

Fluorescent Core–Shell Ag@SiO₂ Nanocomposites for Metal-Enhanced Fluorescence and Single Nanoparticle Sensing Platforms

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The use of fluorescent nanoparticles as indicators in biological applications such as imaging and sensing has dramatically increased since the 1990s.¹ These applications require that the fluorescent nanoparticles are monodisperse, bright, photostable, and amenable to further surface modification for the conjugation of biomolecules and/or fluorophores. Among the many types of fluorescent nanoparticles available today, nanoparticles with core–shell architecture fulfill all these requirements, combining diverse functionalities into a single hybrid nanocomposite.²

In this work, we have developed core–shell (silver core–silica shell) nanoparticles with various shell thicknesses featuring a variety of fluorophores, to show the versatility of the core–shell architecture, and have demonstrated their applicability for two platform technologies, metal-enhanced fluorescence (MEF) and single nanoparticle sensing. We demonstrate the broad potential applications of our technology by employing near-infrared emitting probes (Rh800) for potential applications in cellular imaging and the use of highly photostable long lifetime (μ S) lanthanide probes, probes suitable for off-gating biological autofluorescence. The use of Alexa 647 serves to demonstrate that fluorophores can be readily covalently linked to the core–shell particles also, for metal-enhanced benefits.

MEF is an established technology,^{3a–d} where the interactions of fluorophores with metallic nanoparticles results in fluorescence enhancement, increased photostability, decreased lifetime owing to increased rates of system radiative decay, reduced blinking in single molecule fluorescence spectroscopy,^{3b} and increased transfer distances for fluorescence resonance energy transfer.^{3c} Single-molecule fluorescence spectroscopy is the prime tool in single nanoparticle sensing, and it provides several advantages over ensemble measurements, such as, the elimination of averaging of the spectral properties over all members of the ensemble, which can reveal fundamental features otherwise masked in ensemble experiments.⁴ Accordingly, the use of fluorescent core–shell nanocomposites with single-molecule fluorescence spectroscopy is likely to enhance the capability of single nanoparticle sensing enormously.

The preparation of fluorescent core–shell Ag@SiO₂ nanocomposites was undertaken in three steps (see Supporting Information for details): (1) first, silver colloids are prepared by reduction of silver nitrate by sodium citrate, (2) then a silica shell of various thickness was grown on the colloids, and (3) last, fluorophores (i) (Eu-TDPA, [Tris(dibenzoylmethane) mono(5-amino phenanthroline)europium] or Rh800, Rhodamine 800) were doped or (ii) were covalently linked to the silica shell (Alexa Fluor 647). Figure 1 shows the TEM images of core–shell Ag@SiO₂ nanocomposites

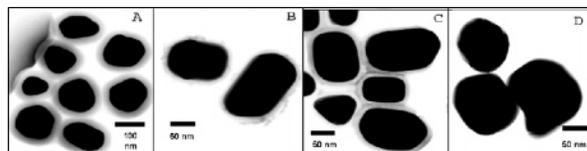


Figure 1. TEM images of Ag@SiO₂. Panels A, B, C, and D show the samples with different thickness of the SiO₂ coating at 35, 15, 11, and 2 nm (± 1 nm), respectively. The diameter of the Ag is 130 ± 10 nm for all the samples.

with different thickness of the SiO₂ coating. The diameter of the silver core was 130 ± 10 nm for all the preparations, a size which has been shown suitable for MEF and the radiating plasmon model.^{3d} The thickness of the silica shell was varied from 2 to 35 ± 1 nm, to optimize fluorescence enhancement and was controlled by the concentration of tetraethoxysilane (TEOS) after alkaline initiation. The surface plasmon resonance peak for silver shifted toward longer wavelengths as the thickness of the silica shell increased (see Table S1, Supporting Information) as expected and observed by others.⁵ The importance of using the silica shell around the silver core is 3-fold: (1) silica layers offer the robustness, chemical inertness, and the versatility needed for the conjugation of biomolecules or fluorophores, (2) it protects the silver core from ions present in biological media, and (3) it allows for the distance dependent MEF phenomenon, which we have determined optimum for shell thicknesses < 10 nm.^{3d}

