Fluorescence Enhancement of Molecules Inside a Gold Nanomatryoshka

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Supporting Information

ABSTRACT: Metallic nanoparticles exhibiting plasmonic Fano resonances can provide large enhancements of their internal electric near field. Here we show that nanomatryoshkas, nanoparticles consisting of an Au core, an interstitial nanoscale SiO₂ layer, and an Au shell layer, can selectively provide either a strong enhancement or a quenching of the spontaneous emission of fluorophores dispersed within their internal dielectric layer. This behavior can be understood by taking into account the near-field enhancement induced by the Fano resonance of the nanomatryoshka, which is responsible for enhanced absorption of the fluorophores incorporated into the nanocomplex. The combination of compact size and enhanced light emission with internal encapsulation of the fluorophores for increased biocompatibility suggests outstanding potential for this type of nanoparticle complex in biomedical applications.

KEYWORDS: Nanomatryoshka, fluorescence enhancement, E-field, Fano resonance, Cy7, IR800

Fluorescence enhancement has been an active topic of research since it was first established that spontaneous emission can be modified by coupling an emitter to a resonant cavity or by positioning it near a metallic surface.¹ ² More recently, fluorescence enhancement has attracted increased interest because it offers the potential for significant improvements in a variety of fluorescence-based biomedical applications, such as in vivo imaging, high-throughput diagnostics, cell sorting, single cell imaging, genomics, and proteomics.³ – ⁹ Of particular interest has been the case where a molecule can be positioned within a few nanometers of the surface of a metallic nanoparticle. Nanoparticle geometry, size, and the distance between fluorophore and metal nanoparticle surface all strongly influence this interaction and can be responsible for either fluorescence enhancement or quenching.¹⁰ Control of these parameters can lead to both enhanced absorption and a reduction in radiative lifetime, resulting in substantial increases in the fluorescence quantum yield of the molecule.

Au nanoshells, composed of a silica core and a metallic shell, have been used extensively for the systematic investigation of fluorescence enhancement due to their geometrically tunable plasmon resonance.¹¹ In particular, Au nanoshells have been used in bioimaging applications to enhance molecular fluorescence in the near-IR, where light has a maximal penetration depth in biological tissue.¹² – ¹⁴ Fluorescence enhancement with nanoshells was obtained by growing a nanoscale dielectric layer around the outer metallic shell layer of the nanoshell, then by adsorbing fluorophores onto the exterior of the coated nanoparticles. The spectral tunability of plasmon resonances in Au nanoshells has enabled a better understanding of nanoparticle antenna-mediated fluorescence enhancement. In larger nanoshells, where scattering dominates the optical response, fluorescence enhancement is maximal when the plasmon resonance is at the same frequency as the fluorophore emission.¹² Near-field mediated absorption enhancement can also be observed with smaller nanoshells, when the plasmon resonance overlaps the absorption frequency of the fluorophore.¹²

Although near-IR fluorescence enhancement with Au nanoshells has proven to create a bright, useful probe for bioimaging, several aspects of this nanoparticle–fluorophore complex limit its widespread use in biomedical applications. The conjugation of fluorophores to the surface of the nanoparticle increases its surface charge, limiting circulation time in the bloodstream.¹⁵ Growth of the dielectric layer around the nanoparticle to separate the fluorophore from the metal surface of the nanoparticle increases its overall size, which can adversely affect biodistribution, particularly in applications involving tumor uptake. To circumvent these limitations, we examine a more compact, yet more complex, plasmonic nanoparticle geometry for fluorescence enhancement: the nanomatryoshka.

A nanomatryoshka consists of a solid Au core surrounded by a thin dielectric (SiO₂) layer, capped with an outer Au shell.
layer. Its plasmonic properties result from the strong interaction between the plasmon modes of the Au core and the Au shell, giving rise to hybridized modes that can be tuned into the NIR, for particle sizes below 100 nm in diameter. These interactions also give rise to a strong Fano resonance in the visible region of the spectrum,\(^{16}\) appearing as an asymmetric modulation in the far-field extinction spectrum of the nanoparticle and prompting an alternate name for these nanoparticles of Fanoshells.\(^{19}\) The Fano resonance results from the coherent transfer of energy between a broad bright (superradiant) mode and a narrow dark (subradiant) mode and results in a dip in the far-field extinction spectrum, referred to as a Fano dip, at a wavelength in between the two modes.\(^{17–20}\) The indirect excitation of the subradiant mode results in a strongly enhanced near field, which can play an important role in the fluorescence enhancement process.

