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Design of Fluorescent Assays for Cyanide and Hydrogen Peroxide Based on the Inner Filter Effect of Metal Nanoparticles

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We have demonstrated the design of a new type fluorescent assay based on the inner filter effect (IFE) of metal nanoparticles (NPs), which is conceptually different from the previously reported metal NPs-based fluorescent assays. With a high extinction coefficient and tunable plasmon absorption feature, metal NPs are expected to be capable of functioning as a powerful absorber to tune the emission of the fluorophore in the IFE-based fluorescent assays. In this work, we presented two proof-of-concept examples based on the IFE of Au NPs by choosing MDMO-PPV as a model fluorophore, whose fluorescence could be tuned by the absorbance of Au NPs with a much higher sensitivity than the corresponding absorbance approach. While the first assay worked in a turn-on mode upon the etching of Au NPs by the analyte, CN^-, the second one functioned in a turn-off mode upon the catalytic growth of Au NPs by the analyte, H_2O_2. As a result, the present IFE-based approach can detect cyanide ranging from 1.0×10^{-6} to 6.0×10^{-4} M with a detection limit of 6.0×10^{-7} M and H_2O_2 ranging from 1.5×10^{-7} to 2.2×10^{-5} M with a detection limit of 8.5×10^{-8} M, respectively. Notably, the present IFE-based approach allows the design of fluorescent assays in a more simple, time-saving, and economical approach when compared with conventional metal NPs-based fluorescent assays, since no modification step of the fluorophore was needed any more.

There is an enormous demand for an optical-based sensing method in many fields such as environmental monitoring, industrial and food processing, biomedical technology, healthcare, and clinical analysis.[1] With the distinct advantage of high sensitivity and ease of operation, development of fluorescence-based assays has received intensive and continuing attention.[2] In recent years, metal nanoparticles (NPs), e.g., Au or Ag NPs, have been favorably adopted as an active unit in the fluorescent assays. For example, based on the ultraefficiently quenching ability of Au NPs through energy transfer or electron transfer processes, numerous fluorescent methods for sensitive detection of biologically or environmentally important analytes have been reported.[3] On the other hand, by virtue of enhanced emission of fluorophores within a certain distance away from metal nanostructures, several recent studies have demonstrated the utilization of Au/Ag NPs to improve the sensitivity of fluorescent assays.[4] It is noteworthy that either quenching or enhancing the emission of fluorophores needs the intermolecular connection of fluorophores with metal NPs in a particular distance or geometry to enable the interaction between them. This meant that in the design of aforementioned metal NPs-based fluorescent assays, fluorophores usually have to be modified or engineered to directly contact with or indirectly be linked to metal NPs, which made the method much more complicated, time-consuming, and consequently restricted their further applications.

In this work, we present an alternative approach to design fluorescent assays based on the inner filter effect (IFE) of metal NPs, where metal NPs function as an absorber to modulate the emission of the fluorophore. The IFE is an important, inevitable, and dominant factor in spectrofluorometry, which results from the absorption of the excitation and/or emission light by absorbers in the detection system.[5] Although IFE is usually considered as an annoying source of error in spectrofluorometry, recent studies have demonstrated its application in developing novel fluorescent assays.[6] Notably, the IFE-based approach does not require the link of the absorber with the fluorophore, which offers considerable flexibility and more simplicity. Moreover, since the changes in the absorbance of the absorber translate into exponential

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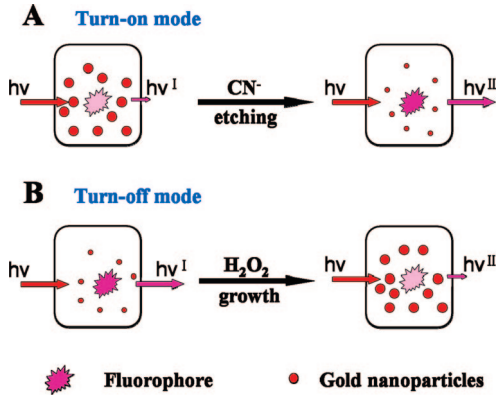
Scheme 1. Schematic Illustration of Fluorescence Assays Based on the Inner Filter Effect of Au NPs[a]



