Enhanced Luminescence of Phenyl-phenanthridine Dye on Aggregated Small Silver Nanoparticles

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Tiopronin-coated silver particles (average diameters of core = 1.6 nm) were prepared by a modified Brust method, and the ligands on the metal core were partially displaced by (2-mercapto-propionylamino) acetic acid-2,5-dioxo-pyrrolidin-1-ylester through ligand exchanges. The particles were bound on amine-pendent polymer backbones by condensation to generate compact aggregates of particles. The aggregated particles displayed a plasmon absorbance rising at 440 nm. Luminescence was enhanced to about 2 times with increasing the plasmon rising by the particle aggregation upon excitation at 400 nm when the polymer was labeled by 3,8-diamino-6-phenyl-phenanthridine.

Introduction

Luminescence spectroscopy can be enhanced when the dye is localized near a metal particle.[1,2] This phenomena, defined as surface enhanced fluorescence (SEF), can be used to develop next-generation biological and chemical sensors.[3–8] The SEF is suggested to occur only when the dye is localized at a certain distance from the metal surface to overcome competitive quenching.[2c–e] An electromagnetic field is believed to exist near the metal core to bring up the SEF.[9] When several particles approach closely, the electromagnetic fields from them are expected to be overlapped to result in a stronger field that leads to a stronger SEF. Meanwhile, the SEF is suggested to be dependent on the core size of the particle. The small particle (diameter <2 nm), which displays only an absorbance decrease from high to low energy wavelength without a plasmon absorbance,[10] is not able to induce a SEF,[7a,11] implying that the plasmon absorbance of the metal particle is a crucial factor in evaluating the electromagnetic field and furthermore bringing up an efficient SEF. On the other hand, the small particle is regarded to possess a larger surface area to cap more functionalized ligands on the metal core relative to the large particle,[10] which is beneficial to improve the sensitivity of detection. In addition, the small particle also displays better solubility, chemical stability, and mobility relative to the large particle in solution. These features are important in developing novel nanomaterials to work as platforms for some biological sensing like in vivo detection. Therefore, it is highly interesting to investigate how to induce the SEF on the small particle.

It was reported that the small particles could bring up a plasmon absorbance rising when they were approached and aggregated compactly.[12] Herein, we wondered whether the luminescence was affected by the plasmon rising by the particle aggregation. The organic monolayer-protected silver particles were employed in this study because of their good chemical stability and high solubility in solution.[10] Silver was used as the metal core because of its relatively weaker quenching capability than gold. *N*-(2-Mercaptopropionyl)glycine (abbrevi-

ated as tiopronin) was used as the ligand because the tiopronin-coated silver particles displayed good solubility in water.[13] The particles were prepared by a modified Brust method, and the core sizes were controlled through the mole ratio of tiopronin/silver salt in the preparation. The tiopronin-coated particles were functionalized with (2-mercapto-propionylamino) acetic acid 2,5-dioxo-pyrrolidin-1-ylester (Scheme 1) through ligand exchanges,[14] and the succinimide-terminated particles were bound on amine-pendent polymers by condensation to achieve the aggregations of particles.

Silver particles can be synthesized using polystyrene mesospheres as templates,[15] implying that the polymer is a good medium to bind the particles. In this study, the amine-pendent polymers were prepared by condensation between poly(methylvinyl-ether-*alt*-maleic anhydride) and diamine derivatives (Scheme 2), including 1,6-diaminohexane (unlabeled polymer) and 3,8-diamino-6-phenyl-phenanthridine (labeled polymer). The succinimide-terminated particles were bound to amine-pendent polymers by condensation, leading to compact aggregation of bound particles along the polymer backbones.[12b] To monitor the absorbance spectral change by the particle aggregation by this model, the unlabeled polymer was first employed to bind the particles, and then the labeled polymer was used to study the dependence of luminescence spectral change with the particle binding. Phenyl-phenanthridine, displaying an absorbance maximum near 400 nm when binding on the polymer backbone, was expected to have sufficient coupling with the plasmon absorbance of the silver particle, so it was used as a fluorophore to study the SEF in the particle aggregation.

