TNT Sensor

DOI: 10.1002/anie.200804066

A Simple Assay for Direct Colorimetric Visualization of Trinitrotoluene at Picomolar Levels Using Gold Nanoparticles**

*Ying Jiang, Hong Zhao, Ningning Zhu, Yuqing Lin, Ping Yu, and Lanqun Mao**

2,4,6-Trinitrotoluene (TNT) is a leading example of a nitroaromatic explosive with significant detrimental effects on the environment and human health.[1] Ever-increasing needs for a secure society and green environment essentially require dramatic improvements in methods for the detection of TNT.[2] Although the emission quenching and redox properties of TNT have so far enabled its detection with elegant fluorescence, luminescence, and electrochemical methods,[3] the pressing need for on-the-spot detection of TNT and its very low concentration substantially necessitate great improvements in the methods used, both in simplicity and sensitivity. However, the complex procedures, high detection limits, and requirement for much instrumentation in the existing methods unfortunately render difficulties in simple, on-the-spot sensitive detection of TNT.

Herein, we report a simple but sensitive method for the colorimetric visualization of TNT at picomolar levels by using gold nanoparticles (Au NPs; see Figure 1). As a result of their interesting optical and electronic properties, for example, the surface plasmon resonance that essentially depends on the size, shape, and distance between the NPs, Au NPs have been widely used in various research and industrial fields.[4] For instance, based on the color change of Au NPs caused by the controllable change in their dispersion/aggregation states, Au NPs have recently been used for colorimetric sensing of phosphatase activity, β-lactamase activity, the mercuric ion, immunoglobulin G, and so forth. This was achieved by rationally tailoring the surface chemistry of the Au NPs through chemical and biochemical approaches in such a way that the target analyte can bind at the functionalized Au NPs through a tailor-made approach that can eventually change the dispersion/aggregation states and thus the color of the Au NPs.[5]

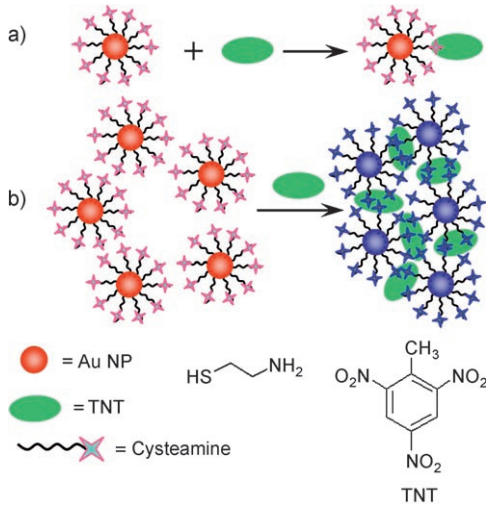

Figure 1. a) D–A interaction between cysteamine and TNT. b) Assay for direct colorimetric visualization of TNT based on the electron D–A interaction at the Au NP/solution interface.

Unlike the binding between, for example, antibody and antigen, complementary oligonucleotides, or guest–host complexes reported thus far, the method demonstrated here is essentially based on the color change of Au NPs induced by the donor–acceptor (D–A) interaction between TNT and primary amines (Figure 1 a). As one kind of electron acceptor, TNT can interact with electron donors, typically primary amines, through D–A interactions.[6] Such an interaction has been recently employed for designing the surface chemistry of nanostructures and electrodes to achieve the selectivity and sensitivity for fluorescent and electrochemical detection of TNT, respectively.[2b–d,3e,h]

In this study, cysteamine was used both as the primary amine and as the stabilizer for Au NPs to facilitate the D–A interaction between TNT and the primary amine at the Au NP/solution interface for direct visualization of TNT, based on the TNT-induced colorimetric nano-gold aggregation phenomenon. Initially, the cysteamine-stabilized Au NPs were well dispersed in distilled water and the color of the uniform suspension was wine red, because of the strong surface plasmon resonance of the Au NPs. The addition of TNT to the dispersion essentially leads to the aggregation of the cysteamine-stabilized Au NPs as a result of the D–A interaction between TNT and cysteamine (Figure 1 b), and the color of the suspension is accordingly changed to violet blue. The clear change in the color of the suspension could be used for the direct colorimetric visualization of TNT. As such a color change can be readily seen by the naked eye, the method demonstrated herein is relatively simple and does not