[a] (A) Turn-on assay for cyanide and (B) turn-off assay for H_2O_2.

changes in the fluorescence of the fluorophore, an enhanced sensitivity for the analytical method is reasonable with respect to the absorbance alone.[6] However, restrictions generally exist in the design of IFE-based fluorescent assays for the limited choice of suitable absorber and fluorophore, because IFE would occur effectively only if the absorption band of the absorber possesses complementary overlap with the excitation and/or emission bands of the fluorophore. Moreover, the conventional absorber usually has a small extinction coefficient, which also restricts the sensitivity of the approach.

In this regard, the recently developed metal NPs should be a favorable choice as an absorber in the design of IFE-based fluorescent assays. Metal NPs are known to have extremely large extinction coefficient (in the order of 10^8 cm^{-1} M^{-1} or more) originating from their surface plasmon absorption, which are much larger than the conventional chromophores.[7] In addition, the surface plasmon absorption of metal NPs is very sensitive to their size, shape, interparticle distance, composition, and environment, which means the absorption spectrum of metal NPs can be facilely tuned to overlap with the excitation or emission spectra of many common fluorophores.[8] Moreover, the rapidly developed nanotechnology in recent years has demonstrated abundant surface chemistry and numerous applications of metal NPs.[9] All these attractive characteristics enable metal NPs to be a potential powerful absorber in IFE-based fluorescent assays. Herein, we present two proof-of-concept examples based on the IFE of Au NPs (Scheme 1). While the first assay works in a turn-on mode upon the etching of Au NPs by the analyte, CN^-, the second one functions in a turn-off mode upon the catalytic growth of Au NPs by the analyte, H_2O_2.

EXPERIMENTAL SECTION

Materials. Poly[2-methoxy-5-(3,7-dimethyloctyloxy)-1,4-phenylenevinylene] (MDMO-PPV) was bought from Sigma-Aldrich. A stock solution of 1.8 mM MDMO-PPV was prepared in tetrahydrofuran (THF). $HAuCl_4 \cdot 3H_2O$, trisodium citrate, potassium cyanide, and other salts were purchased from Beijing Chemical Co. (China). Cetyltrimethylammonium chloride (CTAC) was purchased from Nanjing Robiot Co. (China). All other chemicals were of analytical grade and used without further purification. The water used was purified through a Millipore system. Citrate-stabilized Au NPs were prepared following Frens' method as reported previously.[10] The size of as-prepared Au-NPs was characterized with transmission electron microscopy (TEM), which revealed an average diameter of 13 nm. The concentration of as-prepared Au-NPs was calculated to be approximately 7.1 nM assuming that all gold in the $HAuCl_4$ was reduced.

Fluorescence Investigation on the Interaction between MDMO-PPV and Au NPs. Solution of 3.6×10^{-7} M MDMO-PPV in NaOH−$NaHCO_3$ buffer (0.01 M, pH 10.0) was mixed with different concentrations of as-prepared Au NPs and equilibrated for 2 min. Then the emission spectra were recorded. The fluorescence data were analyzed by plotting the fluorescence intensity decreased (%) at 588 nm versus the concentration of Au NPs. To investigate the effect of excitation wavelength on the fluorescence decrease by Au NPs, a series of fluorescence experiments under the excitation wavelength from 400 to 550 nm was performed.

Turn-On Fluorescent Detection of Cyanide. A 0.12 M stock solution of KCN was prepared in a 0.01 M NaOH−$NaHCO_3$ buffer of pH 10.0, from which various CN^- concentrations were prepared by serial dilution. The stock solutions were used within 3 days. A freshly prepared mixture solution containing 3.6×10^{-7} M MDMO-PPV and 3.6 nM Au NPs in NaOH−$NaHCO_3$ buffer (0.01 M, pH 10.0) was used for CN^- detection, into which KCN with different concentrations was added. The kinetics of the interaction between cyanide and Au NPs showed that the fluorescence increased rapidly in the first 20 min and slowly later. Thus the mixture solution was equilibrated for a fixed time of 20 min before the spectral measurements.

