

Metal-enhanced superoxide generation: A consequence of plasmon-enhanced triplet yields

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The authors report significant enhancements in the generation of superoxide for fluorophores in close proximity to silver nanoparticles. A distance dependence study of the fluorophores from the metallic nanostructures, combined with carefully chosen control samples, confirms that the enhancements in superoxide generation are due to plasmon-enhanced triplet yields, a consequence of the distance dependent sensitizer extent of excitation. This observation strongly agrees with current models developed by the authors. Given that the generation of superoxide and other oxygen species is important for many chemical and biological applications, then we believe that our findings are likely to fuel a wealth of oxygen-based plasmon-enhanced triplet assays. © 2007 American Institute of Physics. [DOI: 10.1063/1.2753718]

Since the first observation of fluorophore-metal interactions in the early 1970's,¹ there has been a wealth of literature describing the photophysical effects of fluorophores/luminophores in close proximity to metallic nanostructures.^{2,3} These early reports of enhanced luminescence intensities described the enhanced emission signatures and decreased lifetimes as due to changes in the luminophores' radiative decay rate, a rate thought to be for the most part constant, and changing only slightly under different environmental conditions.^{4,5} Subsequently, radiative decay engineering⁵ has been useful for numerous biomedical applications, such as DNA hybridization and protein assays.⁶ Realizing that other effects, in addition to radiative rate modifications, such as enhanced absorption, could alter luminophore photophysical characteristics, the phenomenon was renamed as metal-enhanced fluorescence (MEF) in 2002.⁷ However, in 2005, a new more subtle explanation and model for MEF was further postulated by both Geddes and Lakowicz and co-workers, which accounted for the enhanced emission intensities and decreased lifetimes as due to fluorophore-plasmon coupling, the coupling surface plasmons radiating efficiently the photophysical properties of the coupling entity, i.e., radiating plasmon model (RPM).^{8,9} Subsequently, this new description of MEF has led to a resurgence in MEF concepts such as enhanced chemiluminescence¹⁰ and phosphorescence signatures,¹¹ metal-enhanced S_2 emission,¹² and a recent report of enhanced singlet oxygen generation, a consequence of enhanced triplet yields.¹³

In this letter, we subsequently describe the enhanced superoxide anion radical generation for acridine in close proximity to silver island films (SiFs), using a second probe specific to superoxide [dihydroethidium (DHE) probe]. Our

results show that, for the acridine photosensitizer in solution combined with the DHE probe, greater enhancements are observed in superoxide yield as both probes are brought closer to silver. This is consistent with numerous distance dependence measurements of MEF^{3,14} and metal-enhanced triplet yields.^{11,15} Our findings are not only helpful for understanding the photophysics of luminophore-metal interactions, but the practical applications of enhanced superoxide yield are also likely to find numerous and significant applications themselves.¹⁶⁻¹⁹

In this study, SiFs were deposited on glass slides as we have previously published.⁹ DHE, a well known superoxide probe, is highly selective for superoxide.²⁰ In the presence of superoxide, it emits an orange fluorescence (excitation/emission: 473 nm/586 nm). A 300 μ l solution of the fluorophore sensitizer, acridine (50 μ M in ethanol) and DHE (0.845 mM in ethanol), was trapped in a sandwich format between the glass and the silver island films, as shown in the Fig. 1, insert. A control sample, which uses the same glass slides but with no silver deposits, was used to determine the extent of the plasmon-enhanced superoxide generation. Glass slides were deemed appropriate due to the long wavelength absorption of the acridine photosensitizer and DHE probe (430 and 473 nm, respectively) as compared to the intrinsic absorption by glass (<340 nm). SiO_2 coatings, for distance dependence measurements, were deposited using thermal vapor deposition (Edwards Auto 380), where the SiO_2 layers were shown not to perturb the plasmon absorption spectra of the SiFs (data not shown).

