

## Metal-enhanced $S_2$ fluorescence from azulene

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Received 11 September 2006; in final form 31 October 2006

Available online 7 November 2006

### Abstract

In this Letter, we report the first observation of metal-enhanced  $S_2$  emission at room and low temperature (77 K). The  $S_2$  emission intensity of azulene is enhanced by close proximity to silver island films (SiFs). In this regard, a  $\approx 2$ -fold higher  $S_2$  fluorescence intensity of azulene was observed from SiFs as compared to a glass control sample. This suggests that  $S_2$  excited states can couple to surface plasmons and enhance  $S_2$  fluorescence yields, a helpful observation in our understanding the interactions between plasmons and lumophores, and our continued efforts to develop a unified plasmon-lumophore/fluorophore theory.

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### 1. Introduction

The location of electronic energy levels in molecules and the probability and polarization of radiative transitions between ground and excited state can be accurately predicted by quantum mechanical theory [1]. For most molecules, no excited singlet states ( $S_2$ ,  $S_3$ ...) have been observed to emit light upon excitation, the same being true for triplet states ( $T_2$ ,  $T_3$ ...). This is summarized in Kasha's rule: *The emitting electronic level of a given multiplicity is the lowest excited level of that multiplicity* [2].

As the first authentic example of a molecule which violates Kasha's rule, azulene has been the subject of numerous spectroscopic and photophysical investigations [3–6]. Beer and Longuet-Higgins discovered the second excited level emits light upon excitation, producing the anomalous ( $S_2$ – $S_0$ ) fluorescence of azulene in 1955 [6], whereas the normal  $S_1$ – $S_0$  fluorescence and  $T_1$ – $T_0$  phosphorescence were

much too weak to be observed. The absence of  $S_1$ – $S_0$  fluorescence suggests that  $S_1$ – $S_0$  internal conversion is a very rapid process in azulene, this supposition being confirmed by the lifetime of the  $S_1$  state being determined as 1.9 ps [7]. Compared with the analogous spacing of the isomer Naphthalene, azulene has much wider spacing between its zero – point levels of the first two excited singlet states ( $S_1$ ,  $S_2$ ) [8]. The anomalous fluorescence of azulene subsequently occurs because internal conversion fails to take place between the  $S_2$  and  $S_1$  levels, on account of their wide energy separation.

Azulene, with seven-membered rings, is an allelochemical. It can penetrate into cells, interact with cellular components such as DNA-containing organelles or tubulin of the cytoplasm, and selectively fluoresce upon interaction with different cellular compartments [9]. This activity makes it as valuable as other indicator fluorescent dyes. However, the detection is limited by azulene's weak  $S_2$  fluorescence. In this regard, the plasmonic enhancement of fluorescence of azulene is very important, and may facilitate the studying of allelopathic mechanisms at the cellular level.

In recent years, our laboratories have both studied and demonstrated many applications of metal-enhanced fluorescence (MEF) [10–12], metal enhanced phosphorescence [13], metal-enhanced chemiluminescence [14] and surface

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plasmon coupled fluorescence for directional emission [15]. These have included the increased detectability and photostability of fluorophores [16] and chemiluminescent species [14], improved DNA detection [17], the release of self-quenched fluorescence of over labeled proteins [18], enhanced wavelength-ratiometric sensing [19], and the application of metallic surfaces to amplified (ultra fast and sensitive) assay detection [20]. Our laboratory's current interpretation of MEF has been explained by a model whereby non-radiative energy transfer occurs from excited distal fluorophores, to the surface plasmon electrons in non-continuous films (Fig. 1, top), in essence a fluorophore induced mirror dipole in the metal. The surface plasmons in turn, radiate the emission of the coupling fluorophores [10–12]. This explanation has been facilitated by our recent observation of surface plasmon coupled emission (SPCE) [21], whereby fluorophores distal to a continuous metallic film can directionally radiate fluorophore emission at a unique angle from the back of the film. However, all previous MEF and MEP studies were exclusively focused on  $S_1$  and  $T_1$  electronic states, respectively, and their emission.