To probe the anion selectivity, the following salts were used: KCN, CH_3COONa (NaAc), $Na_2B_4O_7$, NaBr, $KBrO_3$, NaCl, $NaClO_4$, KSCN, Na_2CO_3, $K_2C_2O_4$, KF, NaI, KIO_3, $NaNO_2$, $NaNO_3$, Na_3PO_4, Na_2S, Na_2SO_3, Na_2SO_4, $K_2S_2O_8$, and citrate sodium. A 0.12 M stock solution was prepared. Subsequently, the salt solution with appropriate volume was mixed with the above mixture solution. Then the solution was equilibrated for 20 min before the spectral measurements. To assess the practical applicability of the present system, a known amount of KCN was spiked into water samples which were obtained locally from the tap water. An aliquot of a KCN-spiked water sample was added into the solution containing 3.6×10^{-7} M

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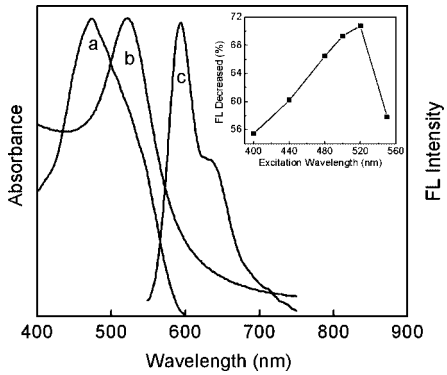

Figure 1. Fluorescence excitation (a) and emission (c) spectra of MDMO-PPV and absorption spectrum (b) of as-prepared Au NPs in $NaOH-NaHCO_3$ buffer (0.01 M, pH 10.0). The inset shows the plots of fluorescence decreased (%) at 588 nm versus the excitation wavelength in the presence of 3.6 nM Au NPs.

MDMO-PPV and 3.6 nM Au NPs in $NaOH-NaHCO_3$ buffer (0.01 M, pH 10.0), incubated for 20 min, and then subjected to spectral measurements.

Turn-Off Fluorescent Detection of H_2O_2. The stock solution of H_2O_2 was freshly diluted from a 30% solution by deionized water. The growth solution consisted of 1.0×10^{-6} M MDMO-PPV, 2.4×10^{-4} M $HAuCl_4$, and 2.4×10^{-3} M CTAC (as a stabilizer) in 0.01 M phosphate buffer solution (PBS, pH 7.5) and different concentrations of H_2O_2. For the catalytic growth of Au-NPs, 3.6×10^{-10} M Au-NP seeds were added into the above solution. The kinetic investigation indicated that the growth finished after an incubation time of 15 min. Thus the spectra were obtained at a fixed time of 15 min after the addition of H_2O_2.

Instruments. Absorption measurements were performed with a Cary 500 UV−vis-NIR spectrometer (Varian). Fluorescence measurements were carried out on a LS-55 luminescence spectrometer (Perkin-Elmer). Each spectrum was the average of three scans. A 1.00 cm path length rectangular quartz cell was used for these measurements. TEM measurements were made on a JEOL 2000 TEM operating at 200 kV. All the measurements were carried out at room temperature (296 ± 1 K).

RESULTS AND DISCUSSION

Effect of Au NPs on the Fluorescence of MDMO-PPV. MDMO-PPV was chosen as a model fluorophore in the study, due to a good spectral overlap of its excitation spectrum with the absorption band of Au NPs (Figure 1).[11] Preliminary experiments suggested a gradual decrease on the fluorescence of MDMO-PPV upon increasing the concentration of citrate-reduced Au NPs (13 ± 1 nm). Since MDMO-PPV is a neutral molecule in the aqueous solution, no complex formation or energy transfer with Au NPs was expected, which was supported by the fact that the plasmon absorption band of Au NPs remained unchanged in the presence of MDMO-PPV. Further investigation showed the extent of fluorescence decrease was strongly excitation wavelength-dependent (Figure S1 in the Supporting Information), and the maximum decrease was obtained upon excitation at 520 nm, which was just near the absorption maximum of Au NPs (seen the inset in Figure 1). This fact further suggested the fluorescence of MDMO-PPV should be mainly decreased by the IFE of Au NPs rather than other possible processes. Upon increasing the concentration of Au NPs, the absorbance of the absorber increased, which would shield the excitation light from the fluorophore. Thus there was less light available to excite the fluorophore, and the emission intensity decreased correspondingly. Notably, in the presence of Au NPs as low as 3.6 nM, the fluorescence intensity of MDMO-PPV decreased by over 70%. This could be attributed to the high extinction coefficient of Au NPs, which was one advantage of using metal NPs as the absorber compared with conventional chromophores. Therefore, upon excitation at 520 nm, the fluorescence of MDMO-PPV can be tuned by the absorbance of Au NPs via IFE in a sensitive and simple approach.