Figure 2 shows the fluorescence emission spectra of a mixture of DHE and acridine solutions on glass and SiFs, before and after UV light exposure. On glass, no fluorescence was detected both before and after light exposure, where exposure (from 10 cm away for 2 min) with a 100 W mercury lamp was used with the acridine photosensitizer for the generation of superoxide. This suggests that too little

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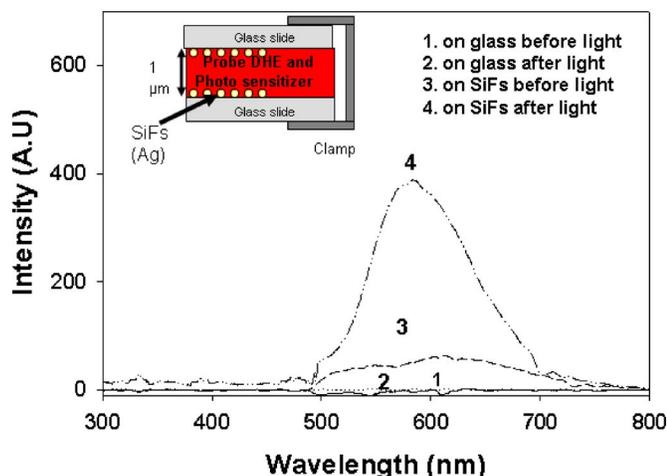


FIG. 1. (Color online) Schematic representation of the sample geometry (insert) and fluorescence emission spectra of a mixture of the DHE probe and acridine on glass and on SiFs, before and after light exposure (2 min) at room temperature. Light source was a 100 W mercury lamp. $\lambda_{\text{ex}}=473$ nm. DHE—dihydroethidium. SiFs—silver island films.

superoxide was generated to be detected in the glass sandwich using the DHE probe and our optical system. However, on SiFs before exposure, one broad peak at 595 nm was observed, which is attributed to the amplified fluorescence peak of DHE. This peak becomes apparent on the SiFs but is not visible on the glass control sample due to the MEF effect, which our group has shown can significantly enhance the emission intensity for nearly every fluorophore tested to date.²¹ After UV light exposure, a significant increase in the fluorescence emission of the DHE probe at 595 nm was evident from SiFs, which strongly indicates enhanced superoxide generation as compared to the glass control sample, which contains no silver nanostructures (Note: we have corrected for the MEF effect on the DHE probe in the absence of acridine). These enhancements can also be evidenced visually from Fig. 2 photographs. On glass, the DHE fluorescence emission was not observed before and after exposure, top left and bottom left, respectively. However, on SiFs, the

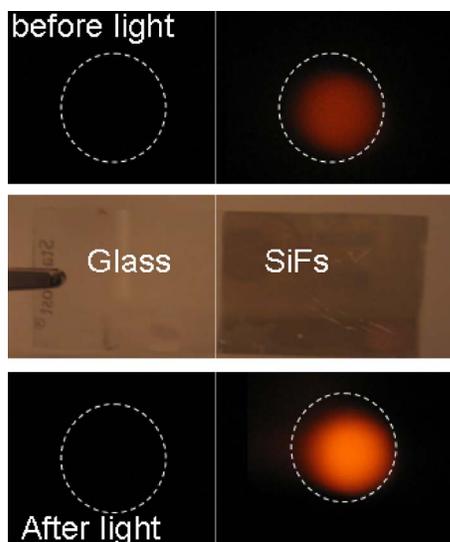


FIG. 2. (Color online) Real color photographs of DHE and acridine emission from glass and SiFs, before and after 2 min light exposure (sensitization). Light exposure source was a 100 W mercury lamp. $\lambda_{\text{ex}}=473$ nm. SiFs—silver island films.

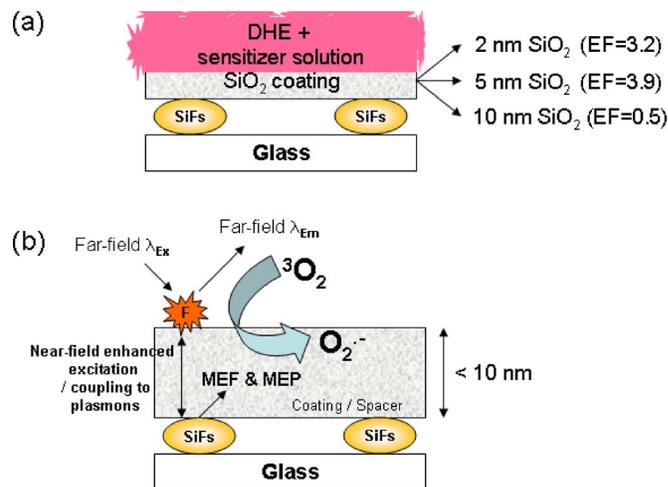


FIG. 3. (Color online) Sample architecture for the distance dependence of metal-enhanced superoxide generation (top), and graphical representation of the interpretation of metal-enhanced superoxide generation with an enhanced and distance dependent excitation rate (bottom). F—fluorophore, MEF—metal-enhanced fluorescence, MEP—metal-enhanced phosphorescence, SiFs—silver island films. EF—enhancement factor = $I_{\text{silver}}/I_{\text{glass}}$.

