Separation distance dependent fluorescence enhancement of fluorescein isothiocyanate by silver nanoparticles†

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Nanocomposites consisting of a metal core, a silica-spacer shell with controlled thickness, and a dye-labelled shell were synthesized and separation distance dependent fluorescence enhancement of fluorescein isothiocyanate by silver nanoparticles was studied; the results indicated an optimum enhancement of 4.8 times with a spacer shell thickness of 21 nm.

Metal nanoparticles (NPs), such as Au and Ag, are known to dramatically change the optical properties of nearby fluorophores.[1] The fluorescence of fluorophores might be enhanced or quenched due to the presence of nearby metallic NPs. The strength of the enhancement or quenching is influenced by many factors, such as size and shape of the metal NPs, the orientation of the fluorophore dipole moments relative to the NPs, the radiative decay rate and quantum yield of the fluorophore.[2] Studies on fluorophores on metal surfaces showed fluorescence quenching is usually observed if the fluorophore is located at a very short distance (<5 nm) from the metal surface.[2] When the fluorophore–metal distance is increased, both fluorescence quenching[3] and enhancement[4] have been reported.

Most studies on fluorescence enhancement are based on silver island films,[1d,4b,4d] which consist of Ag NPs with sub-nanometer sizes on a planar glass substrate. Fluorescence enhancement of tens of folds was obtained using this method.[4b] However, metallic NPs in colloid usually failed to yield fluorescence enhancement, except for some occasional success which was difficult to reproduce.[4a] This might be due to the relatively short fluorophore–metal distances (<16 nm) or flexible spacers employed in these systems. Recently Tovmachenko[2c] *et al.* reported fluorescence enhancement in metal-core/silica-spacer/fluorophore NPs. A 12.5-fold enhancement has been reported with a fluorophore–metal distance of 24 nm. These results suggest that fluorophore–metal distance optimization is important for fluorescence enhancement.

In this work, we investigated the fluorescence enhancement in an Ag-core/SiO_2-spacer/fluorophore system by varying the fluorophore–metal distance from <1 nm to 90 nm. The reaction scheme is presented in Fig. 1. Fluorescein isothiocyanate (FITC) was used as the fluorophore and Ag NPs with an average particle size of 75 ± 5 nm were chosen as the metal core. We found that the fluorescence of FITC was quenched on direct contact with the silver NP. When a silica spacer shell was added between the two layers, fluorescence enhancement was observed instead. An optimum enhancement of 4.8 times occurs with a shell thickness of 21 nm.

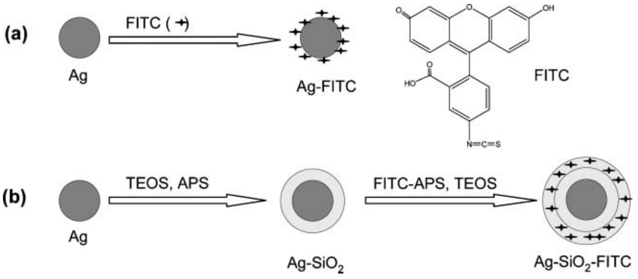

Fig. 1 Schematic illustration of synthesis of Ag–FITC complex and Ag–SiO_2–FITC core-shell NPs. In (a), FITC molecules are directly attached to Ag NPs *via* –SCN groups. In (b), two SiO_2 layers are formed sequentially. FITC are incorporated into the second SiO_2 layer. The fluorophore–metal distance is controlled by adjusting the thickness of the SiO_2 spacer layer. TEOS: tetraethoxysilane; APS: 3-aminopropyltrimethoxysilane; FITC: fluorescein isothiocyanate.

The absorption maxima, fluorescence maxima and fluorescence quantum yield of FITC in basic solutions are 495 nm, 520 nm and ~0.93,[5] respectively. Ag NPs were prepared by a conventional citrate reduction method.[6] Their surface plasmon absorption peaks are centred at 438 nm. Theoretical studies showed that larger metal NPs have better fluorescence enhancement effects[7] compared with smaller ones. To study the interaction of Ag NPs with FITC in the absence of SiO_2 spacer, Ag–FITC nanocomposites were prepared by mixing Ag NPs with FITC directly. The isothiocyanate group (S=C=N–) of FITC directly binds to the surface of a Ag particle, in which the fluorophore–metal distance is less than 1 nm. To investigate the influence of separation distance on fluorescence enhancement, a SiO_2 shell with variable thickness was first grown on the surface of Ag NPs, followed by growth of a second SiO_2 shell in which FITC was incorporated. The fluorophore–metal distance was controlled by changing the thickness of the SiO_2 spacer shell. The details of the synthetic methods are described in the supporting information.