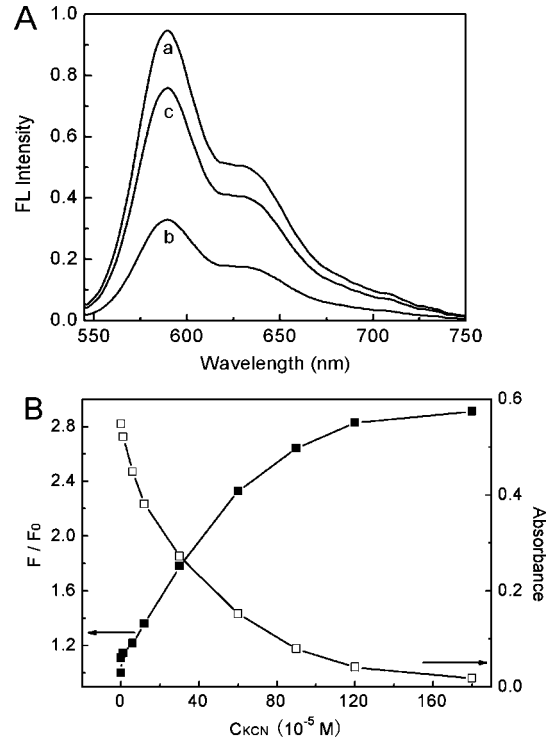

Figure 2. (A) Fluorescence emission spectra of 3.6×10^{-7} M MDMO-PPV alone (a), MDMO-PPV containing 3.6 nM Au NPs in the absence (b) and presence of 6.0×10^{-4} M KCN (c) in $NaOH-NaHCO_3$ buffer (0.01 M, pH 10.0). (B) Signal changes of fluorescence at 588 nm and absorbance at 520 nm as a function of cyanide concentration. The excitation wavelength was 520 nm.

Turn-On Fluorescent Detection of Cyanide Based on IFE of Au NPs. We first designed a turn-on fluorescent cyanide assay based on the analyte-induced decrease of the absorbance of the absorber (Au NPs), which then recovered the IFE-decreased fluorescence of the fluorophore. As shown in Figure 2A, the fluorescence of MDMO-PPV (curve a) decreased in the presence of Au NPs upon excitation at 520 nm (curve b). Upon further addition of cyanide to the above solution, the fluorescence recovered obviously (curve c). Cyanide is known to be capable of dissolving metal NPs in basic aerated solutions.[12] In the

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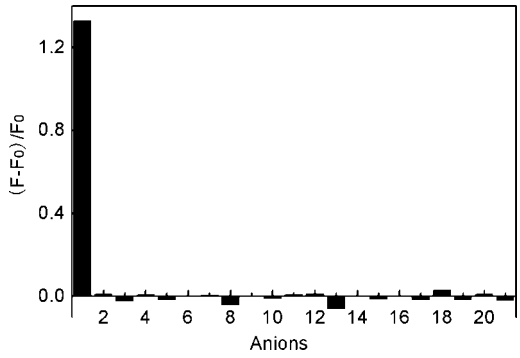

Figure 3. The relative fluorescence intensity variation at 588 nm of the mixture solution containing 3.6×10^{-7} M MDMO-PPV and 3.6 nM Au NPs in the presence of 6.0×10^{-4} M of various anions: (1) CN^-, (2) Ac^-, (3) $B_4O_7^{2-}$, (4) Br^-, (5) BrO_3^-, (6) Cl^-, (7) ClO_4^-, (8) SCN^-, (9) CO_3^{2-}, (10) $C_2O_4^{2-}$, (11) citrate, (12) F^-, (13) I^-, (14) IO_3^-, (15) NO_2^-, (16) NO_3^-, (17) PO_4^{3-}, (18) S^{2-}, (19) SO_3^{2-}, (20) SO_4^{2-}, and (21) $S_2O_8^{2-}$. F and F_0 are the fluorescence intensity in the presence and absence of anions, respectively.