In this Letter, we report our observations on the photo-physical characteristics of azulene, which has well-known  $S_2$  emission, in close proximity to SiFs. We have observed both the enhanced absorption and  $S_2$  emission of azulene from

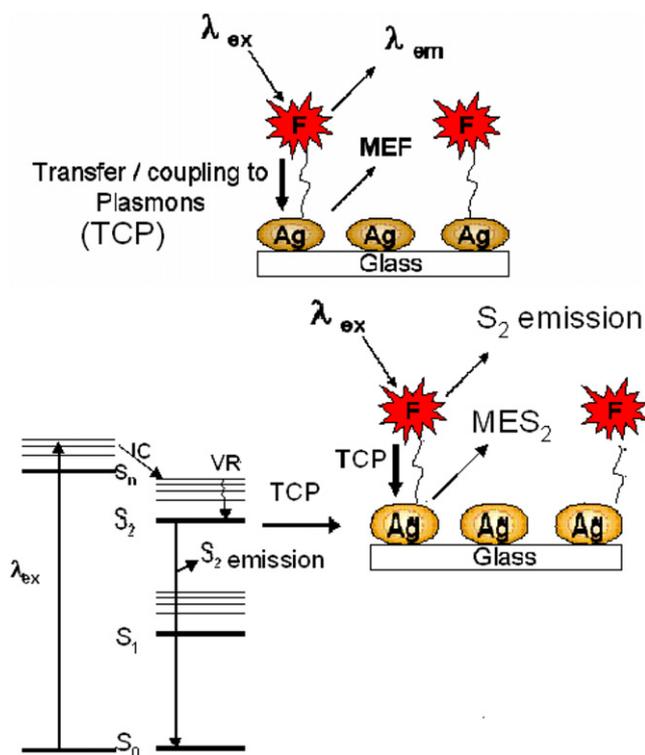


Fig. 1. Graphical representation of our laboratory's current interpretation of metal-enhanced fluorescence (MEF) (top), and metal-enhanced  $S_2$  emission (bottom). IC-internal conversion, VR-vibrational energy relaxation. Ag-Silver nanoparticle (SiFs), MEF-metal-enhanced fluorescence, TCP-transfer/coupling to plasmons,  $MES_2$ -metal enhanced  $S_2$  emission. Energy level spacing not drawn to scale.

SiFs (which is  $\approx 2$ -fold brighter) as compared to a glass substrate, a control sample at room temperature (RT) and at 77 K. Furthermore, a shorter azulene fluorescence lifetime was also observed, consistent with our previous observations of metal-enhanced fluorescence [10–21]. These observations are helpful in our understanding of not only the excitation/relaxation of azulene, but in our laboratories continued efforts to develop a unified theory to explain plasmon-lumophore (and fluorophore) interactions.

## 2. Experimental

### 2.1. Materials

Silver nitrate (99.9%), sodium hydroxide (99.996%), ammonium hydroxide (30%), D-glucose and premium quality silane-prep™ glass slides (75 × 25 mm), Ethanol (HPLC/spectrophotometric grade), azulene, diethyl-ether, isopentane were obtained from Sigma–Aldrich. All chemicals were used as received.

### 2.2. Methods

SiFs were prepared as we have previously published [10]. In a typical SiF preparation, a solution of sodium hydroxide and ammonium hydroxide are added to a continuously stirred solution of silver nitrate at RT. Subsequently, the mixture is cooled down in an ice bath, silane-prep™ glass slides are inserted and a solution of D-glucose is added. As the temperature is increased, the color of the mixture turns yellow-brown and the SiFs-deposited slides are removed from the mixture, washed with water, and sonicated for a few seconds at room temperature. SiFs-deposited glass slides were stored in deionized water until they were used.

