Coupling of Molecular Transition with the Surface Plasmon Resonance of Silver Nanoparticles inside the Restricted Environment of Reverse Micelles

Debabrata Singha, Dillip Kumar Sahu, and Kalyanasis Sahu*

Department of Chemistry, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India

Supporting Information

ABSTRACT: Interaction of molecular transitions of two fluorophores—fluorescein (FL) and safranin O (SAF)—with the surface plasmon resonance (SPR) of silver nanoparticles (AgNPs) inside a water/sodium dioctylsulfosuccinate (AOT)/n-heptane reverse micelle (RM) has been studied using ultraviolet–visible and fluorescence spectroscopies. Here, we exploit the natural capacity of a RM to act simultaneously as a template for nanoparticle formation and host the fluorophores. The fluorophores and reducing agent were loaded together into the water pool; thereafter, silver salt was added, and subsequently, spectral modification and size evolution were monitored by steady-state and time-resolved optical spectroscopy. In the FL–AgNP composite, the SPR band of AgNPs undergoes a strong red shift. Moreover, significant modifications of both the fluorescence intensity and lifetime of FL were found when AgNPs formed inside the RM core. On the contrary, in the SAF–AgNP composite, no such effect was noticed, and the composite system retains the original optical characteristics of their constituents (i.e., both the position of molecular transitions and SPR maximum remain unchanged). This differential effect has been rationalized by the dissimilar plasmon–fluorophore coupling in the two systems, controlled by a combination of spatial distribution and spectral detuning of the molecular absorption maxima of the dyes (455 and 530 nm for FL and SAF, respectively) from the SPR band maximum (~400 nm) of AgNPs.

1. INTRODUCTION

The collective coherent oscillation of conductive electrons on the surface of noble metal nanoparticles (NPs), known as surface plasmon resonance (SPR), can induce a large electromagnetic field around NPs upon irradiation. The enhanced fields may interact strongly with the molecular transition of a closely spaced molecular fluorophore. The near-field interaction of the SPR with the molecular transitions may produce optical properties in the composite, which are entirely different from their constituents.1 The fluorophore–plasmon (FP) coupling can induce a large variation in the absorption and fluorescence intensity, and lifetime of the molecular fluorophore.2–4 The FP composites are convenient multifunctional nanoprobes for optical signal modification,5–6 cellular imaging and photodynamic therapy,7–8 sensing,9 infrared detection,10 molecular switches,11 and so forth. The field strength of SPR depends markedly on the size, shape, and local dielectric environment of the nanostructure.6,12 SPR is reported to generate extinction cross-section up to 100 times higher than the physical cross-section of NPs.13 Nanostructures with various morphologies, for example, thin films,14–16 single nanorod,17 nanorod assemblies,18–19 and spherical NPs,2,16,19 have been utilized to form composites with molecular fluorophores.

The essential requirements of an effective FP coupling are close proximity of the fluorophore to the plasmonic particle and a significant overlap of the SPR with the absorption or emission spectrum of the fluorophore. A strong FP coupling is usually characterized by the appearance of a new hybrid absorption band different from either the molecular absorption or metal NP SPR extinction spectrum.9,13 The energy of the new state can be effectively tuned by modifying the spectral overlap or the distance between the dye and metal nanostructures.3,9,13 Moreover, the presence of a metal nanostructure in the close proximity of a fluorophore can alter the nonradiative decay rates of the fluorophore and can change both the fluorescence lifetime and quantum yield (QY).3 Modulation of the QY particularly depends on the distance between the fluorophore and the nanostructure; at a distance of a few nanometers (>5 nm) from the NP surface, emission enhancement is reported, but at very short distances (<5 nm), fluorescence quenching is usually dominant.3,8,18 Moreover, plasmon can also alter the absorption strength of a molecular absorber, but this phenomenon requires close proximity of the two units. Interestingly, both reduction20,21 and enhancement22 of molecular absorption (or molar extinction coefficient) are reported near metal NPs.
We have chosen two charged fluorophores—FL and SAF—for identifying the FP interactions inside the water pool of the water/sodium dioctylsulfosuccinate (AOT)/n-heptane reverse micelle (RM). The charged fluorophores are expected to be partitioned preferentially inside the RM rather than in the continuous nonpolar phase (n-heptane), and they exhibit a significant overlap with the AgNP SPR (Scheme 1). These fluorophores are extensively used in cellular imaging and in a number of studies involving FP interactions. Jana and co-workers showed that FL coupled with polyacrylate-coated hydrophobic spherical 3–6 nm Ag/Au NPs and hydrophilic Au nanorods synthesized using the Igepal/cyclohexane RM is a robust system for cell imaging and protein detection. They also reported quenching of fluorescence and reduction of fluorescence lifetime when FL is attached to the nanostructures.\textsuperscript{8,23} Similarly, fluorescence quenching of SAF by AgCl NPs inside the AOT RM is also reported.\textsuperscript{2} However, some reports rather claimed a distance-dependent enhancement of QY using structurally analogous fluorescein isothiocyanate bound to silica-coated AgNPs.\textsuperscript{24} Lodeiro et al. showed that FL-functionalized gold and silver NPs can be effectively used as an optical mercury chemosensor.\textsuperscript{25} The binding capacity of FL with silica was used in the synthesis of highly fluorescent FL–silica NPs in Brij35 RM solution.\textsuperscript{26} Moreover, effective photosensitization of TiO\textsubscript{2} by FL in the AOT RM is also reported in the literature.\textsuperscript{27} Interestingly, in spite of the extensive use of the FL dye in FP coupling studies, there are several inconsistencies within different reports available in the literature. Lim et al. claimed 83\% emission quenching when FL interacts with citrate-stabilized AuNPs.\textsuperscript{28} On the other hand, Ragab et al. reported a 3-fold emission enhancement when FL interacts with AgNPs synthesized using the same synthetic protocol of citrate stabilization.\textsuperscript{29} However, Parkin and co-workers found no interaction of FL with citrate-capped AuNPs or AgNPs.\textsuperscript{30} Motivated by these reports and also to obtain further insights, we designed the present system.

