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Time-Resolved Probes Based on Guanine/Thymine-Rich DNA-Sensitized Luminescence of Terbium(III)

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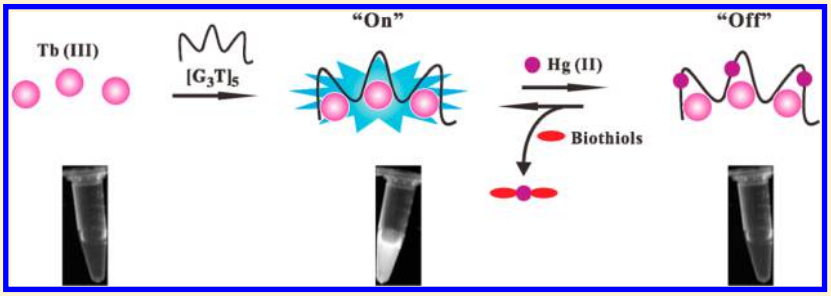
Supporting Information



ABSTRACT: In this study, we have developed a novel strategy to highly sensitize the luminescence of terbium(III) (Tb^{3+}) using a designed guanine/thymine-rich DNA (5′-$[G_3T]_5$-3′) as an antenna ligand, in which $[G_3T]_5$ improved the luminescence of Tb^{3+} by 3 orders of magnitude due to energy transfer from nucleic acids to Tb^{3+} (i.e., antenna effect). Furthermore, label-free probes for the luminescent detection of biothiols, Ag^+, and sequence-specific DNA in an inexpensive, simple, and mix-and-read format are presented based on the $[G_3T]_5$-sensitized luminescence of Tb^{3+} (GTSLT). The long luminescence lifetime of the probes readily enables time-resolved luminescence (TRL) experiments. Hg^{2+} can efficiently quench the luminescence of Tb^{3+} sensitized by $[G_3T]_5$ ($Tb^{3+}/[G_3T]_5$); however, biothiols are readily applicable to selectively grab Hg^{2+} for restoration of the luminescence of $Tb^{3+}/[G_3T]_5$ initially quenched by Hg^{2+}, which can be used for "turn on" detection of biothiols. With the use of cytosine (C)-rich oligonucleotide c$[G_3T]_5$ complementary to $[G_3T]_5$, the formed $[G_3T]_5$/c$[G_3T]_5$ duplex cannot sensitize the luminescence of Tb^{3+}. However, in the presence of Ag^+, Ag^+ can combine the C base of c$[G_3T]_5$ to form C−Ag^+−C complexes, leading to the split of the $[G_3T]_5$/c$[G_3T]_5$ duplex and then release of $[G_3T]_5$. The released $[G_3T]_5$ acts as an antenna ligand for sensitizing the luminescence of Tb^{3+}. Therefore, the $Tb^{3+}/[G_3T]_5$/c$[G_3T]_5$ probe can be applied to detect Ag^+ in a "turn on" format. Moreover, recognition of target DNA via hybridization to a molecular beacon (MB)-like probe (MB-$[G_3T]_5$) can unfold the MB-$[G_3T]_5$ to release the $[G_3T]_5$ for sensitizing the luminescence of Tb^{3+}, producing a detectable signal directly proportional to the amount of target DNA of interest. This allows the development of a fascinating label-free MB probe for DNA sensing based on the luminescence of Tb^{3+}. Results and methods reported here suggest that a guanine/thymine-rich DNA-sensitized luminescence probe of Tb^{3+} represents a new opportunity for versatile background-free biosensing applications.

The sensitive and specific determination of particular analytes is the foundation of bioanalytical chemisty, which is of paramount importance for a broad spectrum of applications, such as clinical diagnostics, forensic analysis, environmental monitoring, etc.[1] To some extent, most assays undergo the issue of a background signal being observed in the absence of an analyte, which is unfavorable to the sensitivity and dynamic range that can be achieved.[2] In this respect, the time-resolved luminescence (TRL) detection technique uses the temporal domain to distinguish long-lifetime luminescence-labeled targets from short-lifetime autoluminescence; that is, detection of the luminescence signal is delayed for a certain time following the termination of pulsed excitation; thereafter only the long-lived luminescence-labeled targets remain visible to the detector, which is an ideal tool to enable the complete removal of background luminescence.[3]

In the past few years, a wealth of long-lifetime luminescence probes has been developed in the shape of metal chelate complexes and nanoparticles, such as lanthanide, phosphorescence, and charge transfer (CT) materials.[4] Trivalent lanthanide ions (Ln^{3+}) present an alternative to organic luminescent stains because of their singular properties, enabling easy spectral and time discrimination of their emission bands which span both the visible and near-infrared (NIR) ranges.[5] Direct excitation of Ln^{3+} to luminesce is difficult because the f−f transitions are Laporte-forbidden, which can be resolved by means of Ln^{3+} coordinated with certain ligands for sensitization of Ln^{3+} luminescence (referred to as antenna effect).[6] The luminescence of Ln^{3+} coordination compounds often roots in the efficient intermolecular energy transfer from the excited triplet state of the antenna ligand to the emitting electronic