Three hundred microlitres of azulene ( $1.0 \times 10^{-3}$  M) in EPA solution (diethyl-ether:isopentane:ethanol = 5:5:2 (v/v)) was dropped in a sandwich format between the glass slides and the silver island films, respectively. Fig. 2 insert

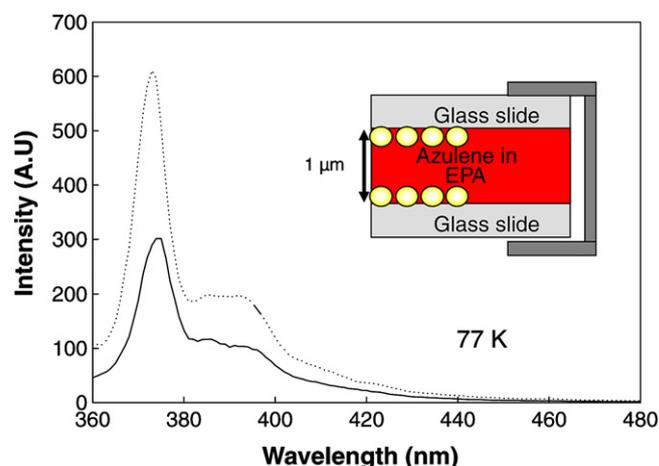


Fig. 2. Absorption spectra of azulene sandwiched between two silvered and unsilvered slides, respectively. EPA, diethyl-ether:isopentane:ethanol = 5:5:2 (v/v). SiFs-silver island films.

shows the experimental sample geometry. The glass/SiFs surfaces were placed in liquid nitrogen for 2 min and used for low temperature (77 K) measurements. Absorbance spectra were taken using a Varian Cary 50 UV–vis Spectrophotometer. Fluorescence measurements were undertaken using a Cary eclipse fluorescence spectrophotometer.

Fluorescence lifetimes were measured using the time-correlated single photon counting technique, using a PicoQuant modular fluorescence lifetime spectrometer (Fluo Time 100) with a PicoQuant LDH-P-C-400 laser as the light source. The intensity decays (Fig. 4) were subsequently analyzed in terms of the multi-exponential model:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \quad (1)$$

Where  $\alpha_i$  are the amplitudes and  $\tau_i$  are the decay times,  $\sum \alpha_i = 1.0$ . The fractional contribution of each component to the steady state intensity is given by:

$$f_i = \frac{\alpha_i \tau_i}{\sum_j \alpha_j \tau_j} \quad (2)$$

The mean lifetime of the excited state is given by

$$\bar{\tau} = \sum_i f_i \tau_i \quad (3)$$

and the amplitude-weighted lifetime is given by

$$\langle \tau \rangle = \sum_i \alpha_i \tau_i \quad (4)$$

The values of  $\alpha_i$  and  $\tau_i$  were determined by non-linear least squares impulse reconvolution, with a goodness-of-fit  $\chi^2$  criterion.

### 3. Results and discussion

Fig. 2 shows the absorption spectra of azulene from between the SiFs and from glass. SiFs and glass without azulene were used as reference backgrounds for the azulene absorption measurements, respectively. From Fig. 2, it can be seen that the azulene has a larger absorbance on the SiFs as compared to glass. The enhanced absorption of dye molecules near to metallic surfaces was first reported by Glass et al. in 1980 and later confirmed by other groups [22–25]. When a lumophore is placed near metal, there is a very strong net absorption effect, a result of the coupling of the molecular dipoles with the localized electromagnetic field of the metallic particle's surface plasmon resonance [12]. In essence, conducting metallic particles can modify the free space absorption condition in ways that increase the photonic mode density and incident electric field felt by a lumophore [10–12]. These enhanced absorption phenomena can lead to surface enhanced luminescence phenomena, such as metal-enhanced fluorescence (MEF) and metal-enhanced phosphorescence (MEP) [13], phenomena described by our groups [10–13].