Here we report the enhancement of molecular fluorescence by nanomatryoshkas when the dye molecules are incorporated within the interstitial dielectric layer between the metallic core and the outer metallic shell. Three molecular fluorophores, Cy7, IR800, and Cy5, were selected to overlap the long-wavelength extinction peak (Cy7 and IR800) or the Fano dip (Cy5) of the far-field extinction spectrum of the nanomatryoshka–dye complex. A strong modification of fluorophore emission, depending on the emission frequency of the fluorophore relative to the various features of the nanomatryoshka extinction spectrum, was observed. For the fluorophores resonant with the long-wavelength subradiant mode close to the extinction maximum, fluorescence was strongly enhanced. Fluorophores with emission overlapping the far-field Fano dip, by contrast, were quenched. Through a simple model, we show that both the enhancement and quenching can be understood in the context of the near-field properties of the Fano resonance of the nanomatryoshka.

**Results and Discussion.**  
**Synthesis of Dye-Encapsulated Nanomatryoshkas.** The synthesis of gold nanomatryoshkas (NM) was performed using a modified version of the technique reported previously.\(^{21}\) This improved method provides precise control over the silica layer thickness and permits a simple, efficient conjugation of Au colloidal nanoparticles and fluorescent dyes by doping the silica layer during synthesis. The dopant, (3-aminopropyl)triethoxysilane (APTES), enables controlled binding of both dyes and Au colloid during synthesis. Uniform silica layer thicknesses were obtained on Au nanoparticles by first growing an initial, oversized silica layer around the nanoparticle (~16 nm, Figure 1A, step 2), then etching the layer to the desired thickness by hydrolysis (Figure 1A, step 3). The etching process was performed during incubation with 1–2 nm Au colloid (see Methods section); after 4 days the width of the silica layer reached 10 nm. During the etching process, the APTES-doped silica became densely covered with the 1–2 nm gold colloid and also became more porous. This increase in porosity (also observed in silica-coated Au nanoshells doped with APTES (Figure S1, Supporting Information)), in conjunction with the presence of amine groups, allowed the incorporation of fluorescent dyes within the silica layer. The nanoparticles at this stage of the synthesis, namely, silica-coated Au nanospheres decorated with ultrasmall Au colloidal nanoparticles, are referred to as seeded precursors in subsequent discussions since they are the synthetic precursors of the final fluorescent nanomatryoshka complexes.

After etching, the seeded precursor nanoparticles were purified (see Methods section), then transferred to an aqueous dye solution. Four variants of seeded precursor nanoparticles were fabricated (Figure 1A, step 4): one was left undoped as a control; the other three were each conjugated with different fluorescent dyes (Cy5, Cy7, or IR800). The nanomatryoshka synthesis was completed with the growth of an Au shell layer, formed by reducing Au\(^{3+}\) onto the seeded precursor nanoparticle in the presence of formaldehyde. (B) Transmission electron microscope (TEM) image of a Au nanosphere with \(r_1 = 20.8 \pm 2.6\) nm. (C) TEM image of a Au nanosphere coated with a silica layer of 16.0 ± 1.3 nm thickness. (D) TEM image of a seeded precursor nanoparticle with etched silica layer (10.5 ± 0.7 nm thick) in the presence of 1–2 nm gold colloid. (E) TEM image of a Au nanomatryoshka of dimensions \(r_1 = 21, 31, 44\) \(\text{nm}\) where \(r_1\) is the radius of the core, \(r_2\) is the silica-coated core, and \(r_3\) is the radius of the nanomatryoshka.