To show the “huge” benefits of using a silver core in the fluorescent core–shell nanoparticles, rather than doping the fluorophores directly onto silica nanoparticles without a silver core, as many other researchers have done,² we have prepared control sample probes *without* the silver core. The control fluorescent probes, subsequently named hollow fluorescent *nanobubbles*, are prepared by dissolving the silver core away (etching) with cyanide from the fluorescent Ag@SiO₂ nanocomposites. Since the fluorophores (Eu-TDPA and Rh800)⁶ employed here are hydrophobic and retained in the hydrophobic pockets of the silica shell or covalently linked to the silica shell (Alexa 647), the etching of the silver core with cyanide did not cause the removal of fluorophores from the shell (thickness > 10 nm). Thus, it is possible to compare the fluorescence emission and lifetime of the fluorescent core–shell Ag@SiO₂ nanocomposites and of the fluorescent nanobubbles in a *quantitative* manner.

It is important to note that the versatility of the preparation technique employed here, allows researchers to incorporate any hydrophobic fluorophore to the silica shell by simply doping fluorophores or by covalently linking those available with suitable amine-reactive groups. Hence, our generic approach allows the use and the benefits of MEF with numerous fluorophores, but in an attractive nanoparticle architecture.

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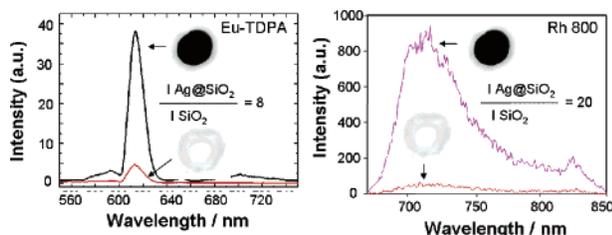


Figure 2. Fluorescence emission intensity of Eu-TDPA-doped Ag@SiO₂ and Rh800-doped Ag@SiO₂ and from the corresponding fluorescent nanobubbles (control samples), Eu-TDPA-doped SiO₂ and Rh800-doped SiO₂. The diameter of the Ag is 130 ± 10 nm and the thickness of the shell is 11 ± 1 nm (optimized) for all the samples.

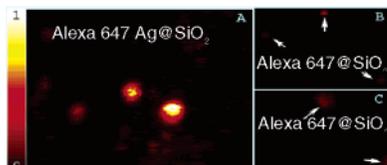


Figure 3. Scanning confocal images (20 μm × 20 μm) of (A) Alexa 647 Ag@SiO₂, (B) Alexa 647@SiO₂, (C) zoomed in version of that shown in panel B. Intensity counts in the scale were normalized to 1.

Figure 2 shows the fluorescence emission intensity from Eu-TDPA-doped Ag@SiO₂ and Rh800-doped Ag@SiO₂ and from the corresponding fluorescent nanobubbles (control samples), Eu-TDPA-doped SiO₂ and Rh800-doped SiO₂. The emission intensity was approximately 8-fold and 20-fold higher for Eu-TDPA-doped Ag@SiO₂ and Rh800-doped Ag@SiO₂ than for Eu-TDPA-doped SiO₂ and Rh800-doped SiO₂, respectively. We also note that the fluorescence emission spectra of the fluorophores were identical in both cases, indicating that the spectral properties of the fluorophores were retained.

