How surface-enhanced chemiluminescence depends on the distance from a corrugated metal film

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Peroxidase labeled streptavidin was immobilized onto the surface of bulk and clusterlike metal films at a distance controlled by a peptide chain with a length between 1.3 and 7.8 nm. Luminol chemiluminescence which occurred at peroxidase vicinity depends on the metal nanostructure. When peroxidase is attached on a bulklike film, chemiluminescence increases monotonously with the distance because of a decrease of the light emission quenching by metal. When peroxidase is attached on a clusterlike film, chemiluminescence undergoes a complex variation with the metal/catalyst distance evidencing a competition between the already mentioned quenching process and a nanostructure-induced catalysis enhancement. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2399934]

Photoluminescence by molecules and atoms is noticeably modified near nanostructured metal surface because of the complex interplay between two opposite phenomena: an enhancing process and a quenching process.[1–3] Enhancement of photoluminescence can be promoted by surface plasmons excited in metal and by a modified density of photon states at nanostructured surface.[4–6] Quenching processes arise from a nonradiative energy transfer between luminescent species and metal.[7–9] In the case of chemiluminescence (CL), gold nanostructures seem to act more as an important enhancement mediation rather than a simple quenching acceptor. This was shown recently for gold particles which not only catalyze the CL of luminol-H_2O_2 system[10] but also emit light under chemical or electrochemically initiated reaction.[11] CL should thus appear as a very attractive experimental tool to quantitatively discriminate between the two opposite effects of a metal nanostructure: quenching and enhancement.

To achieve such a discrimination, we propose to compare the luminol CL at the vicinity of two different types of films, a bulklike and a clusterlike one for different distances between the metal surface and the emitting dye. A bulklike film should only induce quenching, whereas a clusterlike one is expected to promote both quenching and catalysis. In a previous study,[12] we demonstrated that gold clusters could effectively enhance the light emission of the reactions involving peroxidase which was, in the experimental setup chosen (schematic, Fig. 1), the agent that catalyzed luminol CL. Moreover, provided that the strength of catalysis and the ability to quench depend on the distance between the dye and the surface according to different laws, the CL evolution with this distance should clarify the relative contributions of the two mechanisms. This last expectation is justified by the fact that, in our previous study, the CL intensity was found to strongly depend on the type of adsorption of the catalyzing peroxidase (physically or chemically), which actually induces a variation in the film/catalyst distance. In this letter, we carry out additional experiments varying in a systematic and quantitative way the distance between the film and the peroxidase via peptides with different lengths.

Bulklike and clusterlike metal films with a nominal thickness of about 25 nm were deposited onto quartz substrates by pulsed laser deposition technique (Table I).[12] The transition from bulklike to clusterlike thin film can be tuned by controlling the substrate temperature. Clusterlike films of Au and Au/Ag were prepared at 600 and 200 °C separately to present the same absorption properties (inserts in Figs. 2

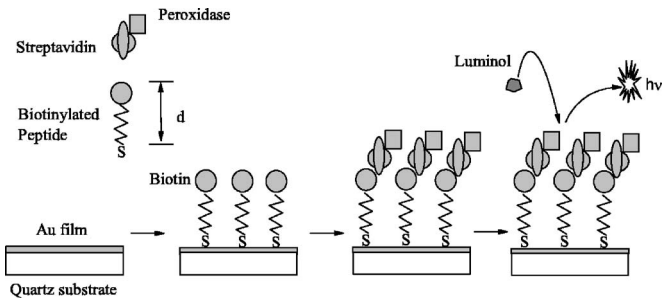

FIG. 1. Schematic representation of the process involved in luminol chemiluminescence induced by peroxidase labeled molecules with different distances from gold film surface.

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TABLE I. Characteristics of the samples studied.

Sample	Substrate temperature during deposition	SPR Peak position (nm)	SPR HWHM (nm)	Roughness (nm)	Particle size (nm)
Flat Au	RT	No peak	No peak	0.3	No particle
Flat Au–Ag	RT	No peak	No peak	0.4	No particle
Rough Au	600 °C	690	110	1.9	30
Rough Au–Ag	200 °C	690	190	4.1	30

and 3), and only differ by their catalytic properties. The metal films made so were used as chip substrates for luminol-H_2O_2 induced CL according to a process described in Fig. 1. First, trithiolated peptides with different lengths modified with a biotin molecule at its *N*-term end were anchored on the Au films by spotting of 1.2 nl drops (1 mg/ml), incubation for 2 h, and final washing. Then, the films were incubated for 30 min with peroxidase labeled streptavidin (1 μg/ml). The peroxidase labeled streptavidin has a globular size of 6–10 nm and the distance between peroxidase and metal is adjusted by the peptide chain length. Four different chain length peptides consisting with 5, 11, 17, and 23 amino acids and denoted as P5, P11, P17, and P23 were used to get separation distances d of about 1.3, 3.5, 5.7, and 7.8 nm, respectively. The treated thin films were dipped into a solution containing the luminol and additives agents favoring its chemiluminescence (220 μM luminol, 500 μM H_2O_2, and 200 μM *p*-iodophenol). Chemiluminescent measurements were taken with a −30 °C cooled charge coupled device camera, allowing to collect the light emitted from the surface. The CL emitted at 425 nm by the luminol brought at the peroxidase vicinity was integrated for 10 s.