[*] Y. Jiang, Dr. N. Zhu, Dr. Y. Lin, Dr. P. Yu, Prof. L. Mao
Beijing National Laboratory for Molecular Sciences
Institute of Chemistry, Chinese Academy of Sciences
Beijing 100080 (P.R. China)
Fax: (+86) 10-62559373
E-mail: lqmao@iccas.ac.cn
Y. Jiang, Dr. H. Zhao
Graduate School of the Chinese Academy of Science
Beijing 100049 (P.R. China)

[**] This research was financially supported by the NSF of China (Grant Nos. 20435030, 20575071, 20625515, 20721140650), National Basic Research Program of China (2007CB935603), Chinese Academy of Sciences, and Center for Molecular Science, Institute of Chemistry.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200804066.

require any instrumentation or smart design and synthesis of fluorescently functional nanostructures or careful modification of an electrode surface. Moreover, the strong D–A interaction between TNT and cysteamine and the good analytical properties of Au NPs described in previous reports substantially enable a picomolar amount of TNT to be visualized by the naked eye. This study essentially offers a new and simple but sensitive method for TNT detection.

As shown in Figure 2a, the sole addition of cysteamine (500 nM) or TNT (5 nM) to the prepared citrate-stabilized suspension of Au NPs (10 nM) did not lead to an observable

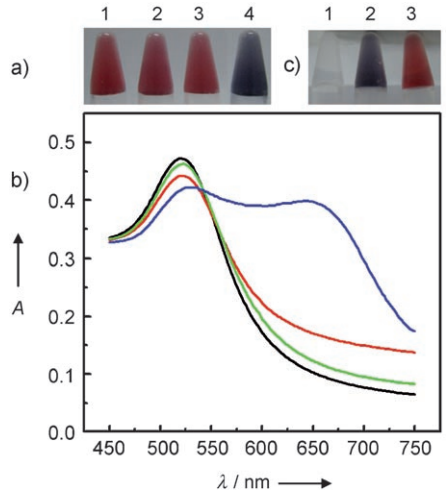

Figure 2. Colorimetric visualization of TNT based on the D–A interaction between TNT and cysteamine at the Au NP/solution interface. a) Direct observation of the color change of Au NPs (10 nM): 1) without any addition; 2) with the addition of 500 nM cysteamine; 3) with the addition of 5 nM TNT; 4) with the addition of 500 nM cysteamine + 5 nM TNT. b) UV/Vis spectra of Au NPs (10 nM): black, without any addition; red: with the addition of 500 nM cysteamine; green: with the addition of 5 nM TNT; blue: with the addition of both 500 nM cysteamine and 5 nM TNT. c) Direct observation of the D–A interaction between TNT and cysteamine amplified by Au NPs: 1) mixture of 500 nM cysteamine + 5 nM TNT; 2) Au NPs (10 nM) with the addition of 500 nM cysteamine + 5 nM TNT; and 3) Au NPs (10 nM) only (without any addition).

aggregation of the Au NPs, and the solution color remained wine red, which suggests that TNT may not induce aggregation of the citrate-stabilized Au NPs. The addition of cysteamine (500 nM) to the initially prepared citrate-stabilized Au NPs essentially gives cysteamine-stabilized Au NPs because of the formation of the Au–S covalent bond. The cysteamine-stabilized Au NPs formed could solubilize into distilled water (Figure 2a). However, when both cysteamine (500 nM) and TNT (5 nM) were added to an aqueous suspension of the Au NPs, the color of the resulting suspension clearly changed from wine red to violet blue, indicative of the aggregation of the Au NPs. UV/Vis absorption spectra (Figure 2b) further demonstrate the aggregation of Au NPs and thus the color change of the Au NPs suspension induced by the addition of TNT: the pure citrate-stabilized suspension of Au NPs (that is, without the addition of any other species) shows an absorption peak at 520 nm, which was ascribed to the surface plasmon resonance of the 13-nm Au NPs. The addition of either cysteamine or TNT does not lead to a remarkable change in the spectra, which suggests that little aggregation of the Au NPs occurred.

