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Metal-Enhanced Chemiluminescence

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Abstract In this short paper we report the interactions of
silver island films with chemiluminescing species. Our find-
ings show that silver island films can increase the detectabil-
ity of chemiluminescent reactions/species, with an approx-
imately 5-fold increase in signal intensity. This finding not
only suggests the use of silver nanostructures to amplify
chemiluminscent signatures in assay platforms, and there-
fore increase the detectability of analytes or biospecies, but
more importantly, suggests that surface plasmons can be di-
rectly excited by chemically induced electronically excited
molecules. This finding is of significance towards our un-
derstanding of fluorophore–metal interactions, a relatively
new near-field fluorescence concept, recently named metal-
enhanced fluorescence and also radiative decay engineering.

Keywords Metal-enhanced fluorescence · Radiative decay
engineering · Silver nanostructures · Metal-enhanced
chemiluminescence · Radiating plasmons · Plasmon
controlled fluorescence

Abbreviations

APS	3-(Aminopropyl)triethoxysilane
MEC	Metal-enhanced chemiluminescence
MEF	Metal-enhanced fluorescence
RDE	Radiative decay engineering
SEF	Surface-enhanced fluorescence
SiFs	Silver island films

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Introduction

The use of light-producing chemical reactions for quanti-
tative detection in biotechnology is rapidly on the increase
[1–7], especially with regard to chemiluminescence based
ligand-binding assays [1–7]. The attractiveness of chemilu-
minescence as an analytical tool lies primarily in the sim-
plicity of detection [8]; the fact that most samples have no
unwanted background luminescence, as is typically observed
in fluorescence-based assays [9]; and the fact that no opti-
cal filters are required to separate the excitation wavelengths
and scatter [8], as is also required for fluorescence-based
detection [9]. Chemiluminescent-based detection is how-
ever currently limited by the availability of chemilumines-
cent probes, which is not a factor governing fluorescence
based detection [9]. Both fluorescence and chemilumines-
cence based technologies do however suffer from an inherent
need for increased sensitivity/detection limits [8,9]. For fluo-
rescence, this is governed by the quantum yield of the tagging
fluorophore, the level of unwanted background fluorescence
and the photostability of the fluorophore [9], whereas, for
chemiluminescence, detection is limited by the quantum ef-
ficiency of the chemiluminescence reaction or probe, and the
time before depletion of the reactants [8]. For both detection
systems, an increased luminescence yield would clearly ben-
efit the overall detectability and therefore, for bioassays, the
sensitivity towards a particular analyte.

In this regard our laboratories have recently developed
a new technology which we have shown can increase the

system quantum yield [10–13], the photostability of the fluorophore [10–13] and by using spatially localized excitation, can readily remove unwanted background fluorescence [14]. To date, we have published numerous papers on metal-enhanced fluorescence (MEF) [10–20] also called radiative decay engineering [21] and surface enhanced fluorescence (SEF) [22], by us. In all of these studies, we have mostly used nanosecond decay time fluorophores in close proximity to a variety of different sized [15] and shaped [16,17] noble metal nanostructures. Until now, we have not considered the effects of silver on chemilumescence-based reactions.

Hence, in this paper, we have used silver island films to enhance the luminescence signatures of standard "glow light stick" solutions, which are readily commercially available. Most profoundly, the lack of an external excitation source to generate metal surface plasmons, suggests that this is the first observation of surface plasmons generated from chemically induced electronically excited states.

Materials and methods

Materials

Silver nitrate (99.9%), sodium hydroxide (99.996%), ammonium hydroxide (30%), trisodium citrate, D-glucose, and premium quality APS-coated glass slides (75 mm × 25 mm) were obtained from Sigma-Aldrich (St. Loius, MO). The blue-glow chemiluminescence sticks used were the "Color Bright" light sticks, obtained from Omniglow (West Springfield, MA).

