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A DNA–Gold Nanoparticle-Based Colorimetric Competition Assay for the Detection of Cysteine

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ABSTRACT

We report the development of a highly sensitive and selective colorimetric detection method for cysteine based upon oligonucleotide-functionalized gold nanoparticle probes that contain strategically placed thymidine–thymidine (T–T) mismatches complexed with Hg^{2+}. This assay relies upon the distance-dependent optical properties of gold nanoparticles, the sharp melting transition of oligonucleotide-linked nanoparticle aggregates, and the very selective coordination of Hg^{2+} with cysteine. The concentration of cysteine can be determined by monitoring with the naked eye or a UV–vis spectrometer the temperature at which the purple-to-red color change associated with aggregate dissociation takes place. This assay does not utilize organic cosolvents, enzymatic reactions, light-sensitive dye molecules, lengthy protocols, or sophisticated instrumentation thereby overcoming some of the limitations of more conventional methods.

As a sulfur-containing amino acid, cysteine plays a crucial biological role in the human body by providing a modality for the intramolecular crosslinking of proteins through disulfide bonds to support their secondary structures and functions.[1] It is also a potential neurotoxin,[2−4] a biomarker for various medical conditions,[5,6] and a disease-associated physiological regulator.[7−9] A variety of methods for detecting cysteine, such as fluorometry based upon fluorescent dyes,[10−13] electrochemical voltammetry,[14−18] and fluorescence-coupled HPLC techniques,[19,20] have been developed. Most of them, however, require complicated instrumentation, involve cumbersome laboratory procedures and are low throughput, which limits the scope of their practical applications. Recently, significant advances have been made in the development of chromophoric colorimetric sensors for detecting cysteine, and they have attracted attention due to their easy readout (often with the naked eye) and potential for high throughput formats.[10,11,21,22] However, they are also limited with respect to sensitivity (limit of detection (LOD) $\geq \sim 1\ \mu$M) and in certain cases not stable or functional in aqueous environments.[10,11,21,22]

Assays based upon oligonucleotide−gold nanoparticle conjugates (DNA−Au NPs) are emerging as alternatives to more conventional chromogenic sensors.[23,24] The DNA−Au NPs are attractive as colorimetric probes because of their intense optical properties, chemical tailorability, distance- and aggregate size-dependent optical properties, unusual hybridization and melting properties, and chemical stability.[23−30] Thus far, DNA−Au NPs have been used to develop assays for a wide variety of analytes, including proteins,[31−34] oligonucleotides,[35−40] certain metal ions[41,42] and other small organic molecules.[43−47] Assays for cysteine based upon *unmodified* Au nanoparticles that rely on the nonselective adsorption of cysteine on the surface of the nanoparticle to effect aggregation and a colorimetric change have been developed. However, while conceptually simple, they lack selectivity and have relatively high LODs ($\geq \sim 7\ \mu$M).[48−51]

We have been developing a new class of assays based upon nanoparticle aggregates formed from DNA duplex interlinks. These DNA-linked Au NP network structures have shown promise for detecting important nucleic acid analytes with single mismatch selectivity,[52,53] probing Hg^{2+} ion at nM levels,[41] identifying triplex promoters,[45] screening duplex DNA intercalators,[46] monitoring enzyme activity and inhibition in real time,[54] and screening the sequence selectivity of DNA-binding molecules[55] in a high throughput manner. The premise behind these assays is that perturbation of the DNA links holding the particles within the aggregates together will lead to a change in the melting temperature (T_m) for the aggregates and therefore the colorimetric transition that takes place when the particles disperse. The Hg^{2+} example is particularly interesting, because it is based upon the concept that a thymidine−thymidine (T−T) mismatch is very selective for Hg^{2+} binding.[41,56] Indeed, when Hg^{2+} binds to the DNA it can increase the T_m by ∼10 °C. The novel cysteine assay reported herein relies on this observation. We inten-