We have also observed that fluorescent core-shell nanoparticles, Rh800 Ag@SiO₂, have a faster decay (0.093 ns) than the corresponding nanobubbles (0.447 ns) and the fluorophore in solution (0.728 ns), (see Figure S3 and Table S2 in Supporting Information). The average lifetimes of Alexa 647-linked Ag@SiO₂, the corresponding Alexa 647 nanobubble, and Alexa 647 in the aqueous solution were 0.64, 1.73, and 1.05 ns, respectively. These observations are in accordance with the previously described MEF phenomenon,^{3,7} where the metal-fluorophore interactions result in an increase in the quantum yield (i.e., emission intensity) of the fluorophore and a decrease in the lifetime of fluorophores owing to two phenomena: an enhanced local electric field and an increase in the intrinsic system decay rate. The first factor provides stronger excitation rates but does not modify the fluorescence lifetime of the molecules. The second factor increases the net nanoparticle quantum yield.

It is interesting to comment on the total detectability of the new MEF nanoparticles, as this is paramount in microscopy and in single molecule studies. While a 20-fold increase in fluorescence/luminescence intensity is clearly beneficial, a reduced particle lifetime also enables the particle to be cycled faster, as the lifetime of a species determines its cyclic rate. Hence, 20-fold increase in intensity coupled with a 10-fold reduction in fluorophore-particle lifetime, provides for a ~200-fold potential increase in particle detectability. In addition, a reduced lifetime, affords for increased fluorophore photostability,^{7–10} as there is less time for excited state photodestructive processes to occur.

Finally, Figure 3 shows representative scanning confocal images of individual fluorescent core-shell nanoparticles, Alexa 647 Ag@SiO₂ (covalently linked), and the corresponding nanobubbles,

Alexa 647 @SiO₂. The bright spots in Figure 3a represent fluorescence emission from the single fluorescent core-shell nanoparticles, while the dimmer spots in Figure 3b,c represent the single nanobubbles. The significant differences in the peak intensities of the two images are immediately evident from Figure 3. For fluorescent core-shell nanoparticles the average value of the peak intensity was approximately 10-fold higher than that of the nanobubbles. This shows that using a silver core results in 10-fold enhancement in the fluorescence emission, which is attributed to the MEF phenomenon.⁵ The heterogeneity in the spots' brightness (Figure 3a) is due to the presence of nanobubbles in the same sample as fluorescent core-shell nanoparticles which were not completely separated after the preparation.

In conclusion, we report the development of highly versatile highly fluorescent core-shell Ag@SiO₂ nanocomposites, which allow researchers to incorporate any fluorophore to the outer-silica shell by two simple methods (i.e., simple doping or covalent attachment) while exploiting the benefits of using a silver core for MEF. To show the generality of the preparation technique, we have developed three different fluorescent probes: an organic fluorophore (Rh800) and a lanthanide probe doped (noncovalently linked), and another organic fluorophore (Alexa 647) covalently linked to the silica shell. When compared to the control sample fluorescent nanoparticles (nanobubbles), fluorescent nanoparticles with core-shell architecture yielded up to 20-fold (with Rh800) enhancement of the fluorescence signal and a potentially 200-fold increase in particle detectability.

Acknowledgment. This work was supported by the National Center for Research Resources, Grant RR008119. Salary support to K.A. and C.D.G. from UMBI is also acknowledged. Authors would like to thank Drs. J. Zhang, J. Lukomska and S. Makowicz for their help with the TEM images and SMFS measurements, respectively.

Supporting Information Available: The experimental conditions for the preparation of fluorescent core-shell Ag@SiO₂ nanoparticles, nanobubbles, and for TEM, steady-state and single molecule fluorescence spectroscopy. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA0680820

Supporting Information for the Manuscript:

“Fluorescent Core-Shell Ag@SiO₂ Nanocomposites for Metal Enhanced Fluorescence and Single Nanoparticle Sensing Platforms” Aslan *et. al.*

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S1. Materials and Reagents

Tetraethoxysilane (TEOS), (3-aminopropyl) triethoxysilane (APS), were obtained from Sigma-Aldrich (St. Louis, MO, USA). The fluorophores Alexa Fluor 647 (Alexa Fluor 647 labeling Kit), Rhodamine 800 (Rh800) and Eu-TDPA [Tris(dibenzoylmethane) mono(5-amino phenanthroline)europium] were obtained from Molecular Probes; Lambda Physik (Fort Lauderdale, FL), and Aldrich (St. Louis, MO, USA), respectively.

