Gold Nanoparticle-Based Monitoring of the Reduction of Oxidized to Reduced Glutathione

ARTICLE *in* LANGMUIR · SEPTEMBER 2007

Impact Factor: 4.46 · DOI: 10.1021/la7011028 · Source: PubMed

Gold Nanoparticle-Based Monitoring of the Reduction of Oxidized to Reduced Glutathione

Xiaorong He,[†,‡] Zhifeng Zhong,[†,§] Yanbing Guo,[†,‡] Jing Lv,[†,‡] Jialiang Xu,[†,‡] Mei Zhu,[†,‡] Yuliang Li,*[,†] Huibiao Liu,[†] Shu Wang,*[,†] Yulan Zhu,[§] and Daoben Zhu*[,†]

Key Laboratory of Organic Solids, Center for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beiijng, 100080, P. R. China, Graduate School of Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing 100080, P. R. China, and Jiangsu Key Laboratory for Chemistry of Low-Dimensional Materials, Huaiyin Teachers College, Huaian 223300, P. R. China

Received April 16, 2007. In Final Form: May 28, 2007

We demonstrate the reduction of oxidized to reduced glutathione in the presence of glutathione reductase enzyme based on the modulation in photoluminescent quenching efficiency between the perylene bisimide chromophore TPPCA and gold nanoparticles (AuNPs). The TPPCA−AuNPs assembly shows a turn-on fluorescent signal in the presence of reduced glutathione, which provides a new concept to measure the balance of glutathione in a creature.

Introduction

In recent years, discovering the synthesis and properties of gold nanoparticles (AuNPs) with well-defined nanostructure has become one of the most highly energized research areas because of potential applications in producing novel photonics, electronics, and biological nanodevices.[1−5] Combination of organic chromophores and AuNPs for producing typical organic/inorganic hybrid materials with unique photophysical and photochemical properties and as building blocks in the growing field of nanotechnology is still a challenge.[6−9] As a photoluminescent quencher, AuNPs can ultraefficiently quench the molecular excitation energy in chromophore−AuNP composites.[10−12] Recently, this superquenching property of AuNPs has been employed by our group to detect copper ions by the modulation of the photoluminescent quenching efficiency of perylene bisimide chromophore and AuNPs.[13]

Glutathione is the most prevalent intracellular thiol-containing tripeptide in nearly all life tissues and plays an essential role in the health of organisms, particularly aerobic organisms.[14−16] It is useful to prevent oxidative stress in cells and helps to trap free radicals that can damage DNA and RNA.[17,18] Glutathione exists as two forms in nature, reduced glutathione (GSH) and oxidized glutathione (GSSG). As an antioxidant, GSH plays an active role in a variety of cellular processes, such as drug metabolism, calcium metabolism, γ-glutamyl cycle, and blood platelet and membrane defense by reacting with electrophiles or toxic agents

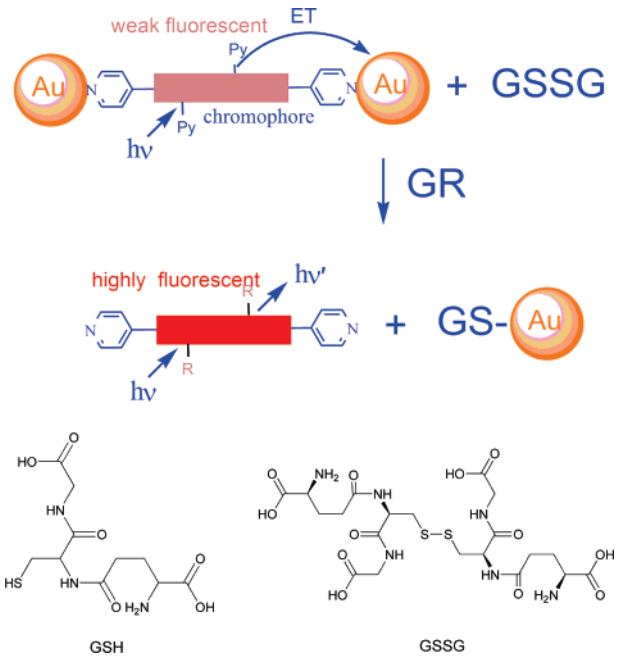


Figure 1. Schematic presentation of reversion process from oxidized to reduced glutathione based on the modulation in photoluminescent quenching efficiency between chromophore and AuNPs. (Bottom) Chemical structure of GSH and GSSG.