Contrarily, a large change in the spectrum of the citrate-stabilized Au NPs was recorded after the addition of both cysteamine (500 nM) and TNT (5 nM): a new and strong absorbance peak appeared at 650 nm, which was ascribed to the absorbance of the aggregated Au NPs. Such a change in the spectrum, which coincides with the change in the solution color displayed in Figure 2a, could be well understood by the TNT-induced aggregation of the cysteamine-stabilized Au NPs through the D–A interaction between TNT and cysteamine at the Au NP/solution interface (Figure 1). Although, as one kind of electron acceptor, TNT could interact with a primary amine electron donor, such an interaction could not be employed for the direct colorimetric detection of TNT down to nanomolar levels (Figure 2c). As displayed in Figure 2a, by using Au NPs as the amplifier and by taking advantage of the D–A interaction between TNT and cysteamine at the Au NP/solution interface, a picomolar level of TNT could be clearly visualized with the naked eye, as described below.

To further demonstrate this simple assay for the direct colorimetric visualization of TNT by the mechanism mentioned above, different amounts of TNT with concentrations down to 5×10^{-13} M (that is, 0.5 pM) were added to aqueous suspensions of Au NPs (10 nM) containing cysteamine (500 nM) and the results are displayed in Figure 3. On increasing the concentration of TNT in the suspension of Au NPs (10 nM) containing cysteamine (500 nM), the color of the suspension gradually changed initially from wine red, then to purple, and finally to violet blue. Even the addition of a

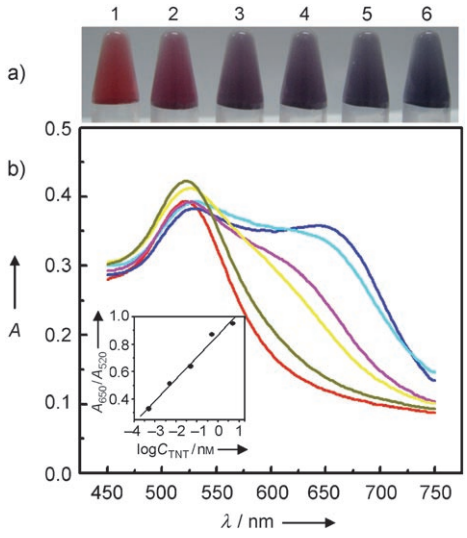

Figure 3. a) Colorimetric visualization of TNT by using Au NPs (containing 500 nM cysteamine). TNT concentrations varied from 5×10^{-13} (2) to 5×10^{-9} M (6). b) UV/Vis spectra of the Au NPs suspension (10 nM) containing 500 nM cysteamine in the presence of different concentrations of TNT: red, 0 M; dark yellow, 5×10^{-13} M; yellow, 5×10^{-12} M; magenta, 5×10^{-11} M; cyan, 5×10^{-10} M; blue, 5×10^{-9} M. Inset: plot of A_{650}/A_{520} against $\log C_{TNT}$ for TNT assay.

picomolar amount (for example, 0.5 pM) of TNT led to a change in the solution color that could be clearly distinguished from that of the initial suspension (10 nM; see Figure 3a). This demonstration reveals that the assay with the Au NPs could be used for direct visualization of TNT down to the picomolar level.

In addition, as shown in Figure 3b, increasing the concentration of TNT in the Au NPs suspension containing cysteamine also results in a clear increase in the absorbance at 650 nm (A_{650}) and a decrease in the absorbance at 520 nm (A_{520}). The ratio of A_{650} to A_{520} was found to be linear with the logarithm of TNT concentration within the concentration range from 5×10^{-9} to 5×10^{-13} M ($r=0.991$), again demonstrating that the assay described here could be used for colorimetric visualization of TNT at ultratrace levels. As far as we know, the detection limit achieved with our method represents the lowest among all values obtained with the methods reported so far.[2b–d,3e–i] Moreover, this method remains much simpler than the existing methods, without the requirements of much instrumentation or designing and synthesizing fluorescently functional nano/microstructures or tailoring the electrode/solution interface to achieve the required sensitivity. These excellent properties substantially enable the practical application of our method for on-the-spot detection of TNT at a very low level.

