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## ARTICLE

## Effect of metal nanoparticles on the photophysical behaviour of dye-silica conjugates.

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Fluorescein has been covalently entrapped into 120 nm silica beads in order to measure the effect of plasmonic gold nanoparticles, having 25 nm diameter, on the radiative processes of the dye. Two distinct regimes of enhancement and quenching of fluorescein emission have been observed depending on the concentration of the metal adsorbed on silica surface and the overlap between the SPR and the fluorescein spectra. At particle concentrations below  $5.0 \times 10^{13}$  nanoparticles/mL, the fluorescence of the dye is enhanced and this effect is more pronounced when the excitation wavelength matches the maximum of the extinction spectrum of the gold nanoparticles. When the concentration of gold is further increased, quenching occurs and it has been attributed to the SPR shift following the aggregation of the gold colloids on the silica surface. The invariance of the fluorescence lifetimes during the whole process indicates that the mechanism of fluorophore-nanoparticle interaction is mainly based on changes in the absorption efficiency of the organic dye.

### Introduction

The design and preparation of multifunctional nanomaterials is a subject that is gaining increasing attention in literature over the past few years.<sup>1-6</sup> In particular, metal based nanostructures are widely investigated since they show remarkable optical properties due to excitation of their surface plasmons by incident radiation, which results in a significant enhancement of the electromagnetic field at the nanoparticle surface. Among metal nanostructures, gold colloids are particularly attractive since their local surface plasmon resonances occur in the Vis region of the spectrum and because their chemical inertia and stability simplify the rationalization of the effects.<sup>7</sup>

This enhanced near field can alter the efficiencies and/or the rate of electronic transitions of chromophoric species placed in close proximity to the metal surfaces. Thus the controlled preparation of multicomponent nanomaterials based on metal and emitting unit can take advantage of the plasmon enhanced electromagnetic field to obtain highly sensitive sensors, contrast agents for high resolution imaging, or antenna systems for optoelectronic devices.<sup>8,9</sup>

The procedure for the preparation of such multifunctional nanostructures and their characterization has already been described in literature<sup>8,10,11</sup> but only few studies are focused on how the simultaneous presence of metal nanostructures and chromophores/emitting units can affect the final properties of the system. In general the emission of a fluorophore can be quenched or enhanced by the presence of a metal in close

proximity. The appearance of one effect or the other is mostly dependent on the size of the gold nanoparticles,<sup>12-14</sup> the distance between the metal and the dye<sup>15-18</sup> and the relative position of the absorption spectra of the two components.<sup>17,19-21</sup> Quenching can basically occur through energy transfer,<sup>22,23</sup> electron transfer<sup>24</sup> or by increasing the radiative rate of the dye;<sup>25</sup> on the other hand, enhancement of the emission is observed either when the surface plasmon of gold nanoparticles intensify the electric field of the incident light<sup>26</sup> (the so called "lightning rod effect") or when the radiative rate of the dye is increased<sup>27,28</sup> or a combination of both effects.<sup>29</sup> The full comprehension of how metal nanoparticles affect the deactivation pathways of an organic dye is crucial to avoid complete quenching of the emission once the two functionalities are assembled together thus limiting the use of the system as imaging tool. In this perspective it has been recently demonstrated by our group that it is possible to completely decouple the fluorescence emission and the photothermal effect of gold by choosing a fluorophore with appropriate optical properties and the optimal size of silica nanoparticles and gold shell thickness.<sup>10</sup>

In order to use the plasmon effects on chromophoric species in a fruitful manner, a deep understanding of their interaction mechanism is necessary.

In the present study, the effect of the addition of cysteine-stabilized gold nanoparticles on the emission of fluorescein, a well-known fluorophore, has been investigated by steady state and time resolved fluorescence measurements. To better control

the metal-dye distance, the fluorophore molecules were covalently linked to silica nanoparticles, which act as inorganic template. The data indicate that two distinct regimes of enhancement and quenching can be identified depending on the concentration of gold added and the relative spectral position between SPR and the electronic transition of fluorescein.

## Experimental Section

### Materials

Sodium citrate tribasic dihydrate ( $\text{Na}_3\text{Ct}\cdot 2\text{H}_2\text{O}$ ,  $\geq 99.0\%$ ), gold (III) chloride trihydrate ( $\text{HAuCl}_4\cdot 3\text{H}_2\text{O}$ ,  $\geq 99.9\%$ ), L-cysteine (97%), fluorescein isothiocyanate (FITC,  $\geq 97.5\%$ ), (3-Aminopropyl) triethoxysilane (APTES,  $\geq 98\%$ ), tetraethylorthosilicate (TEOS, 98%) and ammonium hydroxide solution ( $\text{NH}_4\text{OH}$ , 28.0-30.0%  $\text{NH}_3$  basis) were all purchased from Sigma-Aldrich and used without further purification.