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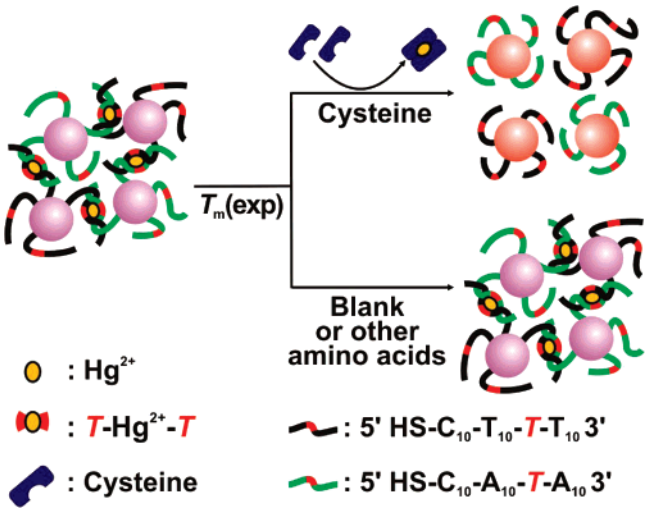
Scheme 1. Colorimetric Detection of Cysteine Using DNA−Au NPs in a Competition Assay Format



tionally prepare Au NP networks interconnected with duplex DNA with strategically placed Hg^{2+}-complexed T−T mismatches (DNA−Au NP/Hg^{2+} aggregates, Scheme 1).[41,56] The cysteine analyte can bind mercuric ion and remove it from the network structure thereby lowering the temperature at which the DNA duplexes dissociate and the corresponding purple-to-red color change takes place. Cysteine at concentrations as low as a 100 nM can be detected with this assay in a colorimetric format.

In a typical assay, two sets of Au NP probes, functionalized with different oligonucleotide sequences (probe **A**, 5′ HS-C_{10}-A_{10}-**T**-A_{10} 3′; probe **B**, 5′ HS-C_{10}-T_{10}-**T**-T_{10} 3′), were prepared using literature procedures (see Supporting Information)[41] and suspended in MOPS buffer (10 mM MOPS, pH 7.5). When aliquots of the red solutions containing the two Au NP probes were combined, they rapidly formed aggregates through DNA-hybridization (confirmed by melting analyses) and turned purple. In general, DNA−Au NP aggregates that contain a single base mismatch dissociate at a temperature lower than that of perfectly complementary particles with a concomitant purple-to-red color change.[52,53] However, it is known that aggregates linked by DNA strands with T−T mismatches will selectively bind Hg^{2+} and form aggregates that are substantially more stable than the Hg^{2+}-free structures.[41] This increased stability is reflected in T_m values with the T_m for the mercuric ion-bound structure being shifted +10 °C compared to the mercuric ion-free structure. When cysteine is added to the solution containing the purple aggregates linked by DNA with Hg^{2+} complexed T−T mismatches, the mercury is sequestered from the aggregate through cysteine complexation, which lowers the T_m of the aggregate and the temperature at which the purple-to-red color change occurs.

The limit of detection and dynamic range of the assay were determined by preparing and studying aqueous cysteine solutions at concentrations ranging from 50 nM to 10 μM. In general, when an aliquot of the solution containing cysteine was combined with the solution containing the DNA−Au NP/Hg^{2+} aggregates to bring the mixture to a cysteine concentration that fell within the range cited above, no change in color was observed at room temperature. Upon heating, however, the aggregates dehybridized with a concomitant purple-to-red color change. The melting transition was obtained by heating the aggregates at a rate of 1 °C/min while monitoring the extinction at 528 nm (Figure 1a), and the T_m was determined from the maximum of the first derivative of the melting transition in the visible region of the spectrum. Importantly, the observed T_m inversely correlates with the concentration of cysteine over the entire range of detectable cysteine concentrations studied (Figure 1a,b). Note that at higher concentrations, the dependence of T_m on cysteine concentration decreases because most of the Hg^{2+} has been removed from the aggregate. The LOD for this system is ∼100 nM in cysteine. Over the region of highest sensitivity (left part of curve in Figure 1b), each 100 nM increase in cysteine concentration results in ∼0.7 °C decrease in T_m. This trend is consistent up to 2 μM cysteine concentration, allowing one to measure cysteine concentration in a quantitative way. Because the total concentration