The three fluorescent dyes chosen for the nanocomplexes were selected in order to investigate the effect of the Fano resonance on fluorescence enhancement (Figure 2). The dyes IR800 and Cy7 were chosen for their emission to be in resonance with the long-wavelength plasmon resonance maximum of the far-field spectrum, while Cy5 emission overlaps with the far-field Fano dip (Figure 2B). IR800 was included in this investigation because the nanomatryoshka–IR800 complex could have potential use as a theranostic probe for simultaneous fluorescence optical imaging and photothermal therapy of cancer at near-IR wavelengths.\(^{14,22}\) Cy5 and
Cy7 have similar chemical structures and quantum yields of 0.20 and 0.30, respectively.23,24

The dyes are most likely bound within the porous silica matrix through both the negatively charged sulfonate group and the N-hydroxysuccinimide (NHS) ester group (Figure S2, Supporting Information). The sulfonate group can form an ionic interaction with the positively charged amine group of the APTES, while the NHS ester moiety can react with the amine group in the APTES to form a covalent amide bond. The extinction spectra of all seeded precursor–dye nanocomplexes exhibited identical plasmon resonances, with maxima at 539 nm, corresponding to identical physical dimensions (Figure S3, Supporting Information). This was also verified by electron microscopy (TEM and SEM), with all nanocomplexes yielding the same dimensions of \([r_1, r_2, r_3] = [21, 31, 44] \text{ nm}\). Green, purple, and blue bars indicate the absorption/emission regions for Cy5, Cy7, and IR800, respectively.

was obtained by normalizing the emission intensities of the nanomatryoshkas with respect to the emission intensities of their respective seeded precursors. The seeded precursors were chosen as a control rather than the free dye in solution because the seeded precursor geometry provides the same chemical environment and dye concentration as in the corresponding nanomatryoshka complex. The fluorescence emission of nanomatryoshka–Cy7 and nanomatryoshka–IR800 were both enhanced by \(~16\times~\) relative to their seeded precursor controls (Figure 3A,B). In contrast, the fluorescence emission of the nanomatryoshka–Cy5 complex was quenched with respect to the corresponding seeded precursor control (Figure 3C).

Both the near-field enhancement and the radiative rate enhancement of molecules inside the nanomatryoshka were calculated to better understand our experimental observations (Figure 4). The radiative rate enhancements were calculated as the ratio of power radiated from a dipole in the nanoparticle relative to the power emitted by a dipole in free space (finite element method, COMSOL Multiphysics 4.1). An orientation-averaged dipole source is placed in the center of the internal silica layer of the nanoparticle. The optical response of the gold was described using an empirical dielectric function,25 and the permittivities of H2O and SiO2 were chosen as 1.77 and 2.92, respectively. The radiated power was calculated by integrating the Poynting vector flux across a spherical surface enclosing the nanostructure. Both extinction spectra and near-field enhance-
Mie scattering spectrum of an [Cy5], were calculated by assuming a SiO2-coated Au nanoparticle permittivities. The properties of the seeded precursor particles is only enhanced by 9×150 emission frequencies of Cy7 and IR800, has a peak value of rate enhancement maximum, which closely matches the complete nanomatryoshka shows a much stronger radiative rate enhancements within a nanomatryoshka relative to a seeded precursor particle, we calculate relative near-field enhancements of 3.9× (Cy5), 86× (Cy7), and 98× (IR800).

These relative near-field enhancements can be understood by examining their relationship with the far-field Fano resonance scattering spectrum (Figure 4C). The energy of the subradiant mode was determined by representing the spectrum as an asymmetric modulation of an underlying Lorentzian resonance as described by Gallinet and Martin.23 With this approach, the energy of the subradiant mode is found to be located at 777 nm, which is also where the near-field enhancement peaks (Figure 4B). The calculated charge plots show that this central frequency exhibits the characteristic antisymmetric response of a subradiant mode, where the charge of the Au core and the Au shell oscillate out of phase and gives rise to a large field enhancement across the dielectric spacer between the two adjacent metallic surfaces.16 In contrast, the charge polarization around the far-field Fano dip and the short-wavelength far-field extinction peak at 565 nm are superradiant in character, with the core and the shell layers exhibiting in-phase charge polarizations with only minimal field enhancements. The maximum near-field enhancements clearly occur for excitation of the subradiant mode.27,29