Figure 2 shows the results of luminol CL on bulklike Au and Au/Ag films. In both cases, the CL intensity increases with the distance and reaches about 2000 in arbitrary units. Assuming that in the absence of clusters, there is no catalytic effect, this increase should be entirely related to the decrease in metal quenching with the distance. Förster transfer theory predicts that such a quenching possesses an inverse fourth power dependence with the distance $d_0^{d\text{-}a}$ between donor and acceptor: $\eta_q \approx (1+(d_0^{d\text{-}a}/d^{d\text{-}a})^4)^{-1}$.[13] In this relation $d_0^{d\text{-}a}$ is a critical distance between donor and acceptor and $\eta_q = I/I_0$ is the ratio of dye fluorescence in presence of a film at the distance $d^{d\text{-}a}$, I, and that in the absence of the film, I_0. Figure 2 shows that in the case of both Au and Au–Ag bulklike films, such a relation is satisfied between the fluorescence intensity I and the distance d between the film and the catalyzing peroxidase. The same fit $\eta_q = [1/((3/d)^4+1)]$ is effectively obtained for the experimental data relative to both

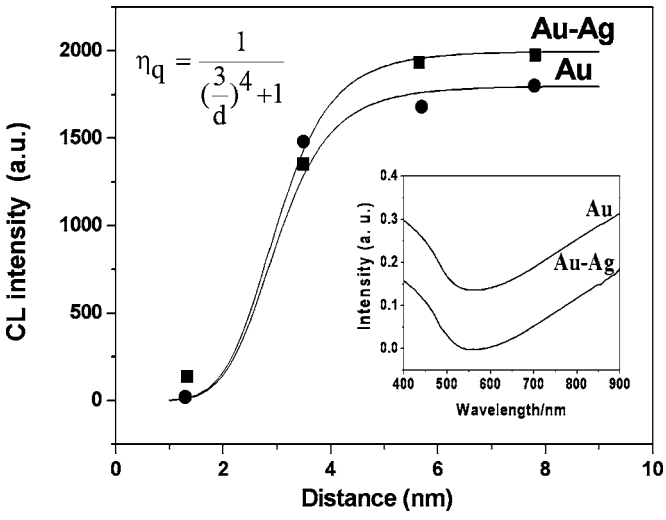

FIG. 2. Luminol-H_2O_2 chemiluminescence induced by peroxidase on *bulklike* Au (circles) and Au–Ag (squares) films with different metal/peroxidase distances. The lines are fits of these data according to a Förster process simulation. Insert: absorption spectra of the *flat* films.