Fig. 3-top shows the  $S_2$  fluorescence emission spectra of azulene from SiFs and from glass at RT, consistent with

previous reports [1–3]. The enhanced fluorescence intensity was  $\approx 1.5$ -fold brighter from the silver, (i.e. ratio of emission from SiFs/glass). It should be noted that the true metal-enhanced fluorescence enhancement factor is  $\approx 37$ -fold. This is because the MEF phenomenon is distance dependent [10–12], where with a sample thickness of  $\sim 1$  micron and an enhanced interaction region  $< 20$  nm [10–12], then only 4% of the sample is within the MEF enhancement region.

In addition to the metal-enhanced  $S_2$  fluorescence at RT, we have observed that temperature can also influence the magnitude of the MEF phenomenon, *hitherto*, an unreported observation. It can be expected that if all other factors remain constant, the fluorescence intensity will increase with decreasing temperature [26]. Fig. 3, bottom shows the fluorescence emission spectra of azulene from between both SiFs and from glass at 77 K. It can be seen that the enhanced fluorescence intensity was  $> 2$ -fold brighter from silver as compared to glass. Table 1 summarizes the fluorescence spectral data for azulene at RT and at 77 K sandwiched between both glass and silver slides. The

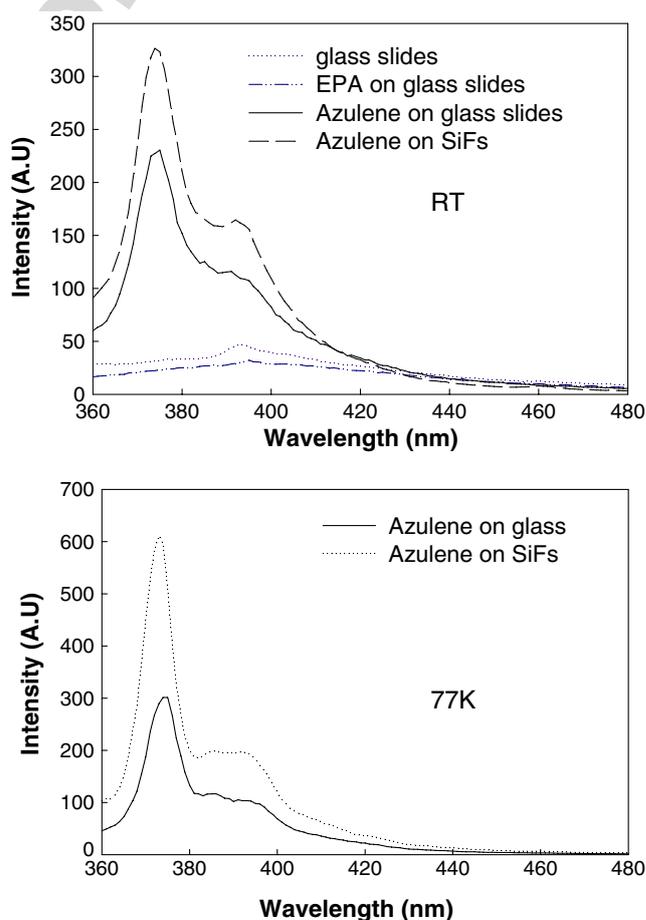


Fig. 3. Fluorescence emission spectra,  $\lambda_{\text{ex}} = 338$  nm, of azulene sandwiched between two silvered (SiFs) and unsilvered slides at room temperature (RT), (top), and the emission spectra at 77 K, (bottom). All spectra are the mean of three separated and independent sample measurements.

enhancement ratio for azulene increases from 1.3 (at RT) to 2.0 (at 77 K). At first, one may consider this increase in ratio to be due to a property of the azulene itself at low temperature. However, this ratio is calculated by the division of the emission intensity of azulene from SiFs by the emission intensity from glass, also measured at 77 K. This suggests that the further increase in MEF at low temperature (i.e. increase in ratio) is indeed a property of the plasmons themselves and the coupling of the azulene to silver surface plasmons at low temperature. Given that the absorption spectrum of the SiFs remains unchanged at low temperature (data not shown), then we speculate that the further enhanced MEF is due to a reduced damping of the coupled  $S_2$  emission. Further low temperature studies are underway in this regard.