We have shown that the near-field enhancement, and concomitantly the central frequency of the Fano resonance, is maximal at the subradiant mode close to the long-wavelength extinction peak of the nanomatryoshka (Figure 4). This is close to the ideal conditions for enhancement where the near-field maximum overlaps the excitation wavelength of the IR800 dye and the scattering peak matches the dye emission. However, the energies of the subradiant and superradiant modes can be readily tuned by changing the dimensions of the NM: for example, by increasing the thickness of the outer Au shell.16 When the outer Au shell is made thicker, the spectral overlap between the subradiant and superradiant modes increases, giving rise to a more symmetric Fano dip in the far-field scattering spectrum and increased field enhancements at the far-field Fano dip (Figure S5, Supporting Information). This geometric control enables tuning of the near-field enhancement maximum to the peak excitation wavelength of the dye while simultaneously overlapping the scattering peak with the dye emission. Matching both the near-field and far-field properties of the NM to the dye is crucial since the overall fluorescence enhancement depends on both the strong local field enhancement and the coupling efficiency of the local emission to the far field through nanoparticle scattering.30,31

To understand why fluorescence quenching is observed in the nanomatryoshka—Cy5 system, we need to account for both the near-field enhancements and nonradiative decay in this nanoparticle geometry. The emission of a fluorophore in the absence of any enhancement/quenching interaction is described in terms of its quantum yield (Q0). The quantum yield can be described in terms of the radiative decay rate (Γr0) and the nonradiative decay rate (Γnr0) by

\[ Q_0 = \frac{\Gamma_{r0}}{\Gamma_{r0} + \Gamma_{nr0}} \]  

(A) Radiative rate enhancement (black line) for a dipole in the center of the silica layer of the seeded precursor (SP) and (B) nanomatryoshka (NM), averaged over all dipole orientations and near-field enhancements (red line) for SP and NM averaged within the silica layer. Insets: electric field enhancements, \(|E/E_0|^2\), calculated at an incident wavelength of 767 nm corresponding to the emission maxima of Cy7. The outer boundary of the NM is indicated for clarity (white dotted line). Green, purple, and blue dotted lines indicate the emission positions of Cy5, Cy7, and IR800, respectively. (C) Analytical Fano resonance model: fitting of the Fano resonance model to the (far-field) Mie scattering spectrum of an \([r_1, r_2, r_3] = [21, 31, 44]\) nm INs. Insets: charge plots. The green solid lines indicates the wavelength of the subradiant mode, which causes the Fano interference and induces the maximum near-field enhancement.

ments were calculated using Mie theory.26 using the same permittivities. The properties of the seeded precursor particles were calculated by assuming a SiO2-coated Au nanoparticle geometry.

The calculated radiative rate enhancements differed strongly between seeded precursor particles and nanomatryoshkas. Seeded precursors yielded only a minor enhancement, with a peak value of 5× found at 560 nm (Figure 4A). In contrast, the complete nanomatryoshka shows a much stronger radiative rate enhancement occurring at 780 nm (Figure 4A). This radiative rate enhancement maximum, which closely matches the emission frequencies of Cy7 and IR800, has a peak value of 150×. At the Cy5 emission band at ~660 nm, the radiative rate is only enhanced by 9×.

The near-field enhancement spectra agree closely with the radiative rate enhancements (Figure 4A). While the radiative rate enhancement calculations provide significant physical insight into the photon emission, they are here calculated for a single spatial point within the silica. In contrast, the average field enhancement represents the field experienced by an ensemble of encapsulated dye molecules distributed throughout the silica layer. The importance of this spatial average can be seen in the near-field distribution of the nanomatryoshka,
which is the probability that the excited fluorophore relaxes by a radiative emission pathway relative to the total relaxation rate. The observed fluorescence emission intensity \( I_0 \) depends on the light excitation intensity \( I_{\text{exc}} \) and the absorptivity \( \epsilon \) of the molecules and can be expressed by

\[
I_0 = I_{\text{exc}}^\epsilon Q_0
\]

When the fluorophore is in the presence of a nanomatryoshka, the near-field enhancement leads to an increased excitation \( \Gamma_{\text{exc}}^\text{NM} \) of the molecule. In addition, the electromagnetic coupling between the molecule and the nanoparticle leads to an enhanced radiative decay rate \( \gamma_{\text{rad}}^\text{NM} \). The near-field enhancement is very close to the Au surface, energy transfer from the molecule to the nanomatryoshka can take place and give rise to fluorescence quenching due to the increase in the nonradiative decay rate \( \gamma_{\text{nr}}^\text{NM} \). This results in a modified expression for the quantum yield of the nanomatryoshka–dye system:

\[
Q_{\text{NM}} = \frac{\Gamma_{\text{exc}}^\text{NM}}{\Gamma_{\text{rad}}^\text{NM} + \gamma_{\text{nr}}^\text{NM}}
\]

and the observed emission of the system is given by

\[
I_{\text{NM}} = I_{\text{exc}}^\epsilon Q_{\text{NM}}
\]