Fig. 2 shows typical TEM images of different nanoparticles. Fig. 2a is a typical image of Ag NPs that were used in this study. The average size of these Ag NPs is 75 ± 5 nm. Fig. 2b shows the structure of Ag–SiO_2 core-shell NPs with a shell thickness of 20 nm. Well defined particles which contained a dark contrast Ag core and a light contrast SiO_2 shell were readily observed. Most of the SiO_2 was found to form around Ag NPs. There were also small fractions of SiO_2 NPs formed alone (<5% according to the TEM images). Fig. 2c displays the structure of Ag–SiO_2–FITC NPs after

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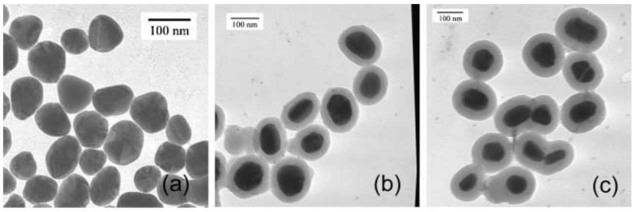

Fig. 2 TEM images of (a) Ag NPs, (b) Ag–SiO_2 core-shell NPs and (c) the corresponding Ag–SiO_2–FITC core-shell nanocomposites.

the second SiO_2 coating. FITC molecules were incorporated into the second SiO_2 shell using a previously reported method.[8] Compared with Fig. 2b, the thickness of the SiO_2 shell increased about 5 nm. Although two SiO_2 shells were formed sequentially, they appeared homogeneous. This shows excellent compatibility of FITC with the SiO_2 matrix. The fluorophore–metal distance was defined as the distance from the centre of the second SiO_2 layer to the edge of the Ag NPs. The FITC–Ag distance is 23 nm for Fig. 2c.

The emission spectra of Ag–FITC and Ag–SiO_2–FITC core-shell nanocomposites, as well as comparison with their FITC reference control are presented in Fig. 3. All fluorescence spectra were measured with an excitation wavelength of 488 nm. For Ag–FITC nanocomposites, in which FITC is directly connected to Ag NPs, the fluorescence of FITC is found to be quenched by a factor of $\sim$3. This result is consistent with the previous report.[9] The fluorescence quenching was believed to be due to resonant energy transfer from the fluorophore to the metal particle.[1] Fig. 3b shows emission spectra of Ag–SiO_2–FITC NPs with a fluorophore–metal distance of 21 nm. Here the control was prepared by dissolving the Ag–SiO_2–FITC NPs in 0.4 M NaOH for 24 h to release FITC molecules from the SiO_2 shell, followed by addition of 50 μL of 2.0×10^{-4} M of $K_3Fe(CN)_6$ solution to dissolve the Ag NPs. 4.8-fold fluorescence enhancement was observed. In addition, the fluorescence maximum of FITC in Ag–SiO_2–FITC core-shell NPs displays a red shift of $\sim$6 nm. It might be due to the dielectric environment changes upon coating an additional SiO_2 shell.[8]

It should be noted that the definition of absolute enhancement factors needs to consider the effects of addition of NaOH and $K_3Fe(CN)_6$ as well as the concentration quenching effect in the nanocomposites due to locally concentrated FITC in the nanoshell. Under our experimental conditions, it was found that addition of NaOH and $K_3Fe(CN)_6$ had opposite effects on the FITC fluorescence intensities: addition of NaOH enhances the FITC fluorescence by 10–25% while addition of $K_3Fe(CN)_6$ quenches the fluorescence by $\sim$15%. The concentration quenching is relatively tricky to characterize due to the difficulty in determining the actual concentration of FITC inside the nanoshell. We performed another control experiment to investigate this effect. In the control experiments, a SiO_2 core was used instead of an Ag core with the same thickness of SiO_2 spacer and dye shells outside to mimic our nanocomposites. These samples were also treated with NaOH and $K_3Fe(CN)_6$. It was found that fluorescence intensities of the samples increase 20–25% upon addition of NaOH to release FITC. This increase is due to the combined effect of the pH effect[10] and suppression of concentration quenching. Further addition of $K_3Fe(CN)_6$ will bring down the fluorescence intensities to the level of only slightly higher than those before the treatment (<10%). These results confirmed that different fluorescence intensities before and after the treatment reflect the enhancement due to the presence of the Ag nanoparticles.

