Modulating DNA-templated Silver Nanoclusters for Fluorescence Turn-on Detection of Thiol Compounds

EXPERIMENTAL SECTION

Materials and Measurements:
Silver nitrate (AgNO₃), 99.9995%, and sodium borohydride (NaBH₄), 98%, were purchased from Alfa Aesar and used without further purification. DNA oligonucleotides were purchased from Sangon (Shanghai, China). All other reagents were all of analytical reagent grade and used as received. Fluorescence measurements were carried out by using a JASCO FP-6500 spectrofluorometer (Jasco International Co., Japan). Electronic absorption spectra were acquired using a CARY 300 UV/Visible spectrophotometer (Varian Inc., Palo Alto, CA). CD spectra were determined using a Jasco 810 (Jasco International Co., LTD., Tokyo, Japan) Nanopure water (18.2 MΩ; Millpore Co., USA) was used in all experiments.

Preparation of fluorescent silver nanoclusters (AgNCs):
The silver nanoclusters were synthesized by first cooling the solution of DNA and AgNO₃ to 0 °C and then adding NaBH₄ followed by vigorous shaking for 2 min. Final concentrations were 15 μM in DNA template, 90μM in AgNO₃ and 90μM in NaBH₄. The reaction mixture was kept in the dark at 4 °C for 3 hours before use. Unless otherwise noted, experiments were carried out in buffer containing 80 mM NH₄Ac with desired pH.

Preparation of Real Samples:
Fresh human blood samples were collected in tubes containing EDTA, and then
centrifuged at 3000 rpm for 15 min. The supernatant solution, which contains proteins and amino acids, was used as the source of plasma. Since most of the thiol compounds in plasma were linked to other thiols and proteins by disulfide bonds, it was necessary to reduce disulfide bonds before analysis and the following procedure according to literatures was carried out.\(^2\) Firstly, 40 \(\mu\)l of HCl (0.2 M) and 20\(\mu\)l of PPh\(_3\) (400 mM in H\(_2\)O-CH\(_3\)CN 20:80 v/v and 2.0 M HCl) were added to 500\(\mu\)l of plasma and incubated for 15 minutes at room temperature to hydrolyze the disulfide bonds. After that, 500\(\mu\)l of CH\(_3\)CN was mixed with 500 \(\mu\)l of hydrolyzed plasma to precipitate plasma proteins followed by centrifugation at 3000 g for 20 minutes. The supernate containing thiol compounds was collected and appropriately diluted with NH\(_4\)Ac buffer (80mM, pH6.9) for further analysis.

**Detection of thiol compound**

In a typical test, DNA-AgNCs (200\(\mu\)l) solution was mixed with certain amounts of analytes. The mixture was incubated at room temperature for 10mins and then fluorescence spectra were recorded under excitation at 580 nm.
Supplementary Material (ESI) for Chemical Communications
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FIGURE

(a) dTGACTAAAACCTTAATCCCC DNA 1
(b) dCACCCACCCACC DNA 2
(c) dCCCTTAATCCCC DNA 3
(d) dCCTCCTTCTCC DNA 4
(e) dCCCTAACCTAACCCTAAT DNA 5
(f) dTAACCCCTCCCCCTTAA DNA 6

FL vs. Wavelength / nm
Figure S1. Fluorescence responses of different DNA-AgNCs to thiol compounds. (a) ~ (j): Fluorescence spectra of DNA-AgNCs in the absence (black line) and the presence of cysteine (500nM, red line), glutathione (500nM, blue line), homocysteine (500nM, green line). For template DNA-1, DNA-3, phosphate buffer (20mM, pH7.0) was used in synthesis according to literature. For template DNA-4, citrate buffer (20mM, pH5.5) was used in synthesis according to literature. For other template DNA, ammonium acetate buffer (80mM, pH6.9) was used in synthesis.
**Figure S2.** Absorption spectra of dC$_{12}$-AgNCs before (black line) and after (red line) adding (a) cysteine (500nM), (b) glutathione (500nM), (c) homocysteine (500nM). The insets are zoom-in images showing the absorption red shift of AgNCs after addition of thiol compounds.

![Absorption spectra of dC$_{12}$-AgNCs](image)

**Figure S3.** XPS spectra of Ag 3d core level of the dC$_{12}$-AgNCs in the presence of cysteine (500nM).
Figure S4. CD spectra of dC_{12} alone (black line), dC_{12}-AgNCs (red line), dC_{12}-AgNCs in the presence of cysteine (500nM, blue line), glutathione (500nM, green line), homocysteine (500nM, purper line).

Figure S5. Fluorescence excitation (black line) and emission (red line) spectra of dC_{12}-AgNCs.
Figure S6. Plots of the enhancement factor \((I - I_0)/I_0\) at 615 nm versus the concentration of added (a) cysteine and (b) homocysteine. The error bars represent the standard deviation of three measurements. Inset is a linear region.
Figure S7. The response time of dC12-AgNCs to different concentration of (a) cysteine (0nM, 50nM, 500nM, 1000nM) and (b) homocysteine (0nM, 50nM, 500nM, 1000nM).
Figure S8. Effect of pH on the dC$_{12}$-AgNCs-based assay for (a) cysteine and (b) homocysteine.
Figure S9. Effect of temperature on the dC_{12}-AgNCs-based assay for cysteine (black), glutathione (red) and homocysteine (blue).

Figure S10. Effect of concentration of DNA-AgNCs on the dC_{12}-AgNCs-based assay for cysteine (black), glutathione (red) and homocysteine (blue). The concentration of the as-prepared DNA-Ag NCs solution is denoted as “1X”.

Figure S11. Fluorescence response of dC$_{12}$-AgNCs in the presence of other amino acids (1-19,10μM), various biologically relevant analytes, 20) K$^+$ (100mM), 21) Mg$^{2+}$ (10mM), 22) Ca$^{2+}$ (10mM), 23) glucose (10mM), 24) citrate (1mM), 25) EDTA (1mM), 26) ascorbic acid (1mM), 27) BSA (2.5μM), 28) HAS (2.5μM), 29) cysteine (1μM), 30) glutathione homocysteine (1μM) and 32) homocysteine (1μM).

Figure S12. Fluorescence response of dC$_{12}$-AgNCs to plasma with or without pretreatment by thiol blocking agent, NEM.
Table 1 Determination of thiol compounds in human plasma.

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<th>Sample</th>
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<th>Added Cys (μM)</th>
<th>Measured (μM)</th>
<th>Recovery (%)</th>
<th>RSD (n=3%)</th>
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