* Corresponding author: Fax: (+86)10-82616576. E-mail: ylli@iccas.ac.cn.
† Key Laboratory of Organic Solids, Chinese Academy of Sciences.
‡ Graduate School of Chinese Academy of Sciences, Chinese Academy of Sciences.
§ Jiangsu Key Laboratory for Chemistry of Low-Dimensional Materials, Huaiyin Teachers College.

(1) Thomas, K. G.; Kamat, P. V. *Acc. Chem. Res.* **2003**, *36*, 888.
(2) Mayer, C. R.; Neveu, S.; Cabuil, V. *Adv. Mater*. **2002**, *14*, 595.
(3) Braun, E.; Echen, Y.; Sivan, U.; Ben-Yoseph, G. *Nature* **1998**, *391*, 775.
(4) Kim, Y.; Johnson, R. C.; Hupp, J. T. *Nano Lett.* **2001**, *1*, 165.
(5) Rao, C. N. R.; Kulkarni, G. U.; Thomas, P. J.; Edwards, P. P. *Chem. Eur. J.* **2002**, *8*, 29.
(6) Kuwahara, Y.; Akiyama, T.; Yamada, S. *Langmuir* **2001**, *17*, 5714.
(7) Lahav, M.; Gabriel, T.; Shipway, A. N.; Willner, I. *J. Am. Chem. Soc.* **1999**, *121*, 258.
(8) Kamat, P. V.; Barazzouk, S.; Hotchandani, S. *Angew. Chem., Int. Ed.* **2002**, *41*, 2764.
(9) Chen, S.; Ingram, R. S.; Hostetler, M. J.; Pietron, J. J.; Murray, R. W.; Schaaff, T. G.; Khoury, J. T.; Alvarez, M. M.; Whetten, R. L. *Science* **1998**, *280*, 2098.
(10) Fan, C. H.; Wang, S.; Hong, J. W.; Bazan, G. C.; Plaxco, K. W.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6297.
(11) Dubertret, B.; Calame, M.; Libchaber, A. J. *Nat. Biotechnol.* **2001**, *29*, 365.
(12) Oh, E.; Hong, M. Y.; Lee, D.; Nam, S. H.; Yoon, H. C.; Kim, H. S. *J. Am. Chem. Soc.* **2005**, *127*, 3270.
(13) He, X. R.; Liu, H. L.; Li, Y. L.; Wang, S.; Li, Y. J.; Wang, N.; Xiao, J. C.; Xu, X. H.; Zhu, D. B. *Adv. Mater.* **2005**, *17*, 2811.
(14) MacMicking, J.; Xie, Q. W.; Nathan, C. *Annu. Rev. Immunol.* **1997**, *15*, 323.
(15) Demmigadams, B.; Adams, W. W. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1992**, *43*, 599.
(16) Kroemer, G.; Dallaporta, B.; Resche-Rigon, M. *Annu. Rev. Physiol.* **1998**, *60*, 619.
(17) Droge, W. *Physiol. Rev.* **2002**, *82*, 47.
(18) Abdilla, N.; Tormo, M. C.; Fabia, M. J.; Chaves, F. J.; Saez, G.; Redon, J. *J. Hum. Hypertens.* **2007**, *21*, 68.