Therefore, the fluorescence enhancement of the nanomatryoshka–dye system relative to the dye in free space is given by

\[
\frac{I_{\text{NM}}}{I_0} = \frac{I_{\text{exc}}^\epsilon Q_{\text{NM}}}{I_{\text{exc}}^\epsilon Q_0}
\]

The excitation intensity enhancement \( \frac{I_{\text{exc}}^\epsilon Q_{\text{NM}}}{I_{\text{exc}}^\epsilon Q_0} \) can be approximated by the near-field enhancement \( \frac{\langle n_p E_{\text{NM}}^2 \rangle}{\langle n_p E_0^2 \rangle} \), where \( n_p \) is a unit vector pointing in the direction of the electric dipole moment \( \langle p \rangle \).

\[
\frac{I_{\text{NM}}}{I_0} = \frac{\langle n_p E_{\text{NM}}^2 \rangle Q_{\text{NM}}}{\langle n_p E_0^2 \rangle Q_0}
\]

which is the product of the near-field enhancement and the quantum yield enhancement. A similar expression can be derived for the fluorescence enhancement in the seeded precursor–dye system:

\[
\frac{I_{\text{SP}}}{I_0} = \frac{\langle n_p E_{\text{SP}}^2 \rangle Q_{\text{SP}}}{\langle n_p E_0^2 \rangle Q_0}
\]

We evaluate the enhancement (following the same procedure used for analyzing the experimental results) by taking the ratio of the fluorescence intensity from the nanomatryoshka–dye system to the reference seeded precursor–dye system:

\[
\frac{I_{\text{NM}}}{I_{\text{SP}}} = \frac{\langle n_p E_{\text{NM}}^2 \rangle Q_{\text{NM}}}{\langle n_p E_{\text{SP}}^2 \rangle Q_{\text{SP}}}
\]

These enhancements depend on the position of the dye in the silica layer because the electromagnetic coupling and energy transfer processes are controlled by the distance between the dye and the Au nanoparticle. These distance-dependent effects can be qualitatively observed in the calculated near-field maps (Figure 4). To account for this distance distribution within the dielectric layer, we approximate the dye molecules as randomly oriented dipoles, then average the near field within the layer. These averaged near fields are shown in Figure 4. Finally, the quantum yield is also averaged resulting in the expression:

\[
\frac{I_{\text{NM}}}{I_{\text{SP}}} \approx \left( \frac{|E_{\text{NM}}|^2}{|E_{\text{SP}}|^2} \right) \left( \frac{Q_{\text{NM}}}{Q_{\text{SP}}} \right)
\]

which was used to describe the experimental fluorescence enhancements. The results of this model show excellent agreement with the experimental results (Figure 5).

Figure 5. Comparison of experimental and theoretical fluorescence enhancement/quenching of the dyes confined inside the nanomatryoshka (NM) versus conjugated to the seeded precursor (SP). Fluorescence enhancements are reported as the fluorescence intensities of the SP–dye and NM–dye conjugates normalized relative to the maximum fluorescence intensities of their respective SP.