Chemiluminescence

The chemiluminescent materials used in this study were obtained from commercial light glow sticks. These glow sticks contain the necessary reacting chemicals encapsulated within a plastic tube. The plastic tube contains a phenyl oxalate ester and a fluorescent probe, where the choice of dye simply determines the color of the luminescence [9]. For our work here, this choice was arbitrary as long as the luminophore emitted in the visible spectral region, consistent with previous reports [10–13]. A glass capsule containing the activating agent (hydrogen peroxide) is placed inside the plastic tube. Activation of the chemicals is accomplished with a bend, snap, and a vigorous shake of the plastic tube which breaks the glass capsule containing the peroxide and mixes the chemicals to begin the chemiluminescence reaction. The hydrogen peroxide oxidizes the phenyl oxalate ester to a peroxyacid ester and phenol. The unstable peroxyacid ester decomposes to a peroxy compound and phenol, the process chemically inducing an electronic excited state. Commercially available chemiluminescence materials were purchased and used to demonstrate the utility of the metal-enhanced chemiluminescence (MEC) approach for the general optical amplification of chemiluminescence signatures.

Formation of silver island films (SiFs) on APS-coated glass substrates

The silver island films were made according to previously published procedures employing the chemical reduction of silver nitrate on glass microscope slides using sodium hydroxide, ammonium hydroxide and glucose [10–13].

Chemiluminescence from SiFs and glass

The chemiluminescence experiments were performed using a blue emission glow stick. After chemiluminescence initiation, approximately 70 μl of the glow stick fluid was placed between two APS-coated microscope glass slides, clamped together. The glass slides contained silver island films on one end and were bare glass on the other end. The bare end of the glass served as the control sample by which to compare the benefits of using the metal-enhanced chemiluminescence phenomenon. Subsequently, the enhancement ratio, the intensity from silver/intensity from glass, could be determined.

Chemiluminescence measurements

Chemiluminescence spectra were collected using an Ocean Optics spectrometer, model SD 2000 (Dunedin, FL), connected to an Ocean Optics 1000 μm diameter fiber with a numerical aperture of 0.22 (Dunedin, FL). The fiber was positioned vertically on top of the slides containing the luminescing material. Spectra were collected with an integration time ranging between 4 and 10 s. The integration time was kept constant between the control and silver island film sample measurements.
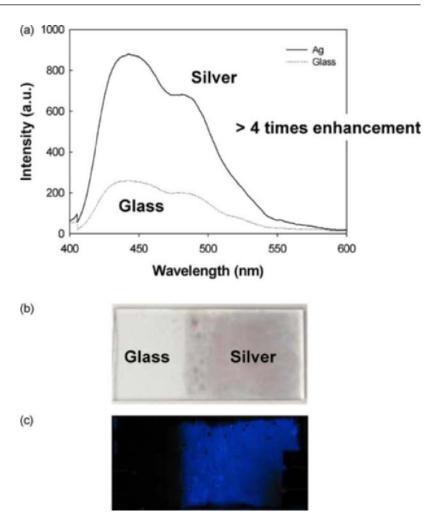
Results

Figure 1(a) shows the luminescence emission spectra from between the silvered glass and glass plates. The emission from the silvered portion of the slide was spatially averaged to be about 4–5 times greater than the glass control side of the sample. In addition, the volume between both the sandwiched glass and silver slides was identical. Figure 1(b,c) shows the photographs of the slides, both before and after the addition of the chemiluminescent material. Approximately 70 μL of the fluid was enough to form a thin coating across both portions of the slide, held by capillary action as the slides were sandwiched. The enhanced chemiluminescence is clearly visible on the silvered portion.

Fig. 1 Metal-Enhanced Chemiluminescence spectra (MEC) on a silvered surface (a) and photographs showing the enhanced luminescence (b) and (c)

Interestingly, the digital camera was not able to capture the blue emission from the thin fluid layer of the glass region of the slide, since the intensity was quite weak as shown in Fig. 1(a).

We also undertook several control experiments to determine the loss of chemiluminescent intensity due to the depletion of the reactants, (Fig. 2). After a period of 60 min, most of the emission from the silvered plates had disappeared. Fig. 2(a). Interestingly, the luminescence emission intensity changed very little in several tens of seconds, Fig. 2(b), which was the time needed to measure the intensity on both silver and glass shown in Fig. 1, making the comparison between silver and glass a valid one.

