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Metal-Enhanced Fluorescence Solution-Based Sensing Platform

Kadir Aslan,[1] Joseph R. Lakowicz,[2] Henryk Szmacinski,[3] and Chris D. Geddes[1,4]

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In recent years our laboratories have reported the favorable effects for fluorophores placed in close proximity to surface immobilized silver nanostructures. These include; greater quantum yields, reduced lifetimes (increased photostability) and directional emission. However, while these findings are likely to find multifarious applications for surface assays based on enhanced fluorescence detection, a solution based enhanced sensing platform has yet to be realized. In this short, note, we show how SiO_2-coated silver colloids, indeed provide for a solution based enhanced fluorescence sensing platform with a 3–5 fold enhancement typically observed.

KEY WORDS: Radiative decay engineering; metal-enhanced fluorescence; solution assays.

Recently our laboratories have fabricated numerous noble-metal surfaces for metal-enhanced fluorescence detection whereby fluorophores can undergo modifications in their radiative decay rate, Γ, Fig. 1 [1–8]. Silver island films (SiFs), formed an APS-coated glass slides by the reduction of silver nitrate by glucose, have proved a versatile surface, providing up-to 10 fold increases in fluorescence signal of appropriate fluorophores [1–5]. Silver colloid coated surfaces have yielded up-to 50-fold enhancements [6], while silver-fractal-like coatings have been shown to increase fluorescein emission, several thousand fold [7,8]. These findings have been most encouraging and suggest the use of metallic nanostructures in surface assays and high-throughput screening plate-well formats. However, to date, little attention has been given to solution-based systems. This is in part due to the complexities associated with working with nano-or picomolar concentrations of silver colloids, their purification, and comparison with an appropriate control system, i.e. a sample with no silver.

In this short note, we show that 3- to 5-fold enhanced fluorescence signals can be obtained from SiO_2-coated silver colloids labeled with Cy3 and by their aggregation in suspension. This inert coating alleviates the close proximity quenching by noble-metals [1,9], as well as provides for a wide variety of chemistries for biomolecule attachment.

The preparation of silica-coated silver spheres and biotinylation of the silica coated silver spheres in suspension is performed in multiple steps. Firstly, the silver spheres were prepared in solution. In this regard, 2 mL of 1.16 mM trisodium citrate solution was added drop wise to a heated (95°C) aqueous solution of 0.65 mM of $AgNO_3$ while stirring. The mixture was kept heated for 10 min, and then it was cooled to room temperature. This procedure yields silver spheres with sizes in the range of 30 to 80 nm. The surface of the silver spheres were modified with 3-(aminopropyl)ethoxysilane (APS) in ethanol. The APS-coated silver spheres were resuspended in a predetermined amount of water and NH_4OH. Then, a solution of

[1] Institute of Fluorescence, Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, Maryland.
[2] Center for Fluorescence Spectroscopy, Department of Biochemistry and Molecular Biology, Medical Biotechnology Center, University of Maryland School of Medicine, Baltimore, Maryland.
[3] Microcosm Inc., Columbia, Maryland.
[4] To whom correspondence should be addressed. E-mail: geddes@umbi.umd.edu

ABBREVIATIONS: TEOS, tetraethylorthosilicate; BSA, bovine serum albumin; MEF, metal-enhanced fluorescence; RDE, radiative decay engineering; Cy 3, N,N'(dipropyl)-tetramethylindodicarbocyanine; TEM, transmission electron micrograph.

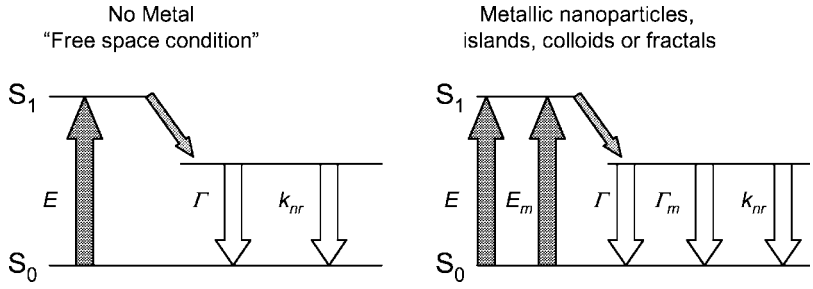

Fig. 1. Classical Jablonski diagram for the free space condition and the modified form in the presence of metallic particles, islands, colloids or silver nanostructures. E-excitation, E_m, Metal-enhanced excitation rate; Γ_m, radiative rate in the presence of metal.

tetraethylorthosilicate (TEOS) was rapidly injected, the reaction continuing for a period of time to control the thickness of the SiO_2 coating layer. The amount of TEOS was calculated based on the total area of silver spheres and the desired shell thickness, assuming complete conversion of TEOS to silica (Fig. 2). Figure 2 (Bottom) shows a typical TEM image of the silica-coated silver spheres. Silica-coated silver spheres were then biotinylated by incubating the silver spheres in a micro molar solution of biotinylated-BSA (BSA-biotin) overnight. The coated silver spheres were separated from the unbound BSA-biotin by centrifugation and were resuspended in deionized water.

