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PREPARATION OF CORE-SHELL SILVER/SILICA NANOPARTICLES AND THEIR APPLICATION FOR ENHANCEMENT OF CYANINE 3 FLUORESCENCE

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Core shell Ag@SiO₂-Streptavidin-Cy3 nanoparticles were prepared. Ag@SiO₂ nanoparticles were synthesized via a sol-gel method. Then, Streptavidin-Cy3 was covalently bonded to the Ag@SiO₂ surface. These core-shell nanoparticles were characterized by steady-state fluorescence spectroscopy and fluorescence scanning. In presence of the silver core, a 2.5-time enhancement of Cy3 fluorescence intensity was obtained. This result shows that these nanoparticles can be potentially helpful in surface analysis based on biochip.

Keywords: Ag@SiO₂; surface enhancement; fluorescence.

1. Introduction

Surface-Enhanced fluorescence (SEF) has provoked much attention for the recent years. SEF appears when a metal is located close to a fluorophore. Indeed, localized surface plasmon resonance (LSPR) can be created via direct illumination of a metal nanostructure. Consequently, sharp spectral absorption and scattering peaks as well as strong electromagnetic near-field can be observed. These properties can affect strongly the fluorescence emission of the fluorophore. The interaction between

near-field of metal nanostructure and fluorophore can be controlled by metal-fluorophore distance, excitation wavelength, and coupling between plasmonic modes and fluorophores.¹⁻³ LSPR of silver and gold show strong SEF effects on organic and inorganic fluorophores.^{1,3} Different kinds of nanostructures have been investigated, including nanoparticles, islands, nanoshells, nanorods. The interesting optical properties of these nanostructures have greatly extended the application of SEF, and have found potential applications in the field of cell

imaging,⁴ photoinduced oxidation,⁵ DNA and RNA detection,⁶ label-free bioassays.⁷

Organic fluorophores (like fluorescein, cyanines, rhodamine) are widely used to label target biomolecules in biological assays. They exhibit many advantages such as a narrow fluorescence emission peak, a high fluorescence intensity, and an easy bioconjugation to biomolecules. Among them, Cyanine 3 (Cy3) is one of the most used. However, its quantum yield is only 4%,⁸ thus, high concentrations of Cy3 must be used in order to obtain efficient labeling.

Recent research reveals that the fluorophore intrinsic quantum yield plays a significant role in SEF. Indeed, the highest enhancement is obtained for low quantum yield fluorophores.⁹ In this case, it has been shown that the expected enhancement can be as large as the inverse of the intrinsic quantum yield.¹⁰

In this work, we fabricated Ag@SiO₂ core-shell nanoparticles in order to enhance the fluorescence signal of Cy3. These nanoparticles are composed of a 60 nm-diameter silver core, coated with a 20 nm-thick silica layer as an optically transparent spacer between silver and Cy3. 60 nm-silver colloids were chosen as core metal because their plasmon band matches well the absorption band of Cy3. A combination of a 60 nm metal core and a spacer thickness of 20 nm was selected considering previous studies.^{3,4} In these conditions, the electric field intensity is sufficiently intense at a distance of 20 nm to the core surface to obtain both a fluorescence enhancement effect on Cy3 and to avoid quenching. Cy3 labeled with Streptavidin was covalently bonded onto the surface of Ag@SiO₂. The structural properties of Ag@SiO₂ and the SEF effect with Cy3 were investigated in detail. At last, fluorescence properties of these samples were determined under steady-state fluorescence spectroscopy and fluorescence scanning.

2. Experimental Section

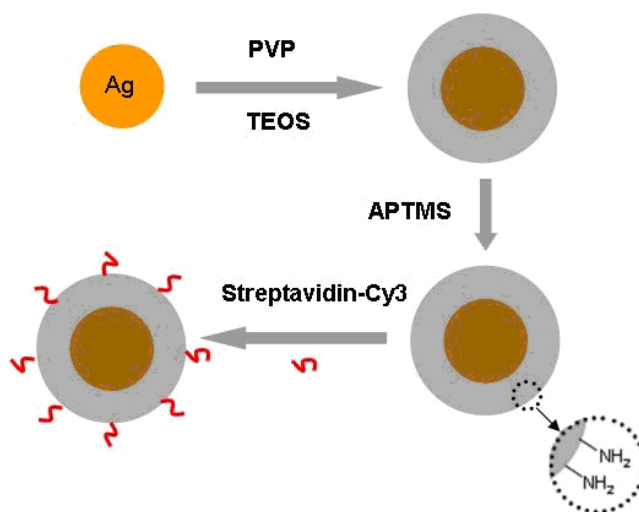
2.1. Chemicals

Tetraethyl orthosilicate (TEOS), 3-aminopropyltrimethoxysilane (APTMS), 3-aminopropyltriethoxysilane (APTES), sodium silicate solution (Na₂O(SiO₂)₃₋₅, 27 wt.% SiO₂), ammonia solution (25%–28%), isopropanol and polyvinylpyrrolidone (PVP10) were purchased from Sigma-Aldrich.