Nanopure water ( $\leq 18.0 \text{ M}\Omega$ ) from a Millipore Milli-Q gradient system and ethanol ( $\geq 99.8\%$ ) from Sigma-Aldrich were used as solvents.

### Synthesis of Au NPs and ligand exchange

Gold nanoparticles were prepared by the standard method described by Turkevich *et al.*<sup>30</sup> Briefly, 2 mL of a 1% w/w solution of  $\text{Na}_3\text{Ct}$  in water were added under vigorous stirring to 40 mL of a 1 mM boiling aqueous solution of  $\text{HAuCl}_4$ . The mixture was kept boiling for 15 min and then cooled to room temperature. Ligand substitution was carried out by addition of 1 mL of a 0.1 M aqueous solution of L-cysteine to 20 mL of the solution of gold colloid followed by gently heating (40-50 °C) of the mixture under stirring for 4 h. To eliminate the citrate exchanged, the resulting solution was centrifuged at 3000 g for 30 min, the supernatant was discarded and the pellet was redissolved in 10 mL of water. This process was repeated three times. The concentration of gold nanoparticles in solution was estimated measuring the total concentration of Au (III) by ICP-OES analysis after complete dissolution of the gold colloid in 1% v/v aqua regia and using the mean diameter value of the nanoparticles obtained from TEM measurements and a gold bulk density of  $19.3 \text{ g/cm}^3$ .

### Synthesis of the FITC-APTES adduct

The fluorophore was covalently coupled to an organosilane following the procedure described by van Blaaderen *et al.*<sup>31,32</sup> 35 mg of FITC was dissolved in 15 mL of ethanol and 50  $\mu\text{L}$  of APTES was added dropwise. The mixture was allowed to react for 16 h and then the resulting FITC-APTES adduct was collected.

### Preparation of FI-SiO<sub>2</sub> nanoparticles

In a 50 mL beaker, 100  $\mu\text{L}$  of FITC-APTES solution and 0.9 mL of TEOS was diluted under magnetic stirring in a mixture containing 22 mL of ethanol and 1.2 mL of water. The addition of 0.7 mL of  $\text{NH}_4\text{OH}$  started the reaction of hydrolysis and co-

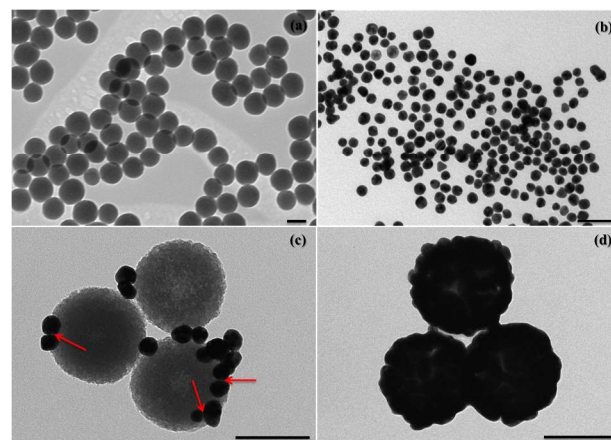
condensation of the two silane reagents. The nanoparticles were collected for centrifugation at 3000 g for 30 min and then redissolved in 25 mL of fresh ethanol. Functionalization of the surface of FI-SiO<sub>2</sub> nanoparticles with amino groups was carried out by adding 40  $\mu\text{L}$  of APTES to 5 mL aliquot of dye-doped silica colloid in ethanol. The solution was left under stirring for 16 h and then centrifuged at 3000g for 30 min. The pellet consisting of the aminated particles was finally dissolved in 10 mL of water.

