Fluorescent gold nanoparticles-based fluorescence sensor for Cu^{2+} ions†

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Received (in Cambridge, UK) 11th November 2008, Accepted 20th January 2009
First published as an Advance Article on the web 11th February 2009
DOI: 10.1039/b820145e

A new fluorescence sensor for the highly selective detection of Cu^{2+} ion with a detection limit of 3.6 nM based on the aggregation-induced fluorescence quenching of the highly fluorescent glutathione-capped gold nanoparticles is reported.

The study of gold nanoparticles (AuNPs) is one of the most exciting research fields in nanotechnology owing to its extensive applications for biosensor fabrication.[1] In this area, a number of colorimetric assays have been developed taking advantage of the extremely high extinction coefficients[2] and the strongly distance-dependent surface plasmon resonance (SPR) absorption[3] of AuNPs. In these assays, AuNPs solution generally exhibits a color change from red to blue because of the shift of the SPR band to longer wavelength when the nanoparticles are in the aggregated state. Reversely, redispersion of the aggregated AuNPs results in a color recovery from blue to red. Based on this state-dependent (aggregation/redispersion) SPR absorption properties, AuNPs can be used as a convenient tool for the sensing of metal ions,[4] enzymes,[5] proteins,[6] oligonucleotides,[7] and other small molecules.[8] In addition to the SPR absorption, the fluorescence properties of AuNPs have also fascinated scientists. Especially, the origin of the observed fluorescence has been intensely discussed.[9] However, applications of this novel fluorophore are greatly limited by the low quantum yields (QYs) ranging from 10^{-5},[9b] to 10^{-3}.[9e] Recently, preparations of fluorescent gold nanoparticles (F-AuNPs) with strong fluorescence emission have been reported.[10] For example, F-AuNPs encapsulated in biocompatible poly(amidoamine) dendrimers exhibit strong emission (QYs between 0.1 and 0.7), and size tunable fluorescence from the UV to the near IR.[10a,b] Introduction of alkanethiol ligands onto the surface of AuNPs can be used in the synthesis of highly fluorescent gold nanoparticles with QYs $\approx$ 0.03.[10e] These novel fluorescent nanoparticles have shown applications in immunoassay[11] and intracellular imaging.[12] However, exploration of F-AuNPs-based fluorescence sensors remains at a very early stage.

In this communication, we develop a highly sensitive and selective fluorescence sensor for the essential biological metal ion Cu^{2+} based on the aggregation-induced fluorescence quenching of glutathione-capped F-AuNPs. Glutathione (GSH), a natural tripeptide, was employed as a stabilizer and a gentle reducing agent in a one-step synthesis of GSH-capped F-AuNPs.[13] TEM images of the as-prepared GSH-capped F-AuNPs, as shown in Fig. 1(a), indicate that the particles are well-dispersed, and the average diameter is 2.1 $\pm$ 0.4 nm. Dynamic light scattering (DLS) measurements show that the hydrodynamic diameter of these particles is 2.5 nm (see Fig. S1a, ESI†). FT-IR spectra of free GSH and GSH-capped F-AuNPs are shown in Fig. S2 (ESI†). The disappearance of the S–H stretching band ($\sim$2525 cm^{-1}) on the surface of GSH-capped F-AuNPs suggests the formation of covalent bonds between thiols and AuNPs. Fig. 2 shows the absorption, fluorescence excitation and emission spectra of GSH-capped F-AuNPs. It is evident that the absorption spectra of GSH-capped F-AuNPs exhibits strong and broad absorption in the UV range with an onset at $\sim$470 nm. The absence of a surface plasmon peak around 520 nm indicates that most of the particles are smaller than 2.5 nm.[14] Strong emission of the GSH-capped F-AuNPs was observed around 561 nm with excitation maxima around 417 nm. The large Stokes shifts (144 nm) and the microsecond-range fluorescence lifetime (1.7 μs) (Fig. S3, ESI†) suggests that the emission might result from a ligand-to-metal charge transfer

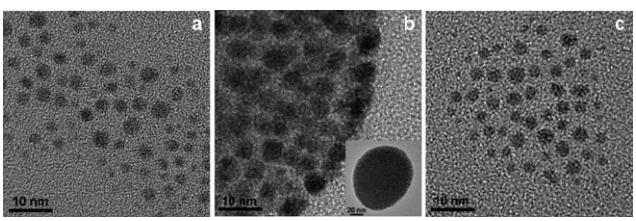

Fig. 1 TEM images of GSH-capped F-AuNPs in different solutions: (a) F-AuNPs in water; (b) F-AuNPs in Cu^{2+} solution; (c) as for (b) + EDTA.