DHE fluorescence emission was much more intense after light exposure in the presence of the acridine photosensitizer, indicating that more superoxide anion radical is generated on SiFs than on the glass slide, cf. top right panel and bottom right panel of Fig. 2. It is interesting to note that the photographs were taken through an emission filter and the intensities observed are not due to backscattering of the excitation light by silver. The middle panel shows a photograph of the silver island films, coated on only half of the glass slide.

At first consideration one may be surprised by the presence of metal-enhanced fluorescence/phosphorescence and metal-enhanced superoxide anion radical generation in the same system, as these processes are effectively competitive and ultimately will provide a route for deactivation of electronic excited states. As recently shown by the authors, simultaneous photophysical mechanisms can be present within the same system when enhanced absorption effects of the fluorophore near to silver are present (i.e., an enhanced excitation rate). In this case, enhanced absorption of acridine near to the plasmon resonant particles facilitates MEF,²¹ MEP,^{11,15} ME¹O₂,¹³ and also metal-enhanced superoxide generation *simultaneously* within the same system. While not shown here, acridine shows an enhanced absorption spectra near to silver, similar to other probes reported by the authors, in essence acridine absorbs more light.¹¹

Finally, it is fairly well known that the metal-enhanced fluorescence phenomenon is distance dependent^{3,5,7,14} with a maximum enhancement factor (for emission intensities) for fluorophores positioned between 5 and 10 nm from the surface.^{5,7} Subsequently, we have investigated whether the generation of superoxide would similarly be influenced by the distance of both the sensitizer and DHE probe from the surface, in an analogous manner to MEF. Using thermal vapor deposition, we have been able to deposit SiO₂ coatings on the surface of the SiFs, effectively distancing the probes from the silvered surface when in a sandwich geometry [Fig. 3(a) correcting for the DHE MEF effect]. Similar to MEF findings,^{5,7} close proximity to silver results in only modest enhancements of superoxide as compared to the glass control sample also supporting SiO₂ layers (enhancement factor EF

=3.2 for 2 nm SiO₂ coatings). For 10 nm SiO₂ coatings the enhancement factor was the smallest, ≈0.5, as compared to 5 nm SiO₂ which yielded an approximately four-fold enhancement in superoxide generation as compared to the glass slide control sample, Fig. 3(a). While at first this finding appears completely consistent with MEF findings and indeed our current interpretation of MEF (RPM model described earlier),⁹ it should be noted that it is thought that an increase in the net system absorption facilitates metal-enhanced superoxide generation, where enhanced S_1 and $T_1 \rightarrow S_0$ emissions are competitive with superoxide generation, which is known to be due to a physical process of an excited state collision with ³O₂ (ground state triplet oxygen). Subsequently, from our distance dependence thickness studies, it appears that the enhanced absorption component of the sensitizer near to silver is also distance dependent, with a maximum value near 5 nm. While the emission of fluorophores near to silver is well known to be distance dependent (efficiency of plasmon coupling²²), this observation strongly suggests that the enhanced excitation component of fluorophores near to silver is also distance dependent, Fig. 3(b). To the best of our knowledge, this is the first report of the distance dependence of fluorophore excitation near to silver, which effectively separates out the enhanced excitation from the emission component. This observation is not only helpful for creating surface architectures for maximum triplet yield and therefore superoxide generation, but is helpful in our laboratories' continued efforts to develop a unified plasmon-fluorophore theory.

In conclusion, we report significant enhancements for the generation of superoxide for fluorophores/sensitizers in close proximity to silver nanoparticles. Superoxide anion radical is generated near to silver surfaces in a distance dependent manner, analogous to reports of metal-enhanced fluorescence. This suggests that the distance dependence of the excitation rate of the acridine sensitizer manifests itself in an increased triplet and therefore superoxide anion radical yield. This is a very helpful observation in understanding the interactions between plasmons and lumophores/fluorophores,

and this approach may well be of significance for enhancing triplet-state reactive oxygen-based assays.

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