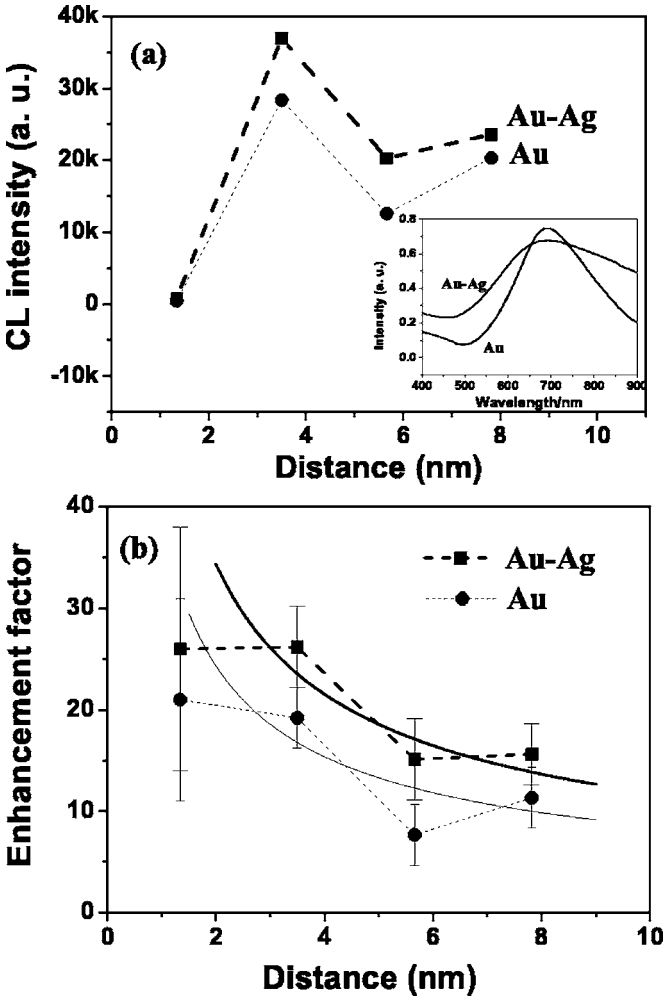

FIG. 3. (a) Luminol-H_2O_2 chemiluminescence induced by peroxidase on *clusterlike* Au (circles) and Au–Ag (squares) films with different distances. Insert: absorption spectra of the *rough* films. (b) Enhancement factor η_e: experimental data (symbols) and data fit (line). The dash lines are guides for the eye.

films with d expressed in nanometers. CL is emitted from excited aminophthalate when the catalyzing peroxidase lies at a distance to the luminol of less than 10 nm.[14] The critical value found $d_0^{\text{quench.}}=3\pm0.5$ nm for both Au and Au–Ag films indicates then that the critical distance $d_0^{d\text{-}a}$ between the film and the luminol is rather in good agreement with the order of magnitude given by Förster for the critical donor/acceptor distance, between 5 and 10 nm. For distances d larger than 6 nm, the fluorescence reaches a plateau at a value which is independent of the chemical nature of the film (1800 for Au and 2000 for Au–Ag). This value is also in excellent agreement with the value relative to freestanding peroxidase (i.e., in the absence of metal surface) measured for peroxidase simply adsorbed on a flat surface of quartz at long distances of the surface.

For both Au and Au–Ag, the CL signal relative to the clusterlike thin films presents a quite different behavior compared to that relative to bulklike films. Figure 3 shows that the CL intensity first strongly increases with the distance between 1.3 and 3.5 nm, and then slowly decreases after 3.5 nm. For a peroxidase/metal distance of 3.5 nm (P11) the intensity reaches high values around 30 000 in the same arbitrary units. This increase of more than one order of magnitude compared to the CL intensity relative to the flat surface confirms the results already obtained in our previous paper.[12] When comparing the intensity obtained with that in the presence of freestanding peroxidase, the increase must be undoubtedly attributed to an enhancement of the luminescence by the presence of clusters. To provide a quantitative information upon such an enhancement, a simple model in which the intensity observed is the product of the quenching factor $\eta_q(\leqslant 1)$ and an enhancement one $\eta_e(\geqslant 1)$ can be proposed, $I=\eta_q\eta_e I_0$. There is no overlapping between luminol emission at 425 nm and the absorption band generated by corrugation. Then luminescence quenching, which could be increased at vicinity of rough surfaces by a modification in photonic mode density,[13] should not depend significantly on the structure of the film. The enhancement factor η_e [Fig. 3(b)] can thus be derived from the relation $\eta_e=I/\eta_q I_0$, where I is given in Fig. 3(a) and $\eta_q I_0$ in Fig. 2.

A conclusion concerning the evolution of η_e with the metal/peroxidase distance can be drawn. As expected, this evolution, which is similar for both compositions, is found to decrease with the distance. However, the decreasing trend is smoother than that relative to quenching. Indeed, when fitting η_e according to a relation similar to that relative to η_q, $\eta_e=1+(d_0^{\text{enhanc.}}/d)^n$, where n is an integer and $d_0^{\text{enhanc.}}$ a critical distance for enhancement; one finds that n is much smaller than 4 the exponent related to quenching and that $d_0^{\text{enhanc.}}$ is much larger than $d_0^{\text{quench.}}\approx 3$ nm. Precisely $\eta_e=1+(230/d)^{0.7}$ for Au and $\eta_e=1+(350/d)^{0.7}$ for Au–Ag with d expressed in nanometers. The relative variations of quenching and enhancement explain the particular behavior of CL intensity with distance observed in Fig. 3(b). At short distance, quenching predominates so that CL is low, whereas when increasing the distance, the enhancing mechanism dominates. That explains the presence of a maximum at a distance of 3.5 nm (for P11) slightly larger than $d_0^{\text{quench.}}$.

Since there is no overlapping between luminol emission and plasmon absorption, the increase in luminol quantum efficiency should be very limited. The most likely reason for the enhancement observed should be then related to a catalysis by metal clusters of reactions involving peroxidase. This interpretation is consistent with the fact that gold clusters were already demonstrated to act as strong catalysts in solution.[10] Here, the catalysis enhancement could arise from a decrease of the redox potential of luminol at vicinity of corrugated gold. Such a shift which facilitates luminol oxidation in the presence of catalyzing peroxidase was indeed already evidenced by our team when luminol is directly fixed on gold clusters.[15] The present experiments (η_e is found to be 30% higher for Au–Ag films compared to Au ones) could then be interpreted as an indication that silver possesses better catalysis properties than gold.

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