To calculate the average quantum yield \( Q_{\text{NM}} \), several approximations were made. When the fluorescent dyes are very close to the surface of the metal, energy transfer from the dyes to the metal particle leads to fluorescence quenching. In this case \( Q \) becomes very small because nonradiative decay increases. We therefore assume that the fluorescence is completely quenched \( Q_{\text{NM}} = 0 \) and \( Q_{\text{SP}} = 0 \) for dyes located within 4.5 nm of either metal surface for the nanomatryoshka or from the surface of the metallic core for the seeded precursor particle. This assumption is consistent with previous studies of single molecule fluorescence on Au nanoparticles. Consequently, only the dye molecules located within a central 1 nm spacer layer (from 4.5 to 5.5 nm from the inner metallic core) are enhanced. For the seeded precursor–dye system, fluorescence enhancements thus only occur in the region 4.5–10 nm outside the metallic core. If we assume that the enhanced quantum yield \( Q_{\text{NM}} \approx Q_{\text{SP}} \approx 1 \), then \( \langle Q_{\text{NM}}/Q_{\text{SP}} \rangle \) can be calculated as the ratio of the volumes of the enhanced molecules in the nanomatryoshka and the seeded precursor complexes: \( \langle Q_{\text{NM}}/Q_{\text{SP}} \rangle \approx 0.153 \). This simple picture well accounts for the observed fluorescence enhancements (Cy7 and IR800) and quenching (Cy5) and are shown in Figure 5. Our analysis suggests that the near-field of the nanomatryoshka, through both absorption enhancement and an increase in the radiative decay rate, is responsible for the fluorescence enhancement that can be observed for dyes confined within the internal layer of the nanoparticle complex.

Conclusions. We report fluorescence enhancement of molecules confined within the inner dielectric layer of a Au nanomatryoshka exhibiting a plasmonic Fano resonance. Our
theoretical calculations are in good agreement with the experimental wavelength-dependent fluorescence enhancements. We have shown that the maximum fluorescence enhancements occurs for excitation of the subradiant mode where also the near-field enhancement is maximal, leading to a ~16× enhancement of NIR fluorescent dyes relative to the seeded precursor particles. These sub-100 nm, highly fluorescent NIR nanomatryoshkas are promising candidates for multifunctional and biocompatible plasmonic nanoparticles for a diverse range of applications, including medical imaging and enhanced photothermal therapy.

**Methods. Notes.** In the fabrication protocols described here, sonication was used to redisperse the nanoparticles after each centrifugation step. Milli-Q grade water was always used unless otherwise specified. Glassware was always cleaned with aqua regia and washed thoroughly with distilled water and Milli-Q water in the last washing step. The 1% (w/v) aqueous chloroauric acid (HAuCl₄·3H₂O, Sigma-Aldrich) solution was used for gold nanoparticle fabrication aged at least 2 weeks before use.

**Coating of Gold Colloid with APTES-Doped Silica.** Au colloid (40 nm citrate NanoXact Gold, nanoComposix) was coated with silica doped with (3-aminopropyl)triethoxysilane (APTES) by a modified Stöber process. APTES was used as a binding site for gold colloids and fluorescent dyes. Twenty-one milliliters of Au colloid (7.0 × 10¹⁰ particles/mL, citrate-capped 40 nm Au sphere, NanoComposix) were added under stirring to an Erlenmeyer flask with a ground glass joint. Next, 180 mL of 200 proof ethanol (Decon Laboratories) and 1.8 mL of ammonium hydroxide (28–30%, EMD Chemicals) were added. Finally, 36 μL of a solution of 10% tetraethoxysilane (TEOS, SIT7110.2, Gelest) in ethanol and 36 μL of 10% APTES (SIA0610.1, Gelest) in ethanol were added. The solution was sealed and stirred 50 min at room temperature followed by stirring 24 h at 4 °C. The solution was transferred to a dialysis membrane (Spectra/Por 6, MWCO = 10000, Spectrum Laboratories) previously washed with Milli-Q grade water to remove residual chemicals and then with ethanol to remove excess water. The solution was then dialyzed in 1 gallon of 200 proof ethanol for at least 12 h at room temperature to remove ammonium hydroxide and the remaining free silanes (TEOS and APTES) from the reaction and therefore decrease aggregation of the nanoparticles during centrifugation. The solution was cooled to 4 °C and centrifuged 45 min at 2000 rcf (the solution was centrifuged in aliquots of ~17 mL using 50 mL plastic tubes). The pellet was redispersed by sonication and using a total volume of 5 mL of ethanol. If the supernatant was still red, the centrifugation was repeated to recoup more particles before combining all the pellets in one solution.

**Fabrication of Seeded Precursor.** Fabrication of the seeded precursor (SP) consists of the functionalization of APTES-doped silica with small gold colloid (1–2 nm) fabricated by the method reported by Duff et al.₃⁶

**Synthesis of Duff Colloid.** Quickly under rapid stirring, 1.2 mL of 1 M NaOH was added to 180 mL of H₂O, followed by the addition of 4 mL of 1.2 mM aqueous tetrakis-(hydroxymethyl) phosphonium chloride (THPC, 80% solution in H₂O, Sigma). After stirring 5 min, 6.75 mL of 1% (w/v) aqueous chloroauric acid (HAuCl₄·3H₂O, Sigma-Aldrich) was quickly added, after which the solution immediately turned brown. The final solution was refrigerated for at least 2 weeks before use.