Experimental Procedures

Chemicals. All reagents (Aldrich), spectroscopic grade solvents (Fisher and Aldrich), and deuterated solvents (Aldrich) were used as received. The RC dialysis membrane (MWCO 8000) was available from Spectrum Laboratories, Inc. (2-Mercapto-propionylamino) acetic acid 2,5-dioxo-pyrrolidin-1-ylester was synthesized as in a previous report.[16]

Amine-Pendent Polymers. Amine-pendent polymers were prepared routinely as in Scheme 2 with an excess amount of diamine to avoid cross-linking. The diamine derivative (1.0

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SCHEME 1: Displacement of Succinimide-Terminated Thiolate Ligands on the Tiopronin-Coated Silver Nanoparticle through Ligand Exchanges
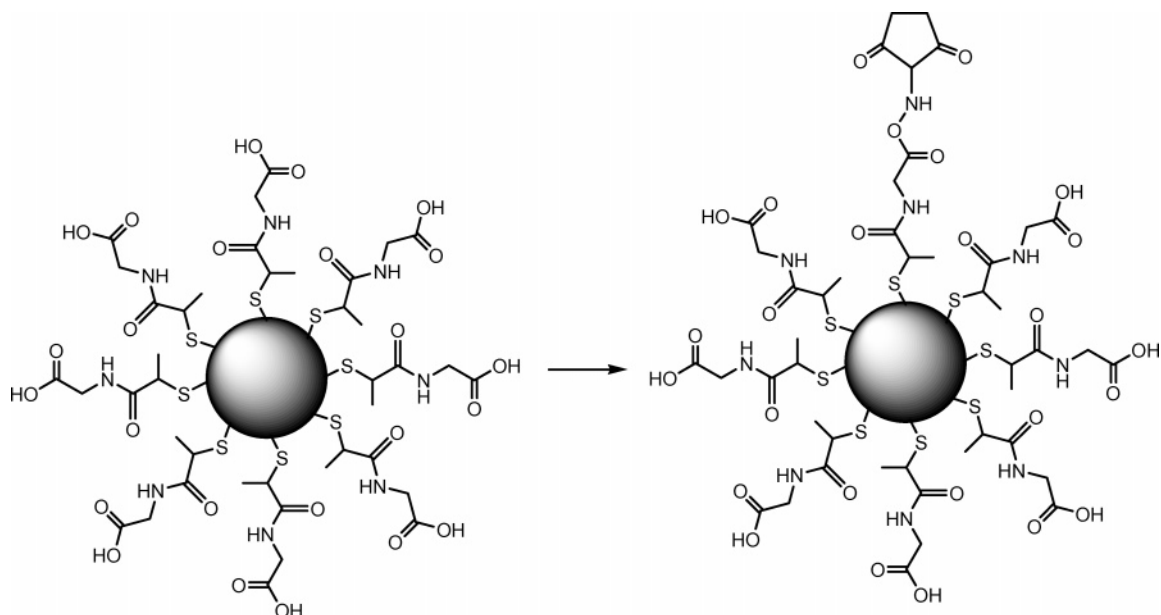
SCHEME 2: Condensation of Diamine Derivative and Poly(methylvinyl-ether-*alt*-maleic anhydride) and Binding of Amine-Pendent Polymer with Silver Particle
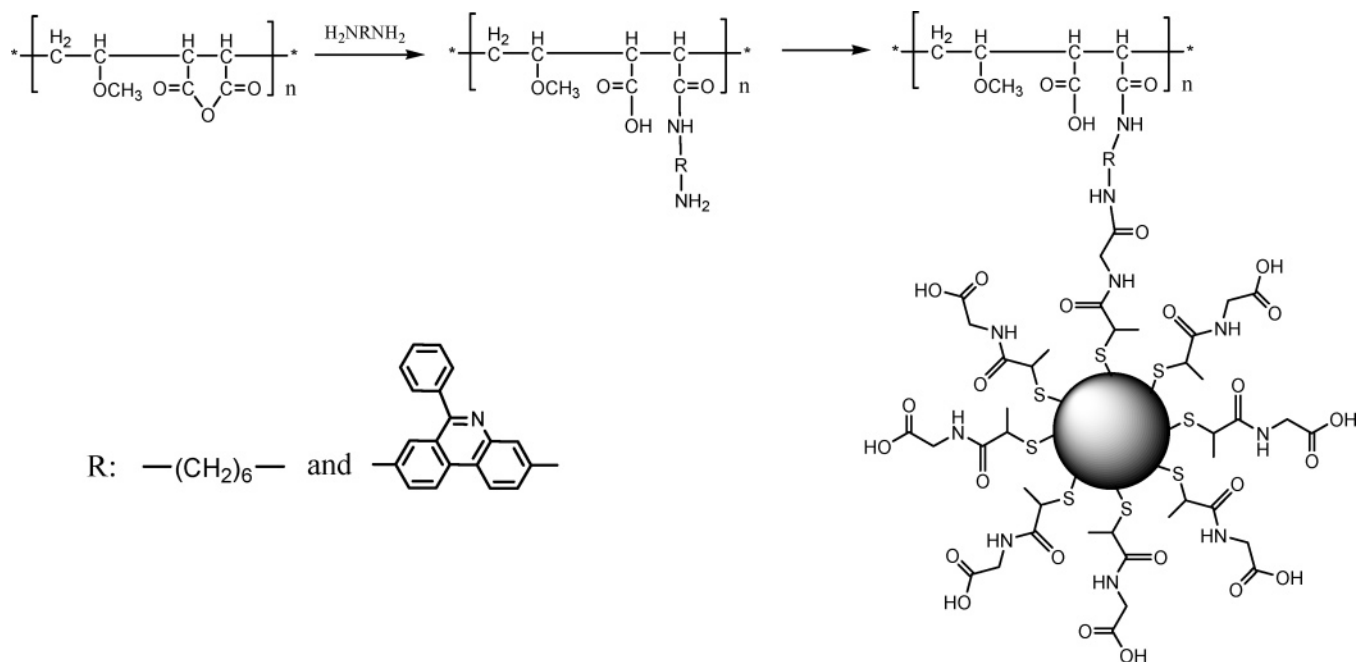
mmol), including either 1,6-diaminohexane or 3,8-diamino-6-phenyl-phenanthridine, was dissolved in 50 mL of acetone, and then poly(methylvinyl-ether-*alt*-maleic anhydride) (average M_w ca. 216 000, 33 mg, 0.2 mmol) in 10 mL of acetone was added slowly at 0 °C. Stirring at room temperature for 1 h, 40 mL of ethanol was added to the solution, and the acetone was removed completely under vacuum. A small quantity of suspends generated by cross-linking between polymer and diamine derivative was filtered, and the solution was dialyzed against ethanol to remove the free diamine compound. The solution was adjusted to pH = 9 by 10 mM NaOH aqueous solution.