Scheme 1. Overlap of the Extinction Spectrum of Silver Nanoparticles (AgNPs) with the Absorption Spectra of Fluorescein (FL) and Safranin, Along with the Chemical Structure of FL and Safranin O (SAF) Molecules

![Scheme 1](image-url)

Figure 1. Evolution of the extinction spectra of different systems with time: (a) AgNPs inside the AOT RM ($w_0 = 6$), (b) AgNPs and SAF in the RM, and (c) AgNPs and FL in the RM. The variation of SPR maxima with time for various systems is shown in plot (d).
Here, for the first time, we will show a novel controlled way to study the FP coupling between the SPR of AgNPs and molecular fluorophores inside the restricted environment of RMs. We have utilized the natural capacity of a RM to act simultaneously as a template for NP formation and to encapsulate the dye. The advantage is that no tricky synthetic skills, for example, surface attachment, electrostatic interaction, or immobilization of the molecular species within a dielectric matrix, are needed to keep the molecule near the NP. However, for an accurate determination of the FP interaction, still several problems may be associated, such as the change in the aggregation state of either the fluorophore or the NP. Thus, the NP–fluorophore system is hard to compare with the unassociated fluorophore or NP system. Here, we have used only ~1 μM of fluorophore, and at this low dye concentration, aggregation may be ignored. Moreover, the dye and reagents required to form the NP are added together into the RM core, and the in situ spectral and size evolutions of the RM system were continuously monitored to detect any spectral or size changes occurring at any instance.

2. RESULTS

2.1. Extinction Properties of the FP Composite Systems. The growth of AgNPs, after dissolution of Ag+ into the water pool of the RM, was monitored by recording the extinction spectra at different time intervals. Figure 1a shows that the SPR of AgNPs gradually develops with time; a clear SPR peak at ~400 nm can be easily visible after 6 min. With time, the SPR band intensity continues to grow for ~18 min, but the SPR peak position remains invariant (Figure 1d).

To study the FP composite systems, the fluorophore concentration was kept low (~1 μM) to ensure that only the monomeric form of the fluorophore prevails inside the RM. As soon as the silver ions dissolve into the water pool of the RM containing a fluorophore, AgNP formation takes place, and FP interaction, if present, may be observed. Both the systems containing AgNPs only and SAF–AgNP composites have a similar reduction time of ~18 min (Figure 1b). The absorption maxima of SAF were at 533 nm inside the RM both in the absence and presence of AgNPs. When the SAF absorption spectra in the RM solution (in the absence of the silver precursor) were subtracted from the absorption spectra of the AgNP–SAF composite at different times, a spectral pattern similar to that of the SPR of AgNPs (in the absence of fluorophore) could be recovered (Figure S1).

In the FL–AgNP composite, the SPR peak also attains the maximum at ~18 min. However, unlike the SAF–AgNP system, with further progress of the reaction, the SPR band of the FL–AgNP composite depletes along with a remarkable red shift (Figure 1c). At the initial time (e.g., ~6 min), the FL–AgNP conjugate shows several bands at different wavelengths designated as λ1 (~402 nm), λ2 (~456 nm), and λ3 (~485 nm). These peaks may be assigned as the SPR of AgNP (λ1) and the monoanionic form of the FL dye (λ2 and λ3).14 These three peaks persisted for up to ~18 min (i.e., time needed to complete the reduction process). The λ1 peak shifting started at ~18 min, the shifting process continued upto ~100 min, and finally, the λ1 peak was shifted by ~30 nm. Saini et al. reported a similar type of depletion along with the splitting of AgNP SPR when the chlorine-P6 dye was gradually added to poly-L-lysine capped AgNPs.18 From the spectral broadening and shifting of the SPR band observed here, it appears that NPs may undergo aggregation. However, structural characterization reveals that FL has a trivial effect on the size and distribution of AgNPs, which will be discussed later in the article.

To represent the FP coupling more effectively, we performed a simple convolution analysis (Figure 2). We assumed that if there is no interaction between the SPR and fluorophore, the extinction spectrum of the SPR–fluorophore composite should be similar in appearance to the sum of the spectra of the two separate systems. It is evident from Figure 2 that the bands of the composite system do appear at the same position like that of the individual systems. However, for the AgNP–FL system, the spectrum of the composite system does not have a significant similarity with the calculated combined spectrum. The combined spectrum shows three peaks at ~430, ~452, and ~485 nm, which are close to the peaks of the AgNPs (400 nm) and FL (455 and 485 nm) alone. The composite system shows a broad peak at ~430 nm and very weak shoulders at ~455 and ~483 nm, indicating the formation of a very different hybrid system.