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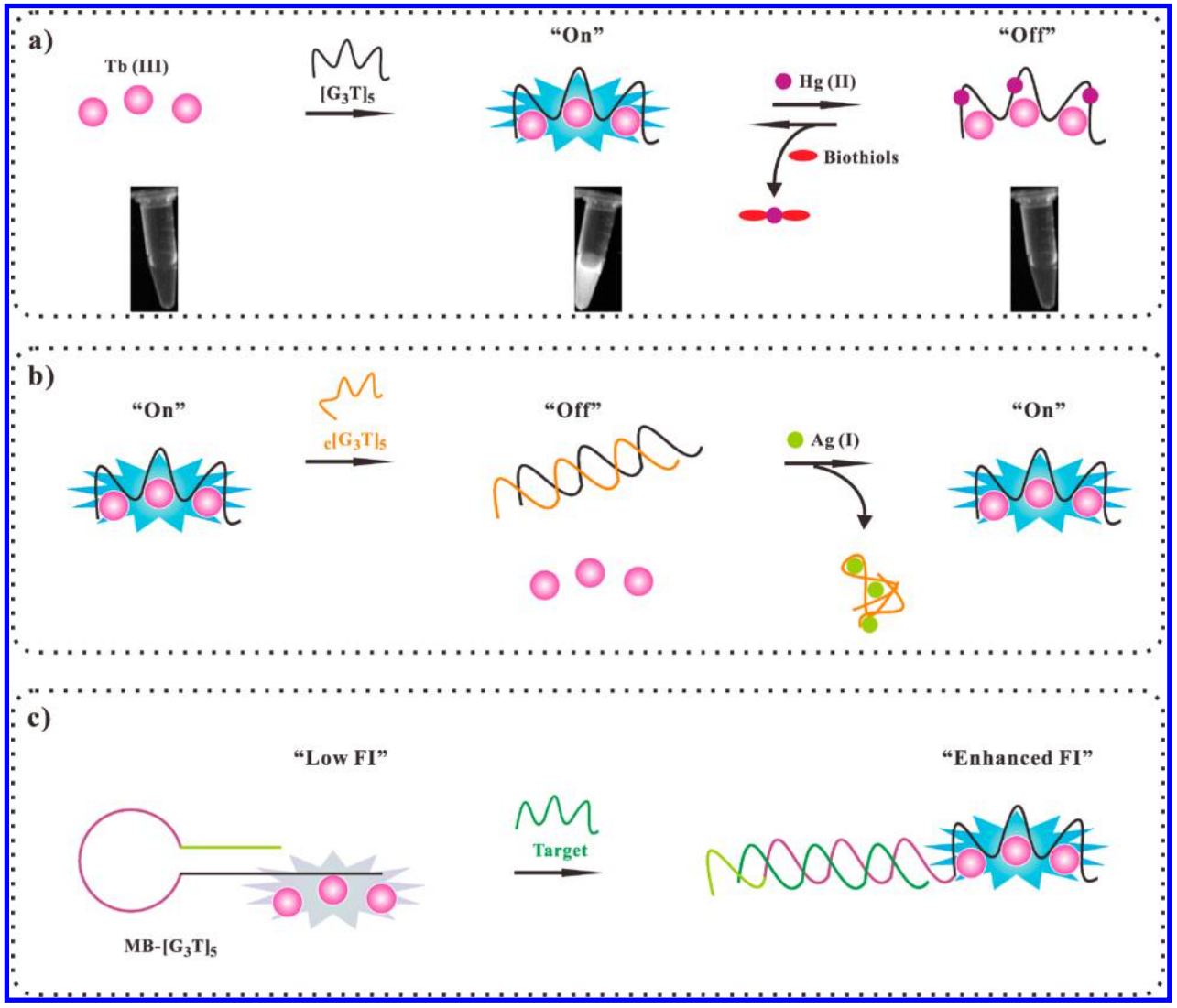
Scheme 1. Schematic Illustration of Our Proposed Time-Resolved Probes Based on Guanine/Thymine-Rich DNA-Sensitized Luminescence of Tb^{3+} for Highly Selective and Sensitive Detection of (a) Biothiols, (b) Ag^{+}, and (c) Sequence-Specific DNA, Respectively



level of Ln^{3+}, such as the terbium ion (Tb^{3+}).[7] Ligand-sensitized, luminescent Tb^{3+} complexes are highly favorable because their unique photophysical properties (sharply spiked emission bands, large Stokes' shifts, and long lifetimes) make them well suited as luminescent probes in TRL bioassays.[8] However, the effective application of a TRL detection technique using Tb^{3+} complexes is restricted by the lack of the target-responsive luminescent Tb^{3+} complexes that can specifically identify various analytes in aqueous solutions.[9] In order to enhance the luminescence, Tb^{3+} must be conjugated to a structure that both shields the Tb^{3+} from the quenching effects of the surrounding medium and has a sensitizing part that absorbs the excitation light and transfers it to the Tb^{3+}.[10] Examples of such structures are lanthanide chelates,[11] cryptates,[12] lanthanide-binding peptides,[13] and sequence-specific DNA.[14]

Since DNA chemical synthesis is convenient for creating DNA with varied sequences and lengths, DNA has been widely employed as sensing or transducing moieties in the development of DNA-based biosensors.[15] It is reported that single-stranded DNA (ssDNA) greatly enhances the Tb^{3+} emission, but double-stranded DNA (dsDNA) does not.[14a] On the basis of these facts, enhanced luminescence resulting from energy transfer from ssDNA to Tb^{3+} has been utilized to investigate the binding of the Tb^{3+} to the bases and nucleotides, and in the detection of single mismatches in duplexes. Zhang et al. reported a signal-on luminescent DNA aptasensor based on Tb^{3+} and target-dependent adaptability of DNA aptamers for label-free detection of ochratoxin A in wheat.[14b] Enlightened by the above facts, it can be envisioned that Tb^{3+}/DNA complex-based luminescent probes can be devised utilizing both the physicochemical properties of Tb^{3+} and the structure-switching of DNA.