3-aminopropyltrimethoxysilane (APTES) was obtained from ABCR. Silver colloids (diameter: 60 nm) were obtained from British BioCell International. Streptavidin-Cy3 Conjugate was obtained from Jackson ImmunoResearch. Coomassie Brilliant Blue G (CBB) was provided by Sigma-Aldrich. 0.01 M PBS (pH 7.4) was prepared by dissolving the content of one pouch of dried powder in 1 L of ultrapure water. Milli-Q water (18.2 MΩ) was used in all the preparations.

2.2. Synthesis of Ag@SiO₂ particles

The synthesis of Ag@SiO₂ particles was achieved following a reported procedure with some modifications (Scheme 1).¹¹ In a typical synthesis, 2 mL of Ag colloids (2.6×10^{10} particles/mL) were mixed with 50 μL of PVP10 solution (12.8 g/L), the reaction mixture was stirred for 24 h at room temperature. The PVP modified Ag colloids were collected by centrifugation and re-dispersed in 14.4 μL of water, 160 μL of 2-propanol and 8 μL of ammonia. After stirring for 10 min, 0.5 μL of TEOS solution (10 vol.% in ethanol) was added. The reaction mixture was then stirred for another 1 h. The product was separated by centrifugation at 10000 rpm for 10 min, and finally dispersed in 2 mL of ethanol.



Scheme 1. Schematic illustration of synthesis process of core-shell Ag@SiO₂ nanoparticles and covalent bonding of Streptavidin-Cy3 to the surface.

2.3. Surface functionalization of Ag@SiO₂ nanoparticles with amino groups

Ag@SiO₂ nanoparticles were functionalized with APTMS, APTES or APDES using a reported method.¹² The amount of APTMS was calculated to be sufficient to provide approximately a 5-monolayer coating on silica particles. 50 μ L of aminosilane solution in ethanol (1 mM) was added into 1 mL of Ag@SiO₂ dispersion. After stirring overnight, the solution was refluxed at 80°C for two additional hours. The amino-functionalized Ag@SiO₂ particles were purified with several centrifugation-washing steps in ethanol. The surface density of amino groups was determined by CBB titration, using a previously reported method.¹³

2.4. Conjugation of NHS-ester Streptavidin-Cy3 to Ag@SiO₂ particles

200 μ L as-prepared Ag@SiO₂-NH₂ nanoparticles were dispersed in a PBS solution. Then 5 μ L of *N*-hydroxysuccinimide (NHS) ester of Streptavidin-Cy3 (0.01 mg/mL) was added and the mixture was allowed to react overnight at 4°C.

2.5. Preparation of reference samples

Silica nanoparticles were selected as reference for fluorescence enhancement measurement. They were prepared using a variation of the method developed by Stöber *et al.*¹⁴ A solution of 3 mL of ammonia was mixed with 50 mL of dry ethanol. Then 1.5 mL of TEOS was added and the solution was stirred overnight. With this method, silica nanoparticles exhibiting a diameter of approximately 100 nm were obtained. The surface amino functionalization and conjugation of Streptavidin-Cy3 were the same as the ones used for Ag@SiO₂ nanoparticles.

2.6. Characterization methods

SEM images were obtained with an Inspect S50 microscope from FEI working at 10 kV. For SEM, samples were prepared by drop-casting 2 μ L of the Ag@SiO₂ nanoparticles dispersion on a silicon wafer substrate. ImageJ 1.40 g software was used for image analysis. UV-Visible absorption spectra were obtained on a SAFAS UV mc2 double-beam

spectrophotometer using a micro-cuve of 1 mm length containing 5 μ L of solution to analyze. Infrared spectra were done in ATR mode with a Nicolet 6700 spectrometer from Thermo Scientific. Fluorescence steady-state spectroscopy was achieved with a SPEX 1681 0.22 m fluorimeter (excitation: 532 nm, detection: 560–700 nm). For fluorescence scanning experiments, 20 μ L of nanoparticles solutions were drop-casted on nonfluorescent glass slides (Schott Nexterion® Glass D). Glass slides were scanned with a GenePix 4100 A microarray scanner (Axon Instruments) at excitation wavelength of 532 nm and detection wavelength of 575 nm for the Cy3 dyes. Images were analyzed with GenePix Pro 6.0 software.

