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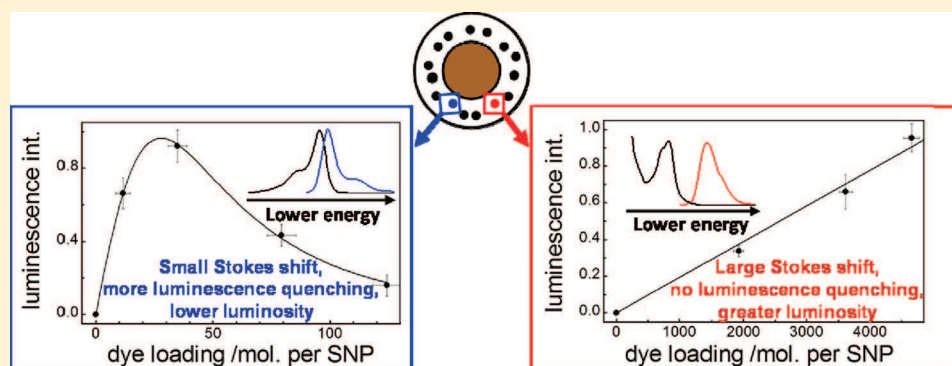
Toward Brighter Hybrid Magnetic-Luminescent Nanoparticles: Luminosity Dependence on the Excited State Properties of Embedded Dyes

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Supporting Information

ABSTRACT:



A series of hybrid, magnetic, and luminescent silica nanoparticles (SNP) have been synthesized and their photophysical properties characterized. The nanoparticles contain, on average, one iron oxide nanoparticle as a magnetic core. The embedded dyes include fluorescein, Alexa Fluor 546, tetramethylrhodamine (TMR), and 4,4-difluoro-5-(2-pyrrolyl)-4-bora-3a,4a-diaza-s-indacene (Bodipy), which are known to have a singlet excited state, and 5-(dimethylamino)naphthalene-1-sulfonyl (dansyl), 7-(diethylamino)-coumarin-3-carboxy (coumarin), and tris(bipyridine)ruthenium(II) dichloride (Rubipy) derivatives, which have a charge transfer excited state. In general, the photophysical properties of the magnetic-core dye-doped silica nanoparticles, SNP^{dye} , where *dye* is one of the seven dyes studied in this project, are dictated by homogeneous energy transfer between dye molecules and by dye aggregation. Molecules with a small Stokes shift and affinity for aggregation upon silica encapsulation (TMR and Bodipy derivatives) can be only sparsely loaded (<50–60 molecules per a 60 nm in diameter nanoparticle) into the nanoparticle matrix before strong fluorescence quenching takes effect. These dyes yield the least bright nanoparticles whose luminosity strongly decreases as the intraparticle dye concentration increases above the rather low maximum brightness loading. Other common fluorophores (Alexa Fluor 546 and fluorescein) can be loaded into the silica matrix with little to no fluorescence quenching until the intermolecular separation becomes less than ~ 5 nm (~ 100 – 200 fluorophores per a 60 nm in diameter nanoparticle). Finally, significantly more (~ 1200 – 4600) dye molecules with a charge transfer lowest excited state and large Stokes shift (Rubipy, dansyl, coumarin) can be loaded into the 60 nm in diameter nanoparticle with no indication of luminescence quenching. The results of this study suggest that the luminosity of a hybrid nanoparticle is highest when the embedded dye has a large Stokes shift and is not susceptible to aggregation, which both guarantee no or little intrananoarticle luminescence quenching. No luminescence quenching by the iron oxide magnetic core has been observed for any SNP^{dye} series.

INTRODUCTION

Multifunctional nanoparticles comprising both luminescent and magnetic components can be of great utility in a variety of biological and security-based applications.^{1–6} Our group is particularly interested in development of iron oxide-core dye doped silica-shell hybrid nanoparticles (SNP^{dye}). Though a recent investigation reported that increasing the number of negative charges on a family of dyes can influence the luminescent properties of the dye-doped silica nanoparticles,⁷ little work has been done to elucidate how molecular properties of the

lowest excited state of the embedded dye affect the luminosity of hybrid nanoparticles. It has recently been reported that thousands of tris(bipyridine)ruthenium(II) dichloride (Rubipy) molecules could be embedded within a silica nanoparticle matrix with little to no deleterious effects on their quantum yield and luminescence lifetime.⁸ Others have reported that the quantum

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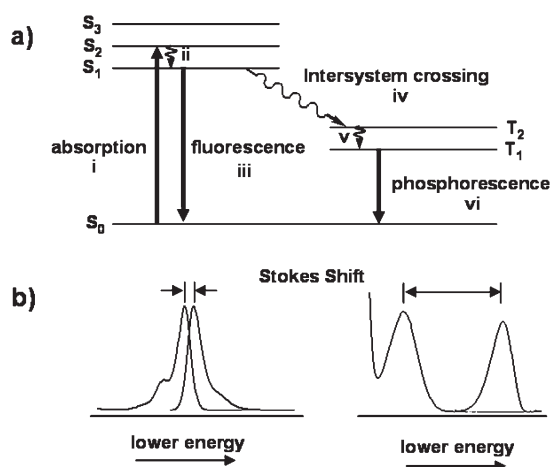


Figure 1. Jablonski diagram (a) describing absorption of a photon (i) to enter an excited state and subsequent relaxation to S_1 (ii) from which fluorescence brings the molecule back to the ground state (iii) or intersystem crossing to an excited triplet state (T_2) (iv). From T_2 , internal conversion relaxes the molecule to the lowest energy triplet state (T_1) (v) followed by phosphorescence that brings the molecule back to the ground state (vi). While emission from the singlet excited state generally results in small Stokes shifts (bi), triplet state emission typically leads to large Stokes shifts (bii). Notable exceptions from this rule are coumarin (in polar solvents) and dansyl discussed in this paper.

yield of cyanine dyes can either decrease⁹ or increase¹⁰ and the quantum yield of rhodamine derivatives may either increase^{11,12} or remain essentially unchanged upon silica encapsulation.⁸ There are also conflicting reports on the effect the iron oxide nanoparticle core has on the luminescence of dye-loaded silica nanoparticles; some studies indicate that the core can quench luminescence,^{13,14} while others suggest that the core has little influence on the emission intensity.¹⁵

Many different types of dyes have been incorporated into silica nanoparticle matrices, but only a few studies have focused on how the luminescence of the embedded molecules is affected by the encapsulation. Ma et al. demonstrated that the molecular interactions between dye molecules and the silica matrix can affect the luminescence of the embedded dye; covalent anchoring of a tetramethylrhodamine molecule to the silica matrix can significantly reduce the nonradiative relaxation rate and results in a large increase in the quantum yield of the fluorophore.⁸ Zaccheroni et al. demonstrated that controlling the pyrene distribution in a silica network can lead to either a monomeric or an excimeric emission depending on the local concentration of molecules.¹⁶ Webb et al. reported that by manipulating the internal architecture of tetramethylrhodamine molecules within silica nanoparticles the photophysical behavior of the embedded dye molecules can be controlled.¹¹ Finally, Acikgoz et al. recently demonstrated that the interaction between excited Bodipy molecules in a hydrogel matrix depends on the separation between the embedded molecules: at distances of ~ 10 nm very little homogeneous FRET occurs, whereas, at separations of 5 nm or less, efficient homogeneous FRET is present.¹⁷ However, there remains a need to comprehensively investigate effects of covalent incorporation of dye molecules into the silica nanoparticle matrix as a function of their lowest excited state properties.

