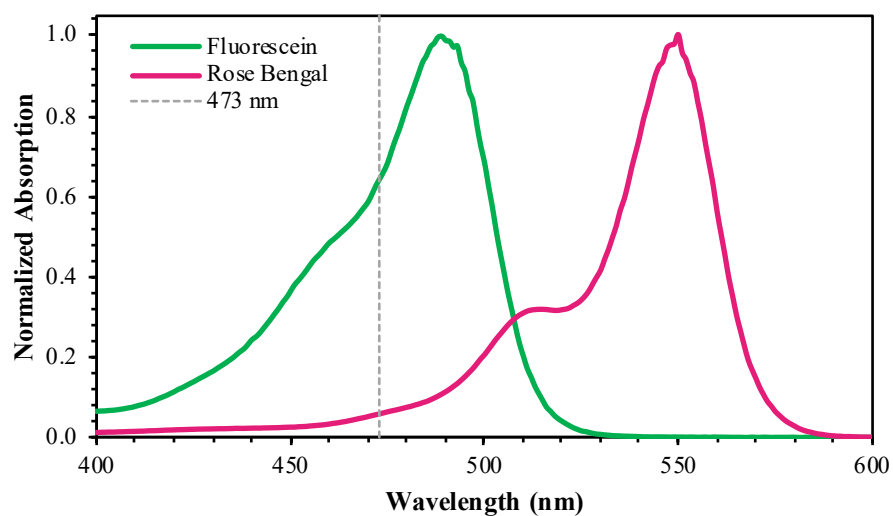


Figure S11. Normalized absorption spectra for fluorescein sodium salt and Rose Bengal dissolved in water. Each spectrum is a single representative spectrum of the three averaged spectra used to determine the extinction coefficients for each fluorophore.

Fluorophore	Wavelength (nm)	ϵ (M ⁻¹ cm ⁻¹)
Fluorescein Sodium Salt (in water)	473	32,000 ± 3,000
	489 (λ_{max})	49,000 ± 5,000
Rose Bengal (in water)	473	6,200 ± 400
	550 (λ_{max})	83,000 ± 6,000

Table S1. Extinction Coefficients (ϵ) of Rose Bengal and Fluorescein Sodium Salt Reported for Different Wavelengths.