In addition to the properties described above, another important aspect of the assay for the colorimetric visualization of TNT is its insensitivity to common interferents. Control experiments with the addition of other explosives and derivatives, such as 2,4-dinitrotoluene (5×10^{-9} M), nitrobenzene (5×10^{-9} M), and toluene (5×10^{-9} M), to the Au NPs (10 nM) suspension containing cysteamine (500 nM) did not result in a change in the color or in the spectrum of the suspension (Figure S1 in the Supporting Information), thus indicating that these species do not interfere with the colorimetric visualization of TNT.

We have also examined the selectivity of this assay against the impurities that could potentially induce the aggregation of Au NPs without the presence of TNT, such as surfactants and salts possibly existing in the sample matrices, for example, soil, groundwater, or surfaces. We found that although the sole addition of sodium dodecyl sulfate, a typical surfactant, to the Au NPs suspension with a final concentration higher than 1.0 mM could not induce the aggregation of Au NPs, the subsequent addition of TNT did not lead to a change in the color of the Au NPs dispersion. This finding suggests that the co-existence of a high concentration of surfactants eventually invalidates this assay for the colorimetric visualization of TNT. Further studies revealed that the co-existence of surfactants at dilute concentrations did not interfere with TNT detection. For example, the co-existence of sodium dodecyl sulfate at final concentrations diluted by more than 100-fold did not result in interference.

We also studied the potential interference from salts, such as sodium chloride, and found that the sole addition of sodium chloride with final concentrations higher than 10 mM to the Au NPs suspension could lead to the aggregation of the Au NPs. Further studies revealed that the co-existence of sodium chloride with final a concentration lower than 3 mM in the suspension did not interfere with TNT detection. Although the possible existence of high concentrations of environmental impurities in 100% groundwater or extracts of soil samples essentially presents difficulties in the routine testing of TNT with methods based on the aggregation of NPs or beads,[2e] and such detection essentially necessitates additional procedures for sample pretreatment, the low detection limit of the assay demonstrated herein may make it possible to detect TNT in diluted samples and thus could enable its application in the selective detection of TNT in environmental matrices.

To further investigate the potential practical application of this colorimetric method, the detection of TNT in a lake water sample and the acetonitrile (ACN) extract of a soil sample was carried out (Figure S2 in the Supporting Information). No change in the color or the spectrum of the Au NPs suspension was observed until the lake water sample and the ACN extract of the soil sample were spiked with 7×10^{-9} and 4×10^{-9} M TNT, respectively. These results were consistent with those reported previously,[2e,3a,j] and suggest the potential application of this method for TNT detection in these environmental matrices.

In summary, by using Au NPs and by taking advantage of the D–A interaction between TNT and cysteamine at the Au NP/solution interface, we have successfully developed a new and simple assay for the direct colorimetric visualization of TNT down to the picomolar level. The method demonstrated herein is relatively simple, without requiring any instrumentation, but possesses the lowest detection limit among all methods reported so far. The striking properties substantially make this method quite promising for on-the-spot sensitive detection of TNT.

Experimental Section

Photographs were taken with a Sony T 70 digital camera. UV/Vis spectra were recorded by a Shimadzu UV-16019c spectrophotometer. Cysteamine and $HAuCl_4$ were obtained from Sigma–Aldrich and trisodium citrate was purchased from Beijing Chemicals Ltd. All chemicals were analytical-grade reagents and used without further purification. Distilled water was used in the study. Unless otherwise noted, the reactions were carried out at room temperature.