S2. Preparation of the Fluorescent Core-Shell Ag@SiO₂ Nanocomposites

S2.1. Preparation of Silver Colloids

Silver colloids were prepared by adding dropwise 10 mL of 38.8 mM sodium citrate aqueous solution within 2 minutes into 490 mL of boiling aqueous solution containing 90 mg of AgNO₃ under vigorous stirring. After boiling for 10 minutes to 1 hour, the reaction solution was cooled to room temperature. The as-prepared silver colloid solution was centrifuged at 500 rpm for 1 hour to remove larger colloids, the remaining silver colloids in solution having an average size of 130 nm.

S2.2. Construction of Core Shell Ag@SiO₂ Nanoparticles with Uniform Silica Layer

Under vigorous stirring, 1 mL of the silver colloids solution was mixed with 250 mL of isopropanol 25 mL of deionized water. Immediately after the addition of 4 mL of 30% ammonium hydroxide, different amounts of tetraethoxysilane (TEOS) were added to the reaction mixture. To obtain different silica layer thicknesses, 100 μ l same amount of TEOS solutions with a concentration between 50% and 100% was added to the suspension. The reaction was stirred at room temperature for 30 minutes and then was allowed to age without agitation at 4°C overnight. Each suspension of silica-coated silver nanoparticles was washed and centrifuged (at 3500 rpm for 30 min) three times with water and ethanol mixture (5:4) at 30 min, followed by resuspension in water. The thickness of the silica layers was determined from TEM images and varied from 2 to 35 nm.

Table S1. The size of Ag colloids, the thickness of the silica shell and the change in absorbance of the Ag@SiO₂ particles. $\Delta\lambda_{\max}$ is the difference between the λ_{\max} of Ag@SiO₂ and Ag colloids themselves.

	Silica Shell (nm)	Ag (nm)	Absorbance (λ_{\max} , nm)	$\Delta\lambda_{\max}$, nm
Ag colloids	-	-	426	-
A	35 \pm 1	130 \pm 10	470	44
B	15 \pm 1	130 \pm 10	443	17
C	11 \pm 1	130 \pm 10	443	17
D	2 \pm 1	130 \pm 10	425	1

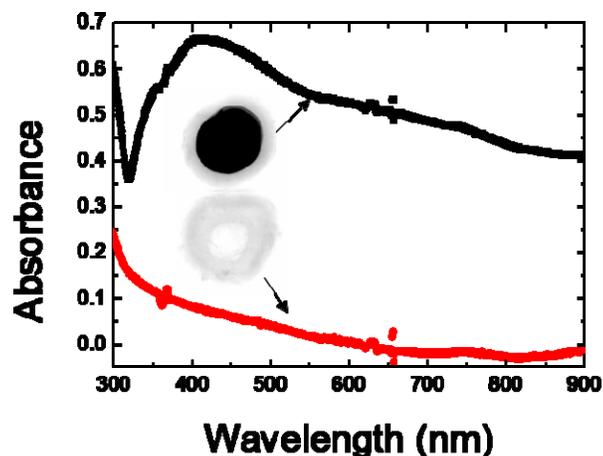


Figure S1 – Absorption spectra of core-shell Ag@SiO₂ nanoparticles and nanobubbles. The nanobubbles show no plasmon absorption band, as the silver has been etched away by cyanide.

S2.3. Construction of the Nanobubbles from the Nanocomposites

The nanobubbles from the Ag@SiO₂ as well as Flu@ Ag@SiO₂ were obtained using the following procedure. 500 μl of 0.1 M sodium cyanide (NaCN, Aldrich) solution was added to the aged suspension (200 μl of Ag@SiO₂) with agitation overnight to dissolve the silver core of the particles. Each suspension of silica-coated silver nanoparticles was washed and centrifuged three times with 1.5 ml water and 1.2 ml ethanol with minute sonication to remove *unreacted* ions at the final stage of preparation, followed by resuspension in water.