Herein, we describe the design of a group of unique Tb^{3+}/DNA complex-based luminescent probes for highly selective and sensitive TRL detection of biothiols, Ag^{+}, and sequence-specific DNA. A designed guanine/thymine-rich DNA (5′-$[G_3T]_5$-3′) was screened and shown to have a significant antenna capability for sensitizing the Tb^{3+} emission. The $[G_3T]_5$-sensitized luminescence of Tb^{3+} (GTSLT) can be effectively quenched by Hg^{2+}; however, in the presence of biothiols, a complex of Hg^{2+}-biothiols is formed due to the high

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affinity of biothiols to Hg^{2+}, which makes the quenching effect from Hg^{2+} to $Tb^{3+}/[G_3T]_5$ inhibited, and thereby, the intermolecular energy transfer from the antenna ligand $[G_3T]_5$ to Tb^{3+} is feasible, and the long-lived Tb^{3+} luminescence is turned on (Scheme 1a). The "off−on" luminescence response of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} system to biothiols can be expected to be used for the highly sensitive and selective luminescence detection of biothiols. With the use of cytosine (C)-rich oligonucleotide $c[G_3T]_5$ complementary to $[G_3T]_5$, the formed $[G_3T]_5/c[G_3T]_5$ duplex cannot sensitize the luminescence of Tb^{3+}. However, in the presence of Ag^+, Ag^+ can combine the C base of $c[G_3T]_5$ to form C−Ag^+−C complexes, leading to the split of the $[G_3T]_5/c[G_3T]_5$ duplex and then release of $[G_3T]_5$. The released $[G_3T]_5$ acts as an antenna ligand for sensitizing the luminescence of Tb^{3+}. Therefore, the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe can be applied to detect Ag^+ in a "turn on" format (Scheme 1b). Moreover, recognition of target DNA via hybridization to a molecular beacon (MB)-like probe (MB-$[G_3T]_5$) can unfold the MB-$[G_3T]_5$ to release the $[G_3T]_5$ sequence for sensitizing the luminescence of Tb^{3+}, producing a detectable enhanced signal directly proportional to the amount of target DNA of interest. This allows the development of a fascinating label-free MB probe for DNA sensing based on the luminescence of Tb^{3+} (Scheme 1c). Most importantly, all of these detection approaches need fewer types of reagents and simple rapid operation, and only label-free oligonucleotides are used during the whole procedure.

EXPERIMENTAL SECTION

Reagents and Materials. The oligonucleotides used in this study were synthesized by TaKaRa Biotechnology Co. Ltd. (Dalian, China) with the following sequences listed in Table S1. The following metal salts, $Mg(NO_3)_2$, $Cu(NO_3)_2$, $Mn(Ac)_2$, $Zn(Ac)_2$, $Cr(NO_3)_3$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, $Co(Ac)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Hg(Ac)_2$, $Ca(Ac)_2$, $CrCl_3$, $Ba(NO_3)_2$, $Al(NO_3)_3$, $NaNO_3$, and KNO_3, were reagent-grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Glutathione (GSH), glutathione disulfide (GSSG), homocysteine (Hcys), and amino acids were purchased from Sigma-Aldrich (St. Louis, MO). Ten × Tris-HAc buffer (100 mM, pH 7.9) was prepared using metal-free reagents in distilled water purified by a Milli-Q water purification system (Millipore Corp., Bedford, MA) with an electrical resistance of 18.2 MΩ. The 1 mM stock solutions of Tb^{3+} were prepared by dissolving pure Tb_2O_3 (Wako Pure Chemical Industries Co., Ltd., Japan) in 2% nitric acid solution and were diluted to desired concentrations with Milli-Q water. An artificial urine solution was prepared according to the recipe reported by Brooks and Keevil.[16] The artificial urine solution contained 1.1 mM lactic acid, 2.0 mM citric acid, 25 mM sodium bicarbonate, 170 mM urea, 2.5 mM calcium chloride, 90 mM sodium chloride, 2.0 mM magnesium sulfate, 10 mM sodium sulfate, 7.0 mM potassium dihydrogen phosphate, 7.0 mM dipotassium hydrogen phosphate, and 25 mM ammonium chloride and all mixed in Milli-Q water, and the pH of the solution was adjusted to 6.0 by the addition of 1.0 M hydrochloric acid. All chemicals used in this work were of analytical reagent grade and obtained from commercial sources and directly used without additional purification.

Instrumentation. Luminescence spectra were measured in a luminescence microplate reader (BioTek Instrument, Winooski, USA) using a black 384 well microplate (Fluotrac 200, Greiner, Germany). The excitation wavelength used was 290 nm for the emission spectra. For the time-resolved luminescence spectra, a delay time of 50 μs and a gate time of 2 ms were used. The solution of Tb^{3+}-$[G_3T]_5$ can be luminescent, and this picture was taken under FR-200A instrument (Shanghai, China).

Investigation of Optimum DNA Ligand to Sensitize the Luminescence of Tb^{3+}. Oligonucleotides with varied sequences were prepared (Table S1). A solution of oligonucleotides (3 μM) and Tb^{3+} (2 μM) was prepared in 10 mM Tris-HAc buffer (pH 7.9). The mixture was incubated for 10 min at room temperature. Then, the time-resolved luminescence excited at 290 nm was recorded; a delay time of 50 μs and a gate time of 2 ms were used.

Assays for Biothiols Using the $Tb^{3+}/[G_3T]_5$-Hg^{2+} Probe. The $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe (Tb^{3+}, $[G_3T]_5$, and Hg^{2+} were used) was prepared in 10 mM Tris-HAc buffer (pH 7.9), and the mixture was incubated for 10 min at room temperature. For the luminescent "on" detection of biothiols, an aliquot of the tested biothiols (Cys, GSH, Hcys) or control samples or Mill-Q water (as blank sample) was added to the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe. The final concentrations of Tb^{3+}, $[G_3T]_5$, and Hg^{2+} were 2 μM, 3 μM, and 20 μM, respectively. The mixture was vortexed to mix all the reagents and then incubated for 10 min at room temperature, and after that an aliquot of 0.1 mL of the mixture was placed in the black 384 well microplate to measure the luminescence intensity (excited at 290 nm, a delay time of 50 μs, and a gate time of 2 ms).