3. Results and Discussion

3.1. Core-shell Ag@SiO₂ nanoparticles

Sol-gel silica coating of noble-metal colloids is a common method used in different situations for several reasons: (i) the optical properties of metal colloids can be tailored by its dielectric constant,¹⁵ (ii) silica is a chemically inert material that can prevent aggregation, (iii) the thickness of silica coating can be controlled at the nanometer-scale by the sol-gel synthesis, (iv) silica is well adapted for further conjugations of biomolecules or fluorophores.¹⁶ However, a main problem when manipulating metal colloids is that their surface stabilizer is very sensitive to their dispersion medium, which can induce coalescence. Several indirect silica coating methods have been developed: for instance, Liz-Marzan used an aminosilane to functionalize gold colloids, then sodium silicate was added to form a thin silica shell, and finally, modified colloids can be easily transferred into an alcohol-water mixture.¹⁷ Graf *et al.* used PVP as a coupling agent to functionalize metal colloids. This amphiphilic nonionic polymer is soluble in water and in many nonaqueous solvents. Thus, it can be adsorbed onto many different surfaces.¹¹ This method is quite general and can be employed for different kinds of metal colloids, such as gold or silver. Herein, we employed Graf's method to elaborate the core-shell Ag@SiO₂ nanoparticles. The commercial silver colloids are initially functionalized with sodium citrate and dispersed in water, thus they bear negative charges. PVP can be adsorbed on their surface after 24 h of

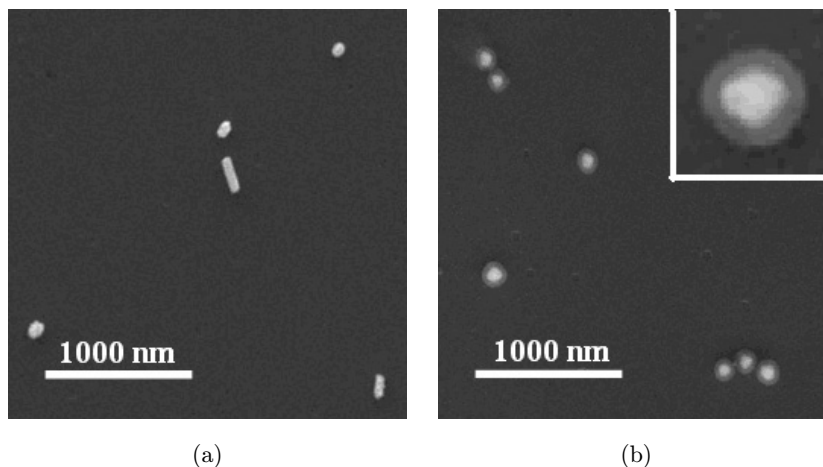


Fig. 1. Ag colloids (a) and core-shell Ag@SiO₂ nanoparticles (b).

reaction at room temperature. Then, the nanoparticles are transferred into 2-propanol, water, ammonia mixture and TEOS as source of silica. The reaction is kept for 2 h at 40°C.

SEM images of commercial Ag colloids and as-prepared core-shell Ag@SiO₂ nanoparticles are shown in Fig 1. The size distribution of commercial Ag colloids is broad, the diameter varies from 60 to 90 nm, and also some nanorods are visible. Different sizes or shapes of metal colloids lead to different plasmon oscillation frequencies.¹⁸ Thus, the plasmon band of these commercial Ag colloids is broad and located between 350 nm and 600 nm, as shown by UV-vis absorption (Fig. 2). After silica layer coating, a homogenous shell of 20 nm of thickness

