Journal of Fluorescence, Vol. 12, No. 2, June 2002 (© 2002)

Editorial

Metal-Enhanced Fluorescence

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Received April 22, 2002; accepted April 25, 2002

KEY WORDS: Radiative decay engineering; intensified fluorescence; reduced lifetime; enhanced photostability.

As fluorescence spectroscopists we are mostly all familiar with doing spectroscopy in bulk samples, where the solutions are typically transparent to the emitted radiation, and the fluorophores emit isotropically into free space and are observed in the far field. There may be changes in the refractive index, such as for a fluorophore in a membrane, but these changes typically have only minor effects on the fluorophores free-space spectral properties.

However, a considerable literature is rapidly emerging [1,2], prompting this informative editorial, which promises to change our perception of how we think about fluorescence. Nearby conducting metallic particles, colloids, or surfaces can modify these *free-space conditions* in ways that increase or decrease the incident electric field, E_m, felt by the fluorophore and also increase and decrease the radiative decay rate, Γ_m. These effects can be described in terms of changes in the photonic mode density, where a large mode density provides more radiative decay pathways and larger radiative decay rates (Fig. 1). The modification of the radiative decay rate, which is the spontaneous rate at which a fluorophore emits photons, is for the most part unchanged or slightly changed by environmental parameters, such as solvent polarity and temperature, and hence is typically thought of as a constant that is primarily dependent on the oscillator strength of the fluorophore [3]. An opportunity to modify this rate is likely to produce a wealth of biomedical and biochemical applications by the direct modification of the fluorescence observables, such as increased quantum yields, decreased lifetimes, increased photostability, and an increase in Förster transfer distances and directional emission. Such opportunities are truly refreshing, because although fluorescence methodology and subsequent applications are routinely practiced in many laboratories around the world, little research on such basic principles has probably hindered fluorescence growth from further revolutionalizing the analytical and clinical sciences. For example, resonance energy transfer (RET) immunosensing has languished of late because of the relatively small D-A R_0 value of probes compared to the size of antibodies and relevant antigens. However, given the increase in transfer distances being reported near metallic islands [2,4], we are now likely to see a resurgence of RET-based immunosensing founded on this new core technology, metal enhanced fluorescence (MEF).

Whereas the demonstrations and applications of MEF are mostly new, the theory of fluorescence enhancement with metallic surfaces and particles has developed since the 1980s, in which the effects are due to at least *three* known mechanisms (Fig. 2). One is energy transfer quenching, k_m, to the metal with a d^{-3} dependence [5]. This quenching can be understood by damping of the dipole oscillations by the nearby metal. A second mechanism is an increase in the emission intensity as a result of the metal increasing the local incident field on the fluorophore, E_m, with a maximum theoretical enhancement effect predicted to be near 140 [6]. This effect has been observed for metal colloids and is appropriately called the "Lightening Rod effect" [7–9]. This enhancement can be understood as being due to the metal particles concentrating the local field and subsequently increasing the rate of excitation. The third mechanism is that a nearby metal can increase the intrinsic radiative decay rate of the fluorophore, Γ_m, that is, to modify the rate at

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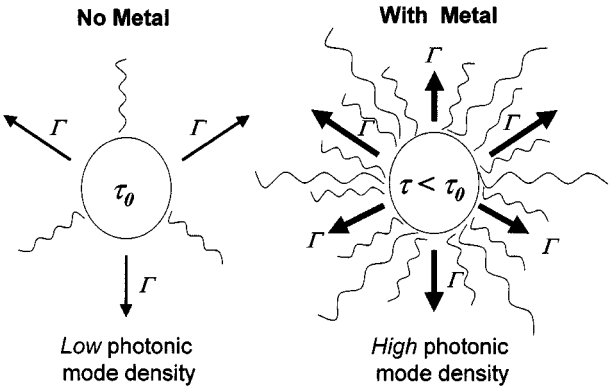

Fig. 1. Low and high photonic mode densities in the absence and presence of metal, respectively.

which a fluorophore emits photons [1,2,10]. To better understand this concept it is informative to consider the Jablonski diagram for fluorophores in the *free-space* condition and the modified form when in close proximity to conducting metallic particles or colloids (Fig. 3).

Absorption of a photon sends the fluorophore to the first excited singlet state (S_1) after which time the excited molecules can emit a photon at a rate, Γ, or return to the ground state by a non-radiative decay process, with a rate k_{nr}. Other deactivation processes are also possible to depopulate the S_1 level, such as quenching processes with a rate k_q. The quantum yield, Q_0, of a fluorophore reflects a competition between radiative decay and non-radiative processes:

$$Q_0 = \frac{\Gamma}{\Gamma + k_{nr} + k_q} \tag{1}$$

The fluorescence lifetime, or decay time, is the average

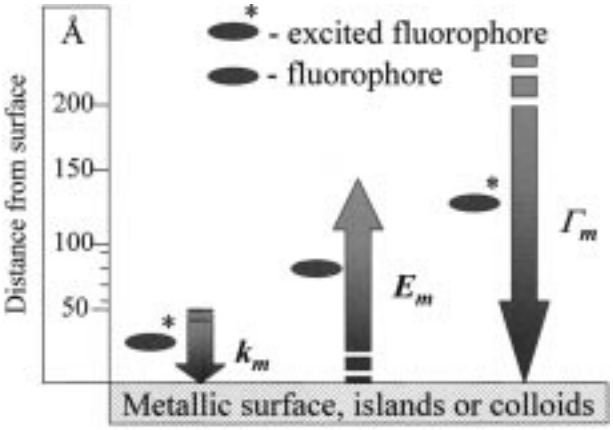

Fig. 2. Predicted distance dependencies for a metallic surface on the transitions of a fluorophore. The metallic surface can cause Förster-like quenching with a rate, k_m, can concentrate the incident field, E_m, and can increase the radiative decay rate, Γ_m.