Following an excitation of a dye molecule from the ground (S_0) to the first excited singlet (S_1) state, two radiative decay

paths, in addition to nonradiative decay channels, are typically possible (Figure 1a). In the first emission pathway, molecules vibrationally relax within the S_1 excited electronic state and decay back to the ground state via emission of a photon in a process called fluorescence. This pathway is typical for many of the most commonly utilized fluorescent molecules including fluorescein, rhodamine, and the cyanine and Alexa Fluor families. The second pathway involves intersystem crossing to and vibrational relaxation within the lowest energy triplet excited state (T_1), followed by decay to the ground state with emission of a photon in a process called phosphorescence. Phosphorescence is a common decay pathway for molecules that undergo metal-to-ligand charge transfer such as tris(bipyridine)ruthenium(II) dichloride derivatives and other metal-based luminescent compounds such as zinc sulfide and strontium aluminate. Though both fluorescence and phosphorescence involve the emission of a photon, there are significant differences between the two processes. For example, the energy difference between the absorption and the emission maxima (Stokes shift) is typically small (10–25 nm) for fluorescing molecules (S_1 emission) but can be quite large (50–200 nm) for phosphorescing molecules (T_1 emission), as highlighted in Figure 1b.

In this project we studied seven SNP^{dye} nanoparticle series, where *dye* is one of the dyes described below. The dyes were selected to cover a wide range of excited state photophysical properties. The set includes commonly utilized fluorophores¹⁸ that span the visible spectrum and emit from singlet excited state such as fluorescein (F), tetramethylrhodamine (TMR), 4,4-difluoro-5-(2-pyrrolyl)-4-bora-3a,4a-diaza-s-indacene (Bodipy) (B), and Alexa Fluor 546 (AF). These molecules have small Stokes shifts (less than 25 nm), and all have significant overlap between the absorption and the emission spectra. We have also investigated tris(bipyridine)ruthenium(II) dichloride (Rubipy), a phosphorescent molecule which emits from a metal–ligand charge transfer excited state. Rubipy has a large Stokes shift (~ 160 nm) and very little overlap between the absorption and emission spectra. Finally, we have incorporated 7-(diethylamino)coumarin-3-carboxylic acid (C) and 5-(dimethylamino)naphthalene-1-sulfonic acid (dansyl) (D) into the silica nanoparticle matrix. These are unique molecules in the sense that they fluoresce but also have large Stokes shifts (~ 54 and 190 nm, respectively) and little overlap between the absorption and emission spectra. It is of interest to determine how the loading of these fluorophores within the silica matrix will affect the luminosity of the nanoparticle.

EXPERIMENTAL SECTION

Chemicals. Tetramethylrhodamine-isothiocyanate (TRITC), 6-(((4,4-difluoro-5-(2-pyrrolyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)styryloxy)acetyl)aminohexanoic acid, succinimidyl ester (Bodipy), Alexa Fluor 546 carboxylic acid, succinimidyl ester, and fluorescein isothiocyanate (FITC) were purchased from Invitrogen Canada. Tetraethoxysilane (TEOS) and 3-aminopropyltriethoxysilane (APTES) (>99% pure) were obtained from Gelest. Bis(2,2'-bipyridine)-4'-methyl-4-carboxybipyridine-ruthenium *N*-succinimidyl ester-bis(hexafluorophosphate) (Rubipy), 7-(diethylamino)coumarin-3-carboxylic acid *N*-succinimidyl ester (coumarin), and 5-(dimethylamino)naphthalene-1-sulfonyl chloride (dansyl) were purchased from Aldrich. Ammonium hydroxide (NH_4OH , 28–30 wt %) and high purity 2-propanol were both obtained from EMD Chemicals. *N,N*-Dimethylformamide (DMF) and ethanol were purchased from EM Science and Commercial Alcohols, respectively.

Scheme 1. A Cartoon Representation of the Protocol Utilized in the Synthesis of the Hybrid Magnetic/Luminescent Nanoparticles

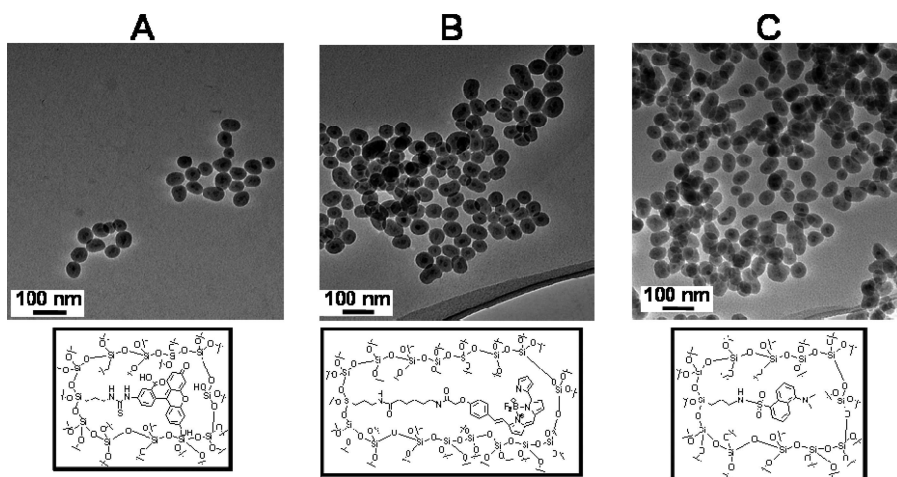
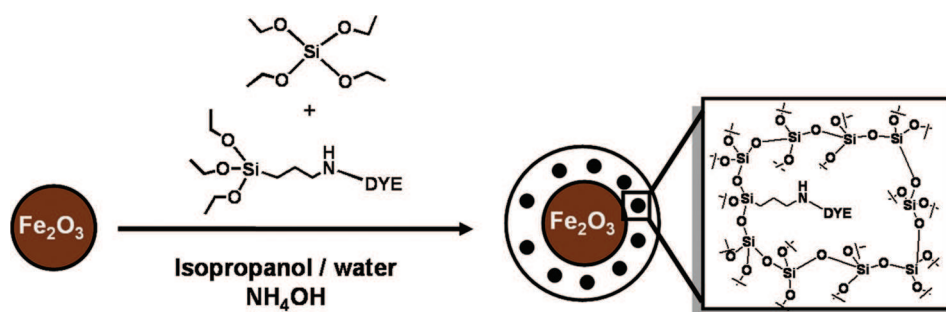


Figure 2. Representative transmission electron microscopy (TEM) images of the hybrid nanoparticles for SNP^F (A), SNP^B (B), and SNP^D (C).

All chemicals were used as purchased. Throughout the preparation of nanoparticles, purified water (Millipore Q-guard 2 purification system, Millipore Corporation, 18 M Ω cm) was used exclusively.

Synthesis of SNPs. Following a protocol developed by van Blaaderen and Vrij¹⁹ and recently extended by others,^{11,20,21} hybrid nanoparticles were prepared through the co-condensation of dye-modified 3-aminopropyltriethoxysilane (dye-APTES) conjugate and the silica network precursor (TEOS) in the presence of water-soluble iron oxide nanoparticles (EMG304 ferrofluid, Ferrotec, USA). The dye-APTES conjugate for each of the seven dyes studied in this work was prepared in the first step of the synthesis. Next, an appropriate amount of the conjugate was added to 45 mL of isopropanol, 4.5 mL of an aqueous solution of iron oxide nanoparticles (Fe₃O₄) and TEOS. Finally, 0.6 mL of a 28% solution of ammonium hydroxide was added to the mixture. Following vigorous mixing on an orbital shaker for 12–18 h, the nanoparticles were repeatedly centrifuged and redispersed in either water or ethanol until the supernatant was no longer luminescent. The purified dye-doped iron oxide-core silica nanoparticles were then redispersed in either water or ethanol, and their absorption and emission spectra were measured. Each SNP^{dye} contained on average one iron oxide nanoparticle. A pictorial representation of the SNP^{dye} synthesis is presented in Scheme 1. A more detailed description of the synthesis for each SNP^{dye} series is provided in the Supporting Information.