The full width half maximum (FWHM) for azulene at RT and at 77 K are also shown in Table 1. FWHM for the fluorescence emission spectra of azulene on SiFs at 77 K was  $\approx 9$  nm which is narrower, as expected, as compared to that measured at room temperature ( $\approx 18$  nm). Also, the emission wavelength maximum at 77 K is identical to that of the spectra measured at RT. Most organic fluorophores also emit phosphorescence when in a rigid medium [6]. It is however, not surprising that phosphorescence was not observed for azulene, because assuming a fluorescence–phosphorescence frequency difference of  $2000\text{--}10000\text{ cm}^{-1}$ , the phosphorescence  $T_1$  energy level of azulene is higher than the lowest excited  $S_1$  singlet level at  $14000\text{ cm}^{-1}$  [27]. The radiationless transition to  $S_0$  couples with the highly forbidden triplet–singlet transition which gives rise to classical phosphorescence [4].

In addition, we studied the intensity decays of azulene in the presence of SiFs. These decays were used to calculate the respective lifetimes shown in Table 2, using non-linear least squares regression analysis. From Fig. 4 and Table 2, we can see both a reduced mean lifetime ( $\tau_{\text{mean}}$ ) and amplitude-weighted lifetime ( $\langle\tau\rangle$ ) for fluorophores near-to silver (0.09 ns) as compared to the glass control sample (0.34 ns). In fact, shorter lifetimes for fluorophores in close proximity to silver nanostructures, coupled with enhanced emission intensities in the same system, is indicative of the MEF  $S_1$  phenomenon, and has been reported and explained by our group many times previously [10–13]. In

Table 1  
Fluorescence spectral data for azulene at RT and 77 K sandwiched between both glass and silvered slides (SiFs)

	Fluorescence	
	FWHM	Enhancement factor (integrated area ratio)
Azulene glass slides, RT	20 nm	–
Azulene glass slides, 77 K	9 nm	–
Azulene SiFs, RT	18 nm	1.3 (1.4)
Azulene SiFs, 77 K	9 nm	2.0 (2.0)

The enhancement factor was calculated as the ‘peak’ emission intensity ratio SiFs/Glass. Integrated area ratio is the ratio of the integrated area under the fluorescence spectra on SiFs and glass. FWHM- full-width half-maximum. SiFs-silver island films.

Table 2  
Fluorescence intensity decay analysis of azulene at room temperature

	$\alpha_1\%$	$\tau_1$ (ns)	$\alpha_2\%$	$\tau_2$ (ns)	$\chi^2$	$\langle\tau\rangle$	$\bar{\tau}_{\text{mean}}$
Azulene on glass	0.74	4.25	99.2	0.31	0.97	0.34	0.67
Azulene on SiFs	0.19	5.00	99.8	0.09	1.01	0.09	0.57

$\alpha_i$  are the amplitudes and  $\tau_i$  are the decay times.  $\langle\tau\rangle$  the amplitude-weighted and  $\bar{\tau}_{\text{mean}}$  is the mean lifetime. SiFs-silver island films.