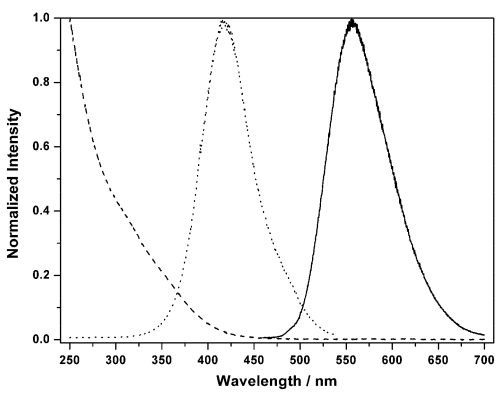

Fig. 2 Normalized absorbance spectra (dashed line), fluorescence excitation spectra (dotted line) and emission spectra (solid line) of GSH-capped F-AuNPs.

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† Electronic supplementary information (ESI) available: Experimental procedures for preparing nanoparticles, Cu^{2+} detection and Fig. S1–S3. See DOI: 10.1039/b820145e

transition.[15] Evaluated QYs of the as-prepared GSH-capped F-AuNPs are 0.01, using quinine sulfate as standard.[16]

The as-prepared GSH-capped F-AuNPs can be used as a highly sensitive and selective fluorescence "turn-off" sensor for Cu^{2+} ion without further modification. It was evidenced that the fluorescence of GSH-capped F-AuNPs was effectively quenched by Cu^{2+} ion (Fig. 3). As indicated in Fig. 4(a), this fluorescence quenching of GSH-capped F-AuNPs is highly selective for Cu^{2+} ion. Upon interaction with different bivalent and trivalent metal ions (2 μM), the GSH-capped F-AuNPs fluorescence intensity shows a substantial decrease for Cu^{2+} ion, whereas the fluorescence intensity of GSH-capped F-AuNPs is nearly unaffected by metal ions other than Cu^{2+}. Fig. 4(b) depicts the sensitivity of GSH-capped F-AuNPs as a Cu^{2+} ion sensor. The Stern–Volmer plot shows a linear relationship (R^2 = 0.996) between the fluorescence decrease with the concentration of Cu^{2+} ion over the range from 8 nM to 2 μM. The limit of detection (LOD)[17] for Cu^{2+} ion was calculated to be 3.6 nM. This value of LOD is three orders of magnitude better than that from a colorimetric AuNPs sensor for Cu^{2+} ion.[4d,f] These results also indicate that GSH-capped F-AuNPs is a more sensitive fluorescence sensor for Cu^{2+} ion than other reported nanoparticles.[18] In addition, unlike most of the organic fluorophores,

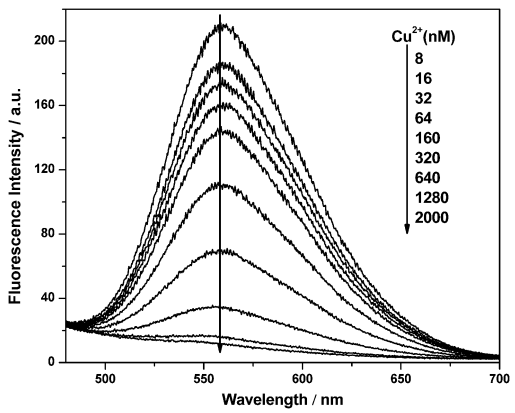

Fig. 3 Quenching of the GSH-capped F-AuNPs by Cu^{2+} ion (performed in pH 5.5, 20 mM MES buffer.)

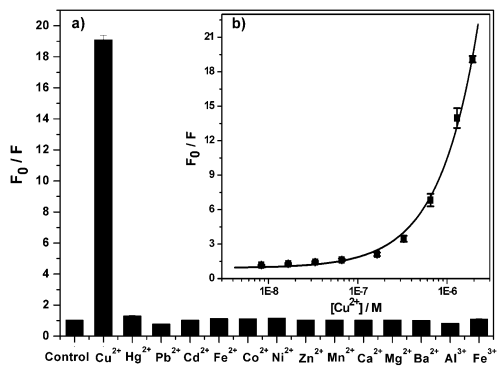

Fig. 4 (a) Selectivity and (b) sensitivity of GSH-capped F-AuNPs toward Cu^{2+} ion (performed in pH 5.5, 20 mM MES buffer; the concentration of all the metal ions in (a) was 2 μM; F_0 and F correspond to the fluorescence intensity of GSH-capped F-AuNPs in the absence and presence of metal ions, respectively.)

the high water solubility of GSH-capped F-AuNPs allows this sensor to be used in aqueous media without the need for organic cosolvents. This high selectivity Cu^{2+} ion sensor may find various applications in environmental and biological samples; furthermore, the long fluorescence lifetime of GSH-capped F-AuNPs enables the use of time-gated detection to reduce the background noise of these samples.