Tiopronin-Coated and Mixed Monolayer Silver Nanoparticles. Tiopronin-coated silver particle (particle **1**) was prepared using a modified Brust reaction[10,17] with tiopronin and silver nitrate in methanol. In a typical reaction, AgNO₃ and tiopronin

were codissolved by a mole ratio of AgNO₃/tiopronin = 1:6 in methanol. Ten times the excess amount of NaBH₄ in methanol was added with rapid stirring at 0 °C. The black suspension was stirred for an additional 1 h, then isolated on a Millipore porous filter (FHLC04700, 0.45 μm), washed with an excess amount of methanol, and dried in air. The residuals were dissolved in water and purified by dialysis (MWCO 8000) against water.

The mixed monolayer nanoparticle (particle **2**) was obtained by stirring a mixing aqueous solution of particle **1** and (2-mercapto-propionylamino) acetic acid 2,5-dioxo-pyrrolidin-1-ylester in a 5:1 mole ratio of tiopronin/thiolate ester for 72 h at room temperature. Water was removed under vacuum, and the residue was washed with methanol to remove uncapped thiolate compound. The composition of the mixed monolayer

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was assessed by ^{1}H NMR spectra with the ratio of methylene on succinimide and methyl on tiopronin.[14a]

Binding Mixed Monolayer Particles on Amine-Pendent Polymers. The amine-pendent polymer was diluted to 100 times in water (the concentration of phenyl-phenanthridine = 5.0×10^{-5} M). The mixed monolayer particle aqueous solution (1 mg/mL) was added quantitatively to the amine-pendent polymer aqueous solution. The mixture was kept stirring for 5 h to make a complete condensation between the succinimide groups on the particle and the amine groups on the polymer.

Spectra. ^{1}H NMR spectra were recorded on GE-QE 300 spectrometer. Absorption spectra were monitored with a Hewlett-Packard 8453 spectrophotometer in a 1 cm cell. Luminescence spectra were recorded with Cary Eclipse fluorescence spectrophotometer. Transmission electron micrographs (TEM) were taken with a side-entry Philips electron microscope operated at 120 keV. 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 the grids.