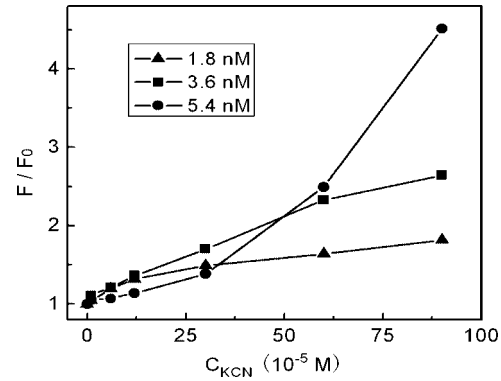

Figure 4. Fluorescence response of the solution containing 3.6×10^{-7} M MDMO-PPV to cyanide in the presence of different concentrations of Au NPs: (▲) 1.8, (□) 3.6, and (●) 5.4 nM. The emission was recorded at 588 nm upon excitation at 520 nm.

presence of cyanide, Au NPs will dissolve by forming a gold cyanide complex, which is accompanied by a gradual decrease on the plasmon absorbance of Au NPs (Figure S2 in the Supporting Information). While the control experiment indicated a negligible effect of cyanide on the fluorescence of MDMO-PPV only, the observed recovery should originate from the decreased absorbance which diminished the IFE of Au NPs. Figure 2B plots the signal changes of fluorescence and absorbance as a function of cyanide concentration. As expected, two opposite optical changes were observed in the presence of cyanide. Since Au NPs absorb the light at the excitation wavelength of MDMO-PPV, it controls the amount of light available to excite the fluorophore. Upon an increase in cyanide concentration, the absorbance of Au NPs decreased, which led to more light available to excite the fluorophore. As a result, increased fluorescence was obtained.

Solutions containing 21 common anions (CN^-, Ac^-, $B_4O_7^{2-}$, Br^-, BrO_3^-, Cl^-, ClO_4^-, SCN^-, CO_3^{2-}, $C_2O_4^{2-}$, citrate, F^-, I^-, IO_3^-, NO_2^-, NO_3^-, PO_4^{3-}, S^{2-}, SO_3^{2-}, SO_4^{2-}, and $S_2O_8^{2-}$) were then tested under the same conditions to evaluate the selectivity of our system (Figure 3). Remarkably, the results indicated no obvious increase of the fluorescence by anions other than cyanide. Hence, the present method is specific for cyanide. The relative concentration of Au NPs in the MDMO-PPV/Au NPs system was found to affect the fluorescence response toward CN^- greatly. As shown in Figure 4, 3.6 nM Au NPs gave the best response in terms of both the sensitivity and linear dynamic range in the present experiment. A higher concentration of Au NPs could weaken the fluorescence more effectively and decrease the background fluorescence, but the response for cyanide in the low concentration range was weak which would deteriorate the detection sensitivity. On the other hand, a lower concentration of Au NPs might enhance the response for cyanide in the low concentration range, but the background fluorescence would be higher which could also affect the sensitivity of the method. In addition, the dynamic range was also found to be narrower.