### Methods

A transmission electron microscope (Philips model 208, operating at 80 kV of beam acceleration) was used to morphologically characterize the samples. A drop of the sample suspensions were deposited in a 400 mesh copper-coated with Formvar support grid and left overnight in a desiccator to allow the solvent to evaporate. The size distributions were obtained by analyzing at least 150-200 nanoparticles for each sample. A Perkin-Elmer Lambda 800 spectrophotometer was used to collect the absorption (or extinction) spectra of the samples. The corrected fluorescence emission and excitation spectra were acquired through a Fluorolog-2 (Spex, F112AI) fluorimeter and the relative emission quantum yields were determined using quinine sulfate in  $\text{H}_2\text{SO}_4$  (1 N,  $\Phi_F = 0.55$ ) as standard.<sup>33</sup> Fluorescence decay times  $\tau_F$  were measured by the single photon counting method using an Edinburgh Instrument 199S setup. A 455-nm nanoLED with a 1.3 ns pulse duration was used as excitation source and the signal was acquired by a Hamamatsu R7400U-03 detector.

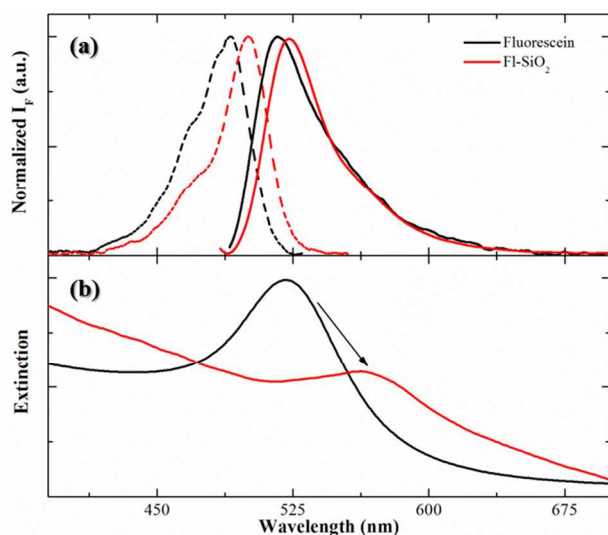
## Results

The hydrolysis and condensation processes of FITC-APTES adduct enable to obtain a colloidal suspension of dye-doped silica beads, which was morphologically and optically characterized. A representative TEM image of FI-SiO<sub>2</sub> sample is reported in Figure 1a. The data demonstrate that spherical silica beads with a mean diameter of 120 nm and a quite narrow size distribution are formed by this procedure.



**Fig. 1** TEM images of bare FI-SiO<sub>2</sub> sample (a), cysteine-stabilized Au NPs (b), FI-SiO<sub>2</sub> sample after addition of a concentration of 5.0x10<sup>13</sup> Au NPs/mL (c) and full silica coverage by Au NPs (d). Scale bar: 100 nm for all images.

Figure 2 shows the fluorescence spectra of a sample of fluorescein doped SiO<sub>2</sub> nanoparticles in water compared with the ones of the free dye in solution. Only slight modifications of the excitation and emission spectra occur when the fluorophore is incorporated inside the silica core and the small shift is most likely due to a “medium effect” resulting from a change in the local microenvironment of the dye molecules. No broadening of the fluorescence spectra is observed and this is an important evidence that aggregation of the dye inside the nanoparticles is negligible.



**Fig. 2** (a) Fluorescence excitation ( $\lambda_{em} = 570$  nm - dashed lines) and emission spectra ( $\lambda_{em} = 460$  nm - solid lines) of fluorescein and FI-SiO<sub>2</sub> sample in water; (b) Extinction spectrum of Au NPs in water (black) and conjugated to the surface of FI-SiO<sub>2</sub> sample at a concentration of 1.5x10<sup>14</sup> NPs/mL.

Fluorescence decays of fluorescein doped-silica particles can be satisfactorily reproduced by a biexponential function. Bimodal decay time distribution models were already proposed for fluorophore adsorbed on silica surfaces;<sup>34,35</sup> in the present system this behaviour is assigned to a distribution of decay times reflecting the fluorophore localization on the silica particles, since aggregate formation had been already excluded (see above). The best fitting of the FI-SiO<sub>2</sub> NPs fluorescence decay gave 2.1 and 4.0 ns decay time values (Table 1). The comparison of these results with literature data suggests the assignment of the longer component to the dye molecules at the particle surface, thus being affected by the solution environment. In fact, the long decay component is close to the decay time values measured for FITC in homogeneous solution. Instead, the shortest component is likely due to dye molecules strongly interacting with silica which can assist side processes, leading to the fluorescence quenching, as previously observed in literature.<sup>36</sup>

**Table 1** Fluorescence lifetimes ( $\tau_F - \lambda_{em} = 530$  nm), and quantum yield ( $\Phi_F$ ) of the dye in solution and inside the nanoparticles.