An important part of the project was the synthesis of dye-free iron oxide-core silica nanoparticles as well as silica-only nanoparticles, which were used to correct absorption spectra to properly quantify SNP^{dye} absorbance and other related quantities.²² The dye-containing, iron oxide-free silica nanoparticles also facilitated the investigation of the core effect on embedded dye molecules. It is important to consider the effect of the iron oxide core when investigating luminescence of SNP^{dye} because the luminescence of dye molecules within close proximity to the core could potentially be quenched.^{13,14}

Characterization. Transmission electron microscopy (TEM) images of the silica nanoparticles were acquired using a Philips CM20 FEG microscope operating at 200 kV. Samples were prepared by dipping a TEM grid into either a water or ethanol solution of the nanoparticles. Diameters as measured from the TEM images of the SNP^{dye} ranged ~50–65 nm. SNP^{dye} typically consisted of a 10 nm iron oxide nanoparticle surrounded by ~20–30 nm thick dye-doped silica shell (Figure 2). While most SNP^{dye} contained one iron oxide nanoparticle, a fraction of them featured multiple iron oxide cores (Figure 2). The multiple core SNP^{dye} were typically larger than the single core ones, indicating that they possibly originated from fusion of single core SNPs. UV–visible absorption spectra were recorded using a Varian Cary 5000 UV–vis–NIR spectrophotometer. Luminescence measurements were all performed with a Horiba Jobin Yvon Fluorolog Tau-3 Lifetime System. The results of the spectroscopic

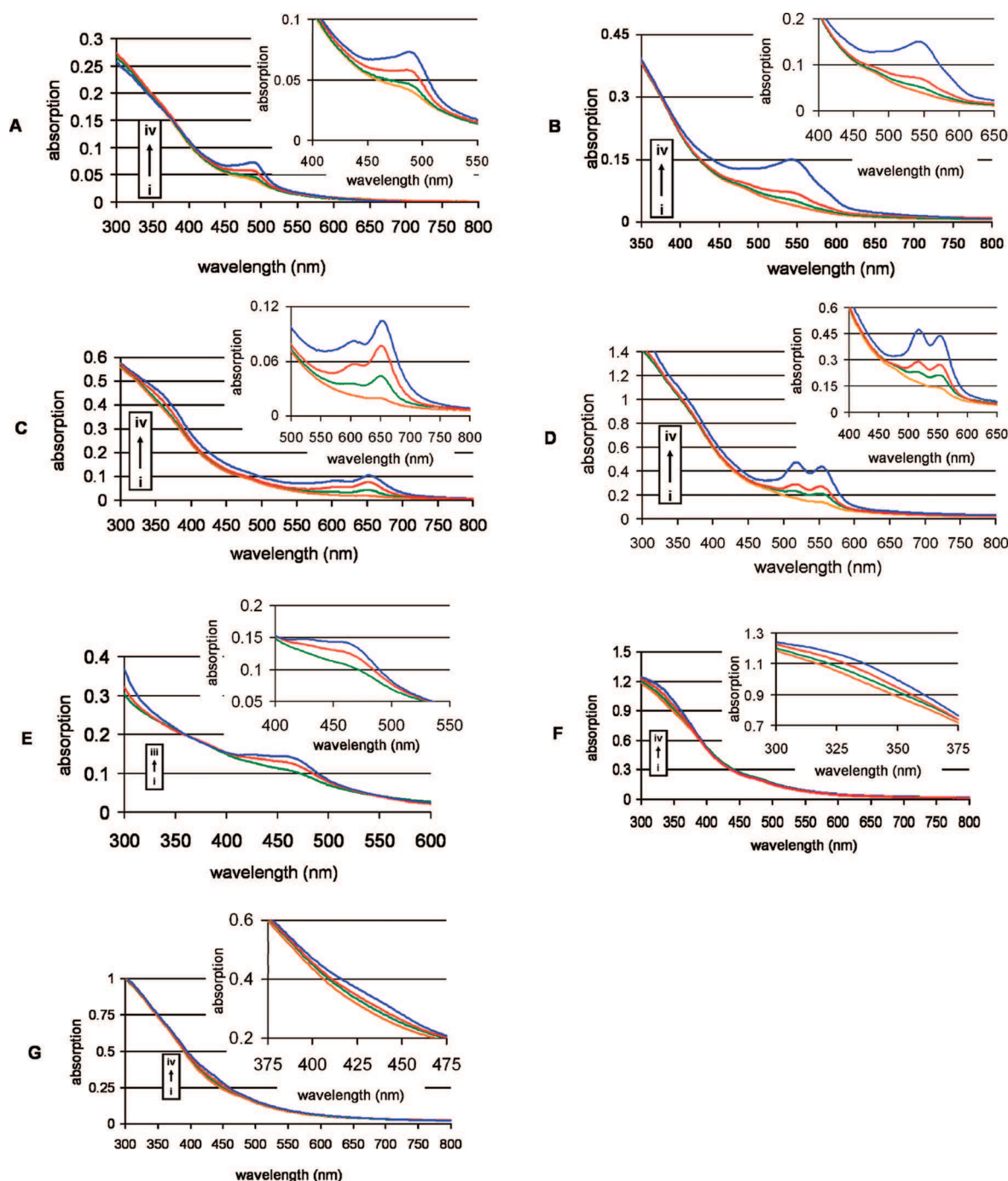


Figure 3. Absorption spectra of SNP^{F} (A), SNP^{AF} (B), SNP^{B} (C), SNP^{TMR} (D), $\text{SNP}^{\text{Rubipy}}$ (E), SNP^{D} (F), and SNP^{C} (G). The insets show magnified spectra of the dye absorption region, and $\text{i} \rightarrow \text{iv}$ indicates an increase in the concentration of the dye-APTES conjugate employed in the synthesis of the corresponding SNP^{dye} , resulting in increasingly higher numbers of dye molecules in SNP^{dye} .

characterization of SNP^{dye} will be discussed in the following section.

RESULTS AND DISCUSSION

First, we focus on SNP^{dye} doped with fluorescein, TMR, Bodipy, and AF. Absorption spectra of the SNP^{F} , SNP^{TMR} , SNP^{B} , and SNP^{AF} are presented in Figure 3. For SNP^{F} and SNP^{AF} the UV–vis absorption spectra generally appear similar to those of free molecules in water except that each of them is superimposed

on the absorption spectrum of the iron oxide nanoparticle and red-shifted by ~ 5 nm (Figure 2A,B). The red shift could be explained by changes in the environment polarity and polarizability of the fluorophores encapsulated in the silica matrix. Similar arguments for the red shift have been presented by Imhof et al.²³ regarding silica nanoparticle matrices and Grauer et al.²⁴ for clay-based matrices. In addition, excitation hopping between embedded dye molecules, which will be discussed later, should also contribute to the red shift.

