# Metal-enhanced bioluminescence: An approach for monitoring biological luminescent processes

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In this letter, the observation of metal (plasmon)-enhanced bioluminescence is reported. Bacteria, which are capable of generating specific bioluminescence signatures upon metabolic changes (general toxicity), have been studied from both glass and silvered glass microwell bottoms, where the silvered microwells have been modified with surface deposited silver island films (SiFs). The presence of the SiFs plasmon amplifies the near-field bioluminescence signatures, $\approx < 50$ nm from the surface, enabling amplified detection of the reporter bioluminescence indicating sample toxicity. Using our approach a greater than fivefold enhancement in *far-field* bioluminescence occurs with much greater enhancements in the *near-field* predicted. © *2009 American Institute of Physics*. [DOI: 10.1063/1.3086283]

Metal-enhanced fluorescence (MEF) has been described in detail over the past five years as a technology for enhancing fluorescence,[1–3] phosphorescence,[4,5] and chemiluminescence[6] signatures by the close proximity of metallic nanostructures. In the near-field, i.e., at distances less than 100 Å from the surface, excited states can nonradiatively induce mirror dipoles in the metallic surface, the surface plasmons, in turn, radiating the coupled quanta efficiently [Fig. 1(a)]. Typically, one observes enhanced far-field radiation (lower detection limits when MEF is applied to immunoassays[7,8]) with considerably shorter luminescent lifetimes, which are though to reflect the very short plasmon lifetimes themselves.[9] Since the lifetimes are considerably reduced, one often observes enhanced luminophore photostability, as the luminescent species spend less time in an excited state and are therefore less prone to destructive excited state processes such as photo-oxidation. Subsequently, MEF affords for ultrabright and ultrastable luminescence probes[10] and detection platforms[7,8,11] to be realized.

For both fluorescent and phosphorescent probes, which generically require an external light source for electronic excitation, an additional *electric field effect* also enhances the far-field luminescent yield by an increase in the absorption cross section of the fluorophore in the coupled fluorophore-metal system. For systems where no external light source is used for excitation, such as for chemiluminescence (chemically induced electronic excited states), dramatic MEF enhancements have also been reported even in the absence of an electric field component, with ≈1000-fold increases in chemiluminescence reported.[6] Subsequently, in this letter, we have explored the use of silver nanostructures to metal-

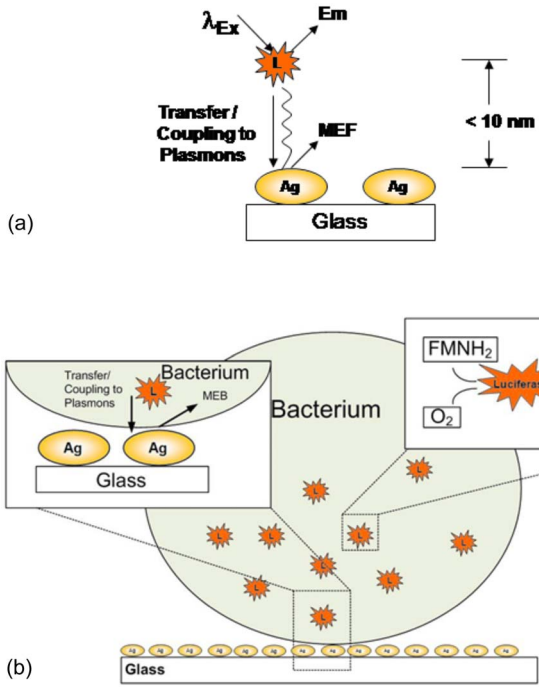


FIG. 1. (Color online) Current interpretation of the mechanism of MEF (a) and of MEB (b).

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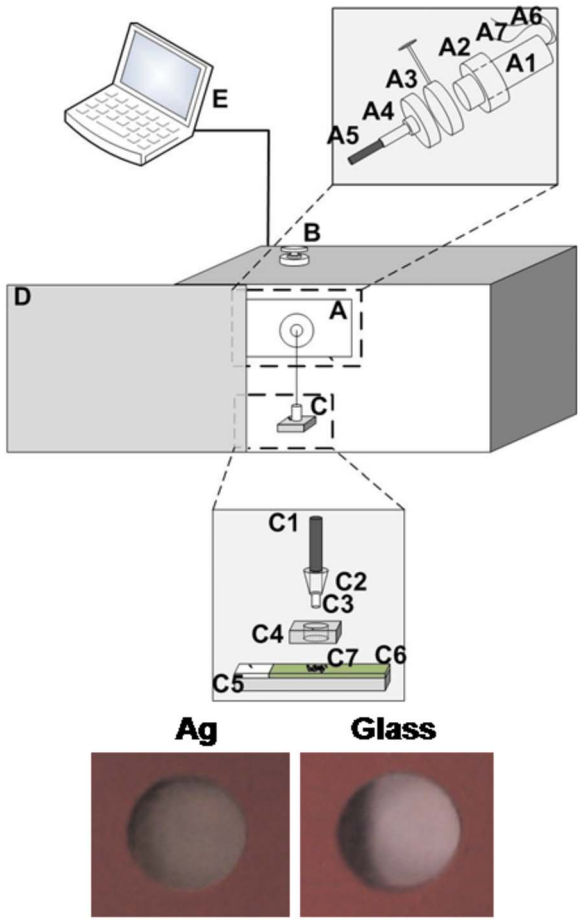