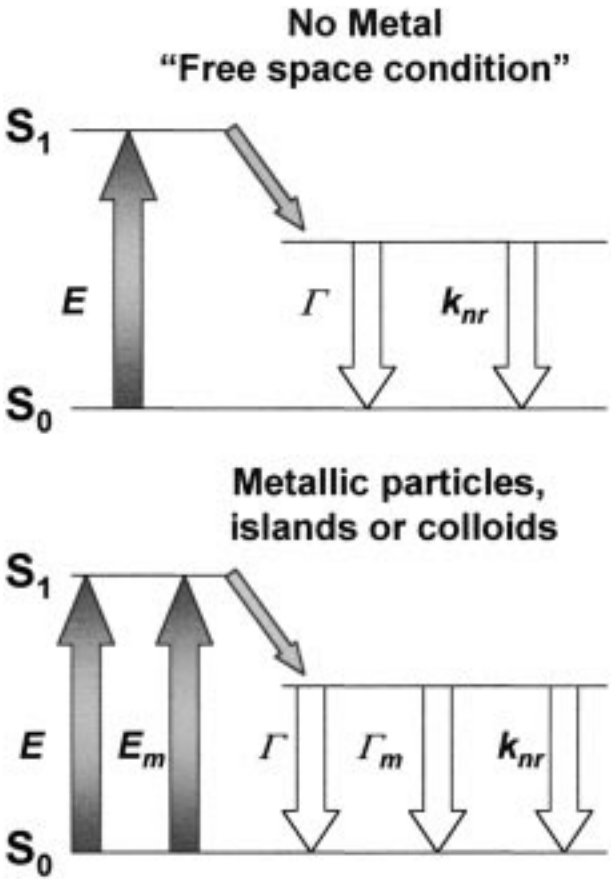

Fig. 3. Classical Jablonski diagram for the free-space condition and the modified form in the presence of metallic particles, islands or colloids. E-excitation, E_m-metal enhanced excitation rate, and Γ_m-radiative rate in the presence of metal.

time an ensemble of fluorescent molecules remain in the S_1 state:

$$\tau_0 = \frac{1}{\Gamma + k_{nr} + k_q} \tag{2}$$

The fluorophores immediate environment strongly influences its fluorescence lifetime and intensity, but does not significantly alter Γ. These changes occur because of changes in k_{nr} or k_q. If Q_0 is relatively small, then $k_{nr} + k_q$ is much larger than Γ. If Q_0 is high, then the Γ is larger than $k_{nr} + k_q$. Hence, we can modify the spectral changes of fluorophores by modifying k_{nr} or k_q. Invariably Q_0 and τ_0 change together.

Modification of Γ is usually not considered because this rate is determined by the transition probability and oscillator strength of the $S_1 \rightarrow S_0$ transition [3]. When fluorophores are placed at suitable distances from metallic particles or surfaces (when we refer to metallic, we mean conductive and not ions or oxides), fluorophores can

undergo modifications to their radiative decay rates, Γ_m, where an increase in Γ_m results in an increase in fluorescence intensity, Q_m, and reduction in lifetime τ_m, which is converse to the free-space condition in which both change in unison.

$$Q_m = \frac{\Gamma + \Gamma_m}{\Gamma + \Gamma_m + k_{nr} + k_q} \tag{3}$$

$$\tau_m = \frac{1}{\Gamma + \Gamma_m + k_{nr} + k_q} \tag{4}$$

An ability to modify and control the radiative decay rate ($\Gamma + \Gamma_m$) can have profound implications for the use of fluorescence in basic research and its applications. The modification and control of the radiative rate has been recently named radiative decay engineering (RDE) by us [1,2], but we have now tended toward using MEF, terminology that additionally accounts for the other so called *lightening rod* fluorescence enhancement effect.

At this point it is informative to consider how modifications of both E_m and Γ_m are likely to influence other well-characterized free-space fluorescence phenomenon.

FLUORESCENCE LIFETIME AND QUANTUM YIELD

We have simulated data based on Eqs. (3) and (4) to demonstrate the effects of increasing the radiative decay rate of low and high quantum yield fluorophores on their lifetime and quantum yield (Fig. 4). The plots have been calculated assuming three fluorophores with a good, 0.5; low, 0.1, and very low quantum yield, 0.01, with an assumed *natural* lifetime of 10 ns, $\Gamma = 10^8\ s^{-1}$. The largest enhancement in *quantum yield* is observed for the weakest fluorophore, where increasing Γ_m has a lesser effect for $Q_0 = 0.5$. At sufficiently high values of Γ_m/Γ, the quantum yield of all fluorophores can be seen to approach unity. It is therefore realistic to envisage fluorophores with quantum yields of about 10^{-3} (practically non-fluorescing) that become highly fluorescent (quantum yield $\sim$ 1.0) near an appropriate metallic surface, with a maximum enhancement factor of $1/Q_0$ [6]. To test this expectation we recently investigated whether metallic particles and surfaces could enhance the intrinsic fluorescence of biologically important low quantum yield species, such as DNA and nucleotides. Amazingly, the intrinsic fluorescence near metallic surfaces was clearly enhanced and therefore measurable [2,11] (Figs. 5 and 6, respectively). An opportunity to effectively make non-fluorescent species fluorescent is likely to fuel a wealth of biotechnology and medical applications, for example, using intrinsic DNA fluorescence for sequencing.