Fig. 4 shows the separation distance dependent fluorescence enhancement of FITC by Ag NPs with fluorophore–metal distance from <1 nm to 90 nm. The corresponding TEM images of the Ag–SiO_2–FITC core-shell NPs are also shown. When the fluorophore–metal distance was increased, the fluorescence

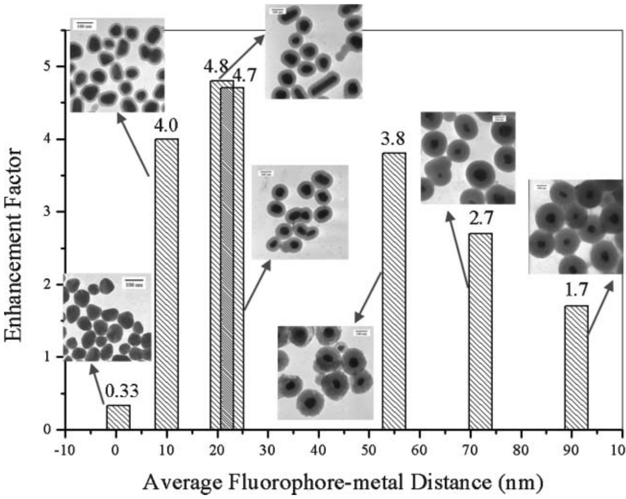

Fig. 4 Distance dependent fluorescent enhancement of the FITC by Ag NPs. Insets are the TEM images of the corresponding Ag–SiO_2–FITC core-shell NPs. The fluorophore–metal distance was defined as the distance from the centre of the second SiO_2 layer to the edge of the Ag NPs.

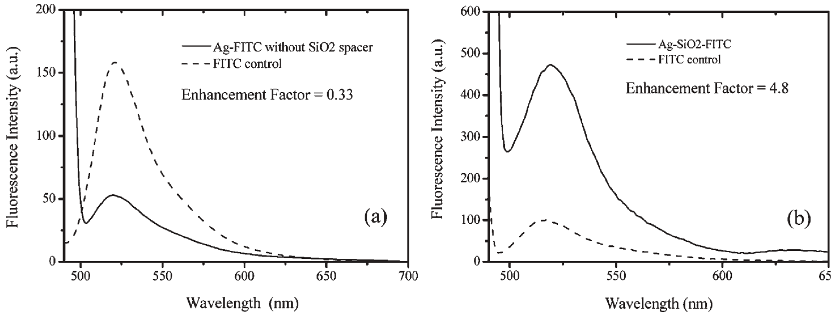

Fig. 3 Emission spectra of (a) Ag–FITC complexes without SiO_2 spacer, (b) Ag–SiO_2–FTIC core-shell NP with a fluorophore–metal distance of 21 nm. The fluorescence enhancement factor is defined as the ratio of the fluorescence intensity of Ag–SiO_2–FITC to the fluorescence intensity of the control.

intensity of FITC was found to be enhanced, in contrast to fluorescence quenching when the fluorophore and the metal core are in direct contact. An enhancement factor of 4.0 was observed with a fluorophore–metal distance of 10 nm. The optimal fluorescence enhancement with an enhancement factor of 4.8 occurred at a fluorophore–metal distance of 21 nm. Further increase of fluorophore–metal distance caused a decrease in the enhancement factor. A fluorescence enhancement factor of 1.7 was still observed at a fluorophore–metal distance of 90 nm.

The mechanism of fluorescence enhancement is still under discussion. The enhancement factor 4.8 at a separation distance of 21 nm corresponds to an apparent yield of 440%. A typical explanation for fluorescence enhancement on a metallic surface is the increase in radiative decay rate.[2] This contribution cannot account for the fluorescence enhancement observed here, since the fluorescence quantum yield of FITC in basic solutions (0.93) is very close to unity. In addition, the increase in radiative decay rate also comes with a strong increase of non-radiative decay, which will counteract the effect of the increase in radiative decay rate. It was previously suggested that amplification of electric fields on the fluorophore near the metal surface could result in fluorescence enhancement.[11] The observed "abnormal" high apparent yield is more likely due to local electric field amplification near the metallic surface and thus enhancement of the absorption. This is also consistent with the fact that the Ag plasmon band has a good spectral overlap with the absorption spectrum of the FITC. Local electric amplification is known to be dependent on the size of the metallic particles.[2b] The fluorescence enhancement and enhancement mechanisms are also expected to be particle size dependent. The optimal separation distance for fluorescence enhancement was found to be 21 nm, which is consistent with the calculation by Tovmachenko *et al.*,[2c] where an optimal spacer thickness of 20–30 nm was predicted.

In summary, a SiO_2 spacer shell was used to vary the fluorophore–metal separation distance from <1 nm to 90 nm to study the fluorescence enhancement of FITC by Ag NPs. The optimal fluorescence enhancement was observed at a fluorophore–metal distance of 21 nm. The fluorescence enhancement is mainly due to the local electric field amplification. The Ag–SiO_2–FITC core-shell NPs have potential applications in bioanalysis as enhanced fluorescence labels.

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