10.1021/la7011028 CCC: $37.00
Published on Web 07/18/2007

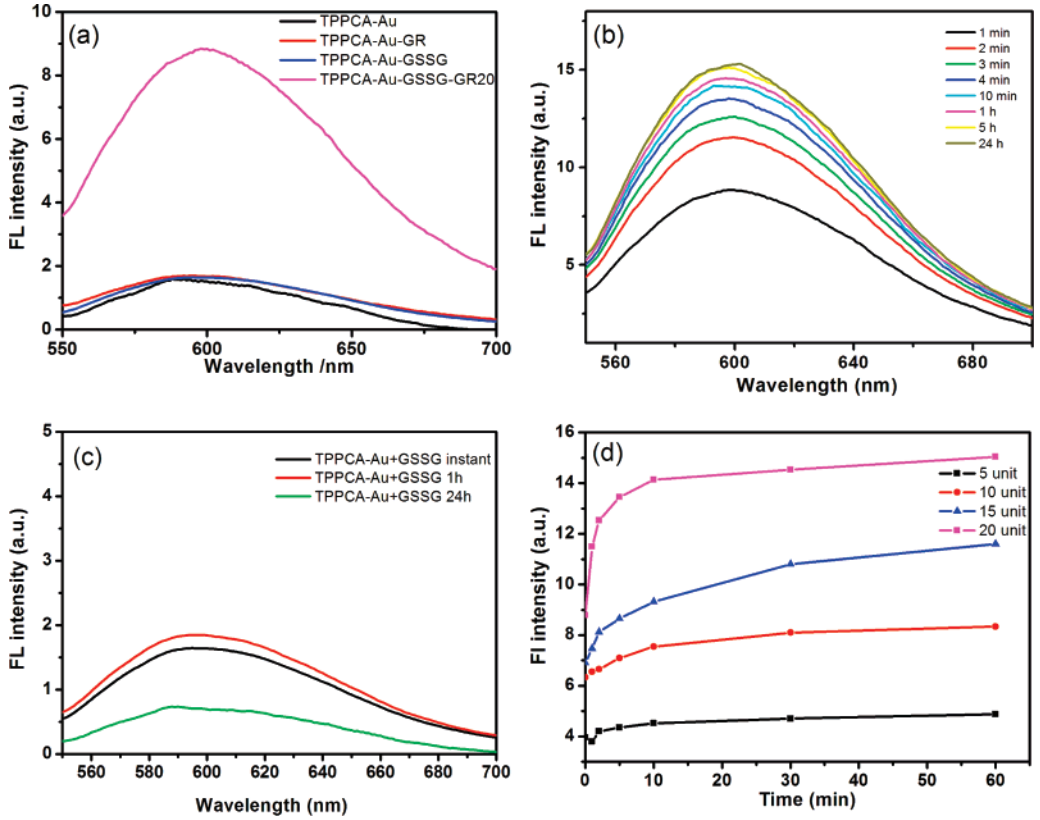
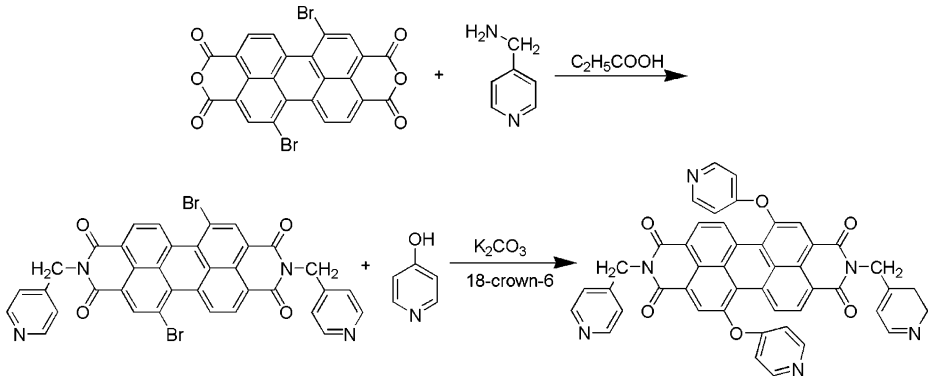


Figure 2. (a) Fluorescence titration of TPPCA−AuNPs after respective adding GSSG, GR (20 units), and a combination of GSSG and GR (20 units). (b) Fluorescence titration of TPPCA−AuNPs by adding GSSG and GR at different time periods. (c) Fluorescence titration of TPPCA−AuNPs by independently adding GSSG at different time periods. (d) Fluorescence titration of TPPCA−AuNPs by adding GSSG and varying amounts of GR in 0.001 M HCl. [TPPCA−AuNPs] = 1.0×10^{-6} M; excitation wavelength is 530 nm.

