Reduced photobleaching of chromophores close to a metal surface

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The photobleaching of chromophores in front of a metal film is measured by recording the emitted fluorescence intensity as a function of time. A strong dependence of the photostability on the distance from the metal surface is found. The experimental data are well described by a classical electromagnetic model with the additional assumption that photobleaching occurs at a constant rate from the excited state. The metal interface influences the photostability of the chromophores in two ways, first by altering the excitation rate by local enhancement of the electromagnetic field and second by altering the electromagnetic decay rate.

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Fluorescence spectroscopy has proven to yield a wealth of information about structure and dynamics on the molecular scale, notably in biological systems. Additional information can be obtained if the sensitivity of the experiment allows for the spectroscopy of single molecules. In this type of experiment it is crucial to obtain as many photons as possible from one molecule before it undergoes irreversible photobleaching. Working at liquid helium temperatures as well as embedding the dyes in a solid matrix, have proved to enhance the lifetime of chromophores by orders of magnitude, whereas photobleaching occurs at a constant rate from the excited state. The metal interface influences the photostability of the chromophores in two ways, first by altering the excitation rate by local enhancement of the electromagnetic field and second by altering the electromagnetic decay rate.

In the work presented here a planar multilayered system is used as a model structure for a quantitative experimental investigation of the effect of a nearby metal surface on the photostability of fluorescing dyes.

The experimental geometry is sketched in Fig. 1(a). The sample used for this study is described in detail elsewhere. In short, an ultrathin gold film (roughness below 1 nm peak to peak) is deposited on a glass slide and covered with a dielectric spacer layer by alternating deposition of positively and negatively charged polyelectrolytes, allowing us to tune the spacer thickness in steps of a few nm. On top of this structure, semiconducting quantum dots are deposited. This sample is measured in a surface-plasmon–fluorescence geometry by index-matching it to a glass prism (refractive index $n_{\text{prism}} = 1.515$), which is mounted on a rotary stage in the Kretschmann configuration. A laser beam (helium–neon, $\lambda = 633$ nm, transverse-magnetic polarization) is directed on the multilayered system through the prism; its angle of incidence $\theta_{\text{ex}}$ can be adjusted continuously. Fluorescence light emitted normally from the prism base is detected with a photomultiplier tube (PMT, Hamamatsu, H 6240 Series), equipped with the appropriate optical filters. In Fig. 1(b) typical measured curves for reflectivity and fluorescence intensity as a function of $\theta_{\text{ex}}$ are displayed.

A related concept has proven experimentally to enhance the brightness of semiconductor quantum dots. Shimizu et al. placed semiconducting quantum dots on a rough metal film. They observed a strongly enhanced luminescence which they ascribed to the enhancement of the electromagnetic emission rate, allowing the quantum dot to emit more photons before entering a dark state. They did not know the local environment of the emitters; therefore, the observed effect could only be discussed qualitatively.

In this case, another approach to increase the number of excitation-emission cycles, proposed in a theoretical paper by Enderlein, should prove to be useful. It relies on the concept of enhancing the electromagnetic decay rate of the molecule by placing it in front of a metallic surface. As a consequence, the molecule will on average stay in the excited state for a shorter time and, assuming that photobleaching happens from the excited state with a constant rate, be able to undergo more excitation-emission cycles before being bleached. As an additional effect, the metal introduces new electromagnetic decay channels to the system, reducing the probability that a photon reaches the far field and is therefore detectable. As a consequence, careful optimization is required to increase the total number of detectable photons for a given chromophore.

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emission wavelengths as well as the nonradiative decay rate of the chromophore as discussed in detail in Ref. 9. The values used in this study are listed in the caption of Fig. 1.

Usually, such measurements are done with laser intensities low enough to ensure negligible reduction of the fluorescence signal over time due to photobleaching of the chromophores. In the study presented here, exactly this decrease of fluorescence intensity with time, induced by a high excitation intensity, is studied. In our previous report it was demonstrated that the classical electromagnetic model as proposed by Chance et al. is appropriate to predict the measured fluorescence intensities for varying spacer thicknesses if photobleaching effects are neglected. For this case, a random distribution of transition dipoles which does not change during the experiment could be assumed.

In the following, the rate equations based on this model are extended to include photobleaching from excited states of the individual chromophores in order to model the experimentally observed bleaching kinetics.

In the following, it is assumed that moderate excitation intensities are used such that the molecule is in the excited state only a very small fraction of the time and that intersystem crossing and saturation effects can be neglected.