Table 1. Dye Loading (or the Number of Dye Molecules per SNP^{dye}) for the Nanoparticles with the Highest Luminosity in the SNP^{dye} Series in the Loading Ranges Studied in This Project, the Corresponding Average Intra-SNP^{dye} Dye Concentration, and the Mean Distance between the Dye Molecules within the Silica Matrix Calculated Assuming a Random and Uniform Dye Distribution

| SNP ^{dye} | dye loading (mol per SNP ^{dye}) | concentration (mM) | mean distance (nm) |
|-----------------------|--|-----------------------|--------------------------|
| SNP ^F | 250 ± 12 | 3.7 | 4.3 |
| SNP ^{TMR} | 57 ± 7 | 0.8 | 7.0 |
| SNP ^B | 35 ± 4 | 0.5 | 8.2 |
| SNP ^{AF} | 142 ± 9 | 2.1 | 5.1 |
| SNP ^{Rubipy} | 4652 ± 124 | 89.8 | 1.6 |
| SNP ^C | 1153 ± 38 | 22.3 | 2.6 |
| SNP ^D | 1233 ± 55 | 23.8 | 2.5 |

In contrast, as the number of encapsulated Bodipy and TMR molecules increases, the absorption spectra of SNP^B and SNP^{TMR}, respectively, evolve from a single band with a blue-side shoulder to a double-band structure. Specifically, the UV–vis spectrum of SNP^B evolves from a single band at 652 nm with a small shoulder at 604 nm for the lowest dye loading SNP^B (Figure 3C-i) to a double band with maxima at the same wavelengths and an approximately 3:2 intensity ratio for the highest loading SNP^B (Figure 3C-iv). Analogously, the SNP^{TMR} spectrum evolves from a band with a maximum at 553 nm and a shoulder at 518 nm at the lowest dye loading (Figure 3D-i), to a double band structure with approximately equal intensity peaks at the same wavelengths at the highest loading (Figure 3D-iv). As previously reported, the evolution of the absorption spectra of the Bodipy²⁵ and TMR²⁶ derivatives could be attributed to formation of dimeric (or multimeric) species. Dimerization, multimerization, or aggregation of embedded dye molecules affect not only absorption, but also luminescent spectra of the multifunctional nanoparticles, as will be discussed later.

The absorption spectra of SNP^{dye} contain contributions from absorption by iron oxide nanoparticles and scattering by silica nanoparticles. These contributions must be subtracted from the SNP^{dye} absorption spectra to accurately determine intraparticle dye concentration, SNP^{dye} quantum yield, and other SNP^{dye} properties. This has been done for all SNP^{dye} using the absorption spectra of the iron oxide-core dye-free nanoparticles and silica-only nanoparticles synthesized at otherwise identical conditions as those for SNP^{dye}. It should be noted that the number of iron oxide nanoparticles used in the original synthesis of the hybrid nanoparticles is known,²⁷ so the approximate number of SNP^{dye} produced is also known (assuming, on average, one iron oxide nanoparticle per SNP^{dye}), which allows the approximate dye concentration in SNP^{dye} to be calculated. These data are included in Table 1 and the Supporting Information.

If there were no concentration-dependent intra-SNP^{dye} luminescence quenching, one would expect a linear relationship between the luminosity of the SNP^{dye} series of hybrid nanoparticles and the average intraparticle dye concentration (or the average number of dye molecules encapsulated within a single SNP^{dye}). As it appears, an approximately linear dependence is observed for the first few lowest dye loading points of SNP^F (Figure 4A) up to ~150 or likely even fewer dye molecules per

nanoparticle, which corresponds to intraparticle fluorophore concentration of ~2.3 mM. Although for both SNP^F and SNP^{AF} fluorescence intensity monotonically increases with increasing dye loading in the entire loading range studied in this project, up to ~250 and ~140 dye molecules per nanoparticle, respectively (Figure 4A,B), a negative (downward) curvature is apparent in both cases, throughout the entire loading range for SNP^{AF} and at the highest loading point for SNP^F.

In contrast, for SNP^B (Figure 4C) and SNP^{TMR} (Figure 4D), luminosity increases with increasing dye loading only in the low loading range, fewer than 35 and 57 molecules per nanoparticle, respectively; at higher loadings luminosity strongly decreases. The intraparticle dye concentrations at the loadings corresponding to the highest emission intensity for both SNP^B (35 dye molecules) and SNP^{TMR} (57 dye molecules) are 0.5 and 0.8 mM, respectively, several times lower as compared with those of SNP^F and SNP^{AF}. As mentioned above, for both SNP^B and SNP^{TMR} the UV–visible absorption spectra show a double-band structure at higher loadings (Figure 3C,D), which indicates a presence of dimerization and/or aggregation. Hypothesizing that the luminosity decrease at higher loading may be due to dimerization and/or aggregation, solution-based mimics connecting Bodipy molecules through a diaminoethyl linker were synthesized, and the luminescence intensity of the dimer was shown to be at least three-fold smaller than that of an equal concentration of the Bodipy monomer (Supporting Information). Therefore, dimerization of embedded Bodipy can be partially responsible for SNP^B luminescence intensity decrease at high loadings.

Aside from loading-dependent dye-quenching effects, loading independent interaction of an embedded dye molecule with the silica matrix may change its quantum yield as compared with that in solution. By liberating the embedded dye molecules from the nanoparticle silica matrix and comparing their luminescence intensity with that of the original SNP^{dye}, one can indirectly measure the effect of silica encapsulation, and in particular dye–silica interactions, on the quantum yield of the dye molecules. In short, if the luminescence intensity of the liberated dye is higher (lower) than the luminescence intensity of the SNP^{dye}, the encapsulation decreases (increases) the dye molecule quantum yield. In this project we liberated dye molecules from SNP^{dye} by dissolving the silica matrix with hydrofluoric acid. The dissolution of the SNP^B series resulted in a decrease of luminescence intensity for the 12-fluorophore SNP^B (Figure 5a) to ~0.8 of the nanoparticle value. An approximately 1.7-, 2.7-, and 9.3-fold increase is observed for the 35, 79, and 124 fluorophore nanoparticles, respectively. This is expected since upon SNP^{dye} dissolution an average distance between dye molecules significantly increases, as compared with distances inside SNP^{dye}. Similar results have been observed for SNP^{TMR} (Figure 5b). The 24 fluorophore SNP^{TMR} fluorescence intensity decreases upon liberation of TMR to ~0.85 of the nanoparticle value. The higher loading SNP^{TMR} (57, 91, and 191 fluorophores per SNP) show approximately 1.5-, 3-, and 10-fold fluorescence intensity increase upon fluorophore liberation, respectively. Thus, the silica matrix encapsulation appears to enhance the quantum yield of both Bodipy and TMR by some 10–20% as compared with the quantum yield in solution at low loading. However, as the loading increases, the quantum yield of the fluorophores encapsulated in the matrix decreases significantly.

To further examine this trend in luminescence intensity (and quantum yield) decrease with increasing dye loading, the relative quantum yield for a SNP^{TMR} at various loadings with respect to