Results and Discussion

To avoid cross-linking between amine-pendent polymer and diamine derivative, the amine-pendent polymer was prepared using 5 times the excess amount of diamine derivative than poly-(methylvinyl-ether-*alt*-maleic anhydride) in the condensation. Two kinds of diamine derivatives were employed, including unlabeled 1,6-diaminohexane (the grafted polymer was abbreviated as unlabeled polymer) and dye-labeled 3,8-diamino-6-phenyl-phenanthridine (the grafted polymer was abbreviated as labeled polymer). The condensation was verified to occur by a solubility change of polymer before and after reaction: poly-(methylvinyl-ether-*alt*-maleic anhydride) was dissolved in acetone and methylene chloride but not in water and alcohol, while the condensed polymer was soluble in all four solvents. The reaction solution was diluted in ethanol, and acetone was removed under vacuum. The residual ethanol solution was dialyzed against ethanol to remove the free diamine derivative and then adjusted to pH = 9 using 10 mM NaOH aqueous solution. Under this condition, the phenanthridine displayed a strong luminescence as well as the silver nanoparticles, which displayed good solubility in water.

Besides the solubility change, the condensation between poly-(methylvinyl-ether-*alt*-maleic anhydride) and 3,8-diamino-6-phenyl-phenanthridine could also be verified by absorbance and luminescence spectral changes when the amine on the dye molecule was transferred to amide in the condensation (Figure 1). The maximum absorbance wavelength was blue-shifted from 417 nm for the free dye to 405 nm after binding to the polymer in acetone, and the luminescence wavelength was red-shifted from 508 to 544 nm upon excitation at 400 nm. Both absorbance and luminescence bands became broaden when binding the dye molecule to the polymer, which was probably due to the mobility restriction of bound dye on the polymer backbone. The concentration of phenyl-phenanthridine was measured to be 5.0 mM in solution through the absorbance, which was close to that of polymer in the preparation, indicating that the free dye was removed completely in dialysis and that the coupled dye was bound on the polymer in a mole ratio of 1:1.

Tiopronin-coated silver particles (particle **1**), prepared by a modified Brust method in a mole ratio of tiopronin/silver salt = 6:1, were dissolved in water at pH = 9.[13] The metal cores were outlined by a TEM image (Figure 2a) showing a poly-dispersion of the core size with an average diameter of 1.6 nm.
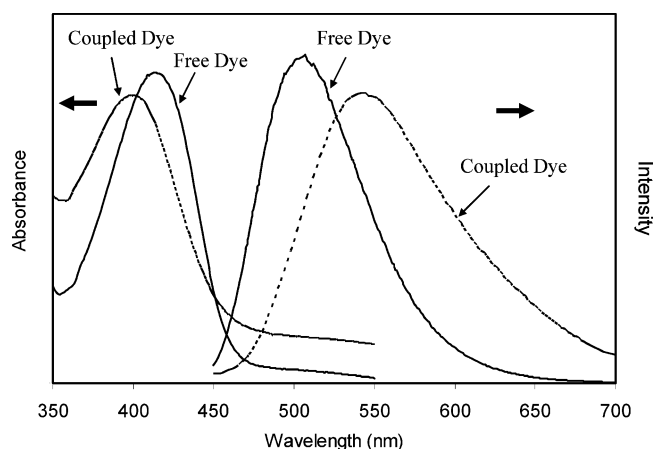

Figure 1. Absorbance and luminescence spectra of free and coupled dye in acetone. Luminescence spectra were monitored upon excitation at 400 nm.
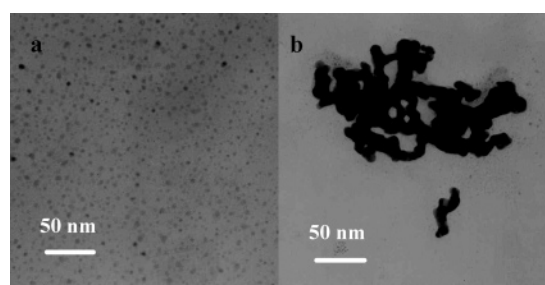