Scheme 1. Synthetic Route to TPPCA



to form conjugates that could be eliminated from the cell.[19,20] In these processes, GSH itself is oxidized to GSSG, and the glutathione reductase then reverts GSSG back to GSH, forming a functional cycle. Obviously, the reduction process is an extremely important pathway for continuing GSH formation and maintaining the balance of GSH and GSSG in cells. The ratio of reduced to oxidized glutathione within cells is often used scientifically as a measure of the health of organisms.[21] Thus, it is necessary to understand and monitor the reduction process of GSSG by glutathione reductase. In this paper, we take advantage of the fluorescence quenching properties of AuNPs to monitor the reversion of oxidized to reduced glutathione in the presence of glutathione reductase, which provides a new concept to measure the balance of glutathione in creature.

Experimental Section

General Information. The chemical reagents were purchased from Across or Aldrich and utilized as received unless indicated otherwise. Glutathione reductase from Baker's yeast was purchased from Sigma. PBS solution ([Na_2HPO_4] = 3.0×10^{-3} M, [K_2HPO_4] = 5.6×10^{-4} M, [NaCl] = 4.2×10^{-2} M, [KCl] = 8×10^{-5} M, ionic intensity = 50 mM, pH = 7.4) was used as the physiological buffer. All solvents were purified using standard procedures. UV−vis spectra were taken on a JASCO V-750 UV/vis/NIR spectrophotometer and fluorescence spectra were measured on a JASCO FP-6600 spectrofluorometer. ^{1}H NMR spectra were obtained on a Bruker Avance DPS-400 spectrometer. MALDI-TOF mass spectra were obtained from a Bruker Biflex MALDI-TOF spectrometer. Transmission electron microscopy (TEM) images were collected on a JEOL JEM 2010 instrument with a 100 keV accelerating voltage.

(19) Seo, J. S.; Lee, K. W.; Rhee, J. S.; Hwang, D. S.; Lee, Y. M.; Park, H. G.; Ahn, I. Y.; Lee, J. S. *Aquat. Toxicol.* **2006**, *80*, 281.
(20) Rayman, M. P. *Lancet* **2000**, *356*, 233.
(21) Reiter, R. J.; Melchiorri, D.; Sewerynek, E.; Poeggeler, B.; Barlowwalden, L.; Chuang, J. I.; Ortiz, G. G.; Acunacastroviejo D. *J. Pineal. Res.* **1995**, *18*, 1.

Preparation of Gold Nanoparticles.[22] $HAuCl_4$ (2.06 mg) and trisodium citrate (1.47 mg) were dissolved in 20 mL of HCl aqueous solution (pH = 3.0) by sonication and then 1.0 mL of ice-cold, freshly prepared 0.1 M $NaBH_4$ was added to the solution while being vigorously stirred at 0 °C. A pink color appeared immediately, indicating the formation of Au nanoparticles, and stirring continued for 1 h.

Preparation of Gold Nanoparticles.[23] The gold nanoparticles were synthesized by the addition of $AuHCl{\cdot}3H_2O$ (10 mg) to a refluxing, rapidly stirred solution of sodium citrate (20 mg) in water (100 mL, pH = 7.4), which was stirred under reflux for an additional 15 min before being allowed to cool. A purple color appeared, indicating the formation of Au nanoparticles.

Synthesis of *N*,*N*′-2,6-Bis(4-aminomethylpyridinyl)-1,7-dibromoperylene-3,4,9,10-tetracarboxylic Acid Bisimide. A mixture of 4-aminomethylpyridine (2.22 mL, 21.8 mmol) and 1,7-dibromoperylene-3,4,9,10-tetracarboxylic acid bisimide[24] (1 g, 1.8 mmol) in 50 mL of metacetonic acid were refluxed for 12 h under argon gas. After cooling down to room temperature, the product was isolated by filtration and washed thoroughly with a large amount of methanol and then dried in a vacuum. The crude product was subjected to column chromatography on silica gel with CH_2Cl_2/EtOAc (v:v = 3:1) as eluent to give a red solid (1.01 g, yield, 76%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 9.54−9.52 (d, 2H, J = 8.20 Hz), 8.97(s, 2H), 8.76−8.74 (d, 2H, J = 8.20 Hz) 8.62−8.61 (d, 4H, J = 5.08 Hz), 7.71−7.51 (m, 4H), 5.50−5.44 (t, 4H, J = 11.88 Hz). MS (MALD-TOF): calcd for $C_{36}H_{18}O_4N_4Br_2$ 727.97, found 728.2.