In this case, the detectable intensity from a single dye molecule $I_{em}$ can be calculated from the excitation rate $P_{ex}$, the nonradiative emission rate $P_{nr}$, the total radiative emission rate $P_{rad,tot}$, and the rate of emission into the solid angle covered by the photomultiplier, $P_{em,PMT}$:

\[
I_{em}(\mu, x) = C_1 P_{ex}(\mu, I(x)) \frac{P_{em,PMT}(\mu)}{P_{rad,tot}(\mu) + P_{nr}}
\]

\[
= C_1 \frac{I(x)}{I_0} P_{ex}(\mu, I_0) \frac{P_{em,PMT}(\mu)}{P_{rad,tot}(\mu) + P_{nr}},
\]

with some constant $C_1$ taking into account that not every photon is detected due to effects such as the quantum efficiency of the detector or losses at the filters. The rates are functions of the orientation of the transition dipole moment $\mu$ and, due to the inhomogeneous illumination $I(x)$, of the lateral position $x$ of the chromophore. This lateral dependence can be explicitly written by introducing $P_{ex}$ for some arbitrary local $I_0$.

Assuming that photobleaching happens exclusively from the excited state, the photobleaching rate $P_{pb}$ is proportional to the time-averaged probability of finding the molecule in the excited state which is directly obtained from the transition rates. Therefore,

\[
P_{pb} = C_2 \frac{P_{ex}(\mu, I(x))}{P_{rad,tot}(\mu) + P_{nr} + P_{ex}(\mu, I(x))}
\]

\[
= C_2 \frac{P_{ex}(\mu, I_0)}{P_{rad,tot}(\mu) + P_{nr}},
\]

with some constant $C_2$ and assuming again sufficiently small excitation rates.

The rates that are influenced by the nearby interface are displayed in Fig. 2 as a function of the layer thickness. In

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**FIG. 1.** (a) Sketch of the optical setup. The path of the laser beam through the prism and index-matched glass slide is displayed by arrows; the fluorescence light is collected by the photomultiplier tube (PMT). The x and z axes of the coordinate system used are indicated; the y axis is perpendicular to the paper plane. The architecture of the multilayered system is drawn schematically. Part (b) displays a typical angle-dependent reflectivity scan (crosses) together with the simultaneously recorded fluorescence (straight line). Here the spacer thickness is $d_{spacer} = 15$ nm. For all calculations in this study the following parameters were used: $\varepsilon_{prism}(\lambda_{ex}) = 2.26$, $\varepsilon_{prism}(\lambda_{em}) = 2.25$; $\varepsilon_{gold}(\lambda_{ex}) = -12.4772 + i \times 1.0441$, $d_{gold} = 41$, $12$ nm, $\varepsilon_{gold}(\lambda_{em}) = -14.94 + i \times 1.1$; $\varepsilon_{spacer}(\lambda_{ex}) = 2.38$, $\varepsilon_{spacer}(\lambda_{em}) = 2.32$.

**FIG. 2.** (a) Calculated modulus squared of the exciting electromagnetic field perpendicular to the sample surface (z axis, triangles) and parallel to it (x axis, squares) for different spacer thickness, normalized to the electric field amplitude of the plane wave incident on the multilayered system. There is no electrical field along y. The incident light has transverse-magnetic polarization and $\theta_{ex}$ is adjusted for maximum electrical field. (b) Total electromagnetic decay rate $P_{em,tot}$ for a radiating dipole oriented parallel (triangles) and perpendicular (squares) to the sample surface as a function of layer thickness. In the inset of (b), the same data are displayed with a logarithmic scale to show the behavior for very small distances.
Fig. 2(a), the squared electrical field strength at the dipole position parallel and perpendicular to the gold surface is shown assuming illumination with unit intensity. Since $P_{\text{ex}}$ is proportional to this quantity, a corresponding variation of the photobleaching rate with distance is to be expected for low excitation rates. For an ensemble of molecules, at spacer thicknesses below 30 nm, the increased electrical field parallel to the surface is on average almost compensated by the decreased perpendicular field. For thicker spacer layers, a decreased photobleaching simply due to decreased excitation rates. For an ensemble of molecules, at spacer thicknesses below 20 nm is seen which should lead to a significantly increased photostability in this regime. After time evolution of this quantity is given by Eq. (1): 

$$I_{\text{tot}} = \int dx \int d\mu \frac{I(x)}{I_0} \cdot n(\mu, x) \cdot C_1 P_{\text{ex}}(\mu, I_0) \frac{P_{\text{em,PMT}}(\mu)}{P_{\text{rad,tot}}(\mu) + P_{\text{nr}}}.$$  