In order to demonstrate that the silica-coated silver spheres can result in metal-enhanced fluorescence upon their close proximity, i.e. a combination of both a radiative rate modification and enhanced electric field effect [1–5], we have utilized the well-known biotin-streptavidin interactions. These interactions occur in relatively fast reaction times (20 min) and is one of the strongest biological interactions found in nature (dissociation constant, $K_D = 10^{-15}$ M). For this purpose, we have aggregated the

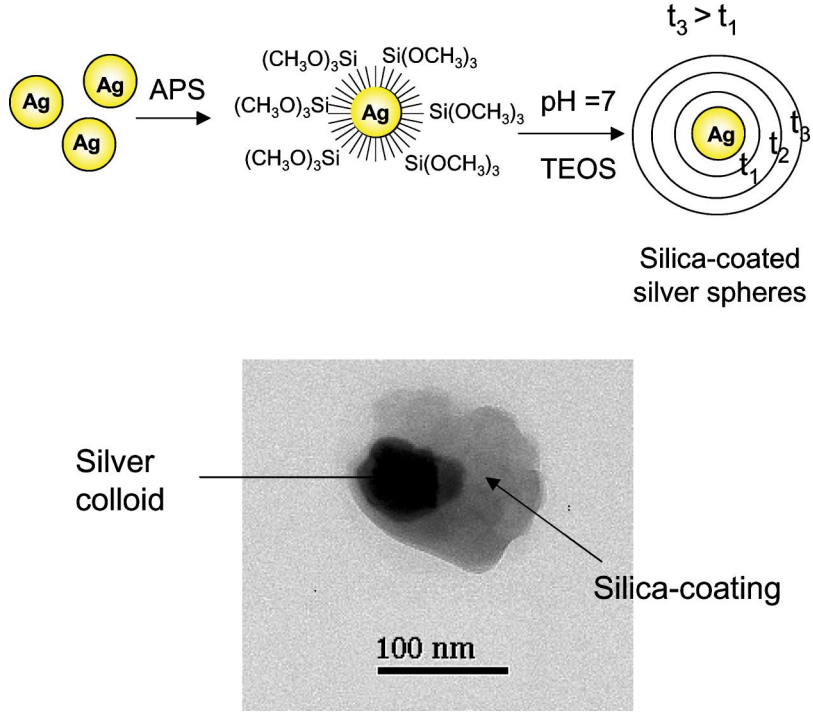

Fig. 2. Experimental procedure for coating of silver spheres with silica (*Top*), and TEM image of silica coated-silver spheres (*Bottom*).

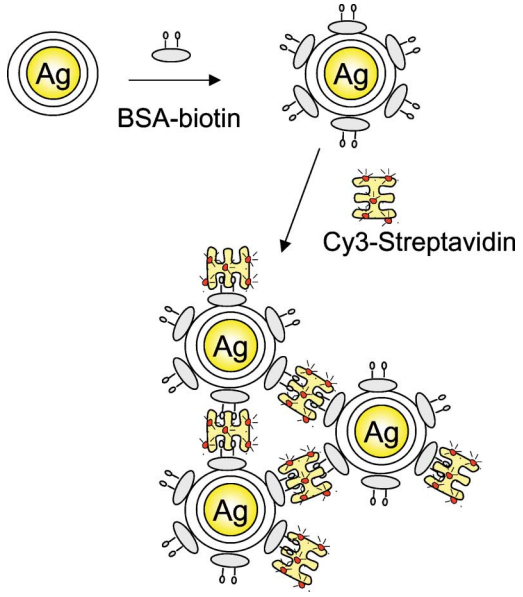

Fig. 3. Experimental procedure for coating of silver spheres with biotinylated-BSA and aggregation of silica-coated silver spheres with Cy3-labeled streptavidin.

biotinylated-silver spheres with Cy3-labeled streptavidin (Fig. 3).

Figure 4 shows the fluorescence emission intensities recorded from the aggregated biotinylated-silver spheres (no label) and from the non-aggregated biotinylated-silver spheres, control samples, C1 and C2, respectively, where can typically see a slight fluorescence signal from the control samples.

However, aggregation of the labeled biotinylated-silver spheres resulted in an approximately 3- to 5-fold higher fluorescence intensity than the non-aggregated system or the aggregated system with no label. This was

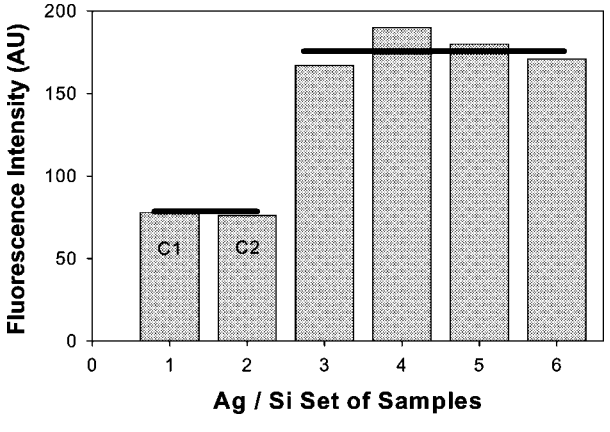

Fig. 4. Fluorescence emission intensity recorded from the aggregated silica-coated silver spheres (Samples 3–6), and control samples (Samples C1-2).

repeated several times, samples 3–6, and in an every case a typically greater signal was observed for the Cy3 labeled aggregated SiO_2 coated colloids.

Interestingly, the Cy3 lifetime was also significantly reduced from ≈1.3 ns to a value too short to measure on the employed instrumentation. An increase in fluorescence signal, accompanied by a reduced lifetime, can only be explained by a radiative rate modification [1–8].

CLOSING REMARKS

In this short note we have reported for the first time how SiO_2 coated silver colloids can be used to provide for a metal-enhanced fluorescence sensing platform. It is likely that other sensing schemes, based on the aggregation and/or disassociation of silver nanostructures can also be realized. The additional use of a SiO_2 coating as described here, provides for both surface nanostructure functionality, protection of the surface plasmon absorption, and a coating to distance fluorophores from close-range metal quenching [1,9].

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