Modification of the spectrum can be attributed to either aggregation of AgNPs or hybridization of the SPR and molecular absorbance via FP coupling. Kitching et al. reported modification of the SPR because of aggregation of AgNPs or AuNPs.33 However, Wang and other researchers claimed that the FP coupling induced a spectral modification.5,9 Therefore, we want to check which of the possibilities is dominant in our system. We want to determine if there is any change in the size distribution of NPs in the presence of FL.
2.2. Structural Characterization of AgNPs and the FL−AgNP Composite System. Figure 3 shows the size distribution of the FL−AgNP composite at different times when AgNPs gradually form within the RM. The details of the average size during the growth of the FL−AgNP composite are available in the Supporting Information (Table S1). For the AOT RM, the hydrodynamic radius of the RM ($r_h$) is correlated with $w_0$ ([water]/[AOT]) as follows\textsuperscript{32}

$$r_h = 0.5w_0 + 1.0$$

(1)

Therefore, at $w_0 = 6$, the hydrodynamic diameter is expected to be $\sim 8$ nm. Our measured hydrodynamic diameter was found to be 6.9 nm, approximately matching with the expected diameter. As seen from Figure 3, after 18 min (time needed for the complete reduction of AgNPs), the hydrodynamic diameter increases from 6.9 to 8.7 nm. The enlargement of the RM may be due to the formation of AgNPs within the core of the RM.\textsuperscript{33} Thereafter, no abrupt change in the hydrodynamic diameter was observed after a longer time delay (Table S1), although a minor increase in the polydispersity was observed after a very long time ($\sim 1000$ min). Thus, time-dependent dynamic light scattering (DLS) measurements give a clear indication that the spectral change in the SPR band in the presence of FL is not merely because of the change in the morphology of AgNPs. DLS measurements were further augmented by the transmission electric microscopy (TEM) measurement of the AgNP-containing RMs with and without FL (Figure S2). Thus, FL has only a trivial effect on the mean size and distribution of AgNPs. The average diameter of the AgNPs was found to be $6.1 \pm 2.6$ and $6.5 \pm 3.1$ nm, respectively, in the presence and absence of FL.

2.3. Emission Properties of FP Composite Systems with Time. To check whether the AgNP formation and the corresponding SPR have any impact on the optical properties of the fluorophore, we measured the excitation (Figure S3) and emission (Figure 4) spectra of the fluorophore inside the RM in the absence and presence of AgNPs. No notable peak shift is observed either for the excitation or emission spectrum when AgNPs are formed inside the RM. Invariance of the peak position gives a clear indication of the invariance in the chemical form of FL and SAF before and after AgNP formation. However, the emission intensity of FL decreases with the gradual formation of AgNPs within the RM (Figure 4).

The fluorescence intensity steadily decreases up to $\sim 40$ min; thereafter, no appreciable change in quenching was observed. This can be rationalized on the basis of the fact that during the growth of AgNPs, the free FL molecule may be adsorbed on the surface of AgNPs. The attachment process of the dye is further boosted by the coexistence of both dye and AgNPs inside the confined environment of RMs. The quenching is more prominent for FL; almost $\sim 62\%$ of the initial fluorescence decreases after AgNP formation. The quenching is almost insignificant ($<10\%$) for SAF in the presence of AgNPs. The quenching of the fluorescence intensity of FL after AgNP formation is very apparent when the systems were viewed
2.4. Time-Resolved Fluorescence Properties of the FP Composite.

Fluorescence anisotropy decays of FL and SAF are shown in Figure 5 before and after the complete growth of AgNPs. It is evident that the anisotropy decay of both the dyes becomes faster upon AgNP formation. The average rotational correlation time ($\langle \tau_r \rangle$) for FL in RMs is found to be $\sim 2.46$ ns (Table 1). This agrees with the average rotational time ($\tau_r = 2.50$ ns) in AOT RMs at $w_0 = 7$, as observed by Dutt.31 The large change in the anisotropy decay of SAF may be associated with the marked reorganization of the RM interface upon AgNP formation. The interface may become less organized upon formation of AgNPs, which may expose SAF more into the external nonpolar phase. This may increase the local motion of the fluorophore, resulting in a faster fluorescence anisotropy decay. Note that the rotational relaxation time of free SAF in a homogenous solvent is quite fast.35 On the other hand, because FL resides in a water pool, it may have a minor change in the microenvironment.

Figure 5 shows the fluorescence decays of both FL and SAF in the RM before and after the complete growth of AgNPs. The decay of SAF almost remains unaffected upon NP formation. This is consistent with the negligible change in the steady-state emission. However, the fluorescence decay of FL becomes much faster after formation of AgNPs. The decay of FL in RMs can be fitted with a biexponential function with time constants of 1.20 ns (16%) and 3.80 ns (84%) (Table 2). Interestingly, in the FL–AgNP system, FL decay becomes more nonexponential, and a reasonable fit needs at least three exponentials with components—0.35 ns (34%), 2.71 ns (28%), and 3.9 ns (38%). The average decay time shortens significantly from 3.40 ns in RMs to 2.20 ns in AgNP-RMs. For SAF, only a marginal decay of SAF was observed by changing the system from RMs to AgNP-RMs.