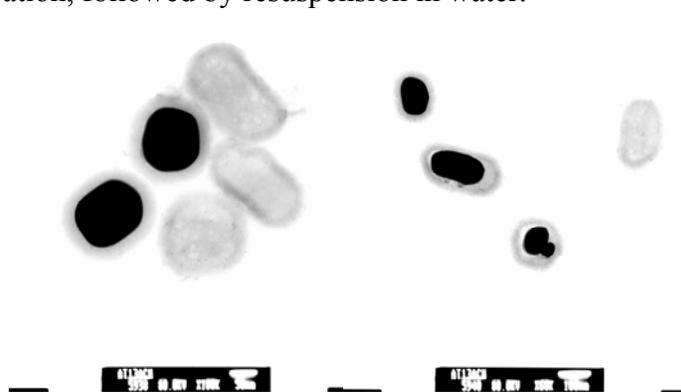


Figure S2 – TEM images of core-shell Ag@SiO₂ nanoparticles and nanobubbles.

S2.4. Construction of the Fluorescent Ag@SiO₂ nanocomposites

Two methods have been developed for the coupling of fluorophores to the core-shell Ag@SiO₂.

One approach is through dyeing (doping) of the Ag@SiO₂ colloids. In this regard, 500 μl ethanol solution of 0.16 mg/ml Eu-TDPA or 0.088 mg/ml Rh800 was added into 500 μl (0.2 mg) of Ag@SiO₂ nanoparticles suspended solution and incubated overnight. The mixture was centrifuged and washed with 1.5 ml H₂O and 1.2 ml ethanol 4 times, respectively. The absorbance and the fluorescence spectra of the washing solution were monitored to ensure the complete removal of the *unadsorbed* fluorophores. For the nanobubbles with fluorophores, cyanide solution

was added before the ethanol washing step and both fluorescent Ag@SiO₂ and nanobubbles followed the exact same procedure. The etching of the silver core with cyanide did not cause the removal of fluorophores from the shell thickness > 10 nm but resulted in removal of most of the fluorophores from a 2 nm shell.

Another approach is through surface derivation and consequent covalent conjugation of fluorophores. In this regard, 1 mg of Ag@SiO₂ nanoparticles was washed consecutively with ethanol, ethanol-toluene (1:1), and toluene, before 20 mL of toluene and 1.6 g of APS was added. The mixture was refluxed for 24 h under nitrogen gas. Then, the nanoparticles were centrifuged and were washed with ethanol and water. A fraction of the above amino-derived nanoparticles was suspended in 1 mL of 0.1 M NaHCO₃ (pH 9.0,) and a solution of amino-active fluorophore, Alexa Fluor 647 in 500 µl DMSO, was added dropwise with stirring. After being stirred at room temperature overnight, the fluorescent core-shell Ag@SiO₂ nanocomposites were centrifuged and washed with water and ethanol, and then stored at 4°C for further applications.

S3. Spectroscopic Measurements

Absorption spectra were measured on a Hewlett-Packard model 8543 spectrophotometer using 1-cm cuvettes. Steady-state fluorescence emission measurements were recorded with a Varian Eclipse spectrofluorometer. The fluorescence intensity decays were measured on a FluoroTime 200 (Picoquant GmbH, Berlin, Germany). Transmission electron micrographs (TEM) were taken with a side-entry electron microscope (Jeol Jem 1200 Ex II Microscope). Samples were cast from water solutions onto standard carbon-coated (200-300 Å) Formvar films on copper grids (200 mesh) by placing a droplet of a ca. 1 mg/mL aqueous sample solution on a grid, waiting 5 min, and removing excess solution by touching a small piece of filter paper to the edge of the grid. The grid was dried in air for 24 h. In some cases, ethanol solutions of the colloids were used and only 3 h were needed for drying.