Au NPs were prepared by a trisodium citrate reduction method as reported before.[7] Briefly, trisodium citrate (5 mL, 38.8 mM) was rapidly added to a boiling solution of $HAuCl_4$ (50 mL, 1 mM), and the solution was kept continually boiling for another 30 min to give a wine-red solution. After filtering the solution through a 0.45-μm Millipore syringe to remove the precipitate, the filtrate was stored in a refrigerator at 4 °C. The concentration of the prepared Au NPs as determined by UV/Vis spectrometry was 10 nM. TNT stock solution was prepared by dissolving TNT (2.7 mg) in ethanol/ACN (4:1, v/v; 4 mL). Stock solutions of 2,4-dinitrotoluene, nitrobenzene, and toluene were prepared with ethanol. The solution of cysteamine was freshly prepared with ethanol before each experiment.

For the direct visualization of TNT with the naked eye, cysteamine (500 nM) was first added to the Au NPs dispersion (180 μL, 10 nM) in distilled water, and then different concentrations of TNT were added to the solution. The total volume of the solution was 200 μL and the final concentrations of TNT in the dispersion were 5×10^{-9}, 5×10^{-10}, 5×10^{-11}, 5×10^{-12}, and 5×10^{-13} M. Photographs were taken for 2 h after TNT was added to the dispersion.

A lake water sample (Summer Palace, Beijing) was used after filtration through a 0.45-μm filter. Extraction from the soil sample (Beijing) was performed by agitating the soil (1 g) and ACN (10 mL) in a capped vial, followed by centrifugation and filtration of the supernatant through a 0.45-μm filter, according to the methods reported previously.[2e,3j] For detection of TNT in the lake water sample and ACN extract of the soil sample, pure and TNT-spiked water sample (10 μL) and ACN extract (10 μL) were added to an aqueous dispersion of Au NPs (190 μL, 10 nM) containing cysteamine (500 nM).

For UV/Vis spectrometric measurements, the dispersions were prepared with the concentrations of Au NPs, cysteamine, and TNT in the solutions essentially the same as those for the direct visualization of TNT. The dispersions were diluted four times with distilled water prior to measurement.

Received: August 17, 2008
Published online: October 7, 2008

Keywords: aggregation · donor–acceptor systems · nanoparticles · sensors · TNT

[1] a) M. Altstein, A. Bronshtein, B. Glattstein, A. Zeichner, T. Tamiri, J. Almog, *Anal. Chem.* **2001**, *73*, 2461; b) R. Hernandez, M. Zappi, C. Kuo, *Environ. Sci. Technol.* **2004**, *38*, 5157; c) J. C. Lynch, K. F. Myers, J. M. Brannon, J. J. Delfino, *J. Chem. Eng. Data* **2001**, *46*, 1549; d) K. C. Crellin, M. Widmer, J. L. Beauchamp, *Anal. Chem.* **1997**, *69*, 1092; e) K. S. Ro, A. Venugopal, D. D. Adrian, D. Constant, K. Qaisi, K. T. Valsaraj, L. J. Thibodeaux, D. Roy, *J. Chem. Eng. Data* **1996**, *41*, 758.

[2] a) T. B. Brill, K. J. James, *Chem. Rev.* **1993**, *93*, 2667; b) C. Xie, Z. Zhang, D. Wang, G. Guan, D. Gao, J. Liu, *Anal. Chem.* **2006**, *78*, 8339; c) D. Gao, Z. Zhang, M. Wu, C. Xie, G. Guan, D. Wang, *J. Am. Chem. Soc.* **2007**, *129*, 7859; d) G. Guan, Z. Zhang, Z. Wang, B. Liu, D. Gao, C. Xie, *Adv. Mater.* **2007**, *19*, 2370; e) G. P. Anderson, S. C. Moreira, P. T. Charles, I. L. Medintz, E. R. Goldman, M. Zeinali, C. R. Tiatt, *Anal. Chem.* **2006**, *78*, 2279; f) S. J. Toal, D. Magde, W. C. Trogler, *Chem. Commun.* **2005**, 5465; g) S. J. Toal, J. C. Sanchez, R. E. Dugan, W. C. Trogler, *J. Forensic Sci.* **2007**, *52*, 79.