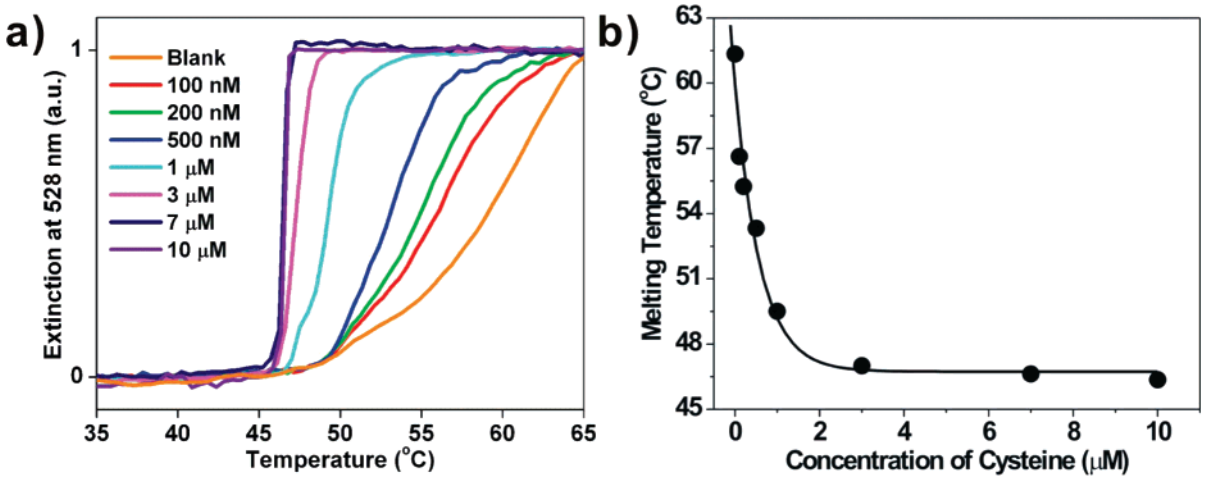


Figure 1. (a) Normalized melting transitions of DNA−Au NP/Hg^{2+} complex aggregates at different concentrations of cysteine. (b) T_m values of the melting transitions in panel a as a function of cysteine concentration.

Figure 2. The colorimetric response of the DNA−Au NP/Hg^{2+} complex aggregates in the presence of the various amino acids (each at 1 μM) at room temperature and 50 °C.

of Hg^{2+} at the start of the experiment was 1 μM, we conclude that the 2 μM concentration value for saturation reflects the formation of the aggregate-free 2:1 cysteine/Hg^{2+} adduct.[57]

To determine the selectivity of this assay, we studied its colorimetric response to the other 19 essential amino acids at a concentration of 1 μM (Figure 2). The color of the aggregate solutions in the presence of these amino acids remained unchanged at 50 °C, the temperature at which the colorimetric transition takes place in the presence of cysteine (Figure 2). The melting behavior of this system was then evaluated in the presence of each amino acid (Figure 3, inset). Only cysteine exhibits a significant perturbation on the Hg^{2+}-duplex interconnects, demonstrating the high selectivity of this system (Figure 3).

There are three factors responsible for the high sensitivity, selectivity, and quantitative output of this assay. First, the high extinction coefficients of the gold nanoparticle−oligonucleotide conjugates (8.1×10^8 cm^{-1} M^{-1} for 20 nm Au NPs)[58] allow one to monitor the changes in duplex T_m at lower concentrations than one can monitor with conventional absorbance-based chromophores (extinction coefficients $\sim 10^4-10^5$ cm^{-1} M^{-1}).[59] The sharp melting transitions for aggregates formed from Au NPs allow one to monitor very subtle differences in T_m effected by cysteine analyte and therefore provide a quantitative measure of cysteine concentration. Finally, the high selectivity and tight binding of cysteine for the Hg^{2+} at the T−T mismatch sites as compared with the other amino acids lead to an assay with high specificity.[57]

It is notable that methionine, the other sulfur-containing amino acid, did not result in a significant change in T_m. The binding affinity of the Hg^{2+} for the thiol in cysteine must be substantially greater than that for the thioether in methionine.[60,61] In addition, Hg^{2+} is known to have an affinity for certain N-type ligands,[62] potentially including basic amino acids such as histidine or lysine. However, in this system if there is such an interaction it is not significant enough to affect the assay. The T−T mismatch site appears to be a strong enough Hg^{2+} binder to effectively compete against all of the amino acids studied other than cysteine.