It is assumed that the absorption and emission dipoles are collinear and the integration is to be taken over all possible dipole orientations. Time evolution of this quantity is given by the time dependence of the population density and can therefore be expressed by 

$$\frac{dI_{\text{tot}}}{dt} = \int dx \int d\mu \frac{I(x)}{I_0} \frac{d}{dt}[n(\mu, x)] \cdot C_1 P_{\text{ex}}(\mu, I_0) \frac{P_{\text{em,PMT}}(\mu)}{P_{\text{rad,tot}}(\mu) + P_{\text{nr}}}.$$  

The temporal evolution of the population density is obtained from Eq. (2) as 

$$\frac{d}{dt}[n(\mu, x)] = C_2 \cdot \frac{I(x)}{I_0} \cdot P_{\text{ex}}(\mu, I_0) \frac{P_{\text{em,PMT}}(\mu)}{P_{\text{rad,tot}}(\mu) + P_{\text{nr}}} n(\mu, x).$$  

The solution of this differential equation would require exact knowledge of the intensity profile of the exciting laser light, which is not trivial to obtain. Nonetheless, the initial change in fluorescence intensity can be extracted without solving Eq. (5). This calculation only requires the initial chromophore distribution, which can be assumed to be homogeneous and isotropic for the as-prepared sample; therefore, $n = n_0$. In this case, Eqs. (4) and (5) yield 

$$\frac{dI_{\text{ges}}}{dt} \bigg|_{t=0} = C_2 \cdot \int dx \left( \frac{I(x)}{I_0} \right)^2 \int d\mu \frac{n_0 \cdot P_{\text{em,PMT}}(\mu)}{P_{\text{rad,tot}}(\mu) + P_{\text{nr}}} \cdot \left( \frac{P_{\text{ex}}(\mu, I_0)}{P_{\text{rad,tot}}(\mu) + P_{\text{nr}}} \right)^2.$$  

This quantity is directly calculated by classical electromagnetic theory. $C_3$ is a constant prefactor.

In order to extract a quantity that is independent of the (arbitrary) concentration of chromophores, the initial intensity change is normalized by the total initial intensity $l_{\text{ges}, t=0}$, which is calculated (up to a constant factor) from Eq. (3) assuming again homogeneously distributed chromophores. The resulting quantity—the relative change in intensity—can be interpreted as an averaged photobleaching rate. This will be the quantity to be compared with the experimental data.

Figure 3 shows the measured intensities as a function of time, normalized to the initial intensity. It was observed in the experiment that the intensity showed some irregular changes like an accelerated decrease or even increase in the range of $\pm 1\%$ during the first 20 s. This may be due to thermal effects in the optical components in the exciting beam, probably heating of the gold layer and the underlying glue. For this reason, in all data sets the first 20 s were not used for the further evaluation; this procedure is justified in the present case since the overall change in intensity during this time is small enough that the assumption of a homogeneous and isotropic dye distribution is still justified. For two values of the spacer thickness, five data sets each are shown, already indicating a slower decay for the sample with $d_{\text{spacer}} = 17.2 \text{ nm}$ compared to the one with $d_{\text{spacer}} = 26 \text{ nm}$. From these data, initial slopes were calculated by fitting an exponential function with an offset to the experimental points and calculating the slope of this function for $t = 0$. This fitting function was chosen for convenience; it describes the initial behavior satisfactorily and allows for a precise determination of the initial slope. It is not based on a physi-
cal model and not suited to describe the experimental data for longer time intervals after significant bleaching has taken place.

The comparison between experiment and theory for the relative initial change in intensity is given in Fig. 4. Good agreement between the theoretical prediction and experimental result is found, with a maximum photobleaching occurring at a spacer thickness of roughly 25 nm. Closer to the interface, metal-enhanced emission leads to a longer chemical lifetime, while for thicker spacer layers the increased photostability can be mainly ascribed to the decreasing excitation rate. The experimental point at a spacer thickness of 9.8 nm is not described by the model; actually, a slight increase of fluorescence intensity with time was observed experimentally, indicating a failure of the classical electromagnetic model. In our earlier study on fluorescence intensities in this multilayered system, a similar disagreement for very thin spacer layers was found. This led already at that point to the conclusion that the classical model is not applicable for layer thickness below 15 nm in our experimental geometry. Currently, efforts in our laboratory have been taken to find an explanation for this experimental fact.

Nonetheless, for larger spacer thicknesses the conclusion can be drawn that the experiment gives quantitative support for the model describing the photostability of organic dyes that was outlined above. Essentially, for spacer layers below 20 nm, the strongly increased electromagnetic decay rate allows the chromophore to undergo more excitation-emission cycles before photobleaching while the longer lifetime for larger spacer thickness is due to a decrease in excitation rate. Based on the effect of increased stability, by increasing the electromagnetic decay rate, more elaborate, three-dimensional metallic structures should be envisaged towards a significant increase of the photons detectable from a chromophore before undergoing irreversible photodegradation.

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