Assays for Ag^+ Using the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ Probe. The $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe (Tb^{3+}, $[G_3T]_5$, and $c[G_3T]_5$ were used) was prepared in 10 mM Tris-HAc buffer (pH 7.9), and the mixture was incubated for 10 min at room temperature. For the luminescent "on" detection of Ag^+, an aliquot of the tested Ag^+ or control samples or Mill-Q water (as blank sample) was added to the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe. The final concentrations of Tb^{3+}, $[G_3T]_5$, and $c[G_3T]_5$ were 2 μM, 3 μM, and 3 μM, respectively. The mixture was vortexed to mix all the reagents and then incubated for 10 min at room temperature, and after that an aliquot of 0.1 mL of the mixture was placed in the black 384 well microplate to measure the luminescence intensity (excited at 290 nm, a delay time of 50 μs, and a gate time of 2 ms).

Assays for Sequence-Specific DNA Using the Tb^{3+}/MB-$[G_3T]_5$ Probe. The Tb^{3+}/MB-$[G_3T]_5$ probe (Tb^{3+} and MB-$[G_3T]_5$ were used) was prepared in a 10 mM Tris-HAc buffer (pH 7.9), and the mixture was incubated for 10 min at room temperature. For the luminescent-enhanced detection of target DNA, an aliquot of the tested DNA or control samples or Mill-Q water (as blank sample) was added to the Tb^{3+}/MB-$[G_3T]_5$ probe. The final concentrations of Tb^{3+} and MB-$[G_3T]_5$ were 2 μM and 3 μM, respectively. The mixture was vortexed to mix all the reagents and then incubated for 60 min at room temperature, and after that an aliquot of 0.1 mL of the mixture was placed in the black 384 well microplate to measure the luminescence intensity (excited at 290 nm, a delay time of 50 μs, and a gate time of 2 ms).

Data Analysis. The GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA) was used to perform the data processing. Each sample was repeated in triplicate, and data were averaged.

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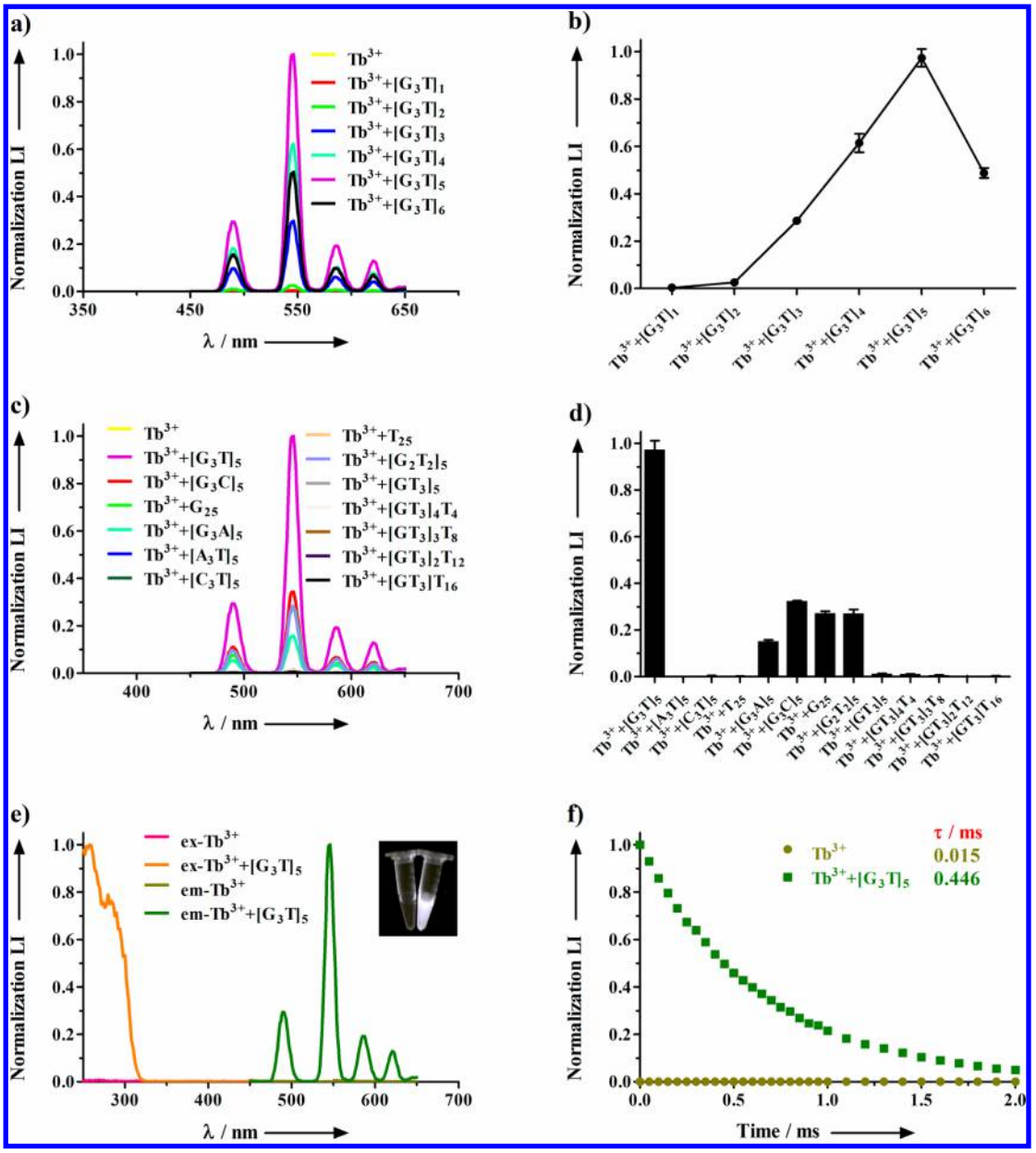