At present, some groups are attempting to sequence a single DNA strand using a single strand of DNA [12,13], where an exonuclease sequentially cleaves single nucleotides from the strand, which are then labeled, detected, and subsequently identified. However, this goal is more difficult than single molecule detection because every nucleotide must be labeled and detected, rather than the simpler task of finding one fluorophore among many. Also, this method requires a 100% labeling efficiency. However, using metal enhanced fluorescence could allow base detection *without labeling*. The released nucleotides could pass through a flow chamber that is specially designed to enhance intrinsic nucleotide fluorescence (Fig. 7). If successful, this approach would allow DNA sequencing using the surface enhanced intrinsic nucleotide fluorescence.

EFFECTS ON COLLISIONAL QUENCHING

When collisional quenching occurs, both the quantum yield and lifetime are reduced [c.f. Eqs. (1) and (2)] by additional rate processes that depopulate the S_1 level, with a typical rate $k_q[Q]$ [14]. It is unlikely that metallic surfaces or colloids can affect this rate, unless the local quencher concentration is changed by the surface.

The well-known Stern-Volmer equation typically describes the fluorescence intensities in the presence, I, and absence, I_0, of a quencher respectively, where k_q is the bimolecular quenching constant and τ_0 is the unquenched lifetime.

$$\frac{I_0}{I} = 1 + k_q\tau_0\,[Q] \tag{5}$$

This equation describes the well-known concept that fluorophores with longer lifetimes are quenched more than those with shorter lifetimes. For a low quantum yield fluorophore in a solution containing quenchers near a metallic surface, then the intensity is expected to increase closer to the surface because of an increase in the radiative rate, which competes more effectively than quenching. Hence, emission from quenched fluorophores will be observed near the metal particles. Interestingly, further increasing the quencher concentration could result in the situation in which only fluorophores near the surface are observed (Fig. 8).

Many biological molecules contain a number of intrinsic fluorophores. For example, suppose a protein has two tryptophan residues and that the exposed surface

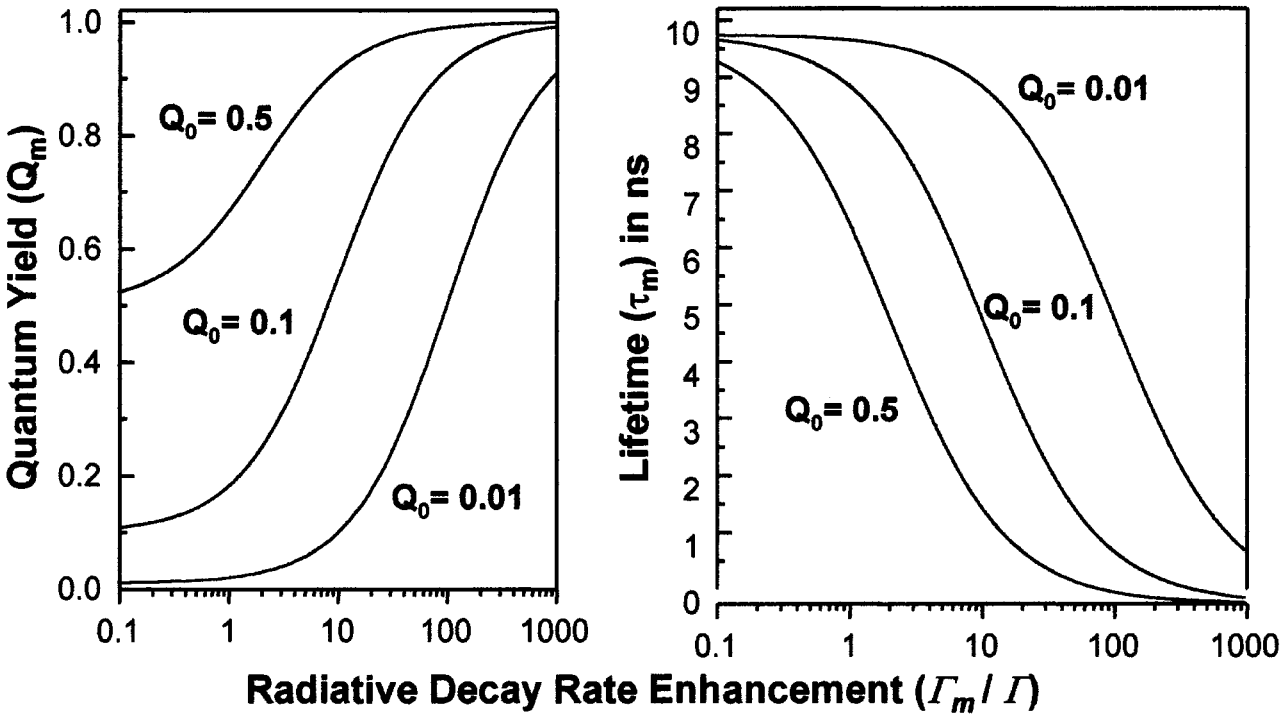

Fig. 4. Metal-induced effects on the fluorescence quantum yield (left) and lifetime (right). Three simulations for quantum yields of 0.5, 0.1, and 0.01 have been assumed with a natural lifetime, τ_N of 10 ns.

is strongly quenched by a quencher. In the absence of metal, emission will only be observed from the higher unquenched residue emitting at shorter wavelengths. However, when the protein is close to the metal, both residues will emit with greater rates but the quenched residue will be increased to a greater extent (Fig. 9), which is likely to alter the spectral shape. Hence, metallic colloids and particles could be used to selectively enhance chromophores of interest in biological systems.