**Seeded Precursor.** First, the APTES-doped silica-coated gold colloids were sonicated for 20 min. Then, in a 50 mL plastic centrifuge tube, 20 mL of Duff colloid solution was added, followed by rapid, simultaneous addition of 300 μL of 1 M NaCl and 1 mL of APTES-doped silica-coated gold colloid (this reaction was repeated until all silica-coated gold colloids were used, usually ~4 reactions per batch). The solution was quickly vortexed and sonicated for 30 min. The resulting solutions were incubated 4 days at room temperature and gently shaken once a day followed by sonication for 20 min. During this time two processes took place: (1) the silica was etched and (2) small gold colloids were attached to the surface of the silica-coated gold colloid. After the incubation, the solutions were sonicated for 20 min, then centrifuged 30 min at 700 rcf. The supernatant was transferred into a new tube, while the pellet was redispersed in 800 μL of water by 5 min of sonication and transferred to a 2 mL centrifuge tube. The centrifugation of the supernatant and recuperation of pellets was repeated three times (in total about 16 pellets were collected, each one distributed separately in a 2 mL tube). All solutions in the 2 mL tubes were centrifuged 30 min at 700 rcf and redispersed in water by sonication for 5 min. Centrifugation was repeated, but particles were redispersed and combined in a total volume of ~4 mL of water. These particles were the seeded precursor used for the conjugation of the fluorescent dyes and then seeded growth of the outer Au shell.

**Functionalization of Seeded Precursor with Fluorescent Dyes.** Three dyes were used for this study, IR800 (IRDye 800 CW NHS ester, 929-70020, LI-COR), Cy7 (sulfo-Cy7 NHS ester, cat 15320, Lumiprobe), and Cy5 (sulfo-Cy5 NHS ester, cat 13320, Lumiprobe). First, the dyes were dissolved in water at 3 mM concentration and immediately added at 0.25 mM final concentration to 1 mL of the SP solution described above. Solutions were incubated at 4 °C for 2 weeks to allow the maximum number of dye molecules to penetrate and interact covalently and electrostatically with the APTES-doped silica. Incubation for 4 h at room temperature was enough to obtain similar results. In particular, the samples reported here were incubated for 2 weeks. SPs were then centrifuged 30 min at 700 rcf followed by redispersion of the pellet in 1 mL of water. This was repeated 3 times, and SPs were redispersed in a final volume of 250 μL of water in the last centrifugation step.

**Note:** another method to attach the dye consisted of adding the dye during the growth of the silica layer. This was achieved by adding only 16 μL of 10% APTES instead of 36 μL of 10% APTES in the silica coating reaction. Then after 2 h a mixture, previously incubated at room temperature under gently shaking for 2 h, composed of 2 μL of APTES, 1.0 mg of sulfo-Cy7-NHS ester dissolved in 60 μL of water, and 125 μL of EtOH, was added dropwise. With this method similar fluorescence enhancements were obtained as when the dye was attached to the seeded precursors, in a step posterior to the silica synthesis.

**Fabrication of Gold Nanomatryoshka.** The synthesis of a metallic shell of gold around the SP was done using a plating solution as a source of Au₃⁵. The plating solution was prepared by mixing 200 mL of water, 50 mg of anhydrous potassium carbonate (K₂CO₃), and 3 mL of 1 wt% aqueous chloroauric gold solution followed by aging for 12–19 h. The reduction of Au₃⁺ into a metallic shell of Au around the SP was done in a 4.5 mL methacrylate cuvette with a plastic cap. A volume of 1.5 mL of plating solution was added into the cuvette followed by 20–
60 μL of SP. Next, 7.5 μL of formaldehyde was dropped inside the cap, and the cuvette was closed followed by a fast shaking of the solution for about 1 min. The solution changed color from red to purple upon the formation of the outer shell. The extinction spectra of gold nanomatryoshkas were measured in a UV–vis–NIR spectrophotometer (Cary 5000, Varian). The plasmon resonance of the nanomatryoshka was controlled by altering the volume of SP in the reaction.