Figure 2. Transmission electron micrograph (TEM) images of (a) particle **1** and (b) particle **4**.
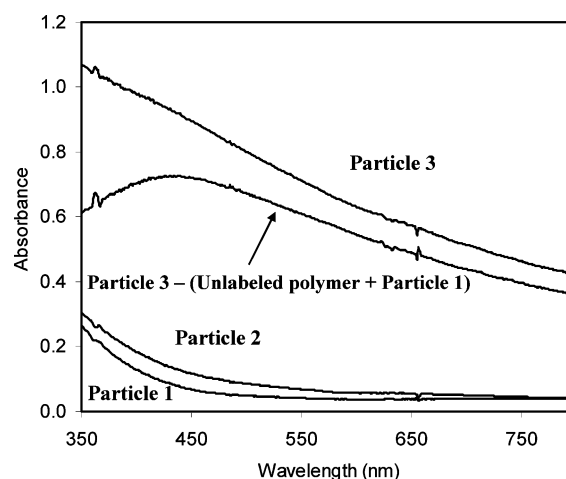

Figure 3. Absorbance spectra of particles **1** (3.0×10^{-6} M), **2** (3.0×10^{-6} M), **3** (3.0×10^{-6} M), and residual spectrum of subtracting the mixture of unlabeled polymer and particle **1** from particle **3** in water.

The composition thus was estimated to be $Ag_{96}(Tio)_{53}$.[10] Particle **1** displayed a sharp absorbance decrease from high to low energy wavelength without a plasmon absorbance (Figure 3), consistent with the feature of small metal crystals.[13]

The tiopronin-coated silver particles could be functionalized with (2-mercapto-propionylamino) acetic acid 2,5-dioxo-pyr-rolidin-1-ylester through ligand exchanges in a 1:1 mole ratio (Scheme 1).[14] The mixed monolayer particles (particle **2**) gave a composition of $Ag_{96}(Tio)_{45}(ester)_8$, determined by ratio of methyl on tiopronin/methylenes on succinimide on ^{1}H NMR spectroscopy.[10] The TEM image showed that the size of metal core was not changed obviously after the ligand exchange reaction. Particle **2** exhibited an identical absorbance feature of

small metal crystals as particle **1** without an obvious plasmon absorbance (Figure 3).[18]

Particle **2** was bound to the amine-pendent polymers by the condensation between the terminated-succinimides on particle **2** and the pendent-amines on the polymers to yield polymer−particle adducts (Scheme 2). Although the particles were expected to have three-dimensional packing along the polymer backbones, they were suggested to aggregate compactly because the neighbor pendent amine groups were separated by four C−C bonds on the polymer backbone, and the distance was estimated to be only ca. 0.6 nm, much shorter than the average diameter of particles (1.6 nm). The compact aggregation of particles could result in an absorbance spectral change.[12] To observe the absorbance spectral change, particle **2** was first bound to the unlabeled polymer. Comparing with a simple codissolved mixture of particle **1** and unlabeled polymer, the unlabeled polymer−particle adducts (particle **3**) displayed a much smoother absorbance decay from high to low energy wavelength (Figure 3), which was probably due to an absorbance rising by the aggregation of the particle. The absorbance rising became more obvious when subtracting the absorbance spectrum of mixture from that of the particle **3** because some particles were believed to be in a status of nonaggregation or incompact aggregation on the polymer backbone. The polymer actually did not show an obvious absorbance spectrum. The residual spectrum displayed an obvious maximum at 440 nm. This wavelength, close to the plasmon wavelength of large silver particle, could be indeed attributed to the plasmon absorbance by the aggregated particles. Meanwhile, the absorbance difference at 440 nm reached saturation at the mole ratio of particle/amine = 0.1. Each particle **2** was known to have an average of eight succinimide groups, so the mole ratio of succinimide/amine was close to 1:1 in the condensation. Therefore, almost all amine groups on the polymer backbones were consumed when binding the particles at the mole ratio of particle/amine = 0.1.

Particle **2** could also be bound on the labeled polymers to produce the labeled polymer−particle adducts (particle **4**), and this kind of adduct was used to study the luminescence spectral change in the particle aggregation. The aqueous solution of labeled polymer (dye concentration = 5.0×10^{-5} M) and particle **2** (particle concentration = 0 to 6.5×10^{-6} M) was kept to stir for 5 h to ensure that the condensation was completed. TEM images showed that the particles were bound so compactly on the polymer backbones (Figure 2b) that it was difficult to figure out the individual particles from the image. When particle **1** and labeled polymer (dye concentration = 5.0×10^{-5} M) were codissolved in water, the absorbance spectra displayed only a simple combination from the dye with a maximum absorbance at 405 nm and the particle with a decrease from high to low energy wavelength when the concentration of particle was $0-6.5 \times 10^{-6}$ M (Figure 4a). However, the bound particles displayed an obvious absorbance rising at near 450 nm with increasing the concentration of the particle (Figure 4b) comparing with the corresponding mixture of labeled polymer and particle **1** (Figure 4a), which was consistent with the situation of particle **3**.