SOLVENT EFFECTS AND SPECTRAL SHIFTS

It is widely accepted that polar fluorophores in polar fluid solvents produce the largest Stokes' Shifts, in which the spectral shifts are due to interactions of the excited state dipole moment of the fluorophore with surrounding polar solvent molecules. For viscous or glassy media, a blue-shifted emission is typically observed because the fluorophore relaxes on a much faster timescale than the time for solvent molecule reorientation. At modest viscosities the emission spectra typically depend on the lifetime of the fluorophore. Hence for polarity-sensitive fluorophores we expect a blue-shifted emission near metallic surfaces because of the reduced lifetime, [c.f. Eq. (4), Fig. 10].

PHOSPHORESCENCE

We typically do not observe phosphorescence from room-temperature solutions because of the quenching by even low concentrations of impurities and because the slow emissive rates cannot compete with the faster k_{nr}. However, if a metallic particle or colloid can increase the radiative rate of the triplet state species, the quantum yield of phosphorescence, in a manner analogous to that of fluorescence, should also increase. The decreased phosphorescent lifetime should also decrease the extent of quenching by oxygen and other quenchers; hence, protein phosphorescence may become commonly observable near metallic surfaces.

MULTIPHOTON EXCITED FLUORESCENCE

In addition to an increase in radiative decay rate, metallic particles are known to concentrate the incident

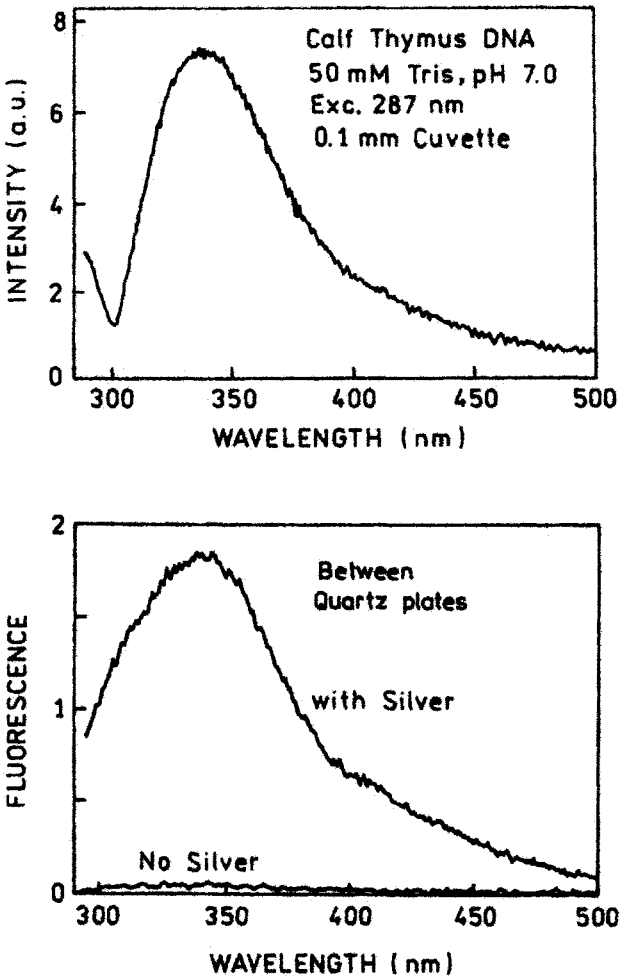

Fig. 5. Emission spectra of calf thymus DNA in a 0.1-mm cuvette (top) and between silvered and unsilvered quartz plates (bottom). The results show that silver islands can enhance the intrinsic or *natural* emission of double helical DNA.

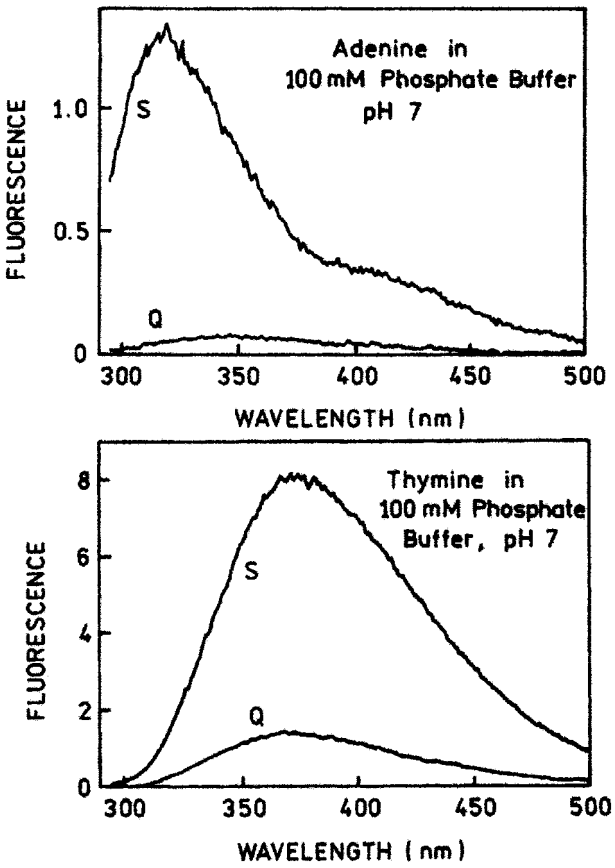

Fig. 6. Emission spectra of adenine and thymine between silvered **S**, and unsilvered **Q**, quartz plates.