The very different anisotropy behavior of the two fluorophores upon AgNP formation can be rationalized on the basis of different locations of the fluorophores inside the RM before AgNP formation. FL mainly remains inside the water pool, whereas SAF predominately resides at the interfacial region of the RM.31,32 The different anisotropy behavior of the two fluorophores upon AgNP formation can be rationalized on the basis of different locations of the fluorophores inside the RM before AgNP formation. FL mainly remains inside the water pool, whereas SAF predominately resides at the interfacial region of the RM.31,32 The difference in the anisotropy behavior of the two fluorophores upon AgNP formation can be rationalized on the basis of different locations of the fluorophores inside the RM before AgNP formation. FL mainly remains inside the water pool, whereas SAF predominately resides at the interfacial region of the RM.31,32 The difference in the anisotropy behavior of the two fluorophores upon AgNP formation can be rationalized on the basis of different locations of the fluorophores inside the RM before AgNP formation. FL mainly remains inside the water pool, whereas SAF predominately resides at the interfacial region of the RM.31,32 The difference in the anisotropy behavior of the two fluorophores upon AgNP formation can be rationalized on the basis of different locations of the fluorophores inside the RM before AgNP formation. FL mainly remains inside the water pool, whereas SAF predominately resides at the interfacial region of the RM.31,32 The difference in the anisotropy behavior of the two fluorophores upon AgNP formation can be rationalized on the basis of different locations of the fluorophores inside the RM before AgNP formation. FL mainly remains inside the water pool, whereas SAF predominately resides at the interfacial region of the RM.31,32

The difference in the anisotropy behavior of the two fluorophores upon AgNP formation can be rationalized on the basis of different locations of the fluorophores inside the RM before AgNP formation. FL mainly remains inside the water pool, whereas SAF predominately resides at the interfacial region of the RM.31,32

2.50 ns) of FL in AOT RMs at $w_0 = 7$, as observed by Dutt.31 The fluorescence anisotropy decay becomes slightly faster upon AgNP formation within the RMs (Table 1). This is in accordance with our earlier report using the anionic C343 probe.32 A marginal decrease in the rotation time was observed when the system changed from RMs to AgNP-containing RMs. On the other hand, for SAF, a substantial decrease in $\langle \tau_r \rangle$ from 3.70 to $\sim 2.07$ ns was observed by changing the system from RMs to AgNP-RMs.

Table 1. Time-Resolved Anisotropy Decay Parameters of Safranin and FL in RMs before and after the Complete Growth of AgNPs

| fluorophore | system | $a_1$ | $\tau_1$/ns | $a_2$ | $\tau_2$/ns | $\langle \tau_r \rangle$/ns | $\langle \chi^2 \rangle$ |
|-------------|--------|-------|-------------|-------|-------------|--------------------------|-----------------|
| safranin    | RM     | 0.16  | 5.97        | 0.84  | 3.27        | 3.70                     | 1.08            |
|             | AgNPs  | 0.20  | 6.01        | 0.80  | 3.07        | 2.07                     | 1.05            |
| FL          | RM     | 0.21  | 6.01        | 0.79  | 2.96        | 2.46                     | 1.16            |
|             | AgNPs  | 0.24  | 0.43        | 0.76  | 2.85        | 2.27                     | 1.07            |

$\langle \tau_r \rangle = a_1 \tau_{1r} + a_2 \tau_{2r}$. 

Figure 6. Fluorescence decays of safranin ($\lambda_{ex} = 510$ nm, $\lambda_{em} = 565$ nm) and FL ($\lambda_{ex} = 405$ nm, $\lambda_{em} = 520$ nm) inside the AOT RM solution before and after AgNP formation.
decrease in the average decay time from 3.00 ns in RM to 2.78 ns in AgNP-RMs was observed.

The radiative plasmon model proposed by Lakowicz shows that the presence of metal NPs near the vicinity of the fluorophore can substantially modify the decay rates of fluorophores. Thus, a strong decrease in the FL decay time indicates modification of either radiative ($k_r$) or nonradiative ($k_{nr}$) pathways of FL in the presence of AgNPs. From the QY and excited-state lifetime measurements, $k_r$ and $k_{nr}$ can be easily calculated as follows

$$k_r = \frac{\phi}{\tau}$$

(2)

$$k_{nr} = \frac{1 - \phi}{\tau}$$

(3)

where $\phi$ and $\tau$ are the QY and lifetime of the fluorophore, respectively. Thus, the observed radiative ($k_r$) and nonradiative ($k_{nr}$) decay rates of FL in the RM solution are 0.081 and 0.27 ns$^{-1}$, respectively. Interestingly, the presence of AgNPs substantially modifies the nonradiative decay rates (from 0.28 to 0.440 ns$^{-1}$). Thus, we infer that the observed changes in the FL emission time is primarily because of the nonradiative energy transfer from the photoexcited FL to AgNPs. The nonradiative energy transfer efficiency ($\eta$) and the rate of energy transfer ($K_{ET}$) can be calculated by using the following equations

$$\eta = 1 - \frac{\tau_{DA}}{\tau_D}$$

(4)

$$K_{ET} = \frac{1}{\tau_{DA}} - \frac{1}{\tau_D}$$

(5)