For the mixture of particle **1** (particle concentration = $0-6.5 \times 10^{-6}$ M) and labeled polymer (dye concentration = 5.0×10^{-5} M), the dye on the labeled polymer displayed a luminescence at 546 nm upon excitation at 400 nm. The emission wavelength did not change, but the intensity decreased with increasing the concentration of particle in solution (Figure 5a), implying that the excited phenyl-phenanthridine was quenched by the silver particle through energy transfer even the particle
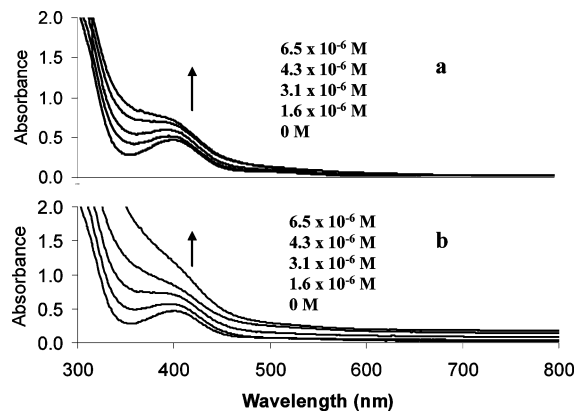

Figure 4. Dependences of absorbance spectrum on the concentration of particles under various conditions: (a) mixing labeled polymer and particle **1** and (b) binding particle **2** to labeled polymer. The dye concentration was 5.0×10^{-5} M in water.
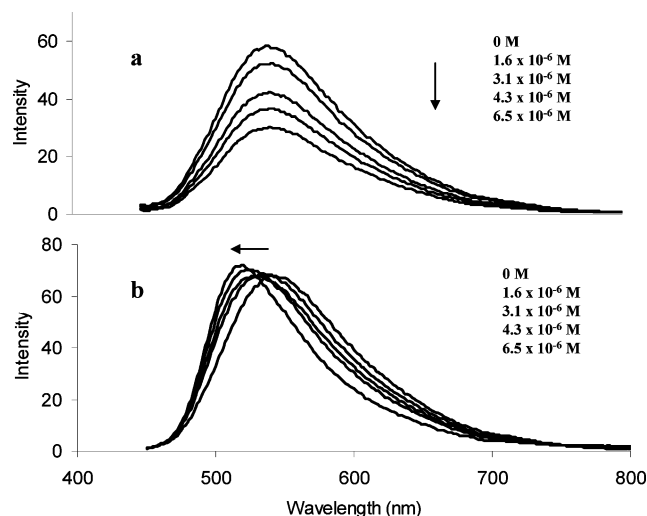

Figure 5. Dependences of luminescence spectra on the concentration of particle upon excitation at 400 nm under various conditions: (a) mixing labeled polymer and particle **1** and (b) binding particle **2** to labeled polymer. The dye concentration was 5.0×10^{-5} M in water.

was not bound on the polymer.[13,19,20] So it was plausible to use the mixture of particle **1** and labeled polymer with the same contraction as a control to study the luminescence spectral change on the concentration of particle in solution. Different from the mixture, the emission wavelength of particle **4** was blue-shifted from 546 to 521 nm with increasing the concentration of particle (Figure 5b), verifying that the particles were chemically bound on the polymer backbones. The luminescence intensity displayed an enhancement compared with the corresponding mixture control. The enhancement efficiency, defined as intensity ratio of aggregate (I_{agg})/mixture (I_{mix}) at the maximum emission wavelength, was plotted against the concentration of the particle (Figure 6), showing an increase with the concentration of particle and reaching to two times at the mole ratio of particle/dye = 0.1. The absorbance difference was estimated from the absorbance subtraction at 440 nm of mixture from the particle **4** and plotted against the concentration of particle (Figure 6). It was interesting to observe that the tendency of enhancement efficiency with the concentration of particle was consistent with that of absorbance difference. Therefore, we supposed that the luminescence was enhanced due to the absorbance rising by the particle aggregation.