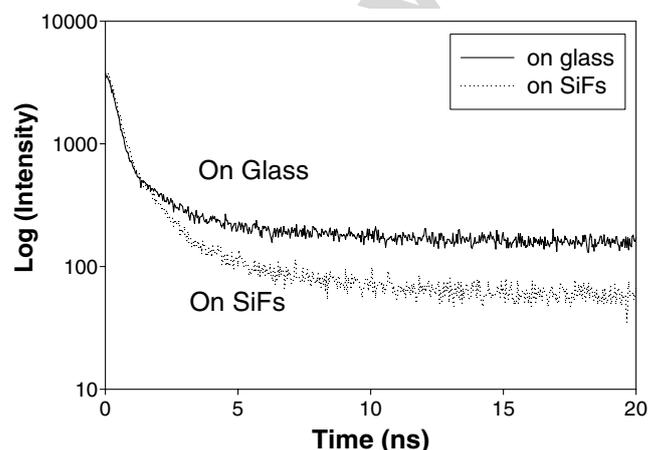


Fig. 4. Fluorescence Intensity decays of azulene from between silvered (SiFs) and unsilvered glass slides at room temperature (RT). Data from least square reconvolution analysis is shown in Table 1.

fluorescence, the spectral observables are governed by the magnitude of  $\Gamma$ , the radiative decay rate, relative to the sum of the non-radiative decay rates,  $k_{\text{nr}}$ , such as internal conversion and quenching. In the absence of metallic particles or surfaces, then the quantum yield,  $Q_0$  and fluorescence lifetime  $\tau_0$  are given, respectively by:

$$Q_0 = \frac{\Gamma}{\Gamma + k_{\text{nr}}} \quad (5)$$

$$\tau_0 = \frac{1}{\Gamma + k_{\text{nr}}} \quad (6)$$

In all of our applications of  $S_1$  MEF to date, we have found that the enhanced fluorescence signals (quantum yields –  $Q_m$ ) of fluorophores in close proximity ( $<10$  nm) to metallic nanostructures could be well described by the following equations [10–12,16]:

$$Q_m = (\Gamma + \Gamma_m)/(\Gamma + \Gamma_m + k_{\text{nr}}) \quad (7)$$

where  $\Gamma$  is the unmodified radiative decay rate,  $\Gamma_m$  is the metal-modified system radiative decay rate and  $k_{\text{nr}}$  are the non-radiative rates, Fig.1-top. Similarly, the metal-modified lifetime,  $\tau_m$ , of a fluorophore is decreased by an increased radiative decay rate:

$$\tau_m = 1/(\Gamma + \Gamma_m + k_{\text{nr}}) \quad (8)$$

From Eqs. (7) and (8), we can see that as the value of the system  $\Gamma_m$  increases, the quantum yield  $Q_m$  increases, while the lifetime,  $\tau_m$ , decreases.

As described in the introduction,  $S_1$ – $S_0$  has a narrow energy gap for azulene and the  $S_1$ – $S_0$  internal conversion

rate is a very rapid process. In this work, we saw no evidence that the  $S_1$  state of azulene can couple or indeed be enhanced by surface plasmons. For the  $S_2$  state, enhanced  $S_2$  emission is currently thought to occur due to the efficient non-radiative transfer to surface plasmons which, in turn, efficiently radiate, similarly to what is observed for many other fluorophores'  $S_1$  emission [10–13]. As shown in Fig. 1, bottom, a highly efficient  $S_2$  emission coupling to surface plasmons, where the plasmons efficiently and quickly radiate the coupled emission, results in a reduced fluorophore lifetime and an enhanced observed  $S_2$  emission intensity. These findings are consistent with Eqs. (7) and (8), which have been used successfully to describe metal-enhanced  $S_1$  emission. To the best of our knowledge, this is the first observation of metal-enhanced  $S_2$  emission.

#### 4. Conclusions

In this Letter, we report the first observation of metal-enhanced  $S_2$  fluorescence. Azulene in close proximity to SiFs can undergo enhanced  $S_2$  fluorescence, a 2-fold increase was observed as compared to an identical control sample containing no silver at 77 K. In addition, the amplitude-weighted lifetime was reduced  $\approx$ 4-fold near to silver. This finding suggests that photon induced electronically excited states at RT, and also at low temperature, can both induce and couple to surface plasmons, facilitating enhanced  $S_2$  fluorescence emission.

#### Acknowledgements

This work was supported by the NIH, NCRR RR008119. The authors also thank UMBI for salary support.

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