where $\tau_D$ and $\tau_{DA}$ represent the lifetimes of the donor (FL) in the absence and presence of the acceptor (AgNPs), respectively. The calculated energy transfer efficiencies and the energy transfer rate for the FL−AgNP pair are 36% and ~1.6 × 10$^8$ s$^{-1}$, respectively (Table 2). For SAF, the energy transfer efficiency and the energy transfer rate are 9% and 0.3 × 10$^8$ s$^{-1}$, respectively. Thus, the energy transfer can be effectively tuned by a precise selection of the molecular dye. From the steady-state and time-resolved data, it is clear that the FL molecule may be located in the close vicinity of AgNPs. Because the rate of energy transfer depends on the spectral overlap between the donor and the acceptor besides the separation distance between the coupling pair, the FL dye satisfies both the criteria, and hence a high rate of energy transfer occurs. Conversely, a low rate of energy transfer for the SAF−AgNP pair can be rationalized on the basis of the fact that SAF is predominantly present in the interfacial region, and hence the spatial separation from the AgNPs formed in the core is quite large. In addition, the spectral overlap also disfavors an effective coupling.

3. DISCUSSION

Our main objective in the present investigation is to probe the modification of the absorption and emission characteristics of a molecular fluorophore induced by the SPR of AgNPs inside the confinement of AOT RMs. AgNP was chosen as its SPR band shows around 5 times stronger excitation coefficient than AuNPs; additionally, AgNPs are also known to exhibit a stronger FP coupling than AuNPs. Usually, the extent of FP coupling depends on the spectral overlap and distance between the molecular absorber and the metal NP. Thus, the two representative pairs, uncoupled (SAF−AgNPs) and coupled (FL−AgNPs), are produced by a judicious selection of fluorophores. Steady-state and time-resolved studies revealed that indeed a strong interaction between FL and AgNPs takes place, but only a trivial interaction was observed for the SAF−AgNP conjugates.

Usually, the interaction of the metal NP with a closely placed molecular absorber occurs via two well-accepted mechanisms: electromagnetic and chemical. The interaction of the electric field of the surface plasmons with the absorption or emission transition dipole of the molecule constitutes the electromagnetic part. On the other hand, mixing of metal states with electronic states of the molecule is considered as the chemical mechanism. Interaction with AgNPs does not alter the excitation or emission maxima of either of the fluorophores, which implies that the energy levels of the fluorophore remain the same before and after coupling with the SPR. However, a strong reduction in the emission intensity and lifetime was noted for FL upon interaction with the NP. These are in accordance with the electromagnetic influence of the FP coupling.

FL may remain in different chemical forms depending on pH of the medium. A concern is that ascorbic acid (AA) used as a reducing agent here may itself change the chemical form or spectral properties of FL. The effect of AA on the optical characteristics of FL in water and in the micellar medium has been investigated in detail by De and Kundu. They demonstrated that AA has a significant effect on the protonation equilibrium of FL in aqueous medium. In neutral water, FL mainly exists in the dianionic form, but with the increase in the AA concentration, monoanionic, neutral, and even cationic forms may dominate at very high concentrations. However, they have not reported any redox reaction of FL in the presence of AgNPs. We studied the effect of AA on FL inside the AOT RMs at $w_0 = 6$ (see the Supporting Information, Figure S5). In the absence of AA, the absorption and excitation spectra of FL show two peaks of similar strengths at 483 and 455 nm (Figure S5). This feature is mainly because of the monoanionic form of FL. With the increase in the AA concentration, the relative contribution of 455 nm peak increases compared to that of the 483 nm peak. In addition, the fluorescence intensity of FL was also quenched significantly with the increase in the AA concentration. Our observations
imply the gradual conversion of the monoanionic form of the FL molecule into a neutral form with increase of AA and is consistent with the observation of De and Kundu. Because we used a fixed concentration (0.1 mM) of AA, we would not expect that the prototropic equilibrium of AA changes appreciably during the NP synthesis. Formation of AgNPs takes place at the expense of AA, and therefore AA concentration gradually decreases but certainly does not increase in the core of RMs, when AgNPs are formed. This is very clear from the excitation spectra of FL recorded before and after the AgNP synthesis inside the RM (Figure S3). We see that the relative contribution of the 483 nm peak increases very slightly after NP formation. This implies that the monoanionic form increases only marginally after AgNP formation.

We can also estimate pH of the medium before and after NP formation. Because FL is a pH-sensitive probe, its excitation and fluorescence spectra may change with the concentration of AA. This may complicate the observed fluorescence quenching. However, it is well-known that the water pool of the AOT RM displays a bufferlike behavior. This means that pH of the internal water pool is relatively insensitive to the externally added acid or base. A number of studies have been devoted to study such a bufferlike action of the water pool of RMs. Using a ratio-metric analysis of the excitation spectrum of FL, Hasegawa showed that the water pool of AOT RMs behaves as a buffer for a wide range of bulk pH (2−12). We estimated pH of the water pool under different conditions from the calibration curve obtained from the FL excitation spectra (Figure S6). We found that pH of the medium changes very slightly (4.8−5.1) in the presence of various additives such as AA (AA−FL) and AgNPs (AgNP−FL).