Sample	$\tau_{F1}$ (ns)	% <sub>1</sub>	$\tau_{F2}$ (ns)	% <sub>2</sub>	$\tau_{mean}$ (ns)	$\Phi_F$
Fluorescein	3.6	100	---	---	3.6	0.97
FI-SiO <sub>2</sub> NPs	4.0	65	2.1	35	3.3	0.60
+ 5.0x10 <sup>13</sup> Au NPs/mL	3.9	61	2.0	39	3.2	---
> 5.0x10 <sup>14</sup> Au NPs/mL	3.8	53	2.0	47	2.9	ca. 0.001

$\tau_{mean}$  is the weighted average of the two decays

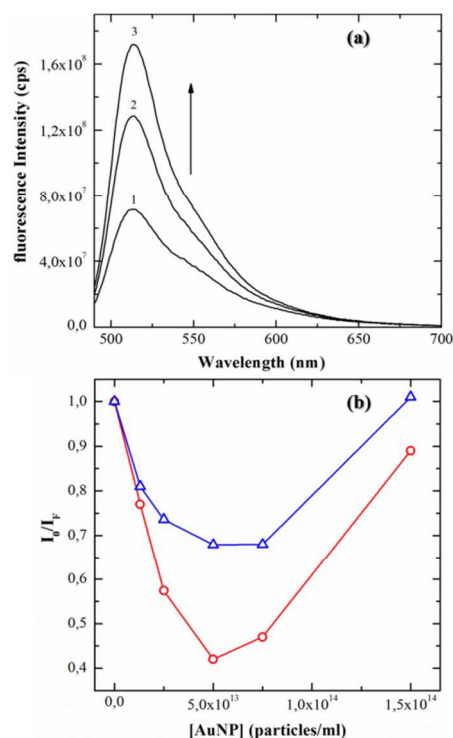
A 40% decrease of the fluorescence quantum yield of the FI-SiO<sub>2</sub> sample (Table 1) indicates that non-radiative deactivation processes become relevant.

Gold nanoparticles have been prepared using the Turkevich method;<sup>30</sup> a mean diameter of 25 nm was obtained for the sample through statistical analysis of TEM images (Figure 1b). The extinction spectrum of the gold colloids (Figure 2b) presents the SPR band centred at 520 nm with a full width at half maximum of 1760 cm<sup>-1</sup>, which indicates a fairly good dimension distribution. In order to avoid the modification of protolytic equilibrium of the dye, the citrate ions, which act as stabilizer on the surface of Au-NP, have been changed with cysteine. The citrate ion is the conjugate base of citric acid which has three acid groups and pK<sub>a</sub> values of 3.1, 4.8 and 6.4.<sup>37,38</sup> In particular, the latter value is similar to the dissociation constant of the carboxylic group of fluorescein (6.43),<sup>39-41</sup> which can result in a perturbation protolytic concentrations of the dye which then affects the fluorescence behaviour. On the other hand cysteine molecules well behave as gold nanoparticle stabilizer<sup>42,43</sup> and they have pK<sub>a</sub> values (1.5, 8.7, 10.2)<sup>44</sup> different from the dye. The stabilizer change does not modify the plasmonic spectrum of the gold colloids.

The cysteine-stabilized gold nanoparticles obtained by ligand exchange were then added to the FI-SiO<sub>2</sub> suspension and let them adsorb on the surface of the silica beads to investigate the effects of plasmonic nanoparticles on the photophysical behaviour of the dye. The present hierarchical assembly of the dye and plasmonic nanoparticles enables to investigate the fluorescence behaviour of the dye in the presence of different concentrations of Au NPs without altering their relative distance.

The effects of the addition on the emitting properties of fluorescein conjugated to the silica beads were monitored through steady state and time resolved fluorescence measurements. Fluorescence spectra obtained by the addition of increasing concentrations of Au NPs to a sample of FI-SiO<sub>2</sub> are reported in figure 3a.

Interestingly, when small amounts of gold colloid are added to the silica beads, a clear enhancement of the fluorescence intensity is observed; the enhancement effect is related to the concentration of gold colloids and reaches its maximum when 5.0x10<sup>13</sup> nanoparticles/mL are present in solution.

