

Fluorescence News

The CFS Engineers the Intrinsic Radiative Decay Rate of Low Quantum Yield Fluorophores

Received January 15, 2002; accepted January 20, 2002

KEY WORDS: Radiative decay rate; intensified fluorescence; surface enhanced fluorescence.

The Center for Fluorescence Spectroscopy (CFS) at the University of Maryland, Baltimore, has recently been investigating the interactions of close-proximity metallic particles, islands, and colloids on the spectral properties of weakly fluorescent fluorophores.

By placing a high quantum yield fluorophore (Rhodamine B, $Q_0 = 0.48$) and a low quantum yield fluorophore (Rose Bengal, $Q_0 = 0.02$) above silver islands deposited on quartz microscope slides, the fluorescence group at Baltimore readily observed a substantial increase in the fluorescence intensity for the low Q_0 species, accompanied by a proportionate reduction in its fluorescence lifetime (Fig. 1b and 2b, respectively). However, for rhodamine B the fluorescence emission intensity is similar in the absence and presence of the silver islands (Fig. 1a), which is explained by the fact that because the quantum yield of rhodamine B is high, its quantum yield cannot be substantially increased by the silver islands. Such effects in fluorescence spectroscopy are *highly unusual* and can be explained by an increase in the radiative decay rate of the weakly fluorescent species.

To explain this new concept in fluorescence, which we have recently termed “radiative decay engineering” [1–4] it is necessary to consider a modification to the classical Jablonski diagram from the *free space* condition, to account for the close proximity of metallic particles or islands. Typically the quantum yield, Q_0 , and lifetime, τ_0 of a fluorophore are given by:

$$Q_0 = \frac{\Gamma}{\Gamma + k_{nr}} \quad (1)$$

$$\tau_0 = \frac{1}{\Gamma + k_{nr}} \quad (2)$$

where Γ is the fluorophores intrinsic radiative decay rate, and k_{nr} are the non-radiative rates. However, in the presence of metallic particles or colloids, an increase in fluorescence intensity, Q_m , can be accompanied by a corresponding reduction in lifetime, τ_m , (Eqs. [3] and [4]), which is converse to the free space condition where Q_0 and τ_0 change in unison, (Fig. 3).

$$Q_m = \frac{\Gamma + \Gamma_m}{\Gamma + \Gamma_m + k_{nr}} \quad (3)$$

$$\tau_m = \frac{1}{\Gamma + \Gamma_m + k_{nr}} \quad (4)$$

Of interest, these equations predict a modification in the radiative decay rate of fluorophores, which has until now mostly been considered as being dependent on the fluorophores’ oscillator strength and only very weakly dependent on its local environment.

Another effect of close-proximity metallic particles is the so called lightning rod effect, in which metallic particles can enhance the electric field incident on a fluorophore by a factor of a 100 or more [5]. This incident field concentration effect, coupled with an increase in radiative decay rate (increased Q_0 and reduced τ_0), which has previously been thought of as constant, is therefore likely to produce some remarkable effects in fluorescence with multifarious applications. It is interesting to predict a few.

- Observe fluorescence emission from low Q_0 species, where the enhancement factor could be as large as $1/Q_0$. For example, one might observe the intrinsic fluorescence from DNA, which is generally considered to be non-fluorescent as $k_{nr} > \Gamma$ resulting in $Q_0 < 10^{-4}$

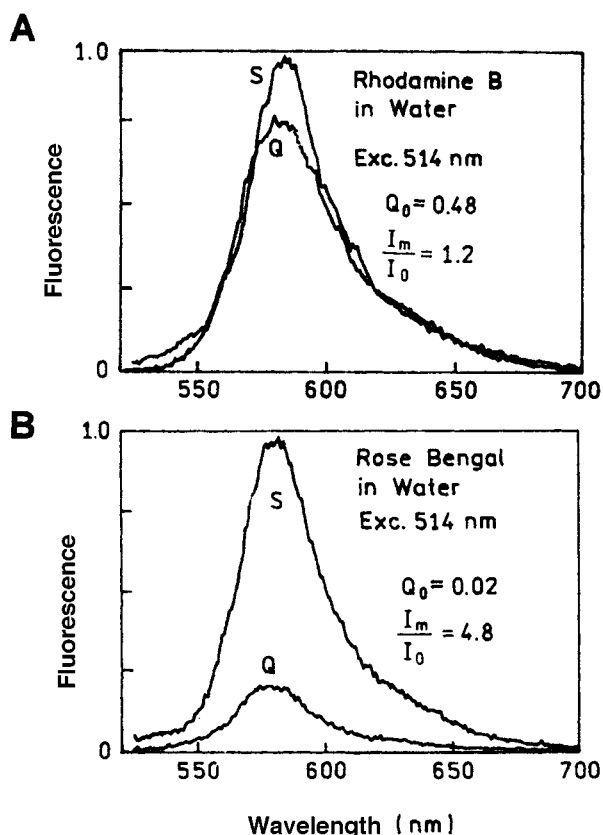


Fig. 1. (A) Fluorescence intensity of rhodamine B and (B) rose bengal, in the presence and absence of silvered quartz slides. S: Silver; Q: quartz slides.