FIG. 2. (Color online) (Top) Descriptive scheme of the setup for kinetic monitoring of bacterial bioluminescence and MEB. (A) Photon counting unit. (A1) Hamamatsu HC135–01 PMT Sensor Module. (A2) PMT fixation ring. (A3) Manual shutter (71430, Oriel). (A4) Fiber holder that prevents the movement of the fiber inside the photon counting unit. (A5) Fiber optic. (A6) Wire connecting PMT to computer. (A7) Electricity cable. (B) The outside handle of manual shutter that enables light access to the PMT. (E) Computer. (D) Door. (C) Bacteria holding unit. (C1) Fiber optic. (C2) Fiber optic holder. (C3) Fiber optic core without jacket. (C4) Well with bacteria. (C5) Glass slide. (C6) Glass slide covering (silver, silver+$SiO_2$, etc.). (Bottom) Photograph of the glass and glass +2.5 min covered silver wells.

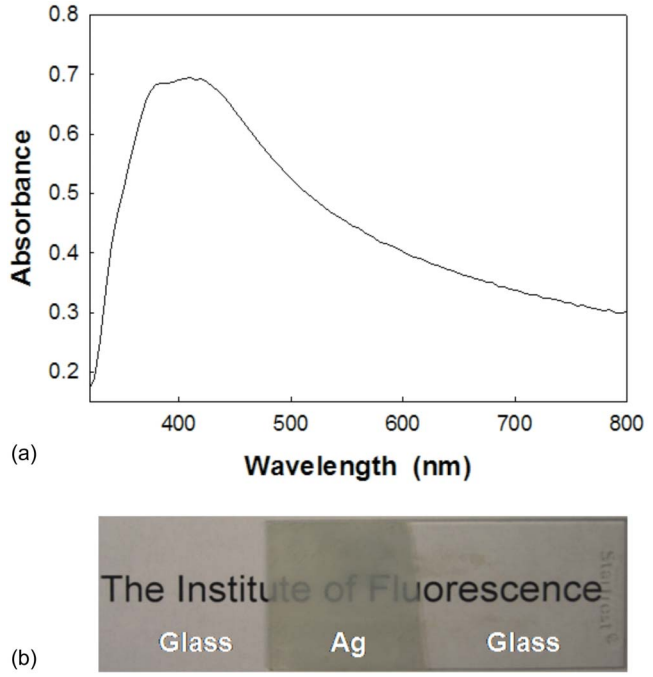


FIG. 3. (Color online) (a) Absorption spectrum of SiFs. (b) Photograph showing the semitransparent nature of SiFs (2.5 min deposition time).

enhanced bioluminescence (MEB) from metabolic changes sensitive bacteria. Traditionally, bioluminescence signatures are relatively weak as compared to fluorescence-based probes with sensitive detectors often employed.[12] These methods suggest activation of the reporter luciferase genes with emission, a readily detectable light signal, which allows the monitoring of bacterial response in real-time by simple luminometry (e.g., fiber optic, luminometers).[13,14] The most commonly used systems are the *luc* gene from the firefly and *lux* genes from bacterial species of the genus *Vibrio*. Expression of the lux luciferase operon produces light without any additions, allowing thereby online monitoring of gene expression, whereas the expression of firefly luciferase genes requires externally added substrate (luciferin) for luminescence. The bacterial lux system is expressed very effectively in different bacterial strains, and this method has been widely applied for different applications.[15–17]

Our results show that similar to fluorescence, phosphorescence, and chemiluminescence based approaches, MEF can also be used to plasmon-enhance bioluminescence signatures [Fig. 1(b)], enabling the much more sensitive detection of bioluminescence with potential multifarious applications in the biosciences.

Silver island films (SiFs) were deposited on glass slides as was described earlier.[18] The bioluminescent *Escherichia coli* strain TV1061 (Ref. 19) used in this study is sensitive to metabolic changes, such as with cytotoxic substances. This bacterium harbors plasmid-borne fusions with a specific heat-shock *grpE* promoter adjacent to the luxCDABE reporter operon.[20] The tested bacteria were induced with a positive control [2% (v/v) ethanol], and after 1.5 h incubation at 26 °C these were placed on ice in order to maintain the bacterial concentration and measured using a field-operable fiber optic photodetector device,[21] as shown in Fig. 2. The effect of three different matrices on bacterial bioluminescence was tested using glass slides, glass slides covered with SiFs, and glass slides with $SiO_2$ covered SiFs. The differences in the light intensity were measured with a Hamamatsu HC135–01 PMT Sensor Module and photographed in real time with a charge-coupled device camera (Retiga-SRV fast 1394, QImaging, Canada).