Synthesis of TPPCA. *N*,*N*′-2,6-Bis(4-aminomethylpyridinyl)-1,7-dibromoperylene-3,4,9,10-tetracarboxylic acid bisimide (73 mg, 0.1 mmol) was added to a mixture of 4-hydroxypyridine (21 mg, 0.22 mmol), K_2CO_3 (45 mg, 0.33 mmol), and 18-crown-6 (174 mg, 0.66 mmol) in 1,4-dioxane solution under N_2 at room temperature. The mixture was stirred at reflux for 5 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was subjected to column chromatography on silica gel with $CHCl_3/C_2H_5OH$ (v:v = 3:1) as eluent to give a red solid (66 mg, yield 86.8%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.74−8.71 (d, 2H, J = 10.7 Hz), 8.64−8.62 (d, 2H, J = 8.64 Hz), 8.58−8.57 (d, 4H, J = 2.72 Hz), 7.67−7.65 (d, 4H, J = 8.32 Hz), 7.49−7.47 (d, 2H, J = 7.25 Hz), 7.42−7.38 (t, 4H), 6.63−6.61 (d, 4H, J = 7.26 Hz), 5.43−5.37 (t, 4H, J = 12.14 Hz). MS (MALD-TOF): calcd for $C_{46}H_{26}O_6N_6$ 758.19, found 759.0. UV−vis λ_{max} [HCl aqueous solution (pH = 3.00), nm]: 404.6; 496.2; 530.2. FL λ_{max} [HCl aqueous solution (pH = 3.0), nm]: 600.0.

Preparation of TPPCA−AuNPs. TPPCA (1 mL, [TPPCA] = 1.0×10^{-4} M) in 0.001 M HCl aqueous solution (pH = 3.0) (or PBS buffer, pH = 7.4) was added into 4 mL of AuNPs solution in the dark. The resulting mixture was stirred for 5 min at room temperature. The pyridine groups were allowed to undergo a place exchange reaction with citrate-protected gold nanoparticles.

The General Procedure for the Displacement Experiment. GSSG (1 mL, [GSSG] = 1.0×10^{-4} M) in deionized water was added to TPPCA−AuNPs ([TPPCA−AuNPs] = 1.0×10^{-5} M) solution in HCl aqueous solution to get the stock solution. It was diluted to 1.0×10^{-6} M, and different amounts of GR were respectively added to the solutions. The fluorescence was measured instantly. The displacement procedure in the physiological media [blood serum: PBS buffer = 1:9 (v:v)] was similar to that in the HCl solution.

Results and Discussion

The sensor system functions are outlined in Figure 1. A new assembly of AuNPs modified by the water-soluble perylene bisimide derivative *N*,*N*′-2,6-bis(4-aminomethylpyridinyl)-1,7-bis(4-hydroxypyridinyl)-3,4,9,10-tetracarboxylic acid bisimide (TPPCA, Scheme 1) was chosen as the functional part. The perylene core was used as a fluorescence transducer, and the pyridine groups were acted as binding sites with AuNPs. As demonstrated before,[13] the fluorescence of TPPCA in the TPPCA−AuNP composite was highly efficiently quenched by AuNPs. Because the interactions between thiol atoms and AuNPs are stronger than those of TPPCA with AuNPs,[26] GSH can displace TPPCA from the TPPCA−AuNP composite in a short time. As a result, the fluorescence of the TPPCA could be recovered. Interestingly, in the case of GSSG, the fluorescence of chromophore could not be recovered in a short time period because steric hindrance from the bulky groups around sulfur atoms of GSSG hamper −SS− group binding to AuNPs effectively.[26] GSSG could be reduced to GSH rapidly, when