Further analytical study under the optimal conditions suggested a linear range of 1.0×10^{-6} to 6.0×10^{-4} M and a detection limit of 6.0×10^{-7} M at S/N = 3. The detection limit was lower than the acceptable cyanide concentration in drinking water (1.9×10^{-6} M) according to the World Health Organization (WHO).[13] The reproducibility of the present method was then evaluated. The relative standard deviation for five repeated measurements of 3.0×10^{-4} M CN^- was 3.7%, which illustrated that the fluorescence response of MDMO-PPV/Au NPs toward cyanide was highly reproducible. The possible interferences of common anions and cations were tested. The results indicated the coexistence of at least 2-fold excess anions did not affect the determination of 3.0×10^{-4} M CN^-. Among the tested cations, K^+, Na^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, and Al^{3+} could be allowed to be at least 1.0×10^{-5} M, and Ag^+, Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Fe^{3+}, and Ni^{2+} could be allowed to be lower than 3.6×10^{-6} M. For a real sample, however, it showed that the interferences from these metal ions were not significant. Several cyanide-spiked tap water samples were then tested to verify the performance of our proposed method. The result showed a recovery of 97.6% (averaged over three separate measurements, as listed in Table S1 in the Supporting Information), which suggested the accuracy and reliability of the present method for cyanide determination in practical applications.

We also examined the possibility of developing an absorbance-based optical cyanide assay upon the dissolution of Au NPs by cyanide. The result showed a linear range of 1.0×10^{-5} to 3.0×10^{-4} M, with a detection limit of 6.0×10^{-6} M. It is obvious the IFE-based fluorescent method possessed a higher sensitivity (1 order of magnitude) and wider ranges than the conventional absorbance method. Such an enhanced response could be explained by the intrinsic relationship between fluorescence and absorbance that the absorber translates the changes in the absorbance into the fluorescence of the fluorophore exponentially.[6c] Therefore, the sensitivity is expected to be greatly improved for the fluorescent assay based on the IFE of metal NPs.

Determination of cyanide has received continuing attention, and many analytical methods have been developed in recent

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years.[14] However, most of these established methods suffered from the requirement of expensive equipments,[14c] complicated operation,[14a,c–f] low sensitivity,[14e–g] or poor selectivity.[14b,g] In contrary, our proposed IFE-based fluorescent method herein is simple, sensitive, and selective, which is expected to provide a reliable and efficient approach for the cyanide determination in real samples.

Turn-Off Fluorescent Detection of H_2O_2 Based on IFE of Au NPs. To further demonstrate the generality of this concept, we then designed a simple fluorescent H_2O_2 assay based on the catalytic growth of Au NPs by H_2O_2, which worked however in a turn-off mode. H_2O_2 is an important intermediate species in many biological and environmental processes, and numerous kinds of O_2-dependent oxidases generate H_2O_2 upon the catalytic oxidation of the respective substrates. Thus sensitive detection of H_2O_2 enables the quantitative assay of not only numerous substrates but also the activity of the respective enzyme. Therefore, development of a sensitive method for H_2O_2 detection is of great importance and broad interest.[15]

As shown in Figure 5A, the fluorescence of MDMO-PPV decreased gradually upon increasing the concentration of H_2O_2 in the growth solution, while the control experiment indicated no obvious effect of H_2O_2 on the fluorescence of MDMO-PPV in the absence of the growth solution. Recent studies have shown that Au NPs can be effectively enlarged by H_2O_2 in the presence of Au NP-seeds,[16] and the absorbance of Au NPs increased correspondingly (Figure S3 in the Supporting Information). Figure 5B plots the signal changes of fluorescence and absorbance as a function of H_2O_2 concentration, which revealed an opposite trend compared with that of Figure 2B. Upon the growth of Au NPs by H_2O_2, the absorbance of the absorber (Au NPs) increased, which led to less light available to excite the fluorophore. As a result, the fluorescence of MDMO-PPV decreased. This method allowed the detection of H_2O_2 in a range of 1.5×10^{-7} to 2.2×10^{-5} M with a detection limit as low as 8.5×10^{-8} M at S/N = 3. It is noteworthy that the present method provided a much lower detection limit than the previously reported absorbance-based method for H_2O_2 detection which was also based on the catalytic growth of Au NPs.[16] Moreover, the present IFE-based fluorescent H_2O_2 assay was more sensitive than other recently published spectroscopic methods for H_2O_2 detection.[15c,17]