Finally, while not shown here, we also measured the *rate of loss* of luminescence from both the silvered and glass portions of the slide. For both, the rate of chemiluminescence was almost identical, suggesting that no chemical interaction between the chemiluminescent reagents and silver occurred, the enhanced luminescence signals observed being due to interactions with surface plasmons as discussed below [23]. Further details in this regard will be published in a full paper elsewhere.

Discussion

Until recently [23], the emission of fluorophores and luminescent species in close proximity to metallic nanostructures was thought to originate solely from the fluorophore, the excited plasmons interacting with fluorophores and changing their free-space spectral characteristics [10–22]. However, only recently our interpretation of metal-enhanced fluorescence has changed to one whereby excited fluorophores can nonradiatively transfer energy and couple to surface plasmons, which in turn, affect the fluorophores photophysical characteristics, in essence, the fluorophore–metal system radiates energy [23]. With the chemiluminescence species shown here, it is thought that a similar effect occurs, where excited species couple to surface plasmons, which in turn affect the photophysical properties of the chemically excited state (Fig. 3). While we present no direct evidence for chemiluminescence induced plasmon emission, the characteristics of the luminophore near the metallic particles observed here are very similar to those observed with nanosecond decay time fluorophores, strongly suggesting the same mechanism for enhanced emission [23].
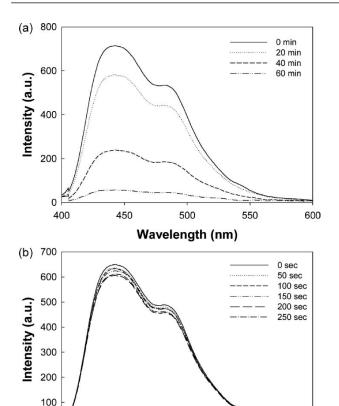
Fig. 2 Metal-enhanced chemiluminescence on a silvered surface as a function of time (a) and the intensity of luminescence in terms of seconds (b)

Interestingly, it is known that surface plasmons can be created by illumination of thin continuous metal films under very unique optical conditions, such as through a prism, or a medium of high dielectric constant and with plane-polarized light [24–25]. However, surface plasmons can also be created by direct illumination of metallic solution based colloids or nanostructures, or even by nanostructures bound
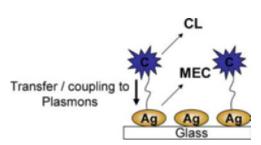

Fig. 3 Proposed model for Metal-Enhanced Chemiluminescence (MEC). The chemically induced electronically excited luminophore (**C**) transfers energy to silver plasmons (a resonance coupling interaction), which themselves radiate the photophysical properties of the excited species. CL—Chemiluminescence, MEC—Metal-Enhanced Chemiluminescence, Ag—Silver

to surfaces [23]. With fluorophores near metallic surfaces, surface plasmons can therefore be generated by both a close-proximity excited fluorophore and by direct illumination. With our chemiluminescent system here, there is no external excitation source for direct illumination, and therefore no possible direct mode of excitation of the surface plasmons. This suggests that the surface plasmons are indeed excited from a chemically induced electronically excited state of a luminophore. To the best of our knowledge this is the first observation of a chemically induced electronic excitation of surface plasmons.

Conclusions

In this paper we have shown the favorable interactions of silvered surfaces with chemiluminescent materials. The presence of the silver nanostructures increases the spatially averaged luminescent intensity approximately 5-fold, with no accelerated depletion of reactants observed, as compared to a sample employing no silver. While we have used commercially available glow sticks as a source of chemiluminescence material and to subsequently project our radiating plasmon hypothesis, silvered surfaces could also readily be used with the many other chemiluminescent reactions and systems employed in biosciences today, to similarly amplify luminescent signal intensities. In this regard, work is underway by our laboratories and will be reported in due course.

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