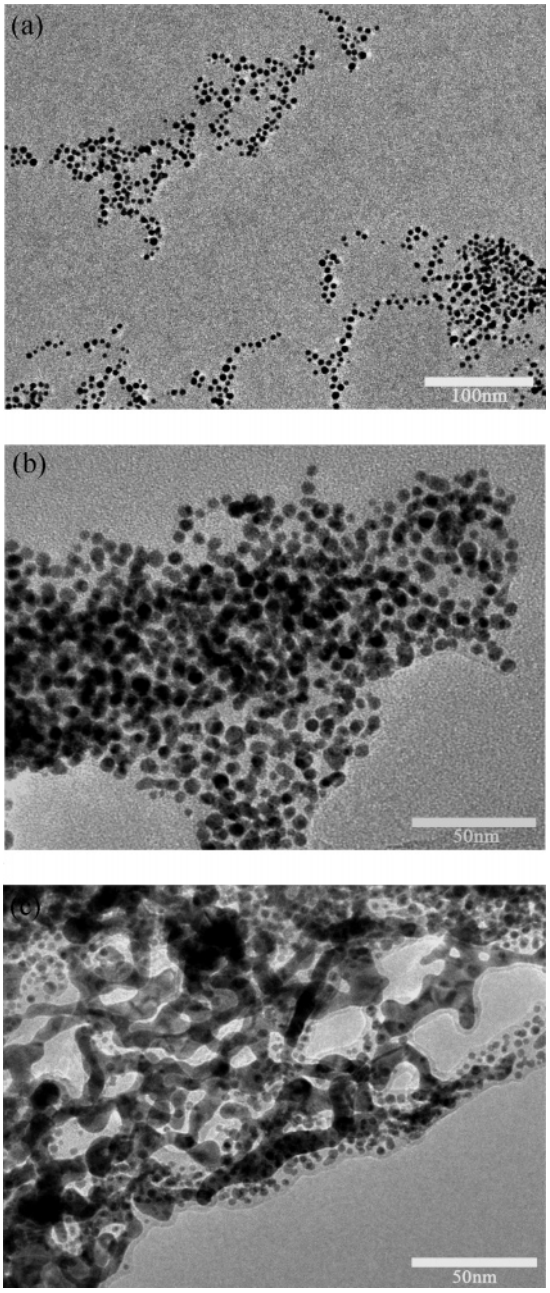


Figure 3. Transmission electron micrographs of monodispersed AuNPs (a), TPPCA−AuNPs (b), and TPPCA−AuNPs after adding GSSG and GR and (c) after doping a corresponding 0.001 M HCl solution on carbon-coated Cu grids.

(22) Jana, N. R.; Gearheart, L.; Murphy, C. J. *Langmuir* **2001**, *17*, 6782.
(23) Shipway, A. N.; Lahav, M.; Gabai, R.; Willner, I. *Langmuir* **2000**, *16*, 8789.
(24) Quinten, M.; Kreibig, U. *Surf. Sci.* **1986**, *172*, 557.
(25) Daniel, M. C.; Astruc, D. *Chem. Rev.* **2004**, *104*, 293.
(26) Chen, S. J.; Chang, H. T. *Anal. Chem.* **2004**, *76*, 3727.

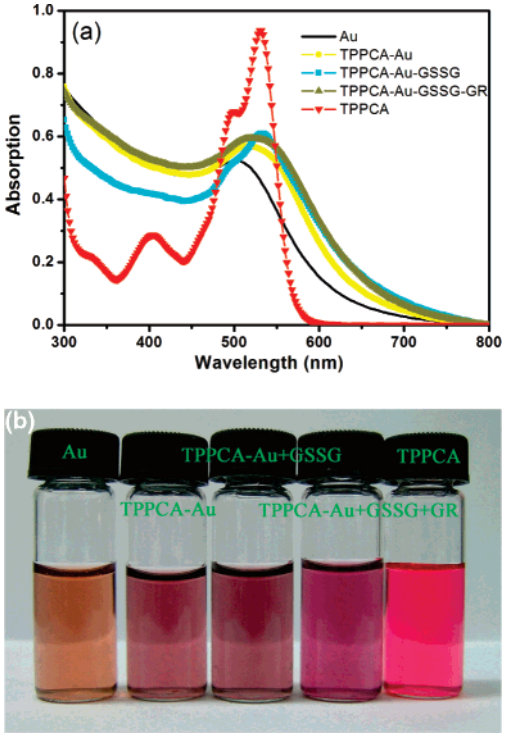


Figure 4. (a) The UV−vis spectra of the AuNPs, TPPCA, TPPCA−AuNPs with, respectively, GSSG and a combination of GSSG and GR in 0.001 M HCl. (b) photographs of such solutions as follows in 0.001 M HCl. From left to right: AuNPs, TPPCA−AuNPs, TPPCA−AuNPs/GSSG, TPPCA−AuNPs/GSSG/GR, TPPCA.