Figure 1. (a) Luminescence responses of 2 μM Tb^{3+} in the presence of a group of 3 μM guanine/thymine (GT)-rich DNA oligos (5′-$[G_3T]_n$-3′, n = 1−6). (b) Plot of luminescence responses of 2 μM Tb^{3+} in the presence of 3 μM $[G_3T]_1$, $[G_3T]_2$, $[G_3T]_3$, $[G_3T]_4$, $[G_3T]_5$, and $[G_3T]_6$, using the luminescence enhancement at 546 nm to monitor the interaction. (c) Luminescence responses of 2 μM Tb^{3+} in the presence of a group of 3 μM $[G_3T]_5$, $[G_3C]_5$, G_{25}, $[G_3A]_5$, $[A_3T]_5$, $[C_3T]_5$, T_{25}, $[G_2T_2]_5$, $[GT_3]_5$, $[GT_3]_4T_4$, $[GT_3]_3T_8$, $[GT_3]_2T_{12}$,and $[GT_3]T_{16}$. (d) Bars represent luminescence responses of 2 μM Tb^{3+} in the presence of 3 μM $[G_3T]_5$, $[A_3T]_5$, $[C_3T]_5$, T_{25}, $[G_3A]_5$, $[G_3C]_5$, G_{25}, $[G_2T_2]_5$, $[GT_3]_5$, $[GT_3]_4T_4$, $[GT_3]_3T_8$, $[GT_3]_2T_{12}$, and $[GT_3]T_{16}$ using the luminescence enhancement at 546 nm to monitor the interaction. (e) Excitation and emission spectra of a 2 μM Tb^{3+} solution in the absence and presence of 3 μM $[G_3T]_5$ and corresponding emission under a common FR-200A instrument with 365 nm UV lamp (inset). (f) Luminescence lifetime of 2 μM Tb^{3+} in the absence and presence of 3 μM $[G_3T]_5$ in aqueous solution.

RESULTS AND DISCUSSION

Guanine/Thymine-Rich DNA-Sensitized Luminescence of Tb^{3+} (GTSLT). It is reported that the natural nucleobases of DNA and RNA are not optimal for sensitization of most Ln^{3+}s;[17] however, Tb^{3+} luminescence can be sensitized in a base sequence and structure dependent manner.[14a,18] What's more, ssDNA can greatly enhance the Tb^{3+} emission, but dsDNA does not.[14a] Topal et al. showed that Tb^{3+} could be sensitized by DNA oligos with especially efficient sensitization by guanine (G)-rich sequences.[19] Guanine can greatly enhance the Tb^{3+} emission because its triplet energy state overlaps with the resonance energy levels of Tb^{3+}.[20] Morever, quadruplex-forming DNA bearing human telomeric repeat sequence 5′-$G_3(T_2AG_3)_3$-3′ (htel21) has been reported for Tb^{3+} luminescence enhancement;[21] thus the DNA with a specific sequence or conformation, such as G-quadruplexes, may be an important factor.[22] On the basis of the above facts, in this work, we prepared a series of DNA oligos with various sequences (Table S1), aiming to investigate an optimum DNA ligand for effective sensitizing of the luminescence of Tb^{3+}. First, a group of guanine/thymine (GT)-rich DNA oligos (5′-$[G_3T]_n$-3′, n = 1∼6) was incubated with Tb^{3+}, and there were significantly different sensitizing responses of the luminescence of Tb^{3+} by such GT-rich DNA oligos, following the order $[G_3T]_5$ >

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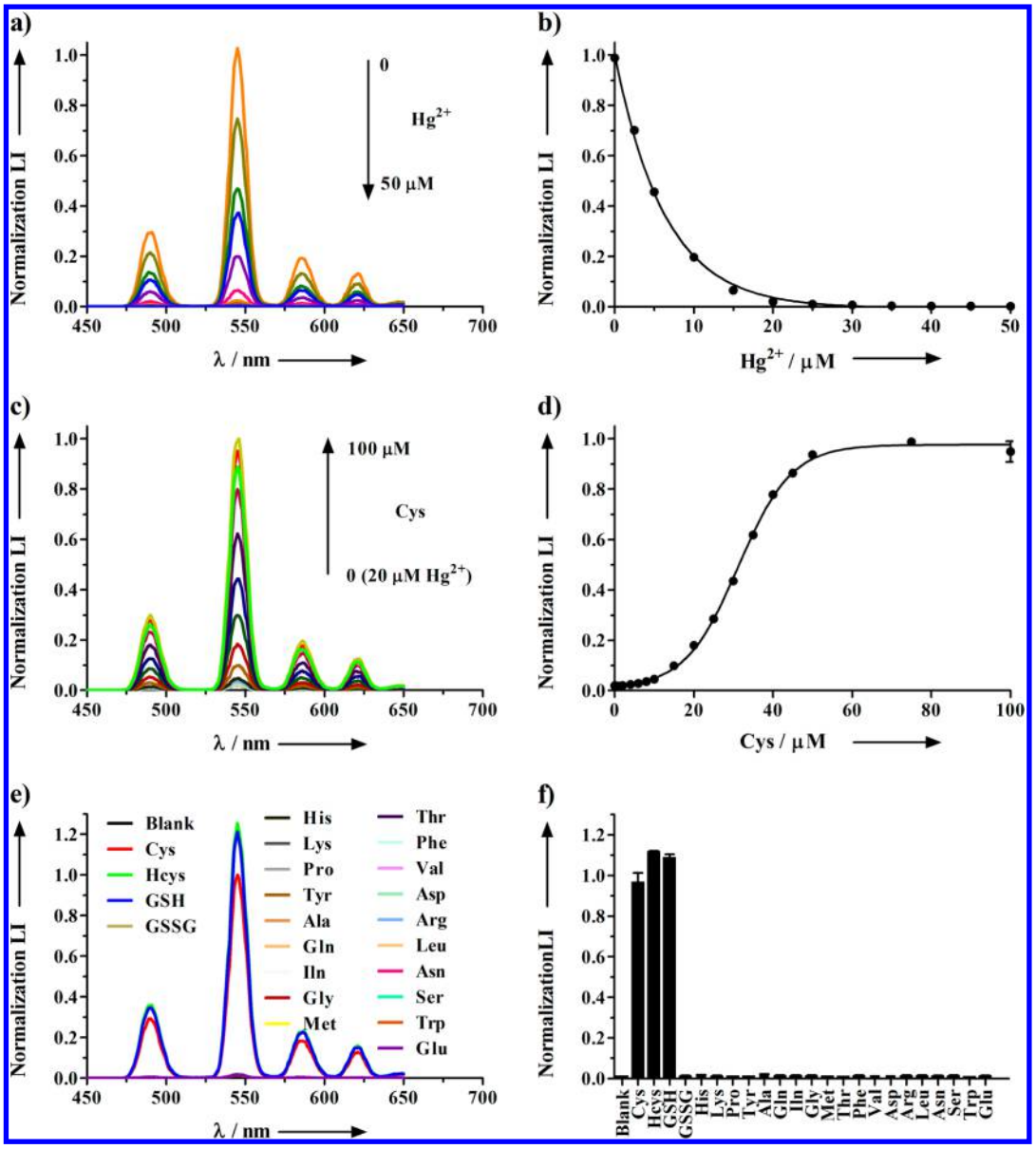