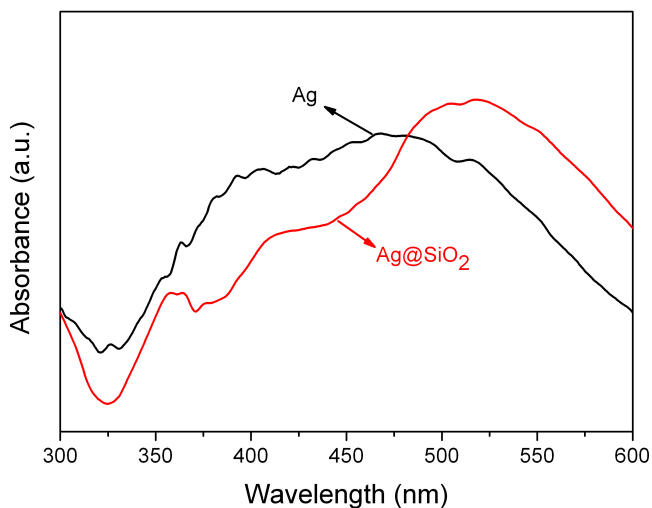


Fig. 2. UV-Visible absorption spectra of Ag colloids and core-shell Ag@SiO₂ nanoparticles.

has been achieved. Consequently, the plasmon band red shifted, due to the increase of the local refractive index around the Ag colloids.¹⁹

3.2. Conjugation of Streptavidin-Cy3 to Ag@SiO₂ nanoparticles

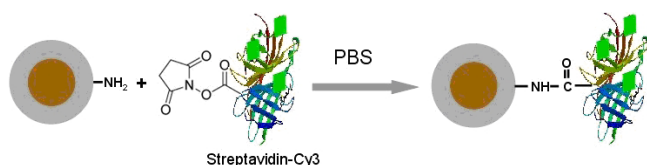
Ag@SiO₂ nanoparticles were firstly amino functionalized. Three aminosilane molecules were selected, as shown in Table 1. The density of amino groups on the silica surface was evaluated by colorimetric titration with CBB.¹³ From Table 1, we can see that with APTMS and APTES almost the same density of amino groups is obtained on silica surface, while with APDES, the density is weaker. Indeed, APTMS has three very reactive alkoxy functions, APTES has three less reactive alkoxy functions and APDES has only one weakly reactive function.

In the process of immobilization, after the alkoxy hydrolysis, the siloxane function reacts with hydroxyl groups on the silica surface, thus more reactive functions the molecule owns, more immobilization capability it has. Thus, APTMS and APTES are easier to immobilize onto silica surface than APDES. In the following, we used APTMS for the high density of amino groups it provides.

Finally, Streptavidin-Cy3 was conjugated to the amino groups. A schematic representation of Streptavidin-Cy3 conjugation to amino-modified Ag@SiO₂ with APTMS is presented in Scheme 2. Streptavidin-Cy3 includes an activated ester function which can react with amino group to form an amide covalent bond.²⁰ The conjugation of Streptavidin-Cy3 should be performed in PBS at 4°C

Table 1. Aminosilane molecules chemical formula and amino density immobilized on silica surface.

Molecule	Density
APTMS (3-aminopropyltrimethoxysilane)	1.08 molecules/nm ²
APTES (3-aminopropyltriethoxysilane)	1.06 molecules/nm ²
APDES (3-aminopropyldimethylethoxysilane)	0.84 molecules/nm ²

Scheme 2. Schematic illustration Streptavidin-Cy3 conjugation to Ag@SiO₂ nanoparticle.

under stirring because higher temperature will induce the loss of activity of activated ester function. A controlled amount of Streptavidin-Cy3 molecule was added, equal to the total amount of amino groups onto silica surface.

4. Metal-Enhanced Fluorescence Intensity

To prove that the fluorescence enhancement is due to the presence of metal core, a reference consisting in pure silica nanoparticles conjugated with the same amount of Streptavidin-Cy3, was prepared. Figure 3 shows the fluorescence emission intensity of Ag@SiO₂-Streptavidin-Cy3 nanoparticles compared to the SiO₂-Streptavidin-Cy3 reference. The emission intensity of Ag@SiO₂-Streptavidin-Cy3 was approximately 2.5 times higher than the reference sample.