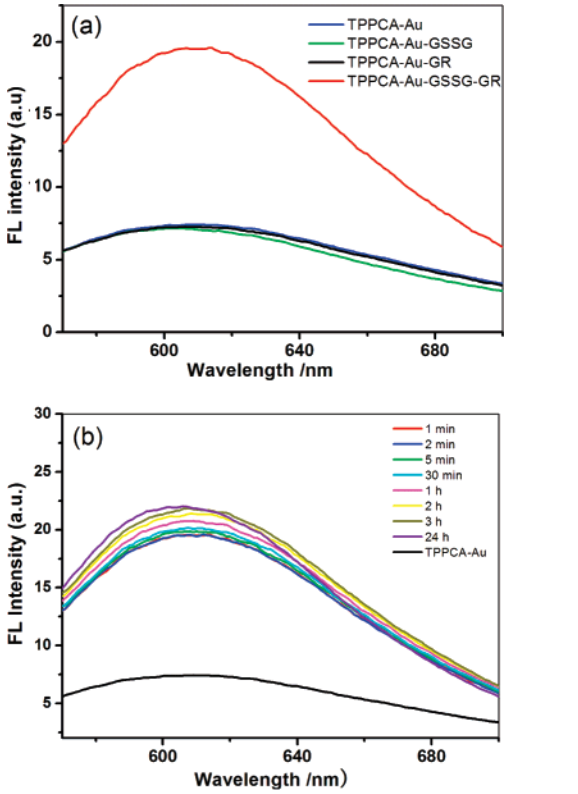


Figure 5. (a) Fluorescence titration of TPPCA−AuNPs after respectively adding GSSG, GR (20 units), and a combination of GSSG and GR (20 units) in PBS buffer [blood serum:PBS buffer = 1:9 (v:v)]. (b) Fluorescence titration of TPPCA−AuNPs by adding GSSG and GR over different time periods in PBS buffer [blood serum:PBS buffer = 1:9 (v:v)] [TPPCA−AuNPs] = 1.0×10^{-6} M; excitation wavelength is 540 nm.

glutathione reductase is added. GSH coordinates with AuNPs effectively and TPPCA is displaced from the chromophore. The fluorescence is thus turned on. It is possible to monitor the reduction of GSSG to GSH based on the turn-on fluorescence response of TPPCA in TPPCA−AuNP composite.

TPPCA was obtained by the condensation reaction of *N*,*N*′-2,6-bis(4-aminomethylpyridinyl)-1,7-dibromoperylene-3,4,9,10-tetracarboxylic acid bisimide with 4-hydroxypyridine in dioxane in the presence of K_2CO_3 as the base and 18-crown-6 as the phase catalyst. Monodispersed gold nanoparticles were prepared in presence of trisodium citrate by reduction of $HAuCl_4$ with $NaBH_4$ in HCl aqueous solution (pH = 3.0), followed by modification with TPPCA through a place exchange reaction of pyridine groups with citrate protectors.

The instant changes in the fluorescent spectra of TPPCA−AuNPs/GSSG composite were monitored in the presence of GR as shown in Figure 2a. As expected, TPPCA in the TPPCA−AuNPs composite emitted almost no fluorescence. The fluorescence spectra of TPPCA showed little change upon addition of GSSG. The steric hindrance from the bulky groups around sulfur atoms of GSSG maybe hampered −SS− group binding to AuNPs effectively. (Figure 1)[26] After 20 units of glutathione reductase were added, the fluorescence intensity of TPPCA recovered clearly and about 4.5-fold enhancement of TPPCA emission intensity was observed. As a control experiment, in the absence of GSSG, almost no emission increase was observed upon adding GR. These results clearly exhibited that the emission increase of TPPCA in the TPPCA−AuNPs composite originated from the reduction of GSSG to GSH in the presence of GR. In order to illustrate the reduction process, the fluorescence changes versus incubating time of GR were carried out. As shown in Figure 2b, the fluorescence intensity of the composite was time-dependent and reached a plateau after about 1 h. The corresponding fluorescence changes of independent presence of GSSG were also performed, as shown in Figure 2c. The fluorescence changed little within a 1 h period while decreasing obviously after 24 h. The turn-on fluorescence signal in Figure 2b was thus considered to be induced by the reduction of GSSG to GSH in the presence of GR. To cast light on the catalysis efficiency of glutathione reductase in such a system, photoluminescence monitoring of different amounts of GR versus incubating time was performed (Figure 2d). The fluorescence enhances as the amount of GR increases. The decrease of the amount of GR gives rise to a slow initial cleavage reaction rate and low level of fluorescence recovery. It was difficult to detect fluorescence changes when only 5 units of GR were added, while the fluorescence recovered clearly in 1 min when 20 units were added.