Figure 2. (a) Luminescence responses of $Tb^{3+}/[G_3T]_5$ solution to Hg^{2+}. The luminescence emission spectra are shown for various Hg^{2+} concentrations of 0, 2.5, 5, 10, 20, 30, 40, and 50 μM. (b) Plot of luminescence responses of $Tb^{3+}/[G_3T]_5$ solution to the various concentrations of Hg^{2+} indicated. (c) Luminescence responses of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe to Cys. The luminescence emission spectra are shown for various Cys concentrations of 0, 0.1, 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, and 100 μM. (d) Plot of luminescence responses of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe to the various concentrations of Cys indicated. (e) Luminescence responses of $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe to biothiols (Cys, GSH, and Hcys), various amino acids, and GSSH. (f) Bars represent the luminescence responses of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe to 40 μM biothiols (Cys, GSH, and Hcys), 100 μM various amino acids, and GSSH, using the luminescence enhancement at 546 nm to monitor the responses.

$[G_3T]_4 > [G_3T]_6 > [G_3T]_3 > [G_3T]_2 > [G_3T]_1$ (Figure 1a and b). $[G_3T]_5$ improved the luminescence of Tb^{3+} by 3 orders of magnitude due to energy transfer from nucleic acids to Tb^{3+}. Thus, the $[G_3T]_5$ displays the best quality for effective sensitizing of the luminescence of Tb^{3+}. We further evaluated whether G/T bases of $[G_3T]_5$ are necessary for its effective sensitizing capacity to the luminescence of Tb^{3+}. In Figure 1c and d, all of the G-containing DNA oligos can sensitize the luminescence of Tb^{3+}, while, it should be noted, the G/T-containing DNA oligo ($[G_3T]_5$) works with especially efficient sensitization, following the order $[G_3T]_5 > [G_3C]_5 > [G_2T_2]_5 > G_{25} > [G_3A]_5 > [GT_3]_5 > [GT_3]_4T_4 > [GT_3]_3T_8 > [GT_3]_2T_{12} > [GT_3]T_{16} > [C_3T]_5 > T_{25} > [A_3T]_5$ (Figure 1d). Tb^{3+} solution showed a very weak luminescence emission under excitation with 290 nm light (Figure 1e); however, in the presence of $[G_3T]_5$, the luminescence intensity of Tb^{3+} was increased significantly (approximately 2000 times), which is significantly higher than the reported htel21-sensitizated luminescence of Tb^{3+} under the same conditions (Figure S1). It is also worthy of mention that $[G_3T]_5$-sensitized luminescence of Tb^{3+} (GTSLT) can even be observed by naked eyes under a common FR-200A instrument with a 356 nm UV lamp (inset of Figure 1e). The four emission peaks at 491, 546, 585, and 620 nm are typically the characteristic peaks of Tb^{3+}. The luminescence intensity of Ln^{3+} depends on the efficiency of energy transfer from the ligand to Ln^{3+}. If there is efficient intermolecular energy transfer, Ln^{3+} can be excited more effectively, resulting in an enhanced luminescence of Ln^{3+}.[23]

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The results indicate that an optimum DNA ligand ($[G_3T]_5$) was identified in this work for effective sensitizing of the luminescence of Tb^{3+} via efficient intermolecular energy transfer from the $[G_3T]_5$ ligand to Tb^{3+}. We also investigated the luminescence lifetime of Tb^{3+} in the absence and presence of $[G_3T]_5$. Figure 1f shows that the emission lifetime of Tb^{3+} increased from 0.015 to 0.446 ms in the presence of $[G_3T]_5$. This fits in with that longer emission lifetimes of Ln^{3+} can be realized with a decrease in the rate constant for nonradiative deactivation through O−H vibration of coordinated H_2O molecules[14a] and with a reduction in energy loss of the Tb^{3+} complexes.[24] The stoichiometry of binding of Tb^{3+} to $[G_3T]_5$ was determined by titrating $[G_3T]_5$ with 2 μM Tb^{3+}, using the luminescence enhancement at 546 nm to monitor the interaction. Figure S2 shows that 2 μM Tb^{3+} fully binds to about 3 μM $[G_3T]_5$.