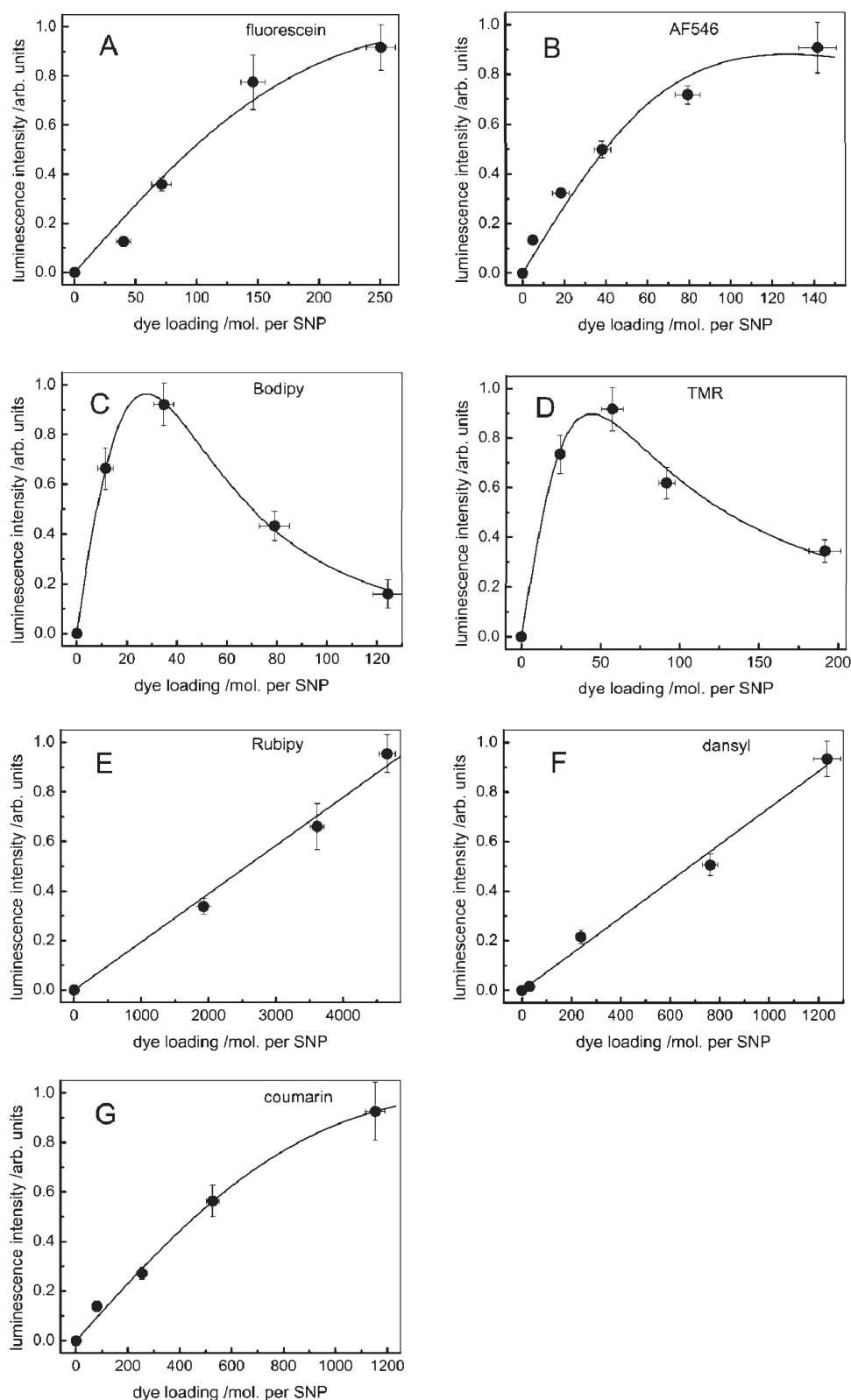


Figure 4. Luminescence intensity as a function of dye loading (number of dye molecules per nanoparticle) for SNP^{dye} . The embedded dye is indicated in each panel. The curve through the data points in each graph is a model fitted to describe the loss in emission intensity as the dye loading increases.

the quantum yield of TMR-APTES in solution was determined.²² For each SNP^{TMR} in the series, the fluorescence intensity at 575 nm (upon excitation at 550 nm) versus

absorbance at 553 nm was plotted, and the relative quantum yield was determined by comparing the slope of the best fitting straight lines for the SNP^{TMR} data to the slope for TMR-APTES.

Again we observed that the relative quantum yield increased with loading for the lowest loading, but strongly decreased when TMR concentration was further increased. These data are presented in the Supporting Information. In contrast, a similar analysis for the SNP^{F} series indicated that the quantum yield of embedded fluorescein was approximately the same as that of free FITC-APTES in solution except for the highest loading SNP^{F} (~ 250 molecules per SNP), for which the average separation between embedded fluorescein molecules was ~ 4.3 nm, and the relative quantum yield was ~ 0.7 that of the free fluorescein-APTES. A similar trend was observed for SNP^{AF} .

It is also noteworthy that the quantum yield appears not to be affected by the presence of the iron oxide core, as for both fluorescein and TMR containing SNP^{dye} synthesized either with or without the iron oxide cores under otherwise identical conditions the relative quantum yield was the same (see Supporting Information).

The maximum intraparticle dye concentration that can be achieved before fluorescence quenching becomes apparent ranges for the fluorophores discussed above (Bodipy, TMR, fluorescein, and AF) from ~ 0.5 – 3.7 mM. This concentration range corresponds to ~ 35 – 250 fluorophores per nanoparticle (Table 1). In contrast, working with Rubipy, Wang and co-workers²⁸ and Ma and co-workers^{8,27,29} were able to electrostatically embed upward of 10 000 molecules within a 60 nm in diameter silica nanoparticle, which corresponds to an intraparticle concentration approaching 100 mM. Such a dramatic loading difference inspired us to include in our investigation Rubipy as well as coumarin and dansyl, which all emit from a charge transfer state and show large Stokes shift.

It should be pointed out that, like all of the other dyes studied here, Rubipy was covalently embedded into the silica matrix, and thus our $\text{SNP}^{\text{Rubipy}}$ synthesis method differed from the one previously reported by Wang and co-workers, who utilized electrostatic interactions to trap Rubipy inside silica nanoparticles.²⁸ In the entire loading range studied in this work, up to ~ 4650 Rubipy molecules per nanoparticle, $\text{SNP}^{\text{Rubipy}}$ luminescence intensity appeared to be proportional to the dye loading (see Figure 4E). Attempts to further increase Rubipy-APTES loading resulted in the generation of Rubipy-doped bulk silica with embedded iron oxide nanoparticles rather than nanoparticles. Thus, covalently bonding Rubipy to silica matrix limited the maximum dye concentration in silica nanoparticles to $\sim 46\%$ of that reported by Wang and co-workers,²⁸ where dye molecules were trapped electrostatically. Although the bulk silica composite was not a subject of this study, we noted that the luminosity versus absorbance linear relationship observed for $\text{SNP}^{\text{Rubipy}}$ extended to the bulk, suggesting that even brighter composites containing a greater concentration of Rubipy can possibly be generated. The number of dye molecules in the highest loading $\text{SNP}^{\text{Rubipy}}$ is ~ 21 times greater than that for the brightest SNP^{dye} loaded with singlet emitting dyes. The average separation between the Rubipy molecules in the maximum loading $\text{SNP}^{\text{Rubipy}}$ is only ~ 1.6 nm (Table 1), which is several times smaller than the average distance in the brightest singlet emitting dye SNP^{dye} .

As mentioned above, the key feature that distinguishes Rubipy from TMR, AF, fluorescein, and Bodipy is the nature of the emitting excited state. Unlike the singlet emitters, excited Rubipy undergoes a metal-to-ligand charge transfer, resulting in a large energy difference between the absorption and the emission maxima corresponding to the Stokes shift of ~ 145 nm. The role of a dye molecule large Stokes shift in synthesis of high luminosity SNP^{dye} was further explored using two popular dyes,

coumarin and dansyl, which, like Rubipy, have large Stokes shifts, 52 and 190 nm, respectively, but 1 to 2 orders of magnitude shorter radiative lifetimes.

The coumarin-loaded (SNP^{C}) and dansyl-loaded (SNP^{D}) nanoparticles showed weak absorption, which is an inherent property of the dyes. Analogous to $\text{SNP}^{\text{Rubipy}}$, the luminosity of both SNP^{C} and SNP^{D} appeared approximately proportional to the number of embedded dye molecules in the entire dye loading range (Figure 4G,F, respectively). To verify whether dimerization could affect the linear growth of SNP^{D} luminosity at still higher loadings, we synthesized dansyl dimer and observed that its absorption and emission spectra were nearly identical to those of the monomer (see the Supporting Information). Because the monomers can be put in such close proximity with no apparent luminescence quenching, it is reasonable to believe that higher concentrations than observed in the scope of this study can be achieved without any deleterious effects on the luminosity of SNP^{D} .

Similarly to the singlet-emitting dye SNP^{dye} , the luminescence of coumarin in SNP^{C} is not affected by the presence of the iron oxide core, as we experimentally determined by measuring the luminosity of purposefully synthesized coumarin-loaded iron oxide-free silica nanoparticles (see the Supporting Information).