The fluorescence quenching of GSH-capped F-AuNPs in the presence of Cu^{2+} ion was attributed to nanoparticle aggregation induced by the complexation between GSH and Cu^{2+} ion. Previous investigation of GSH self-assembled monolayers (SAMs) revealed that GSH molecules which adsorbed onto the gold electrode could combine with Cu^{2+} ion resulting in the formation of 2 : 1 (GSH : Cu^{2+}) complexes.[19] Thus, it is reasonable to speculate that Cu^{2+} ion might be able to stimulate the aggregation of GSH-capped F-AuNPs, resulting from the metal–ion-based particle linking process.[4a,e,h] This speculation was proved by TEM and DLS measurements. Typical TEM images (Fig. 1(b)) show that GSH-capped F-AuNPs are cross-linked to each other forming a compact structure of >100 nm the presence of Cu^{2+} ion. DLS data show that the average hydrodynamic diameter of these linked particles is 180.6 nm (Fig. S1b, ESI†), which is much higher than that of the individual nanoparticles before treatment with Cu^{2+} ion (Fig. S1a, ESI†). These results of DLS measurements imply that the observed TEM image of aggregation did not result from the solvent drying process during TEM specimen preparation, and further support the Cu^{2+}–ion-induced aggregation of GSH-capped F-AuNPs.

That complexation between GSH molecules and Cu^{2+} ion induced aggregation and fluorescence quenching of GSH-capped F-AuNPs was further confirmed by a competition test. In this test, ethylenediaminetetraacetate (EDTA), a strong metal ion chelator, was used in competition with GSH-capped F-AuNPs for Cu^{2+} ion, and in regulating the aggregation/redispersion of GSH-capped F-AuNPs, and thus the quenching/recovering of the fluorescence of GSH-capped F-AuNPs. As expected, adding an equivalent amount of EDTA results in the dispersion of the GSH-capped F-AuNPs aggregates and isolated nanoparticles were once again observed by TEM (Fig. 1(c)). DLS measurements further support the redispersion of the aggregated nanoparticles (Fig. S1c, ESI†). Correspondingly, the quenched fluorescence resulting from the aggregation of GSH-capped F-AuNPs is recovered. Fig. 5 shows both decreasing of the fluorescence intensity of GSH-capped F-AuNPs as a function of Cu^{2+} ion titrated and restoring of the quenched fluorescence intensity of GSH-capped F-AuNPs followed by titration of EDTA. Notably, the fluorescence intensity is completely restored at [Cu^{2+}] = [EDTA], and above that concentration the fluorescence intensity stays nearly unchanged. In a control experiment, EDTA showed no influence on the fluorescence of GSH-capped F-AuNPs in the absence of Cu^{2+} ion, further confirming that the observed fluorescence recovery results from the complexation between EDTA and Cu^{2+}.

In summary, we have demonstrated GSH-capped F-AuNPs can be used as a fluorescence "turn-off" sensor for Cu^{2+} ion. The high sensitivity and selectivity response might be attributed to the excellent sensitivity of fluorescence

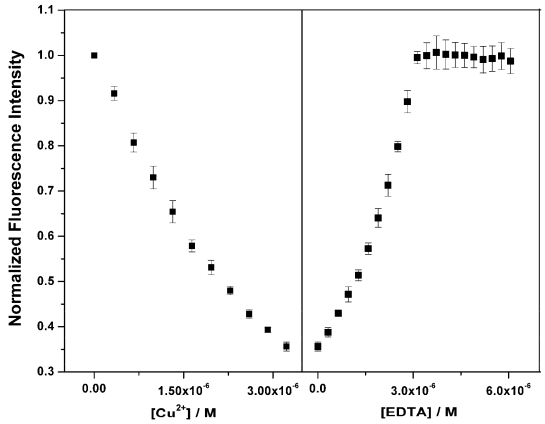

Fig. 5 Fluorescence response of GSH-capped F-AuNPs to the sequential addition of Cu^{2+} ion followed by EDTA.

spectroscopy and the high complexation constant of the amino acid moiety with Cu^{2+} ion which is larger than that with other metal ions.[20] This highly sensitive and selective sensoring results from the aggregation of GSH-capped F-AuNPs induced by the cross-link complexation between GSH and Cu^{2+}. Therefore, this strategy can in principle be extended to fluorescence sensors for other metal ions by the modification of the GSH-capped F-AuNPs with selective binding groups. Moreover, the carboxylic acid and amino functionality of the capped GSH molecules would make this further modification much easier. In addition, the observed reversible fluorescence recovery suggest that GSH-capped F-AuNPs/Cu^{2+} aggregates could be useful as a ''turn-on'' fluorescence sensor for anions or proteins which competitively complex with Cu^{2+} ion.

This work was supported by the National Natural Science Foundation of China (20675068, 20835005). We thank Prof. G. B. Han and Prof. Y. Zhang for the DLS and fluorescence lifetime measurements.

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