- Observe an increase in photostability from fluorophores because a reduction in τ_0 implies that fluorophores will spend less time in the excited state and therefore have less chance for photochemical reactions.
- One may reduce the photochemical damage on samples that can occur at high illumination intensities, because near metallic particles we can observe the same emission intensity using a lower incident power.
- We can observe a higher photon flux from fluorophores. The maximum number of photons that are emitted by a fluorophore each second is roughly limited by the inverse of the lifetime. For example, a 1- μ sec lifetime can yield about 10^6 photons per second per fluorophore. If the close proximity of a metal reduces the lifetime ten fold, the incident intensity can increase tenfold before depletion of the ground state.
- There may also be applications in biological imaging using this phenomenon. For example,

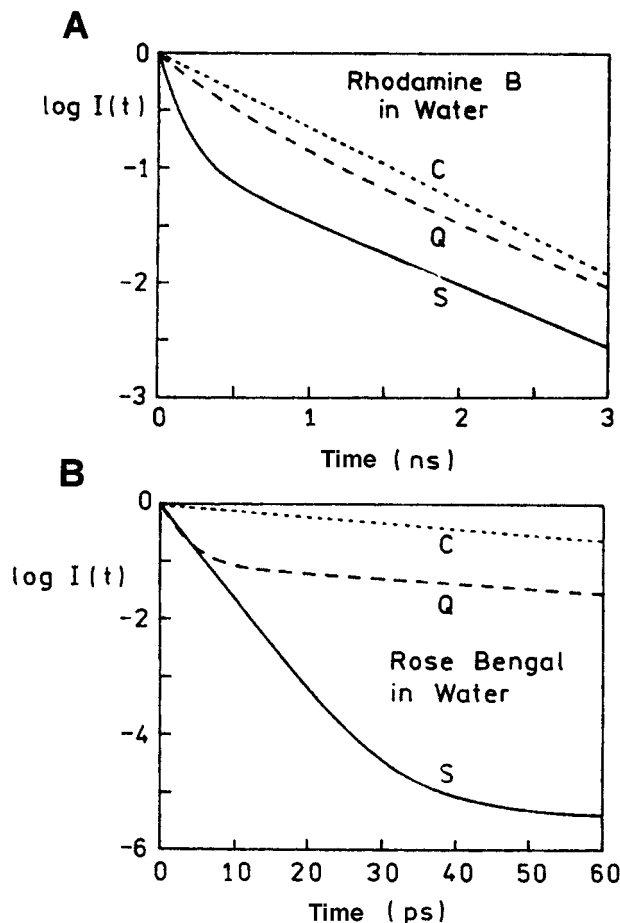


Fig. 2. Intensity decays for (A) rhodamine B and (B) rose bengal in cuvettes, C, between unsilvered quartz slides, Q, and between silvered quartz slides S. For further details see [2].

in two-photon excitation, the rate of excitation depends on the square of the incident intensity. This means that two-photon excitation may be dramatically increased near metallic particles.

The CFS expects metal-enhanced fluorescence to have numerous applications in analytical chemistry, diagnostics, genomics and proteomics.

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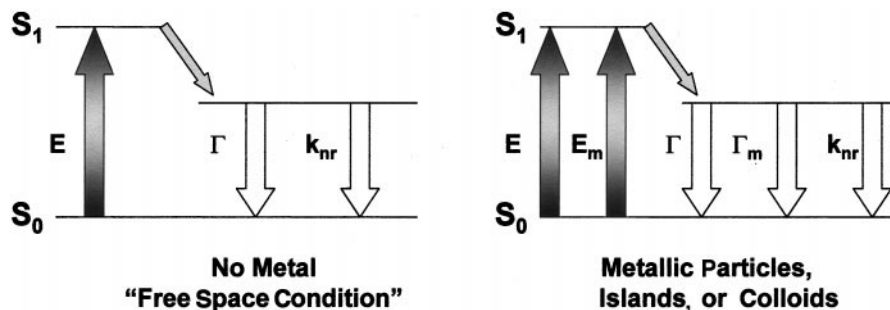


Fig. 3. Classical Jablonski diagram for the free space condition and the modified form in the presence of metallic particles, islands, or colloids. E : Excitation, Γ_m radiative rate in the presence of metal.

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