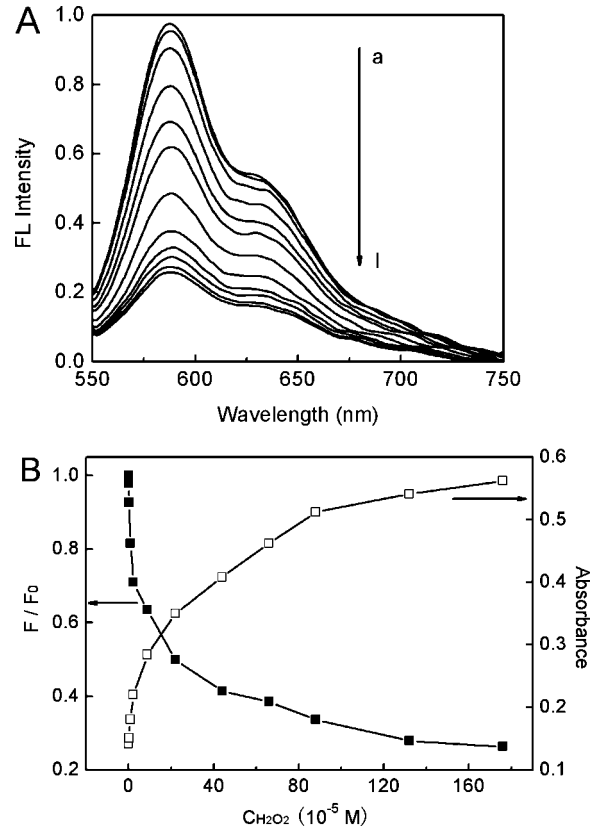

Figure 5. (A) Fluorescence emission spectra of MDMO-PPV in the growth solution containing 3.6×10^{-10} M Au-NP seeds, 2.4×10^{-4} M $HAuCl_4$, and 2.4×10^{-3} M CTAC in phosphate buffer (0.01 M, pH 7.5) in the presence of increasing concentrations of H_2O_2 (from a to l). (B) Signal changes of fluorescence at 588 nm and absorbance at 520 nm as a function of H_2O_2 concentration. The excitation wavelength was 520 nm.

The possible interferences of foreign coexisting substances on the H_2O_2 detection were then tested. The results showed that coexistence of common cations like K^+, Na^+, Ag^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, or Al^{3+} produced negligible influence at concentrations up to 1.0×10^{-5} M. Among the tested anions, Br^-, BrO_3^-, Cl^-, ClO_4^-, CO_3^{2-}, F^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_3^{2-}, or SO_4^{2-} remained inert for the detection at concentrations up to 0.1 mM. The relative standard deviation for five repeated measurements of 2.2×10^{-5} M H_2O_2 was 4.1%, which illustrated that the response of the present system was reproducible. Therefore, a simple and sensitive fluorescent method for the detection of H_2O_2 can be fabricated based on the IFE of Au NPs. Furthermore, since many kinds of O_2-dependent oxidase generate H_2O_2, the present sensing scheme can also be facilely extended to detect various substrates such as glucose[17d] and cholesterol[18] by using the corresponding oxidases in the growth solution.

CONCLUSIONS

In conclusion, we have demonstrated in this work the feasibility of developing a new type fluorescent assay based on the inner filter effect of metal NPs for the first time. By employing cyanide and H_2O_2 as the model analytes, we show that metal NPs can function as a powerful absorber in the IFE-based fluorescent

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assays to tune the emission of the fluorophore with higher sensitivity than the absorption-alone approach. Compared with conventional metal NPs-based fluorescent assays, the present IFE-based strategy allows the design of fluorescent assays in a more simple, time-saving, and economical approach, since no modification step of the fluorophore was needed. Moreover, it is anticipated that the present concept can be generalized to design fluorescent assays for sensing a wide range of metal NPs-involved events such as interparticle aggregation/de-aggreagation, shape/size evolvement, surface adsorption/desorption, and so on.

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SUPPORTING INFORMATION AVAILABLE

Fluorescence data of MDMO-PPV in the presence of Au NPs at different excitation wavelength, absorption spectra of Au NPs upon etching by cyanide, absorption spectra of Au NPs upon the catalytic growth by H_2O_2, and recovery data for the detection of cyanide in spiked tap water samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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