Of all dyes studied here, silica-encapsulated coumarin showed the greatest quantum yield enhancement, a ~ 10 fold increase as compared with coumarin quantum yield in water. This observation is consistent with that recently reported by Herz et al.³⁰ It has been proposed that the increase in quantum efficiency is due to a two-fold enhancement in the radiative rate and a reduction in the nonradiative rate due to the rigid silica environment restricting the rotational mobility of the dye.³⁰ As for dansyl and Rubipy, we found that the quantum yield of dansyl was unchanged upon incorporation into the silica matrix, and Wang et al.²⁸ and Ma et al.^{8,29,31} came to a similar conclusion regarding Rubipy. Interestingly, the fact that the quantum yield for embedded coumarin increases so dramatically (10-fold) in comparison to the free dye in solution means that the maximum luminosity of SNP^{C} may be greater than or comparable to that for some other SNP^{dye} discussed above, despite the fact that the other dyes have quantum yields and extinction coefficients significantly higher than those of coumarin. To illustrate the point, we estimate maximum luminosities of SNP^{C} (quantum yield $\Phi = 0.2$, maximum loading $N_{\text{max}} = 1154$, and relative extinction coefficient with respect to fluorescein $\epsilon_{\text{F}} = 0.226$), SNP^{F} ($\Phi = 0.92$, $N_{\text{max}} = 146$, and $\epsilon_{\text{F}} = 1$), and SNP^{B} ($\Phi = 0.46$, $N_{\text{max}} = 28$, $\epsilon_{\text{F}} = 1.229$) as $L = \Phi N_{\text{max}} \epsilon_{\text{F}}$ to obtain 52, 230, and 16, respectively. Thus, in the loading range studied in this project, although the brightest SNP^{C} is not as bright as SNP^{F} , it is over 3 times brighter than the brightest SNP^{B} despite the superior extinction coefficient and quantum yield of Bodipy.

The results of our studies indicate that four factors must be considered when optimizing dye selection and loading to maximize luminosity of a hybrid SNP^{dye} : molecular interactions between dye monomers, monomers and multimers, and monomers and aggregates, which can result in resonance energy transfer and luminescence quenching; dye aggregation resulting in the presence of nonluminescent absorbers; an absorption–emission Stokes shift of the embedded dye, which affects the significance of the two previous factors; and the effect of the silica matrix on the quantum yield of the embedded dye, which can result in quantum yield enhancement for the embedded dye molecules.

Even though the seven SNP^{dye} series investigated in this work show widely varying luminescence properties, it is possible to

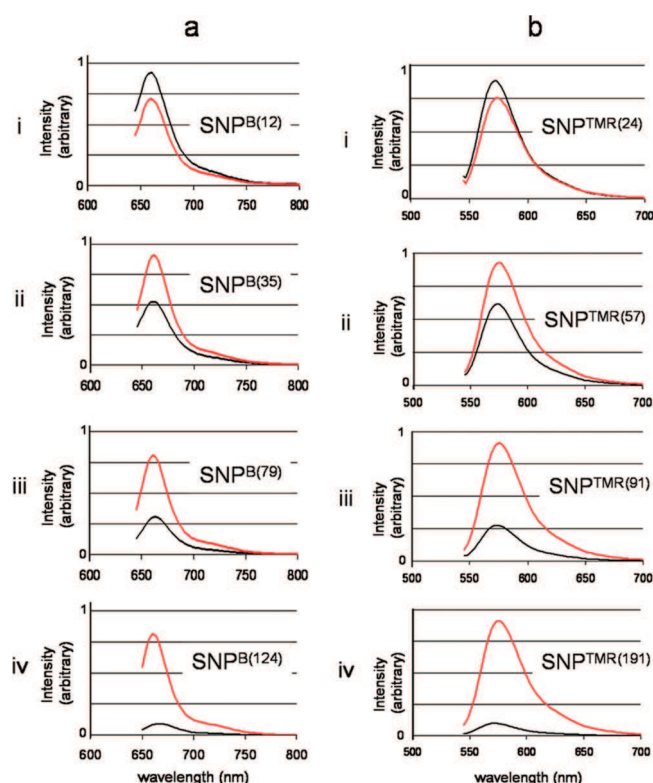


Figure 5. Emission spectra of Bodipy (a) and TMR (b) before (black) and after (red) addition of hydrofluoric acid to liberate the fluorophores from the silica matrix of SNP^{B} and SNP^{TMR} , respectively.

describe the luminosity as a function of dye loading for all SNP^{dye} using one simple model. For all SNPs but those loaded with Rubipy and dansyl, the SNP^{dye} luminosity shows, at some loading, a departure from linearity versus dye loading. This indicates a presence of a nonradiative process or processes, which depend on dye loading and, therefore, on intermolecular distances of embedded dye. The Förster resonance energy transfer (FRET) may be such a process.¹⁷ In addition, dimerization and/or aggregation of Bodipy and TMR is evident from the absorption spectra and may play a role in the decrease of luminosity at higher loadings in SNP^{dye} loaded with Bodipy and TMR as well as other dyes.

Out of three possible FRET processes, energy hopping, singlet annihilation, and triplet annihilation, we expect the hopping to be the leading cause of emission intensity decrease in our case. Because the luminescence spectra were recorded at the weak excitation limit ($\sim 1 \times 10^6$ counts per second), the singlet annihilation could be ruled out (regardless of how large the overlap between monomer emission spectrum and singlet excited monomer absorption spectrum is) since the probability of finding two dye molecules in a close proximity, both of them in the singlet excited state, would be negligible even at the highest dye loadings. As for the triplet annihilation, we expect it should not play an important role either since the longest lifetime observed here for the three highest loading dyes is only a few hundred nanoseconds (Rubipy,⁸ which incidentally does not show any sign of luminescence quenching) and thus, given the weak excitation, the probability of finding a pair of nearby embedded dye molecules both in a triplet state would be rather small.

As stated before, absorption spectra of SNP^{B} and SNP^{TMR} indicate that the number of dimers and/or aggregates within the

silica matrix relative to the number of monomers increases with dye loading. Bodipy was found to efficiently form nonfluorescing H dimers in the silica matrix, which can quench monomer fluorescence via resonance energy transfer.²⁴ Unlike other dyes studied in sol–gel glasses which can form J dimers and higher aggregates, Bodipy exclusively and almost completely forms H dimers. Various rhodamines are known to form both H and J dimers as well as trimers and higher aggregates depending on the environment. In aqueous solutions rhodamines favor the H dimer structure due to the hydrophobic character of their substituents. While rhodamine B appears to form dimers, but not larger multimers, in aqueous solutions, rhodamine 6G efficiently forms dimers as well as trimers, with trimers more effectively quenching monomer fluorescence. Analogous to other xanthene dyes, fluorescence quenching in rhodamines by the dimers and trimers is induced by resonance energy transfer between monomers and aggregates.³² By considering FRET (hopping) to be the primary mechanism of energy transfer between dye monomers as well as monomers and aggregates in SNP^{dye} and recognizing that, in addition to monomer nonradiative decay channels, quenching by dimers, trimers, and possibly higher aggregates contributes to fluorescence depletion, we developed a simple model describing the variation of luminosity with dye loading for all seven types of SNP^{dye} studied here.

