**Supporting Information for the Manuscript:**
"Fluorescent Core-Shell Ag@$SiO_2$ 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) triethoxylsilane (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@$SiO_2$ 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 $AgNO_3$ 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@$SiO_2$ Nanoparticles with Uniform Silica Layer*

Under vigorous stirring, 1 mL of the silver colloids solution was mixed with 250 mL of iso-propanol 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 μl 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@$SiO_2$ particles. Δλmax is the difference between the λmax of Ag@$SiO_2$ and Ag colloids themselves.

| | Silica Shell (nm) | Ag (nm) | Absorbance (λmax, nm) | Δλmax, nm |
|---|---|---|---|---|
| Ag colloids | - | - | 426 | - |
| A | 35±1 | 130±10 | 470 | 44 |
| B | 15±1 | 130±10 | 443 | 17 |
| C | 11±1 | 130±10 | 443 | 17 |
| D | 2±1 | 130±10 | 425 | 1 |

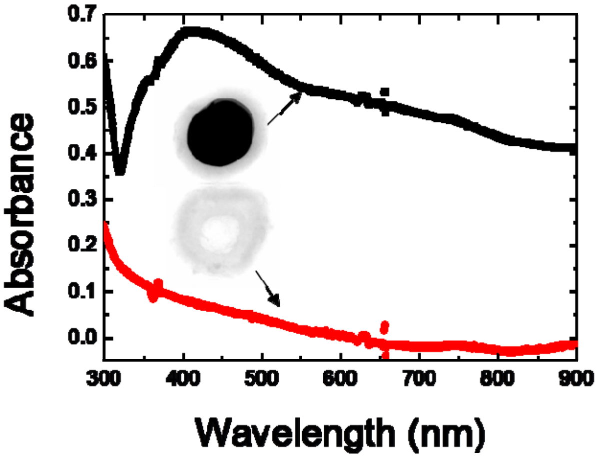

**Figure S1** – Absorption spectra of core-shell Ag@$SiO_2$ nanoparticles and nanobubbles. The nanobubbles show no plasmon absorption band, as the silver has been etched away by cyanide.

### *S2.3. Construction of the Nanobubbles from the Nanocomposites*

The nanobubbles from the Ag@$SiO_2$ as well as Flu@ Ag@$SiO_2$ 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@$SiO_2$) 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.

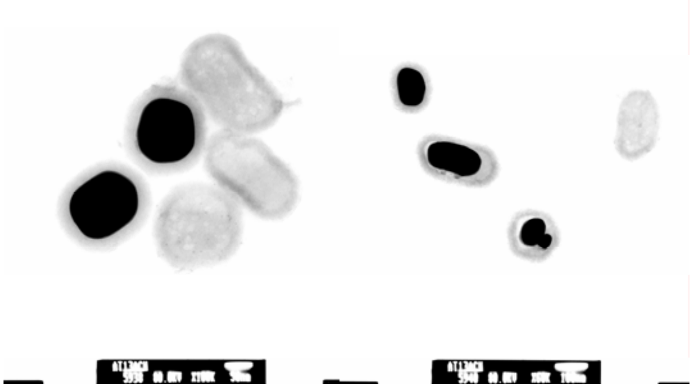

**Figure S2** – TEM images of core-shell Ag@$SiO_2$ nanoparticles and nanobubbles.

### *S2.4.Construction of the Fluorescent Ag@$SiO_2$ nanocomposites*

Two methods have been developed for the coupling of fluorophores to the core-shell Ag@$SiO_2$.

One approach is through dyeing (doping) of the Ag@$SiO_2$ 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@$SiO_2$ nanoparticles suspended solution and incubated overnight. The mixture was centrifuged and washed with 1.5 ml $H_2O$ 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@$SiO_2$ 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@$SiO_2$ 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 $NaHCO_3$ (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@$SiO_2$ 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.

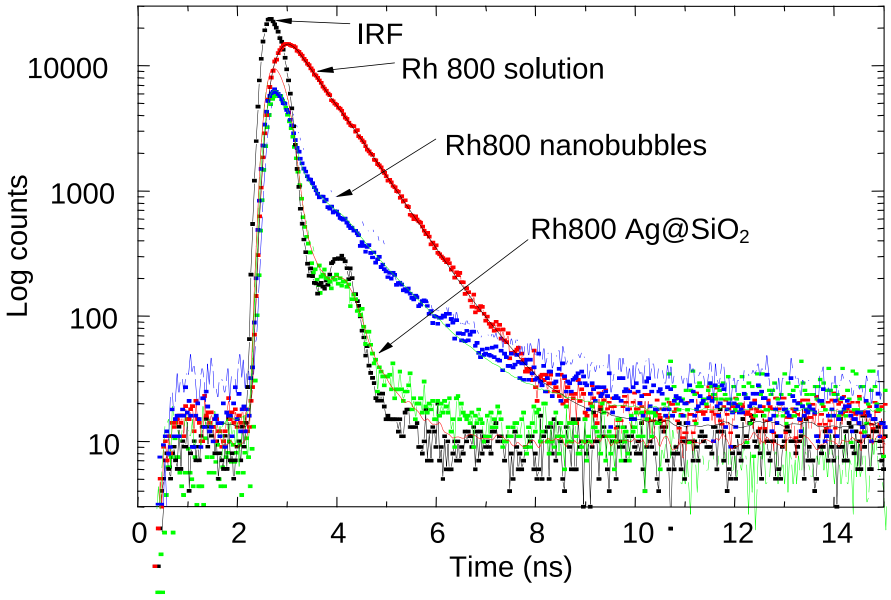

***Figure S3.*** Intensity time decay of Rh800 in solution, Rh800-doped Ag@$SiO_2$ and Rh800-doped $SiO_2$. The instrument response function (IRF), is also included.

**Table S2.** Lifetime data for Rh800 and Alexa Fluor 647 conjugated Ag@SiO$_2$, Nanobubbles and in free solution.

| | $\tau_1$ (μs) | $A_1$ % | $\tau_2$ (μs) | $A_2$ % | $\tau_{av}$ | $\chi^2$ |
|---|---|---|---|---|---|---|
| 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 μs, 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 647core-shell Ag@SiO$_2$ 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@SiO$_2$ 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 μm images. The samples were excited with a 645 nm solid state laser.