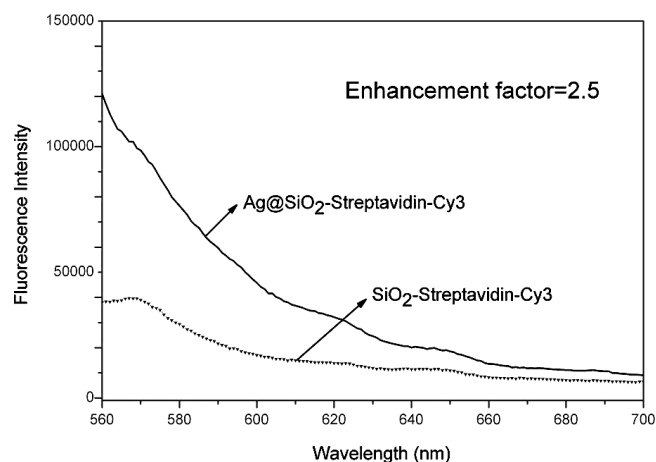
Lakowicz proposed that the fluorescence enhancement is due to the increase of fluorophore quantum yield.⁹ The intrinsic quantum yield (Q_0) of fluorophore is given by:

$$Q_0 = \frac{\Gamma}{\Gamma + k_{nr}}$$

Here, Γ and k_{nr} represent respectively radiative decay rate and nonradiative decay rate. In the presence of a metal, the radiative decay rate near the metal is increased and is given by $\Gamma + \Gamma_m$, Γ_m is the additional radiative decay rate due to the metal, the quantum yield Q_m of fluorophores near the metal thus become:

$$Q_m = \frac{\Gamma + \Gamma_m}{\Gamma + \Gamma_m + k_{nr}}$$

The metal-induced change to $\Gamma + \Gamma_m$ results in an increase of the quantum yield, due to an increase of the weight of the radiative processes compared to the nonradiative processes. Thus, the quantum yield enhancement (Q_m/Q_0 ratio) is more efficient for fluorophores exhibiting a low intrinsic quantum yield Q_0 .

Fig. 3. Fluorescence emission spectra of Ag@SiO₂-Streptavidin-Cy3 nanoparticles (black) and reference sample (red).

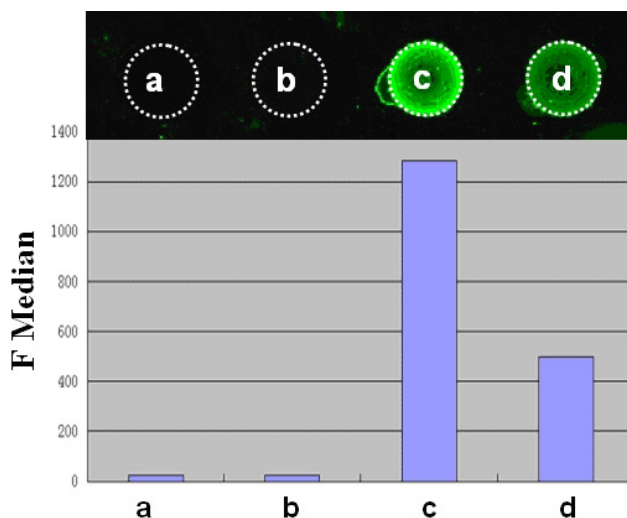


Fig. 4. Fluorescence image and median value of fluorescence intensity (F Median) for (a) only glass slide, (b) Ag@SiO₂, (c) Ag@SiO₂-Streptavidin-Cy3, and (d) SiO₂-Streptavidin-Cy3.

Finally, the fluorescence properties of Ag@SiO₂-Streptavidin-Cy3 nanoparticles were evaluated on a glass slide in order to test them in “real” bioassay conditions. Different samples were dropped on glass slides (Fig. 4) and their fluorescence intensities were compared on a fluorescence scanner, in the excitation and emission conditions generally used for Cy3. We can see that there is no fluorescence intensity for the blank consisting of only glass slide [Fig. 4(a)] and Ag@SiO₂ nanoparticles [Fig. 4(b)]. The fluorescence intensity of Ag@SiO₂-Streptavidin-Cy3 is brighter than SiO₂-Streptavidin-Cy3. The calculations show that the median fluorescence value is increased by 2.6-times. Thus, these silica-coated metal-fluorescent nanoparticles are an effective way to obtain a fluorescence enhancement, and will probably find their application potential in biochips.

5. Conclusion

In conclusion, we have synthesized Ag@SiO₂ nanoparticles, which can be conjugated to Streptavidin-Cy3 molecules after silica surface modification. The fluorescence properties of Ag@SiO₂-Streptavidin-Cy3 nanoparticles were determined under steady-state fluorescence spectroscopy and fluorescence scanning. A 2.5-time enhancement of fluorescence was achieved. Further work will be to optimize different parameters such as the nature and shape of the metal core, the metal-fluorophore distance to improve the fluorescence enhancement.

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