light [7–9]. The incident intensity is the square of the incident field strength; thus, it is possible that one can obtain remarkable increases in fluorescence intensity for multiphoton excitation. To test this prediction, we recently investigated the multiphoton excitation of aqueous 1-anilinonaphthalene-8-sulfonic acid (ANS) $Q_0 < 0.01$. In the absence of silver metal, no fluorescence was observed; but in the presence of silver island films, a significant 2-photon enhancement in fluorescence intensity was observed (Fig. 11a). Whilst it could be argued that this effect is due to an increase in the radiative decay of the weakly fluorescing species, further control studies with rhodamine B (high quantum yield, $Q_0 = 0.48$) have revealed significant 2-photon enhancements in the presence of metal, compared to both non-metal containing samples (Fig. 11b) and also compared to that using 1-photon excitation (Fig. 11c). Additionally, recent results have also shown that 2-photon excitation is indeed *localized* and results in an increased probe *photostability* [2,15].

RESONANCE ENERGY TRANSFER (RET)

At present there are only a few experimental results on the effects of metal particles or surfaces on resonance energy transfer, although there are some interesting theoretical predictions [16,17]. In the absence of metal particles, the rate of energy transfer depends on the donor, **D**, and acceptor, **A**, distance, i.e.,

$$k_T^0 = \frac{1}{\tau_0}\left(\frac{R_0}{r}\right)^6 \tag{6}$$

If the D and A are located along the long axis of an ellipsoid with the dipoles also orientated along this axis, then significant enhancements in the rates of RET are predicted (Fig. 12), where the enhancements are given

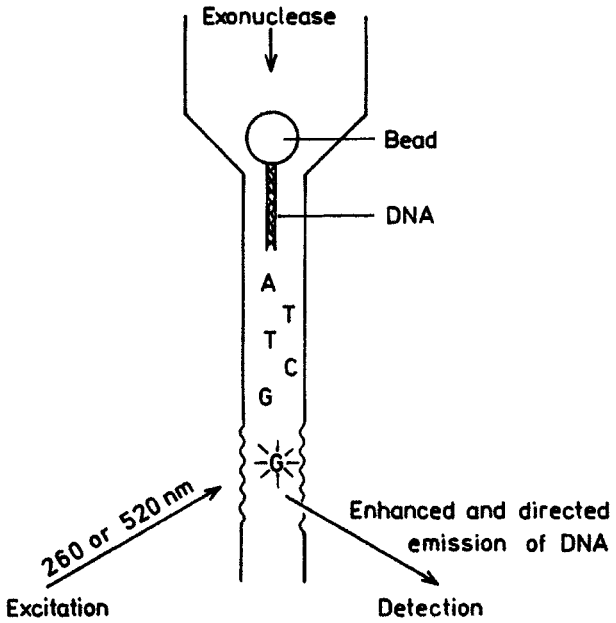

Fig. 7. The possibility of single-strand DNA sequencing using metal enhanced fluorescence (MEF). MEF may also provide for directional emission [1], which may further push the envelope of sensitivity for single base detection and identification.

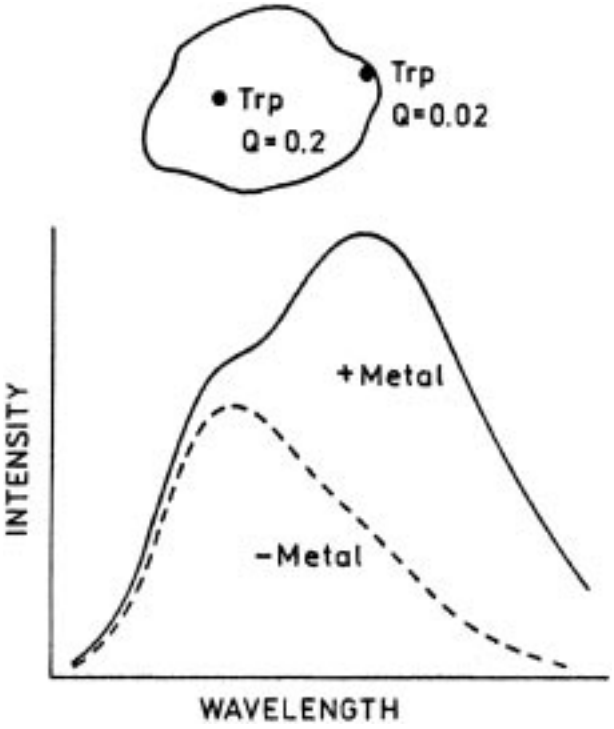

Fig. 9. Effect of an increased radiative rate on a protein with two tryptophan residues, one with a low quantum yield.

as a ratio of the rates of transfer in the presence, k_T^m, and absence, k_T^O, of metal. Enhancements of up to 10^4 have been predicted, where the enhancement depends on the transition energy being in resonance with the particle. A smaller but still significant enhancement is found for a less resonant particle (Fig. 12, *lower curve*) and also on wavelength [1,16,17]. The enhanced rate of RET persists for much greater than typical Förster distances. Although these simulations are for dipoles on the long axis and orientated along this axis, the enhancements are still large when the donors and acceptors have different orientations and locations around the metallic particles [17].