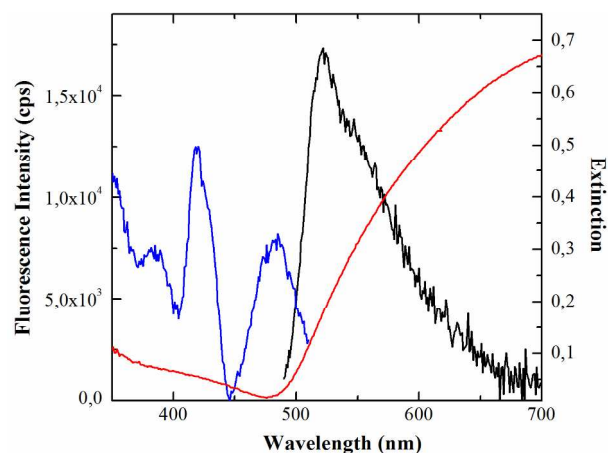


**Fig. 3** (a) Fluorescence emission spectra of FI-SiO<sub>2</sub> sample in presence of 0 (1), 2.5×10<sup>13</sup> (2) and 5.0×10<sup>13</sup> Au nanoparticles/ml (b) Stern-Volmer plots for FI-SiO<sub>2</sub> sample obtained from the emission intensity values at 520 nm exciting the sample at 485 nm (red circles) and 455 nm (blue triangles).

Upon addition of higher concentrations of Au NPs (up to 1.5×10<sup>14</sup> nanoparticles/mL), the emission intensity of the dye decreases back to the original intensity. In these latter conditions the extinction spectrum of the suspension does not resemble the features of isolated Au NPs, but it appears red-shifted and broader (Figure 2b, red line) mostly due to the coupling among gold nanoparticles, although the occurrence of scattering phenomena cannot be excluded. The shape of fluorescein spectrum is not affected by the addition of gold nanoparticles (Figure 3a), indicating that the observed effects are not due to changes of the emitting states. The spectral measurements carried out using different excitation wavelength revealed that the enhancement factor strongly depends on the excitation energy. The fluorescence intensities as function of gold nanoparticle concentrations are presented in Figure 3b using the formalism of the Stern-Volmer model in order to make the comparison easier. The data demonstrate that the effect on the emission of the dye is more pronounced when the excitation wavelength is closer to the maximum of the extinction spectrum of the gold nanoparticles (Figure 2). In particular, an enhancement factor of 2.5 is reached upon excitation at 485 nm while almost half of the increase is observed when the samples are excited at 455 nm. Since no spectral differences are noticed at the two excitation wavelengths and the effects are related to the presence of gold nanoparticles, the enhancement can be attributed to the coupling between the electronic transitions of the dye molecules and the SPR of gold colloids. At 485 nm a higher

overlap between the energy of the localized surface plasmon of the metal surface and of the lowest electron transition of the dye occurs leading to an intensification of the effect.<sup>19</sup> Since the increase of the emission intensity could result from a mixed population of fluorophores at different distances from the gold colloids, the maximum value of the enhancement factor obtained at this point for clusters of gold nanoparticles adsorbed on the silica surface could be higher as previously observed in literature.<sup>45</sup> This hypothesis is further supported by the morphological characterization of the hybrid sample with 5.0×10<sup>13</sup> Au NPs/mL; TEM images (Figure 1c) indicate that the plasmonic Au NPs tend to accumulate on the surface of the doped silica particles. The dye molecules close to the silica surface are able to interact with the gold colloids. When the amount of Au NPs is increased, the degree of agglomeration advances resulting in an interparticle coupling effect, which shift the SPR spectrum of the metal assemblies<sup>46,47</sup> as shown in Figure 2b. In these conditions the fluorescence of the dye is quenched.

When the amount of reduced metal on the surface of dye-doped silica beads is further increased till to have a complete coverage, the extinction spectrum of the hybrid nanostructures is further red shifted and it resembles the one of a gold nanoshell (Figure 1d and 4).<sup>10,48</sup> In this case, the fluorescence spectrum of the dye becomes very weak (Figure 4) resulting in a emission efficiency value of 0.1%. In these conditions the excitation spectrum of the dye is completely distorted. In order to have a deeper understanding of the interactions between the dye and the metal nanostructures, fluorescence decay curves were recorded.



**Fig. 4** Fluorescence excitation (blue line) and emission spectra (black line) of FI-SiO<sub>2</sub> completely covered with Au NPs in water together with the extinction spectrum (red line) of the coupled gold nanostructures.