[3] a) H. Sohn, R. M. Calhoun, M. J. Sailor, W. C. Trogler, *Angew. Chem.* **2001**, *113*, 2162; *Angew. Chem. Int. Ed.* **2001**, *40*, 2104; b) H. Sohn, M. J. Sailor, D. Magde, W. C. Trogler, *J. Am. Chem. Soc.* **2003**, *125*, 3821; c) A. Rose, Z. Zhu, C. F. Madigan, T. M. Swager, V. Bulovic, *Nature* **2005**, *434*, 876; d) T. L. Andrew, T. M. Swager, *J. Am. Chem. Soc.* **2007**, *129*, 7254; e) R. Tu, B. Liu, Z. Wang, D. Gao, F. Wang, Q. Fang, Z. Zhang, *Anal. Chem.* **2008**, *80*, 3458; f) H. X. Zhang, A. M. Cao, J. S. Hu, L. J. Wan, S. T. Lee, *Anal. Chem.* **2006**, *78*, 1967; g) B. Filanovsky, B. Markovsky, T. Bourenko, N. Perkas, R. Persky, A. Gedanken, D. Aurbach, *Adv. Funct. Mater.* **2007**, *17*, 1487; h) M. Riskin, R. Tel-Vered, T. Bourenko, E. Granot, I. Willner, *J. Am. Chem. Soc.* **2008**, *130*, 9726; i) S. A. Trammell, M. Zeinali, B. J. Melde, P. T. Charles, F. L. Velez, M. A. Dinderman, A. Kusterbeck, M. A. Markowitz, *Anal. Chem.* **2008**, *80*, 4627; j) S. Hrapovic, E. Majid, Y. Liu, K. Male, J. H. T. Luong, *Anal. Chem.* **2006**, *78*, 5504.

[4] a) For a typical review, see: M. C. Daniel, D. Astru, *Chem. Rev.* **2004**, *104*, 293; b) J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **1998**, *120*, 1959.

[5] a) R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, C. A. Mirkin, *Science* **1997**, *277*, 1078; b) J. M. Slocik, J. S. Zabinski, J. D. M. Phillips, R. R. Naik, *Small* **2008**, *4*, 548; c) D. Li, A. Wieckowska, I. Willner, *Angew. Chem.* **2008**, *120*, 3991; *Angew. Chem. Int. Ed.* **2008**, *47*, 3927; d) Y. Choi, N.-H. Ho, C.-H. Tung, *Angew. Chem.* **2007**, *119*, 721; *Angew. Chem. Int. Ed.* **2007**, *46*, 707; e) X. Y. Xu, M. S. Han, C. A. Mirkin, *Angew. Chem.* **2007**, *119*, 3538; *Angew. Chem. Int. Ed.* **2007**, *46*, 3468; f) C. Guarise, L. Pasquato, V. De Fillippis, P. Scrimm, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 3978; g) A. Laromaine, L. Koh, M. Murugesan, R. V. Ulijin, M. M. Stevens, *J. Am. Chem. Soc.* **2007**, *129*, 4156; h) Z. F. Ma, S.-F. Sui, *Angew. Chem.* **2002**, *114*, 2280; *Angew. Chem. Int. Ed.* **2002**, *41*, 2176; i) Y. Tang, F. Feng, F, He, S, Wang, Y, Li, D. Zhu, *J. Am. Chem. Soc.* **2006**, *128*, 14972.

[6] a) R. Liu, Z. Wang, D. Gao, F. Wang, Q. Fang, Z. Zhang, *Anal. Chem.* **2008**, *80*, 3458; b) N. R. Walker, M. J. Linman, M. M. Timmers, S. L. Dean, C. M. Burkett, J. A. Lloyd, J. D. Keelor, B. M. Baughman, P. L. Edmiston, *Anal. Chim. Acta* **2007**, *593*, 82; c) C. F. Bernasconi, *J. Org. Chem.* **1971**, *36*, 1671.

[7] K. G. Grabar, R. G. Freeman, M. B. Hommer, M. J. Natan, *Anal. Chem.* **1995**, *67*, 735.