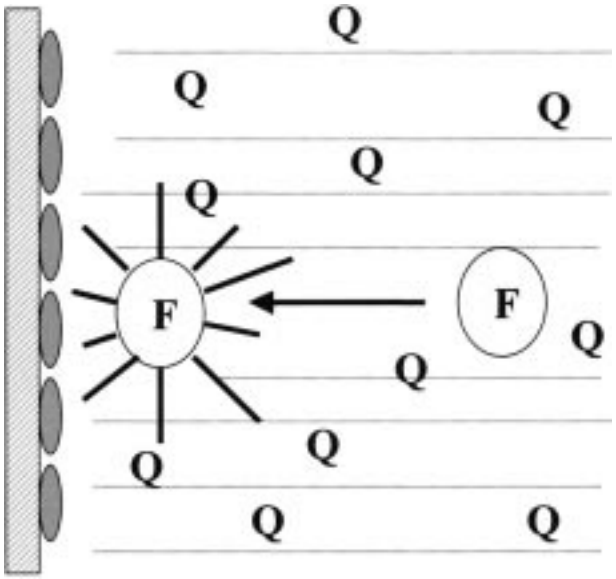

Fig. 8. An increase in fluorophore intensity as the fluorophore approaches the metallic surface. The quencher concentration is constant at all distances. **F**: fluorophore; **Q**: quencher species.

Experimentally, we have observed a dramatic increase in RET between donors [DAPI (4′, 6-diamidino-2-phenylindole)] and acceptors [(propidium iodide (PI)] bound to double helical DNA located above metallic silver islands. Our recent results suggest the presence of at least two populations of D-A pairs, with the pairs close to the silver islands displaying at least a 5-fold increase (165 Å) of the Förster distance, compared to the observed free-space condition value of 37.4 Å [2,4].

To place these exciting results in context it is important to realize that in nearly all cases of RET, its usefulness as a spectroscopic ruler is determined by the magnitude of the Förster distance, R_0. For the most favorable case of high spectral overlap and high quantum yield, the maximum value of R_0 is near 55 Å for organic fluorophores and up to 90 Å for lanthanide donors [18,19]. Hence, because of this rather short upper limit of R_0, it

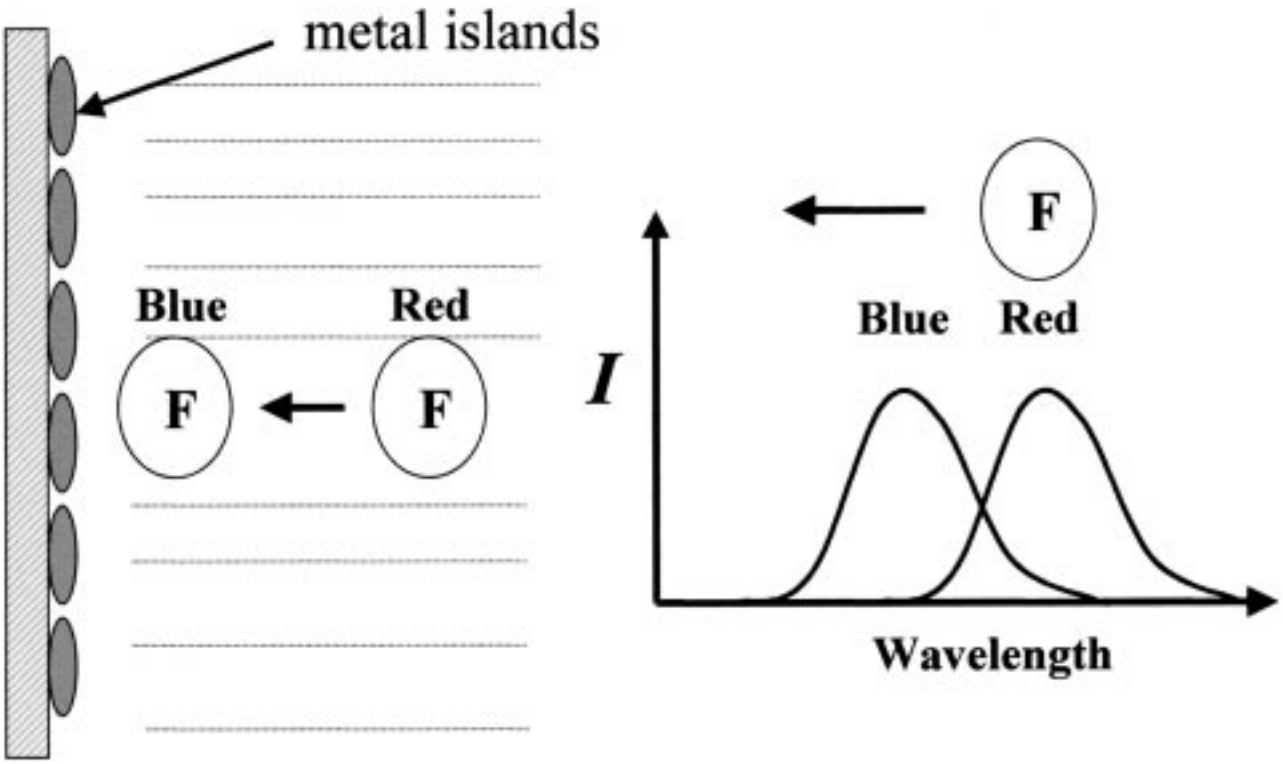

Fig. 10. Effect of an increased radiative rate (reduced lifetime) on the emission spectra of a polarity-sensitive probe. **F**: fluorophore.