It is well known that thiolated molecules such as dithiothreitol (DTT) remove thiolated oligonucleotides from Au surfaces.[63] Therefore, we considered the possibility of such displacement by cysteine through a ligand-exchange process, which could result in irregular functionality of the NP probes and impede assay performance. To verify the stability of the DNA−Au NPs toward cysteine, we investigated the number of oligonucleotides bound to the nanopar-

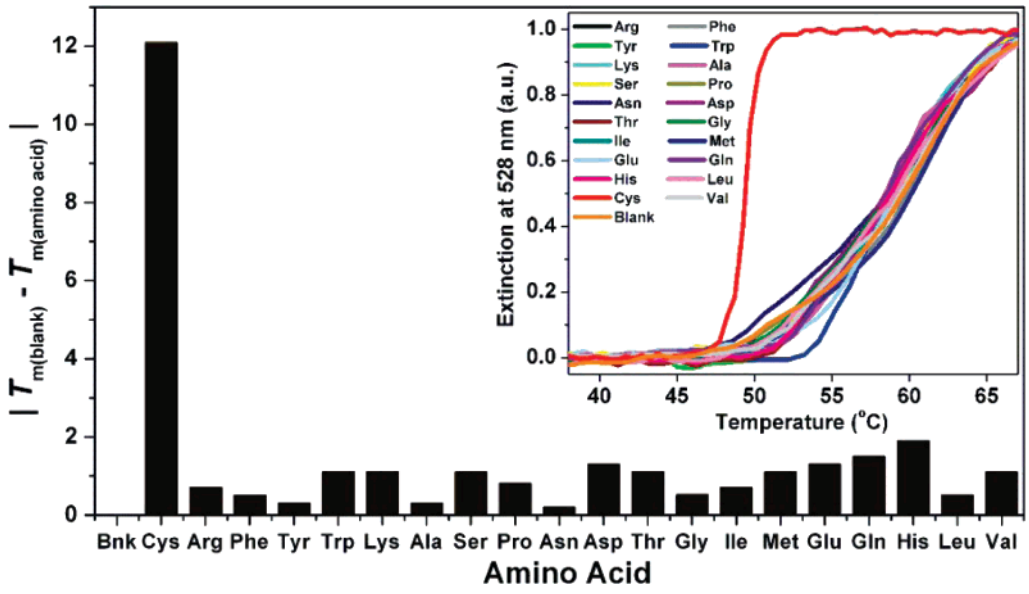


Figure 3. The difference of the T_m values of the blank and the amino acid samples (each at 1 μM) and their normalized melting profiles (inset).

Table 1. The Average Number of Fluorophore-Labeled DNA Strands Per Particle before and after Being Exposed to Cysteine at 25 or 50 °C for 1 h

temperature (°C)	cysteine concentration (μM)		
	0	1	10
25	126.2 ± 1.3	121.7 ± 4.5	118.5 ± 5.3
50	117.3 ± 2.6	112.5 ± 5.4	106.2 ± 11.2

ticle probe using fluorescence spectroscopy before and after conducting the assay. In this study, a thiol-modified oligonucleotide sequence labeled with a fluorophore (F-probe **A**, 5′ HS-C_{10}-A_{10}-T-A_{10}-(6-FAM) 3′) was used to functionalize Au NPs (20 nm in diameter). The DNA−Au NPs with a fluorophore were incubated with various concentrations of cysteine (0, 1, and 10 μM over 1 h at 25 °C and 50 °C). The number of DNA strands before and after incubation with cysteine was determined by measuring the fluorescence from the DNA released by DTT.[63,64] This surface-loading assay shows that there are approximately 126 oligonucleotides per as-prepared particle. After soaking in a 10 μM aqueous solution of cysteine for 1 h at room temperature, 90% of the oligonucleotides remain on the particles (113 strands/particle, Table 1). Even at elevated temperatures (50 °C),[65] the average number of DNA strands per particle is ∼106 (Table 1), indicating that the probes survive the conditions of the assay.

In conclusion, this manuscript describes a new type of rapid, highly selective, and sensitive colorimetric assay for detecting cysteine using gold nanoparticle−oligonucleotide conjugates. This assay is based on the thiophilicity of Hg^{2+} and the unique optical properties and the sharp melting properties of DNA−Au NPs in a competition assay format. Importantly, the assay described in this work is easily read out with the naked eye or a UV−vis spectrometer by monitoring the temperature at which the purple-to-red color change takes place, providing a quantitative measurement of cysteine concentration in a high throughput manner. The LOD is 100 nM, which is to the best of our knowledge a new record as a colorimetric cysteine detection system. The assay is also particularly attractive because it does not rely on organic cosolvents, enzymatic reactions, light-sensitive dye molecules, lengthy protocols, and sophisticated instrumentation. Considering the physiological link between cysteine and a variety of diseases and disease status,[1−9] this assay may become useful in the biomedical research community.

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Supporting Information Available: Materials and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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