Assays for Biothiols Using the $Tb^{3+}/[G_3T]_5$-Hg^{2+} Probe. As mentioned above, we presented a strategy to highly sensitize the luminescence of Tb^{3+} using the G/T-rich DNA ($[G_3T]_5$) as an antenna ligand. It is reported that nucleic acids can interact with metal ions through nucleobases and the phosphodiester backbone.[25] It is well-known that Hg^{2+} can specifically interact with thymine bases to form strong and stable thymine−Hg^{2+}−thymine complexes (T−Hg^{2+}−T).[26] In this work, the luminescence response investigations of $Tb^{3+}/[G_3T]_5$ to various metal ions indicate that the luminescence of $Tb^{3+}/[G_3T]_5$ can be quenched by some metal ions, such as Hg^{2+}, which may result from the interaction between $[G_3T]_5$ with these metal ions that weakens the sensitizing responses of luminescence of Tb^{3+} by $[G_3T]_5$ (Figure S3a). Furthermore, we also tested the luminescence responses of $Tb^{3+}/[G_3T]_5$ with metal ions toward cysteine (Cys). It was found that Cys captures Hg^{2+} through the interaction between thiol groups and Hg^{2+}, which recovers the luminescence of $Tb^{3+}/[G_3T]_5$ that was initially quenched by Hg^{2+} (Figure S3b). The results demonstrated that the solution of $Tb^{3+}/[G_3T]_5$ containing Hg^{2+} ($Tb^{3+}/[G_3T]_5$-Hg^{2+} probe) can potentially be used for "turn on" detection of Cys. The determination of Cys is significant because Cys is an important precursor in the pharmaceutical, food, and personal care industries.[27]

To demonstrate the feasibility of our proposed approach, the experiments begin with the use of Hg^{2+} to quench the luminescence of $Tb^{3+}/[G_3T]_5$, to construct the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe for Cys sensing. Figure 2a illustrates how the addition of increasing concentrations of Hg^{2+} in the range of 0 to 50 μM results in the gradual luminescent quenching of $Tb^{3+}/[G_3T]_5$ solution. Also, the change of the quenched luminescence intensity was sensitive to Hg^{2+} ions in a concentration-dependent manner, and we observed an obvious decrease of luminescence at the point of 20 μM (Figure 2b). The luminescence quenching of $Tb^{3+}/[G_3T]_5$ by Hg^{2+} is fast, which can be finished within 10 min (Figure S4). Then, we further investigated the feasibility of the quantitative detection of Cys on the basis of the above $Tb^{3+}/[G_3T]_5$ solution containing Hg^{2+}. A $Tb^{3+}/[G_3T]_5$ solution containing 20 μM Hg^{2+} was prepared, i.e.. the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe. The $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe was weakly emissive in aqueous buffered solution. However, after adding Cys with increasing concentrations, a gradual luminescence increase in the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe was observed (Figure 2c). The sensing behavior of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} toward Cys may be due to the presence of a thiol-containing compound, which can effectively absorb Hg^{2+} by the formation of the strong Hg^{2+}−S bond. From Figure 2d, it can be seen that the luminescence enhancement of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe is sensitive to the concentration of Cys, where the fitting range is from 0 to 100 μM with a Boltzmann sigmoidal equation. The limit of detection of Cys based on 3σ was approximately 0.5 μM. The kinetics luminescence increase of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe toward Cys was monitored, and it can be finished within 10 min (Figure S5). Apart from Cys, we also utilized the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe to monitor other biothiols including GSH and Hcys (Figure S6). The specific response of the probe to biothiols (Cys, GSH, and Hcys) was evaluated by examining the luminescence signal of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe in the presence of various amino acids and GSSH under the same conditions. As shown in Figure 2e and f, only the addition of biothiols (Cys, GSH, and Hcys) could induce a prominent increase in the luminescence emission of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe, whereas the addition of a 2.5-fold 19 other amino acids and GSSH caused only very slight luminescence changes. Thus, a highly sensitive time-resolved luminescence detection method for biothiols was developed by using $Tb^{3+}/[G_3T]_5$-Hg^{2+} as a luminescent probe. In order to test the feasibility of our proposed method in real samples, we studied the possible applicability of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe for the direct measuring of biothiols in urine and serum. Urine is a variable fluid, both between individuals and in the same individual over time; thus we prepared a stable artificial urine according to the reported literature, which is a suitable replacement for normal urine for use in a wide range of experiments.[16] Recovery of an added known amount of Cys to the artificial urine and serum samples was in general larger than 95% (Table S2), which indicated that the present method has promise in practical application with great accuracy and reliability.

Compared with other probes for biothiols (Table S3), our proposed label-free probe not only has comparable detection limits, a low cost of detection, and an easy preparation of the probe but also does not require complex chemical modifications. More importantly, the background autoluminescence, often present in biological samples, can be efficiently removed via the time-resolved luminescence technique.