The possibility of aggregation of FL in the presence of AgNPs may be another important concern. Thus, we deliberately take a very low concentration of fluorophore (∼1 μM) to avoid any aggregation. Simple calculation (Supporting Information, Table S2) reveals that the number of RMs is 2−3 orders of magnitude higher than the number of FL molecules. Thus, it is very unlikely that two FL molecules may reside in the same core of RMs, when AgNPs are formed. This is very clear from the excitation spectra of FL recorded before and after AgNP formation. The possibility of aggregation further supported that no aggregation of FL takes place (Supporting Information, Figure S3).

Now, we have to consider the distribution of NPs and fluorophores inside the RM system. As residual positive charges may exist on the AgNP surface, surface attachment with negatively charged FL molecules is highly probable (Scheme 2). It was reported that the nucleation process for the AgNP formation inside the RM takes place via Ag4 clusters that are finally converted into AgNPs. Furthermore, invariance of time-resolved anisotropy decays suggests that FL molecules may not penetrate the AOT surfactant barrier when AgNPs are formed; in other words, most of the FL molecules are attached to the AgNP surface. As the residual positive charges on the AgNP interface are stabilized by the negatively charged capping agent AOT, after formation of AgNPs inside the RMs, no water pool practically exists. It is unlikely that the SAF molecule penetrates the negatively charged surfactant layer and attaches with the AgNP surface; rather, it may be present in the interfacial region (Scheme 2). Note that the interfacial region is the place where maximum perturbation is expected when AgNPs are formed inside the RM core. A significantly faster anisotropy decay of SAF after AgNP formation may arise from its presence in the interfacial region. A minor decrease (~10%) in the QY of SAF indicates a trivial interaction with the AgNPs, and the reduction of the QY may be due to the emitted photons reabsorbed by AgNPs by the inert filter effect. This is also supported by the lifetime measurement, where no change in fluorescence decays was observed. On the basis of the above discussion, Scheme 2 was designed showing probable distribution of dyes and FP interaction of AgNPs inside the AOT RM system.

The remarkable modification of the extinction spectrum of the SPR band of AgNPs during the progress of the FL−AgNP composite can be due to the FP coupling change of refractive index around the NPs or aggregation of NPs. Because we do not observe any appreciable difference in the TEM image of the composite system from that of the AgNPs in the RM, we may exclude the possibility of aggregation. However, we cannot rule out the possibility of nanoscale aggregation of small AgNPs (say <3 nm) within the RM core induced by FL because TEM and DLS are not sensitive to these microscopic aggregations. A strong coupling of the SPR band of the metal NPs with the excitation state of the fluorophore may generate two new states with upper and lower energies. A significant change in the local refractive index around NPs may occur when a molecular fluorophore binds to the NP surface. In all of the aforementioned cases, usually a red shift was noticed in the FP composite system. Because we can only resolve the low-energy SPR band (at ~430 nm) but not the high-energy one, we may ascribe the shift of the SPR to the change in the refractive index. However, because of significant spectral broadening, we cannot rule out the presence of a high-energy peak, and hence other possibilities may also prevail.

4. CONCLUSIONS

In conclusion, we have demonstrated an easy and convenient way to study the in situ FP coupling both by confining the fluorophore and gradually developing the NPs inside the RMs. For the two fluorophores, FL and SAF, we observed very different spectral modifications. For the SAF−AgNP composite, the extinction spectrum was simple superposition of the SPR band of AgNPs and the absorption spectrum of SAF. However, for the FL−AgNP composite, the extinction spectrum
modulates strongly; the original SPR band at ∼400 nm depletes strongly, whereas a new peak develops at ∼430 nm. However, the absorption, excitation, and emission spectra of FL do not show any shift of molecular transition frequencies in the presence of AgNPs. Moreover, TEM and DLS measurements revealed very less morphological change of the AgNPs in the presence of FL. Thus, the modification of the extinction spectrum of the SPR band may not be because of the aggregation of AgNPs but attributed to the FP coupling. In the FL–AgNP composite system, quenching of the fluorescence intensity and the decrease of the lifetime of FL was attributed to the energy transfer from the fluorophore to the metal NP. The very different spectral behavior of the FL–AgNPs and SAF–AgNPs was due to a combination of different FP distance distribution inside the RM and different spectral detuning of the molecular transitions from the SPR band of AgNPs.

5. EXPERIMENTAL SECTION

5.1. Materials Used. FL, SAF, and n-heptane (UV spectroscopic grade) were purchased from Merck. AOT (98%), silver nitrate (99.9999%), and L-AA were purchased from Sigma-Aldrich Chemicals. Millipore water with resistivity (18.2 MΩ cm) was used for all solution preparations. AOT was dried overnight at 80 °C in an oven before use to remove the adsorbed moisture. All studies were performed at room temperature (25 °C).

5.2. Preparation of RM Solutions Containing Dyes. RM solution at w_o = 6 (w_o = [water]/[surfactant]) was prepared by adding the requisite amount of aqueous solution of AA into 0.09 M AOT solution in n-heptane. To incorporate the charged dyes into the RM solution, the required amount of a stock solution of the fluorophores in ethanol was spread in a 3 mL glass vial and allowed to evaporate completely. Then, the residual dyes were redispersed in 3 mL of pure RM solutions. The concentration of the dyes was kept at ∼1 μM.