To obtain insights into the mechanism of the modulation in the photoluminescence recovery of TPPCA by AuNPs, the transmission electron microscopy (TEM) analysis of AuNPs was carried out (Figure 3). The average diameter of gold nanoparticles without modification of TPPCA was 5 nm (Figure 3a). After modification with TPPCA, TEM measurements indicate the aggregation of TPPCA−AuNPs colloids (Figure 3b) due to complexation between pyridine and AuNPs with an average diameter of 5 nm. Figure 3c shows the image of TPPCA−AuNPs upon adding GSSG and GR. Due to stronger complexation interaction with GSH, Au nanoparticles tend to form a complex with GSH, leading to reorganized configurations.

Colorimetric monitoring of the reduction process of GSSG by GR was also possible, since the absorption maximum of TPPCA−AuNPs was changed in the presence of GSSG upon addition of GR. The AuNPs itself displayed the typical plasmon peak at 502

nm. TPPCA showed the characteristic absorption maximum at 530 nm. TPPCA−AuNPs composite exhibited the sum of the absorptions of AuNPs and TPPCA with a maximum peak at 516 nm. Upon adding GSSG to TPPCA−AuNP composite, the solution displayed little color changes showing a maximum absorption peak at 518 nm. When GR was further added, TPPCA and AuNPs were separated from each other and the solution displays the characteristic absorption of TPPCA at 532 nm (Figure 4a). The plasmon peak of AuNPs was undetectable due to strong absorbance of TPPCA in the region from 500 to 550 nm. In this case, the red-shift of solution absorption resulted in a violet-to-purple color change[4] (Figure 4b). It should be pointed out that it would be better to show the differences between the solutions by direct visualization rather than a picture.

To validate this method for biological applications, fluorescence titration experiments were performed in a physiological PBS buffer (ionic intensity = 50 mM, pH = 7.4) with blood serum [blood serum: PBS buffer = 1:9 (v:v)]. The instant changes in the fluorescent spectra of TPPCA−AuNPs/GSSG composite were monitored in the presence of GR as shown in Figure 5a. The control experiments showed that TPPCA−AuNPs, TPPCA−AuNPs/GSSG, or TPPCA−AuNPs/GR emitted almost no fluorescence. While 20 units of glutathione reductase were added to the solution of TPPCA−AuNP/GSSG, about 3-fold enhancement of TPPCA emission intensity was observed. The fluorescence changes versus incubating time of GR were also carried out. As shown in Figure 5b, the fluorescence intensity of TPPCA enhanced instantly and clearly. These results clearly exhibited that the emission increase of TPPCA in the TPPCA−AuNPs composite could be applied to monitor the reduction process of GSSG to GSH in the presence of GR in a biological sense.

Conclusions

In conclusion, we have developed a simple approach to monitor the reversion of oxidized to reduced glutathione in the presence of glutathione reductase. The TPPCA−AuNPs assembly shows a turn-on fluorescent signal in the presence of reduced glutathione, which provides a new concept to measure the balance of glutathione in vivo. The approach will enable us to design and build novel biology devices and other devices based on these functional materials.

Acknowledgment. This work was supported by the National Natural Science Foundation of China (20531060, 10474101, 20571078, 20418001, 20473102) and the Major State Basic Research Development Program (2006CB806200, 2006CB932100).

LA7011028