Following an excitation, a dye molecule embedded in SNP^{dye} can emit a photon, transfer excitation energy to another monomer or an aggregate, or relax nonradiatively, in which case two possibilities are considered: nonradiative relaxation via monomer channels and relaxation via aggregate channels should another molecule or molecules be close by so a de facto dimer, trimer, or an aggregate can be said to exist. While the aggregate to which energy is transferred is expected to quickly relax nonradiatively to the ground state, the monomer can either emit a photon or further transfer the excitation energy. Integrating the luminescence over an observation time interval we can write the luminescence contribution from the originally excited molecule (F_1) as $F_1 = A_1 \Phi P (1 - E_{\text{RET}})$, where Φ is the quantum yield of the SNP^{dye} embedded molecule (as a monomer), A_1 is the average number of direct excitations of the selected dye molecule, E_{RET} is the FRET efficiency, and P is the probability of the molecule not being a part of a nonradiating dimer, trimer, or a larger aggregate. The luminescence contribution of a monomer to which energy has been transferred from the originally excited molecule (F_2) can be similarly written as $F_2 = A_2 \Phi P (1 - E_{\text{RET}})$, where A_2 , the average number of secondary excitation can be calculated from the equation for F_1 as $A_2 = A_1 \Phi P E_{\text{RET}}$. F_i , the luminescence contribution in the i -th energy hop, can thus be written as $F_i = A_1 (\Phi P)^i E_{\text{RET}}^{i-1} (1 - E_{\text{RET}})$. Summing over all luminescence contributions, F_i , with $i = 1, \dots, \infty$ and over all dye molecules in the SNP^{dye} yields the total luminescence of the SNP^{dye} , F_{SNP} , equal to $F_{\text{SNP}} = A_1 N \Phi P (1 - E_{\text{RET}}) / (1 - \Phi P E_{\text{RET}})$. A similar model for dye-loaded silica nanoparticles has previously been discussed by Nooney et al.³³

The FRET efficiency, E_{RET} , is expressed by a well-known equation $E_{\text{RET}} = 1 / (1 + (r_{\text{mean}}/R_0)^6)$, where r_{mean} is a mean distance between dye molecules in SNP^{dye} and R_0 the Förster radius in SNP^{dye} . It should be pointed out that the determination of distance distribution of N points (here molecules) in various n dimensional solids is an active area of research in geometric probability and, to the best of our knowledge, there exists no analytical formula for such distribution in a three-dimensional shell of outer diameter d_{out} and inner diameter d_{in} , which

Table 2. Results of the Model Fits for Seven SNP^{dye} Series^a

| dye | A_1 | N_0 | α | Φ | R_0 (nm) | $R_{0, \text{free}}$ (nm) |
|-------------|------------------------|------------|---------------------------|--------|------------|---------------------------|
| Bodipy | $524(15) \times 10^3$ | 30.8(31) | $0.95(17) \times 10^{-3}$ | 0.46 | 8.5 | 5.7 |
| TMR | $728(81) \times 10^4$ | 44.1(80) | $1.6(20) \times 10^{-3}$ | 0.14 | 7.6 | 4.4 |
| AF | $750(82) \times 10^2$ | 58.5(78) | [0] | 0.79 | 6.9 | 6.8 |
| fluorescein | $147(20) \times 10^3$ | 100(26) | [0] | 0.92 | 5.8 | 4.4 |
| coumarin | $700(41) \times 10^2$ | 1530(170) | [0] | 0.20 | 2.3 | |
| dansyl | $1893(63) \times 10^2$ | very large | [0] | 0.07 | | |
| Rubipy | $820(30) \times 10$ | very large | [0] | 0.05 | | |

^a Numbers in parentheses indicate one standard deviation in the units of the last quoted digit of the parameter. R_0 was estimated assuming random and uniform distributions of dye molecules in SNP^{dye}. See text for definitions of the quantities.

approximates the dye-loaded silica shell in SNP^{dye}. Nevertheless, assuming random and uniform distribution of embedded dye molecules, we can expect that in the first approximation $r_{\text{mean}} \sim N^{-1/3}$. In general, R_0 in SNP^{dye} is expected to be different from the Förster radius in an anisotropic solution, and its determination may not be straightforward as, for example, the relative orientation of embedded dye molecules may be far from random. However, for any SNP^{dye} we can define N_0 as a dye loading at which the mean distance between the embedded dye molecules equals R_0 . Thus, we can write $R_0 \sim N_0^{-1/3}$ with the same proportionality constants as for r_{mean} (see above). Therefore, we can express the efficiency of the homo FRET between embedded dye molecules as $E_{\text{RET}} = 1/(1 + (N_0/N)^2)$.

For a random and uniform distribution of dye molecules in SNP^{dye} the probability of a selected molecule not being a part of a nonradiating dimer, trimer, or a larger aggregate (not having a nearest neighbor within an “aggregation distance”) can be defined as $P = e^{-\alpha(N-1)}$, where N is the number of dye molecules in SNP^{dye} and α is an aggregation affinity defined as $\alpha = (2a/d)^3$, with a being an aggregation radius (a distance at which non-radiating aggregates can be formed) and d the diameter of SNP^{dye}. If dimerization is expected to be the leading aggregation process, a should be of the order of an intermonomer distance in a dimer. In general, however, a (and thus α too) is expected to be an effective parameter characterizing aggregation affinity. For example, when aggregation is aided by the silica matrix, as it has been observed for the dimerization of Bodipy, a is expected to be significantly larger than the intermonomer distance. Substituting the expressions for E_{RET} and P into the equation for F_{SNP} we obtained, after simplifications, $F_{\text{SNP}} = (A_1 N \Phi e^{-\alpha(N-1)}) / (1 + (1 - \Phi e^{-\alpha(N-1)})(N/N_0)^2)$.

Using this model, we fitted the SNP^{dye} luminescence intensity versus dye loading data for all seven dyes studied in this work, determining best estimates for N_0 , A_1 , and, for selected dyes only, α (see Table 2). We also present the fitted data in Figure 4. The model, although a rather simple one, allows for satisfactory fits for all seven SNP^{dye} series (Figure 4). However, because of the model simplicity, a small number of data points for some of the SNP^{dye} series should be treated as effective values, where the true value would have to take into account additional contributions not explicitly considered here (i.e., changes in quantum yield for each loading, aggregate size, etc.). While the SNP^B and SNP^{TMR} series cannot be satisfactorily fit with the α parameter set to zero, the parameter is rather poorly defined and strongly correlated with N_0 . As mentioned, for dansyl and Rubipy we have observed no sign of loading-dependent luminescence quenching, thus only the A_1 parameter could be determined. For the other five dyes, the fits yielded N_0 and, for SNP^B and SNP^{TMR} only, α parameters.

To relate N_0 to intermolecular distances in SNP^{dye} we assume uniform and random distribution of dye molecules and neglect the presence of the iron oxide core, which occupies less than 5% of the SNP^{dye} volume. In this case, the mean near neighbor distance between N dye molecules in SNP^{dye} of diameter $d_{\text{out}} = 60$ nm can be expressed as r_{mean} (nm) = $26.8N^{-1/3}$.³⁴ Using this equation we converted N_0 parameters to corresponding mean near neighbor distances shown in Table 2 as R_0 . For Bodipy, TMR, and fluorescein the R_0 distance in SNP^{dye} is somewhat larger as compared with a corresponding Förster radius for free dye molecules in solution. The differences seem too large to be explained by increasing quantum yield Φ or the orientation parameter (κ^2) in the silica matrix. Given the size and precision of each SNP^{dye} series data set we were unable to identify additional luminescence quenching processes and better define Förster radius in the SNP^{dye} silica matrix. The aggregation affinity parameter, α , for both SNP^B and SNP^{TMR} is approximately two orders of magnitude larger than expected in the case of random formation of dimers and aggregates, which is consistent with the affinity of both Bodipy and TMR for aggregation as shown in the UV–vis spectra of SNP^B and SNP^{TMR}.