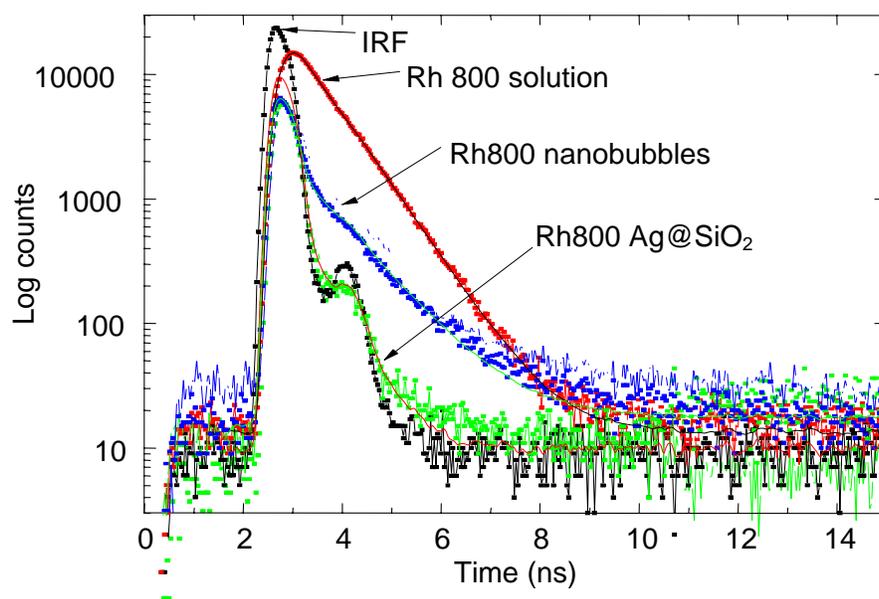


Figure S3. Intensity time decay of Rh800 in solution, Rh800-doped Ag@SiO₂ and Rh800-doped SiO₂. The instrument response function (IRF), is also included.

Table S2. Lifetime data for Rh800 and Alexa Fluor 647 conjugated Ag@SiO₂, Nanobubbles and in free solution.

	τ_1 (μ s)	A ₁ %	τ_2 (μ s)	A ₂ %	τ_{av}	χ^2
Rh800 in solution	0.728	100	-	-	0.728	1.178
Rh800 Ag@SiO ₂	0.05	99.2	0.562	0.8	0.093	1.484
Rh800 nanobubble	0.04	96.5	0.954	3.5	0.447	1.759
Alexa 647 Solution	0.26	20.7	1.10	79.3	1.050	1.396
Alexa 647 Ag@SiO ₂	0.31	474.3	0.995	135.3	0.640	0.942
Alexa 647 nanobubble	1.73	100	-	-	1.730	1.023

The lifetimes of EuTDPA@Ag@SiO₂ and EuTDPA@Nanobubble and in aqueous solution were measured to have an average lifetime of 25.3, 17.2, 2085 μ s, respectively. The observation is in accordance with the earlier experiments on lanthanide complexes and for the lifetimes (Meng Wu et al., Journal of Fluorescence, **2005**, 15(1), pp.53-59).

S4. Single Molecule Fluorescence Spectroscopy (SMD)

Single Alexa 647 core-shell Ag@SiO₂ fluorescence measurements were obtained using a scanning confocal microscope (Picoquant MicroTime 200). The excitation laser was reflected by a dichroic mirror to a high numerical aperture (NA) oil objective (100x, NA 1.3) and focused to a diffraction limited spot (~300 nm) on the sample surface. Fluorescence emission from Alexa 647 core-shell Ag@SiO₂ was collected by an avalanche photodiode through the dichroic beam splitter and a band-pass (650-720 nm, Chroma) filter. Integration times of 3 ms per pixel were used to obtain 512 x 512 pixel raster scanned 20 x 20 μ m images. The samples were excited with a 645 nm solid state laser.