**Fluorescence Enhancement Experiment.** Each batch of SP–dye conjugate was first tested to fabricate nanomatryoshkas as described previously. Once the right volume of seeded precursor to make the nanomatryoshka was known, then equal volumes of seeded precursor were used to prepare the controls (seeded precursor–dye conjugates). The controls were prepared in a manner similar to the nanomatryoshka, adding all reagents except the formaldehyde to be consistent with the solvent present in the nanomatryoshka. All samples (nanomatryoshkas and controls) were prepared simultaneously, and when the outer nanomatryoshka shell layer was complete (~3 min after adding formaldehyde), water was added to the samples to bring the solution to a total volume of 2.5 mL. Next, fluorescence was measured using a spectrofluorometer (Fluorolog-3 Horiba JobinYvon). The Cy5 dye was excited at 640 nm, and emission was collected from 652 to 750 nm. Cy7 was excited at 745 nm, and emission was collected from 760 to 850 nm. IR800 was excited at 765 nm, and emission was collected from 852 to 950 nm. IR800 was excited at 765 nm, and emission was collected from 852 to 950 nm.

**Transmission Electron Microscope (TEM) Imaging.** Samples were drop cast on TEM grids (CF200-Cu mesh copper grids, Electron Microscopy Sciences). Most samples were imaged using a JEOL 1230 high contrast transmission electron microscope. For improved imaging of the core inside the NM, the NM samples were imaged using a JEOL 2010 transmission electron microscope.

**Scanning Electron Microscope (SEM) Imaging.** First, silicon wafers (p-type/boron-doped silicon, Silicon Valley Microelectronics) were functionalized with PVP (poly(4-vinylpyridine), Sigma-Aldrich) by immersion in 1% (w/v) ethanolic solution for 24 h. Silicon wafers were washed with ethanol to remove excess PVP on the surface. Wafers were dried in a stream of nitrogen gas, then the solution was dropcast onto the silicon wafer and allowed to interact with the substrate for 1–4 h. The remaining solution was removed in a water rinse, and the sample was again dried with nitrogen. SEM imaging was performed using a QUANTA 650 FEG SEM. Nanoparticle dimensions were determined from SEM images with a custom MATLAB sizing program based on edge detection with a Hough transform.

**Mie Theory Calculations.** Mie theory calculations were performed to obtain the theoretical extinction spectrum of the NM and SP that closely matched the experimental extinction spectrum for the dimensions [r1, r2, r3] = [21, 31, 45] nm. The dielectric constant of SiO2 (2.92) required to match the experimental spectrum was higher than pure silica (2.04), likely due to a combination of factors such as doping of APTES in the silica, attachment of dyes, and filling of small gold colloid in the cracks of the silica, which would lead to an effective medium with an elevated refractive index.37,38 The average field enhancements within the silica layer were obtained using Mie scattering theory

\[
\left\langle \frac{E}{E_0} \right\rangle = \int_{\text{silica}} \sum_{n=1}^{\infty} \frac{2\pi}{2n+1} n^2 (n+1)^3 r^2 dr \times \left\{ \frac{n}{n+1} \left[ j_n^2(kr) + j_{n+1}^2(kr) \right] \right\} + \left( \frac{n}{n+1} \right) j_{n+1}^2(kr) \left[ h_n^2(kr) + h_{n+1}^2(kr) \right]^{-1}
\]

where \(a_n, b_n, c_n\) and \(d_r\) are coefficients of the spherical harmonic vectors for the silica layer.

- **ASSOCIATED CONTENT**

**Supporting Information**
Experimental demonstration of porosity of APTES-doped silica layer in Figure S1; schematic diagram illustrating the conjugation of fluorescent dyes in Figure S2; experimental extinction spectra and TEM images of seeded precursors in Figure S3; experimental extinction spectra and SEM images of the nanomatryoshkas in Figure S4; theory calculated extinction, absorption, scattering, and central frequency of Fano resonance of nanomatryoshkas with different Au shell thicknesses in Figure S5; comparison of experimental and theoretical emission spectra in Figure S6. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**
The authors declare no competing financial interest.

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