CONCLUSIONS

The absorption and luminescence of hybrid luminescent and magnetic nanoparticles can be affected by a variety of factors. Our study demonstrates that the main factor influencing the overall luminosity of SNP^{dye} is resonance energy transfer between monomers and other monomers as well as monomers and aggregates leading to luminescence quenching, the efficiency of which strongly depends on spatial separation of dye molecules within the nanoparticle matrix, which is mainly determined by dye loading. The resonance energy transfer effects can be minimized or even avoided by utilizing dyes with large Stokes shift, such as Rubipy, dansyl, and coumarin. Dye aggregation can also limit SNP^{dye} luminosity, so dyes showing affinity for aggregation, such as Bodipy and TMR, should be avoided. Silica matrix confinement of dyes can increase their quantum yield, as is the case for coumarin, and this effect can be used to increase SNP^{dye} luminosity. Overall, these results are valuable as they provide information that can be utilized to either enhance or totally quench the luminescence from a dye-doped SNP^{dye}. It can be of interest to develop quenched SNP^{dye} where certain conditions may lead to the liberation and subsequent “turn-on” of fluorescence following molecules liberation from the silica matrix. Specifically, one could imagine a dosimeter, where an increase in emission intensity could signal the presence of specific ions or molecules that can dissolve the silica matrix. Conversely, in labeling studies that employ SNP^{dye}, it would be beneficial to

choose SNP^{dye} that exhibit the highest luminosity. This investigation suggests that the brightest nanoparticles are those that exploit dyes that either do not aggregate within the nanoparticle environment or do not homogeneously quench luminescence through resonant energy transfer processes when present in high concentrations/close proximity to other dye molecules.

■ ASSOCIATED CONTENT

S Supporting Information. Full details of the synthesis and characterization of all of the nanoparticles. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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■ REFERENCES

- (1) Lu, C.-W.; Hung, Y.; Hsiao, J.-K.; Yao, M.; Chung, T.-H.; Lin, Y.-S.; Wu, S.-H.; Hsu, S.-C.; Liu, H.-M.; Mou, C.-Y.; Yang, C.-S.; Huang, D.-M.; Chen, Y.-C. *Nano Lett.* **2007**, *7*, 149–154.
- (2) Koch, A. M.; Reynolds, F.; Kircher, M. F.; Merkle, H. P.; Weissleder, R.; Josephson, L. *Bioconjugate Chem.* **2003**, *14*, 1115–1121.
- (3) Lee, J.-H.; Jun, Y.-W.; Shin, J.-S.; Cheon, J. *Angew. Chem., Int. Ed.* **2006**, *45*, 8160–8162.
- (4) Kim, J.; Lee, J. E.; Lee, S. H.; Yu, J. H.; Lee, J. H.; Park, T. G.; Hyeon, T. *Adv. Mater.* **2008**, *20*, 478–483.
- (5) Veisheh, O.; Sun, C.; Gunn, J.; Kohler, N.; Gabikian, P.; Lee, D.; Bhattarai, N.; Ellenbogen, R.; Sze, R.; Hallahan, A.; Olson, J.; Zhang, M. *Nano Lett.* **2005**, *5*, 1003–1008.
- (6) Gao, J. H.; Li, L. H.; Ho, P. L.; Mak, G. C.; Gu, H. W.; Xu, B. *Adv. Mater.* **2006**, *18*, 3145–3148.
- (7) Herz, E.; Ow, H.; Bonner, D.; Burns, A.; Wiesner, U. *J. Mater. Chem.* **2009**, *16*, 6341–6347.
- (8) Ma, D.; Kell, A. J.; Tan, S.; Jakubek, Z.; Simard, B. *J. Phys. Chem. C* **2009**, *113*, 15974–15981.
- (9) Bringley, J. F.; Penner, T. L.; Wang, R.; Harder, J. F.; Harrison, W. J.; Buonemani, L. *J. Colloid Interface Sci.* **2008**, *320*, 132–139.
- (10) Burns, A. A.; Vider, J.; Ow, H.; Herz, E.; Penate-Medina, O.; Baumgart, M.; Larson, S. M.; Wiesner, U.; Bradbury, M. *Nano Lett.* **2009**, *9*, 442–448.
- (11) Larson, D. R.; Ow, H.; Vishwasrao, H. D.; Heikal, A. A.; Wiesner, U.; Webb, W. W. *Chem. Mater.* **2008**, *20*, 2677–2684.
- (12) Sokolov, I.; Naik, S. P. *Small* **2008**, *4*, 934–939.
- (13) Kim, J.; Kim, H. S.; Lee, N.; Kim, T.; Kim, H.; Yu, T.; Song, I. C.; Moon, W. K.; Hyeon, T. *Angew. Chem., Int. Ed.* **2008**, *47*, 8438–8441.
- (14) Zhang, L.; Liu, B.; Dong, S. *J. Phys. Chem. B* **2007**, *111*, 10448–10452.
- (15) Gu, H.; Xu, K.; Yang, Z.; Chang, C. K.; Xu, B. *Chem. Commun.* **2005**, 4270–4272.
- (16) Rampazzo, E.; Bonacchi, S.; Montalti, M.; Prodi, L.; Zaccheroni, N. *J. Am. Chem. Soc.* **2007**, *129*, 14251–14256.
- (17) Acikgoz, S.; Aktas, G.; Inci, M. N.; Altin, H.; Sanyal, A. *J. Phys. Chem. B* **2011**, *114*, 10954–10960.
- (18) Lavis, L. D.; Raines, R. T. *ACS Chem. Biol.* **2008**, *3*, 142–155.
- (19) Van Blaaderen, A.; Vrij, A. *Langmuir* **1992**, *8*, 2921–2931.
- (20) Wang, L.; Tan, W. *Nano Lett.* **2006**, *6*, 84–88.
- (21) Kim, S.; Ohulchanskyy, T. Y.; Pudavar, H. E.; Pandey, R. K.; Prasad, P. N. *J. Am. Chem. Soc.* **2007**, *129*, 2669–2675.
- (22) Martini, M.; Montagna, M.; Ou, M.; Tillement, O.; Roux, S.; Perriat, P. *J. Appl. Phys.* **2009**, *106*, 094304.
- (23) Imhof, A.; Megens, M.; Engelberts, J. J.; de Lang, D. T. N.; Sprik, R.; Vos, W. L. *J. Phys. Chem. B* **1999**, *103*, 1408–1415.
- (24) Grauer, Z.; Avnir, D.; Yariv, S. *Can. J. Chem.* **1984**, *62*, 1889–1894.
- (25) Tleugabulova, D.; Zhang, Z.; Brennan, J. D. *J. Phys. Chem. C* **2002**, *106*, 13133–13138.
- (26) Marmé, N.; Friedrich, A.; Denapate, D.; Hackenbeck, R.; Knemeyer, J.-P. *Chem. Phys. Lett.* **2006**, *428*, 440–445.
- (27) Ma, D.; Veres, T.; Clime, L.; Normandin, F.; Guan, J.; Kingston, D.; Simard, B. *J. Phys. Chem. C* **2007**, *111*, 1999–2007.
- (28) Wang, L.; Wang, K.; Santra, S.; Zhao, X.; Hilliard, L. R.; Smith, J. E.; Wu, Y.; Tan, W. *Anal. Chem.* **2006**, *78*, 646–654.
- (29) Ma, D.; Guan, J.; Normadin, F.; Denommee, S.; Enright, G.; Veres, T.; Simard, B. *Chem. Mater.* **2006**, *18*, 1920–1927.
- (30) Herz, E.; Marchincin, T.; Connelly, L.; Bonner, D.; Burns, A.; Switalski, S.; Wiesner, U. *J. Fluoresc.* **2010**, *20*, 67–72.
- (31) Ma, D.; Jakubek, Z.; Simard, B. *J. Nanosci. Nanotechnol.* **2006**, *6*, 3677–3684.
- (32) Lopez Arbeloa, F.; Riuz Ojeda, P.; Lopez Arbeloa, I. *J. Chem. Soc., Faraday Trans.* **1988**, *84*, 1903–1912.
- (33) Nooney, R. I.; McCahey, C. M. N.; Stranik, O.; Le Guevel, X.; McDonagh, C.; MacCraith, B. D. *Anal. Bioanal. Chem.* **2009**, *393*, 1143–1149.
- (34) Li, M.; Ghosh, S.; Richmond, O.; Weiland, H.; Rouns, T. N. *Mater. Sci. Eng. A* **1999**, *265*, 153–173.