However, the enhancement efficiency by the aggregated small particles was much lower than that on the solid silver substrate
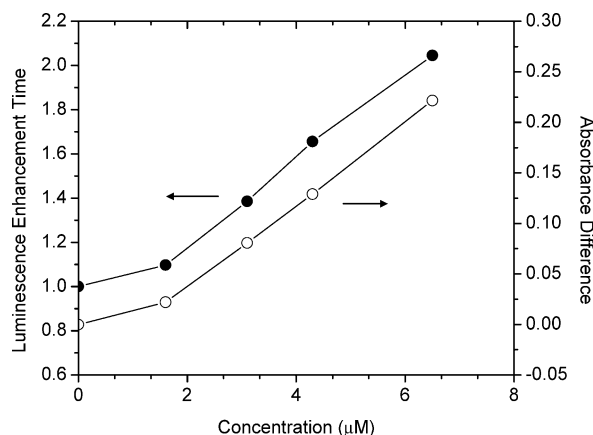
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Figure 6. Plots of the luminescence intensity ratio of aggregate over mixture (I_{agg}/I_{mix}) at maximum wavelength and the absorbance difference between aggregate and mixture at 440 nm against the concentration of particle in water.

(1 order of magnitude).[2] This value was even lower than that on the large silver particles (average diameter 5 nm) dissolved in solution.[21] It could be ascribed to two factors: the core size of particle and distance from the dye to metal core surface. The SEF was recognized as a size-dependent factor: the small particle can quench fluorescence, but the large particle can enhance fluorescence.[22] From the current results, it was noted that even though the aggregation of the small particle could bring up a luminescence enhancement, the enhancement efficiency could not be as high as that on the large particle. Meanwhile, the SEF was dependent on the distance from the dye to the surface of silver core, and the optimistic distance was suggested to be about 10 nm.[8,9] If the dye was very close to the metal core, the enhancement would be weakened by a competitive quenching. In the current model, the distance from dye to silver surface was estimated to be the linker length, including two C—C binds, three C—N binds, one C—S bind, and one S—Ag bind, which was ca. 1 nm. This length was much shorter than the optimistic distance for the maximum SEF. Hence, it was not surprising that the enhancement efficiency was low on the aggregated small particles.

However, the aggregation of particles still brought up a luminescence enhancement to some extent, indicating that the enhancement on the aggregated particle could not be explained by an ordinary competitive mechanism of quenching and enhancement. Because the luminescence was enhanced accompanying with the simultaneous plasmon absorbance rising, the luminescence enhancement on the aggregated small particle was believed to be relevant to the absorbance change. In the other words, the compact aggregation of small particles brought up a rising plasmon absorbance, and such an absorbance change could overcome a competitive quenching to induce a luminescence enhancement, even though the distance from the fluorophore to the metallic surface was much shorter than the optimal distance for the SEF.

Conclusion

Amine-pendent polymers were synthesized by condensations between poly(methylvinyl-ether-*alt*-maleic anhydride) and diamine derivatives (1,6-diaminohexane or 3,8-diamino-6-phenyl-phenanthridine). Tiopronin-coated silver nanoparticles with an average diameter of 1.6 nm were prepared using a modified Brust method, and the tiopronin ligands were partially displaced by (2-mercapto-propylamino) acetic acid 2,5-dioxo-pyrrolidin-1-ylester. The succinimide-terminated particles were chemi-

cally bound to the amine-pendent polymers leading to compact aggregation of these particles on the polymer backbones. The aggregated particles could bring up a plasmon absorbance rising at near 440 nm. If the polymer was labeled by 3,8-diamino-6-phenyl-phenanthridine, the luminescence was enhanced by 2 times when using a mixture of labeled polymer and tiopronin-coated particle as control. This result suggested that the small particles could induce a fluorescence enhancement when they were aggregated compactly, but the enhancement efficiency was not as high as that of the large particle. It was observed that although the dye was quite close to the metal core relative to the optimal distance for the maximum enhancement, but the luminescence was still enhanced accompanied by a simultaneous plasmon absorbance rising. Because the organic monolayer-coated particles can be controlled to displace by multiple dye and other functionalized ligands, these small particles are expected to have potential applications in in vivo luminescence detection using their good solubility and chemical stability in solution as well as high penetration capability through liposome or cell film.

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