5.3. Synthesis of AgNPs inside Dye-Loaded AOT RMs. The synthesis of AgNPs inside dye-loaded RMs was performed by slight modification from our earlier works. First, a stock solution of silver nitrate in ethanol was made, and the required amount of the solution was spread in a 5 mL glass vial and allowed to dry completely by gentle heat. Thereafter, the dye-loaded RM solution was added to the silver nitrate containing vial and stirred. The overall concentrations of both AgNO3 and AA were 0.1 mM. Upon addition of the dye and AA-loaded RM solution, a bright yellow-to-reddish color develops gradually with time, depicting the formation of AgNPs.

5.4. Instruments Used. Absorption spectra were measured using a PerkinElmer LAMBDA-750 spectrophotometer and fluorescence emission spectra were recorded in a Jobin Yvon FluoroMax4 spectrofluorometer. All absorption and emission measurements were performed using a standard quartz cuvette of 10 mm path length. The QY measurements were carried out by taking FL in 0.1 M NaOH (QY = 0.95) and SAF in CH3CN (QY = 0.24) (refs43 and 44). The QY was calculated from the equation

\[ Q = \frac{I}{A} \times \frac{A_R}{A} \times \frac{n_R^2}{n} \]

where Q is the QY, I refers to the integrated fluorescence intensity, A stands for absorbance, and n is the refractive index of the solvent. The unsubscripted letters and the subscript “R” represent the sample and reference, respectively.

The time-resolved fluorescence spectra were recorded with a time-correlated single-photon-counting (TCSPC) setup (HORIBA Instruments) using picosecond laser diodes, DeltaDiode-405L and 510L, with excitation wavelengths of 405 and 510 nm, respectively. The full width at half-maximum of the setup was typically ∼90 ps. The fluorescence decays were fitted using DAS6 software. The fluorescence decays were recorded by keeping the analyzer at the magic angle (55°) with respect to the polarizer. To measure the fluorescence anisotropy decays, the analyzer was rotated at regular intervals to obtain parallel \( I_\parallel \) and perpendicular \( I_\perp \) decay components of a fluorescence decay separately. The anisotropy function, \( r(t) \), was constructed using the expression

\[ r(t) = \frac{I_\parallel(t) - GL_\parallel(t)}{I_\parallel(t) + 2GL_\parallel(t)} \]

The G value of the setup was determined at 520 nm emission wavelength of FL and was found to be 0.72. Fluorescence anisotropy decays were also fitted using DAS6 software.

TEM measurements were carried out in a JEOL JEM 2100 electron microscope operating at a maximum accelerating voltage of 200 kV. TEM samples were prepared by spreading a drop of the AgNP dispersion onto a copper grid coated with carbon. TEM images were analyzed by ImageJ software with PSAR12 plug-in; the mean and standard deviations were calculated for 500–600 particles. DLS measurements were carried out in a Malvern Nano ZS 90 instrument using a 50 mW DPSS laser (λ = 532 nm) at a 90° scattering angle. The solutions were filtered with poly(tetrafluoroethylene) syringe filters having 0.2 μm pore size before the DLS measurements.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b00902.

Mathematically generated difference spectra, TEM image of AgNPs before and after incorporation of FL, steady-state excitation spectra of safranin and FL before and after AgNP formation, photographs of the solutions in normal light and under UV irradiation, effect of AA on the optical properties of FL inside AOT RMs, estimation of pH inside the water pool of AOT RMs, summary of DLS measurements, and mathematical estimation of the number of RMs and NPs (PDF)

AUTHOR INFORMATION

Corresponding Author
*E-mail: ksahu@iitg.ernet.in (K.S.).

ORCID
Kalyanasis Sahu: 0000-0002-6594-303X

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Central Equipment Facility (CIF), IIT Guwahati for instrumental support for TCSPC and TEM measurements. The work is financially supported by the Department of Science and Technology (DST), India (EMR/2014/000011). We thank the Central Equipment Facility (CIF), IIT Guwahati for instrumental support for TEM measurements.
FL, fluorescein; SAF, safranin O; AgNP, silver nanoparticle; AgNP–RM, silver nanoparticle containing reverse micelles; QY, quantum yield; FP, plasmon–fluorophore; NPs, nanoparticles.