The effect of Au NPs on the emission decay curves is negligible at all concentrations of gold colloid added. Indeed in all cases the decay curves could be satisfactorily fitted by bi-exponential functions resulting in fitting parameters (Table 1) similar to those obtained for FI-SiO<sub>2</sub> NPs. The lack of observing any remarkable changes in the decay time values

between the regimes where intensity enhancement or quenching has been observed suggests that Au NPs would not modify the deactivation rates of fluorescein emitting state. However at the present stage the possibility that the contributions of quenched and enhanced fractions of dye molecules balance in the decay time cannot be completely ruled out and deeper investigations and analysis of fluorescence decay times are currently in progress.

This observation together with the data on the fluorescence intensities at different Au NPs concentrations allows to propose the hypothesis that in the present system the enhancement of fluorescein emission occurs by an intensification of the local electric field of the incident light and thus of the excitation rate.<sup>49-51</sup> The effect is more pronounced when the dye molecules on the silica surface are located between two adjacent gold nanoparticles as indicated by the red arrows in figure 3c; in this position the local plasmon field is particularly intense as demonstrated by theoretical calculations and literature experimental data.<sup>52-56</sup> When the density of the Au NPs on the silica surface becomes high enough, interparticle coupling occurs causing the bathochromic shift of their extinction spectrum (Figure 2b), which then falls out-of-resonance with the absorption spectrum of the dye. In this regime, fluorescence quenching is observed probably due to reduced absorption efficiency. In conclusion, for the present system in which the dye-metal distance is fixed, a dependence of the fluorescence intensities on the relative spectral position of the dye and the metal has been detected.

## Conclusions

The effects of plasmonic gold colloids on the fluorescence behavior of fluorescein have been investigated by steady state and time resolved fluorescence measurements. In particular, the fluorophore molecules were covalently linked to silica nanoparticles, which act as inorganic template and it enables to fix the distance between the metal and the populations of fluorophores differently exposed to the external medium; the preparation resulted in silica particles with an average diameter of 120 nm, which show a bright fluorescence whose excitation and emission spectra are similar to the ones of the dye in homogeneous solution. Cysteine-stabilized gold nanoparticles having an average diameter of 25 nm and the extinction spectrum with maximum at 520 nm have been prepared in order to evaluate the effects of increasing concentrations of gold colloids on the emission properties of fluorescein-doped silica particles.

The data indicate that two distinct regimes of enhancement and quenching can be identified depending on the concentration of gold added and the relative spectral position between SPR of the metal nanostructures and the electronic transitions of fluorescein. The lack of observing any changes in the fluorescence decay times of the dye suggested that the modifications in the fluorescence intensities are likely due to

effects on the absorption efficiency induced by the plasmonic field.

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## Notes

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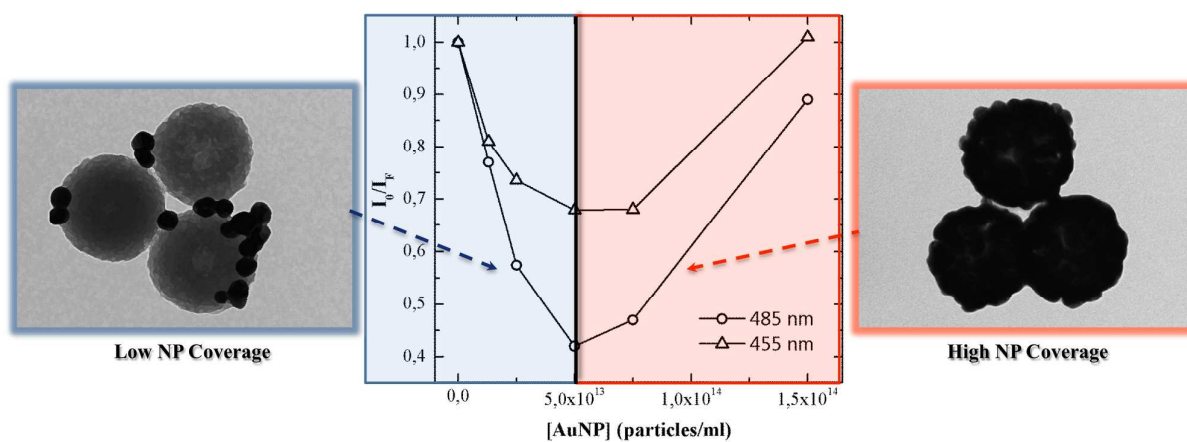
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## Effect of metal nanoparticles on the photophysical behaviour of dye-silica conjugates.

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The controlled adsorption of gold colloids on dye-doped silica particles enables to distinguish a fluorescence enhancing or quenching regime depending on the position of metal plasmon bands.