has been difficult to use RET for cases in which D and A molecules are more than 100 Å apart and in DNA, where the donors and acceptors are separated by more than 30 base pairs. However, it appears that these "common," but rather short, free-space Förster distances may now be substantially increased by placing the donor and acceptors pairs above silver island films. This is likely to result in further applications of RET in biochemical and biomedical research, such as in metal enhanced energy transfer with DNA arrays or gene chips or increasing the efficiency of light-harvesting assemblies based on RET [20].

Quantifying the Overall Enhancements Resulting from Metallic Surfaces

We have shown that by modifying E_m and Γ_m we can modify the spectral observables of well-characterized free-space fluorescence phenomena. It is now informative to speculate on both the overall enhancements in terms of photostability and detectability and the potential increases in emission intensity resulting from the three known effects caused by near-by metal surfaces and particles.

Fluorophores can undergo a finite number of excitation and emission event cycles before photodecomposition. This is a particularly important consideration in single molecule detection and fluorescence microscopy, in which the signal level is limited by the photodecomposition. For photostable molecules such as tetramethylrhodamine, photodecomposition occurs after about 10^5 event cycles; less stable fluorophores will degrade after significantly fewer event cycles. It is therefore intuitive to comment on how metals can modify the number of photons detected per fluorophore.

First, considering the d^{-3} quenching effect, which occurs for fluorophores positioned less than 50 Å from the surface. Assuming there is no change in the radiative rate, $\Gamma_m = 0$, the emission intensity will decrease as a result of quenching near the surface. However, it is known that quenching by RET results in an increased photostability, because the fluorophores spend less time in the excited state, avoiding potential opportunities for photochemical reactions. In fact, the increased photostability above silver-island films has been reported [21]. Given the increase in photostability, the fluorophore is expected to undergo more excitation/emission event cycles before photodecomposition, but the reduced quenched lifetime is expec-

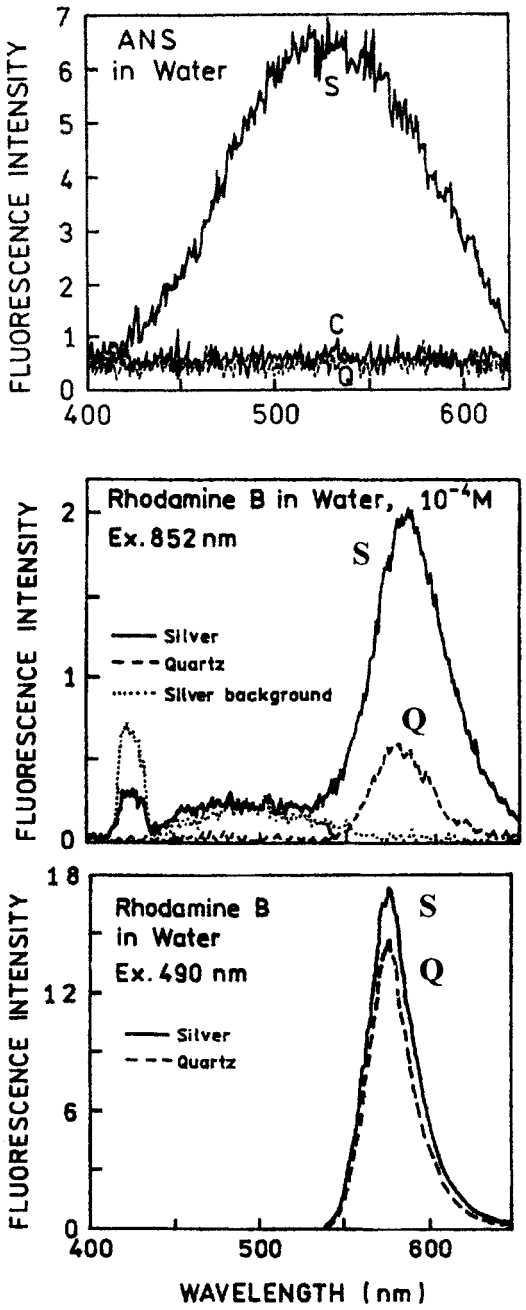

Fig. 11. Multiphoton excited fluorescence of aqueous ANS between silvered, **S**, and quartz **Q**, slides and in a 0.1-mm, cuvette, **C** (a). Interestingly, the MPE fluorescence above silver is substantially more intense than that observed in the 0.1-mm cuvette. Two-photon enhanced rhodamine B emission (b) and, the 1-photon excitation of aqueous rhodamine B (c).

ted to cancel out this effect, resulting in a similar number of observed photons or fluorophore detectability.