Figure 3(a) shows the plasmon absorption spectra of SiFs where the surface plasmon absorption is well known to be characteristic of the shape, size, and type of metal of the nanoparticles immobilized on the glass surface.[22] In this case, the shape of the plasmon absorption band was similar to the shapes typically observed on the glass substrates, with a peak near 425 nm.[2,23] The SiFs are noncontinuous, semitransparent silver nanodeposits,[2,23] as shown in Fig. 3(b).

The interaction of fluorophores with metallic particles has been the subject of several publications.[24,25] However, the interaction of the bacterial luminescence with metallic particles has hitherto been ill-explored. Figure 4 visually demonstrates the benefits of the MEB. Bioluminescent emission observed from bacteria located on SiFs was ≈1.5-fold higher in comparison to nonsilvered coated glass [Fig. 4(b)]. The color photograph insets provide visual evidence for enhanced bacterial luminescence from the silver film. This phe-

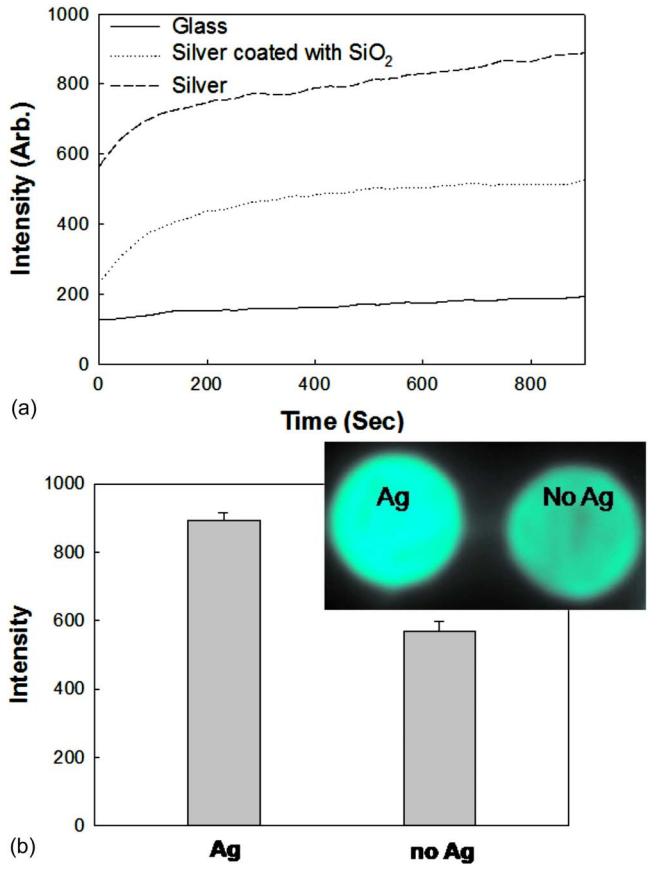


FIG. 4. (Color online) (a) Bioluminescent bacteria emission intensity from silvered, silvered glass coated with $SiO_2$, and glass wells as a function of time. (b) Light intensity of bioluminescent bacteria on silver coated glass in comparison to noncoated glass. (b-insert) The intensity of light is much higher in silver coated wells.

nomenon is due to the enhancement in bioluminescence emission by the silver nanoparticles (that is, MEB); excited bacterial luciferase molecules that are located in close proximity to the silvered surface transfer their energy to the silver nanoparticles where the energy is coupled efficiently and the emission from the bacteria-silver "system" becomes greater than the bioluminescence signal from bacteria alone, i.e., it is plasmon amplified. Kinetic measurements [Fig. 4(a)] support these findings. While in this case, there is up to fivefold difference in the light intensity between bacteria placed on the silvered and nonsilvered coated glass surfaces. Similar to MEF, MEB demonstrates a clear distance dependence of excited state coupling: as the distance between silver islands and bacteria increases (such as by adding an inert $SiO_2$ layer), the far-field total light enhancement decreases [Fig. 4(a)]. Whole cell optic biosensors have been already proposed for use in many environmental applications.[26,27] Thus, increasing the light intensity will invariably increase the sensitivity and eventually the attractiveness of these constructs in the future.

In conclusion, we report the observation of MEB. Bioluminescence signatures generated in close proximity to the metallic surfaces demonstrate enhanced emission as compared to a nonsilvered coated glass control sample containing the same number of bacteria. The bacteria are unperturbed by the silvered surface and show a greater than fivefold enhancement in the far-field bioluminescence detected as compared to the control sample. Interestingly, this enhancement originates from less than 10 nm of bacterial sample, i.e., MEF interaction coupling distance, suggesting that the *near-field* enhancements are considerably larger. We have also studied the effects of protecting the bacteria from silver using an $SiO_2$ layer to demonstrate that MEB is a through space interaction, similar to other metal-enhanced phenomena reported by our laboratory.[1–6] The simplicity of our approach suggests that metallic substrates can readily be used for observing a whole host of bioluminescence processes. Further work in underway by our laboratories and will be reported in due course.

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