REFERENCES

(1) Fofang, N. T.; Park, T.-H.; Neumann, O.; Mirin, N. A.; Nordlander, P.; Halas, N. J. Plasmonic Nanoparticles: Plasmon–Exciton Coupling in Nanoshell–J–Aggregate Complexes. Nano Lett. 2008, 8, 3481–3487.
(2) Pramanik, S.; Bhattacharya, S. C.; Imae, T. Fluorescence Quenching of 3,7-Diamino-2,8-Dimethyl-5-Phenyl Phenazinium Chloride by AgCl and Ag Nanoparticles. J. Lumin. 2007, 126, 155–159.
(3) Su, H.; Zhong, Y.; Ming, T.; Wang, J.; Wong, K. S. Extraordinary Surface Plasmon Coupled Emission Using Core/Shell Gold Nanorods. J. Phys. Chem. C 2012, 116, 9259–9264.
(4) De Luca, A.; Dhamra, R.; Rashed, A. R.; Coutant, C.; Ravaïne, S.; Barois, P.; Infusino, M.; Strangi, G. Double Strong Exciton–Plasmon Coupling in Gold Nanoshells Infiltrated with Fluorophores. Appl. Phys. Lett. 2014, 104, 103103.
(5) Ni, W.; Yang, Z.; Chen, H.; Li, L.; Wang, J. Coupling between Molecular and Plasmonic Resonances in Freestanding Dye–Gold Nanorod Hybrid Nanostructures. J. Am. Chem. Soc. 2008, 130, 6692–6693.
(6) Lee, K.-C.; Lin, S.-J.; Lin, C.-H.; Tsai, C.-S.; Lu, Y.-J. Size Effect of Ag Nanoparticles on Surface Plasmon Resonance. Surf. Coat. Technol. 2008, 202, 5339–5342.
(7) Schwitzgebel, J.; Wiehe, A.; Gräfe, S.; Gitter, B.; Eppl, M. Calcium Phosphate Nanoparticles as Efficient Carriers for Photodynamic Therapy against Cells and Bacteria. Biomaterials 2009, 30, 3324–3331.
(8) Saha, A.; Baisiruddin, S. K.; Sarkar, R.; Pradhan, N.; Jana, N. R. Functionalized Plasmonic–Fluorescent Nanoparticles for Imaging and Detection. J. Phys. Chem. C 2009, 113, 18492–18498.
(9) Ni, W.; Chen, H.; Su, J.; Sun, Z.; Wang, J.; Wu, H. Effects of Dyes, Gold Nanocrystals, Ph, and Metal Ions on Plasmonic and Molecular Resonance Coupling. J. Am. Chem. Soc. 2010, 132, 4806–4814.
(10) Choi, Y.; Kang, T.; Lee, L. P. Plasmon Resonance Energy Transfer (PRET)-Based Molecular Imaging of Cytochrome C in Living Cells. Nano Lett. 2009, 9, 85–90.
(11) Zheng, Y. B.; Yang, Y.-W.; Jensen, L.; Fang, L.; Juluri, B. K.; Flood, A. H.; Weiss, P. S.; Stoddart, J. F.; Huang, T. J. Active Molecular Plasmonics: Controlling Plasmon Resonances with Molecular Switches. Nano Lett. 2009, 9, 819–825.
(12) Singha, D.; Barman, N.; Sahu, K. A Facile Synthesis of High Optical Quality Silver Nanoparticles by Ascorbic Acid Reduction in Reverse Micelles at Room Temperature. J. Colloid Interface Sci. 2014, 413, 37–42.
(13) Rodarte, A. L.; Tao, A. R. Plasmon–Exciton Coupling between Metallic Nanoparticles and Dye Monomers. J. Phys. Chem. B 2017, 121, 3496–3502.
(14) Zhao, J.; Das, A.; Schatz, G. C.; Silar, S. G.; Van Duyne, R. P. Resonance Localized Surface Plasmon Spectroscopy: Sensing Substrate and Inhibitor Binding to Cytochrome P450. J. Phys. Chem. C 2008, 112, 13084–13088.
(15) Pan, S.; Wang, Z.; Rothberg, L. J. Enhancement of Adsorbed Dye Monolayer Fluorescence by a Silver Nanoparticle Overlay. J. Phys. Chem. B 2006, 110, 17383–17387.
(16) Yang, Z.; Ni, W.; Kou, X.; Zhang, S.; Sun, Z.; Sun, L.-D.; Wang, J.; Yan, C.-H. Incorporation of Gold Nanorods and Their Enhancement of Fluorescence in Mesosstructured Silica Thin Films. J. Phys. Chem. C 2008, 112, 18895–18903.
(17) Ni, W.; Ambjörnsson, T.; Apell, S. P.; Chen, H.; Wang, J. Observing Plasmonic–Molecular Resonance Coupling on Single Gold Nanorods. Nano Lett. 2010, 10, 77–84.
(38) De, S.; Kundu, R. Spectroscopic Studies with Fluorescein Dye—Protonation, Aggregation and Interaction with Nanoparticles. J. Photochem. Photobiol., A 2011, 223, 71–81.

(39) Marques, B. S.; Nucci, N. V.; Dodevski, I.; Wang, K. W. C.; Athanasoula, E. A.; Jorge, C.; Wand, A. J. Measurement and Control of pH in the Aqueous Interior of Reverse Micelles. J. Phys. Chem. B 2014, 118, 2020–2031.

(40) Hasegawa, M. Buffer-Like Action in Water Pool of Aerosol OT Reverse Micelles. Langmuir 2001, 17, 1426–1431.

(41) Petit, C.; Lixon, P.; Pileni, M. P. In Situ Synthesis of Silver Nanocluster in AOT Reverse Micelles. J. Phys. Chem. 1993, 97, 12974–12983.

(42) Zhao, J.; Jensen, L.; Sung, J.; Zou, S.; Schatz, G. C.; Van Duyne, R. P. Interaction of Plasmon and Molecular Resonances for Rhodamine 6G Adsorbed on Silver Nanoparticles. J. Am. Chem. Soc. 2007, 129, 7647–7656.

(43) Broglia, M. F.; Gómez, M. L.; Bertolotti, S. G.; Montejano, H. A.; Previtali, C. M. Photophysical Properties of Safranine and Pheno safranine. J. Photochem. Photobiol., A 2005, 173, 115–120.

(44) Brannon, J. H.; Magde, D. Absolute Quantum Yield Determination by Thermal Blooming Fluorescein. J. Phys. Chem. 1978, 82, 705–709.