Secondly, it is predicted that suitably fabricated metallic particles, with regard to shape and size, can

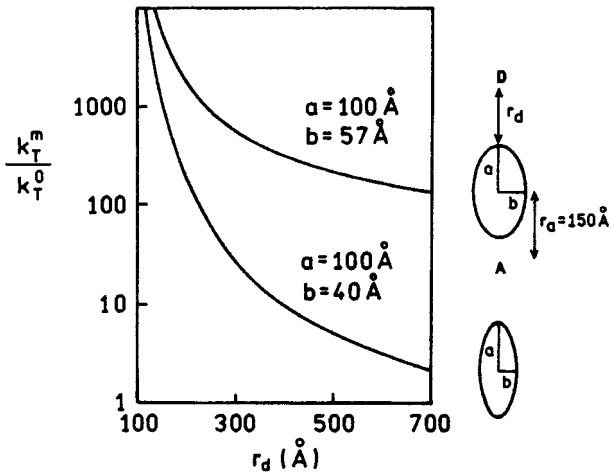

Fig. 12. Enhancements in the rate of energy transfer in the presence of a silver particle.

result in a maximum 140-fold amplification of the *local* intensity [6], which is likely to result in a larger observed fluorescent intensity at a given illumination. Given that the radiative rate is not altered, the lifetime will remain the same. Also, because there is no quenching (lifetime remains unchanged), we expect the same overall number of photos until the fluorophore degrades. One possible effect is that the fluorophore may display ground state depletion at lower incident intensity of the excitation light. However, we have not found any reports of increasing rates of intersystem crossing or blinking resulting from thus, metallic particles. The intensity is proportional to the squared field strength; appropriately designed metallic particles may result in an additional 10,000-fold increase in fluorescence intensity. Enhanced excitation rates are likely to increase the extent of fluorophore photodecomposition. However, given the possible enhancements, one may be able to observe the same emission intensity from fluorophores, but by using a 10,000 times weaker power excitation source, which may be desirable in some biological studies.

Thirdly, it has been predicted that the location of a fluorophore at a suitable distance from a metallic surface can result in over a 10^3 increase in radiative decay rate [5,7,22] (Fig. 13), which, at the same illumination intensity and combined with an enhanced excitation rate, could produce a remarkable cumulative 10^7 increase in fluorescence intensity.

In terms of photostability and detectability, an increase in the radiative decay rate is expected to result in dramatic increases in the number of photons observed from each fluorophore. The decreased lifetime is expected to increase the photostability of fluorophores and therefore allow a greater excitation/emission cyclic rate.

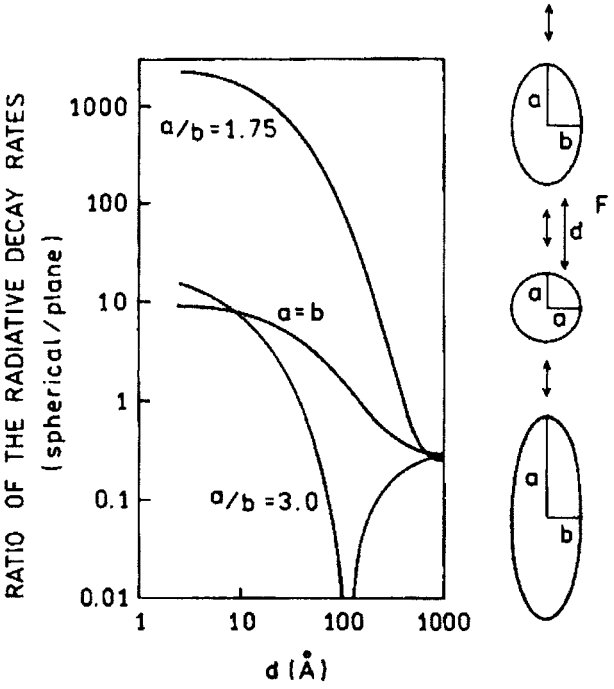

Fig. 13. Effect of a metallic spheroid on the radiative decay rate of a fluorophore.

Hence, the combined effect of an increased quantum yield and reduced lifetime is likely to result in substantially more detected photons per fluorophore.

Finally, directional emission, which can be characteristic of surface enhanced fluorescence [1], could allow for an additional factor of 10 or more, (typical spectrophotometers only detect about 1% of all isotropic fluorescence emission [1]), which, combined with a further potential 100-fold enhancement when using 2-photon excitation, may allow incredible enhancements of $\approx 10^{10}$ in fluorescence emission intensity to be observed. Such remarkable enhancements will most likely facilitate future single molecule detection or, even more profoundly, allow the *visual detection* of single molecules.

CLOSING REMARKS

We have seen that the close proximity of fluorophores to specific metallic geometries can modify the free-space spectral properties of fluorophores in ways that increase quantum yields, Q_m, decrease lifetimes, τ_m, increase rates of energy transfer, k_T^{m}, and increase excitation rates, E_m. However, the fabrication of nanometer scale surfaces/devices to exploit these through-space phenomena is by no means simple at this time and will require the multidisciplinary efforts of physicists, surface chemists, and spectroscopists alike. We think that although this concept is still new and therefore currently unfamiliar to most fluorescence workers, over time, MEF is likely to develop into a field in its own right, in a manner analogous to the evolution of surface enhanced Raman scattering with respect to Raman spectroscopy.

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