Assays for Ag^+ Using the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ Probe. Silver compounds play vital roles in daily life and pharmaceutical applications; however, their extensive use will cause serious environmental pollution issues.[28] Additionally, Ag^+ is considered to be one of the heavy metal ions that can cause environmental pollution for water resources.[29] Therefore, it is necessary to develop new techniques and methods to monitor Ag^+ with rapidity and selectivity. Ag^+ can specifically interact with cytosine bases to form strong and stable cytosine−Ag^+−cytosine complexes (C−Ag^+−C).[30] Heretofore, various methods for Ag^+ detection have been developed by our group and other researchers according to DNA-Ag^+ base pair complexes.[1c,26a,b,30] Our previous work has revealed that a designed C-base containing ssDNA can undergo a conformational alteration due to the formation of C−Ag^+−C complexes in the presence of Ag^+.[1c] In this part, we describe our ongoing effort to develop a facile and label-free luminescence assay for Ag^+ based on competition strategy, in which $Tb^{3+}/[G_3T]_5$ complexes act as a signal indicator and $[G_3T]_5/c[G_3T]_5$ duplex as a target-responsive element. We introduced a C-rich oligonucleotide $c[G_3T]_5$, which is complementary to $[G_3T]_5$. And the resultant $[G_3T]_5/c[G_3T]_5$ duplex does not sensitize the luminescence of Tb^{3+} (Figure S7). This result is due to the fact that dsDNA cannot enhance the Tb^{3+} emission.[14a]

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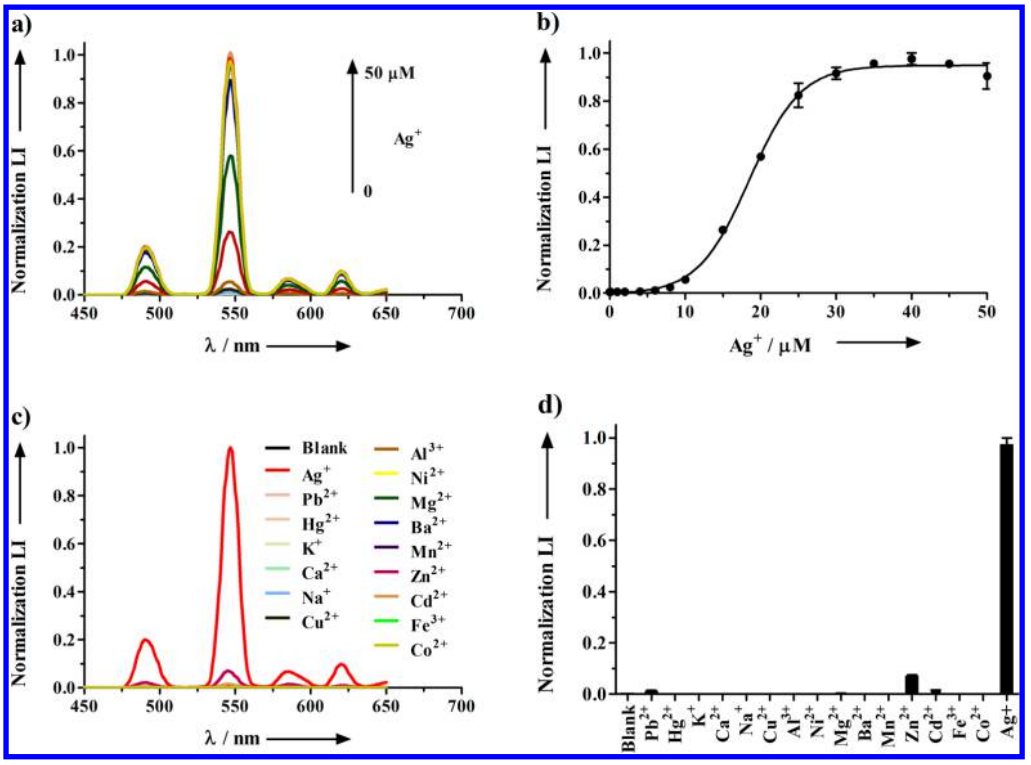

Figure 3. (a) Luminescence responses of the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe to Ag^+. The luminescence emission spectra are shown for various Ag^+ concentrations of 0, 0.01, 0.1, 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, and 50 μM. (b) Plot of luminescence responses of the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe to the various concentrations of Ag^+ indicated. (c) Luminescence responses of the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe to various cations (Pb^{2+}, Hg^{2+}, K^+, Ca^{2+}, Na^+, Cu^{2+}, Al^{3+}, Ni^{2+}, Mg^{2+}, Ba^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Fe^{3+}, Co^{2+}, and Ag^+). (d) Bars represent the luminescence responses of the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe to various cations, using the luminescence enhancement at 546 nm to monitor the responses.

However, in the presence of Ag^+, the as-prepared $[G_3T]_5/c[G_3T]_5$ duplex can be split by the formation of C–Ag^+–C complexes in $c[G_3T]_5$, and the released $[G_3T]_5$ acts as an antenna ligand for sensitizing the luminescence of Tb^{3+} (Scheme 1b). As depicted in Figure 3a, with the addition of an increasing concentration of Ag^+ to the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe, an obvious increase in the luminescence of Tb^{3+} was clearly detected. The sensitivity of the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe for Ag^+ detection was investigated. From Figure 3b, it can be seen that the increasing luminescence of Tb^{3+} is sensitive to the concentration of Ag^+, where the fitting range is from 0 to 50 μM with a Boltzmann sigmoidal equation. The limit of detection of Ag^+ based on 3σ was approximately 0.01 μM. The detection of Ag^+ using the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe is rapid and can be finished within 6 min (Figure S8). To test selectivity, competing stimuli including 15 100 μM cations were examined under the same conditions as in the case of 30 μM Ag^+ (Figure 3). It was found that Ag^+ results in an obvious change in the luminescence of Tb^{3+}, while there was a nearly negligible luminescence change in the presence of other stimuli. The results demonstrated the excellent selectivity of this approach applied in Ag^+ detection over cations (Figure 3c and d).

In order to examine the feasibility of our proposed $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe in real samples, we studied the possible applicability of the $Tb^{3+}/[G_3T]_5/c[G_3T]_5$ probe for the direct measuring of Ag^+ in real water samples. Recovery of an added known amount of Ag^+ to the real water samples was in general larger than 95% (Table S4), which indicated that the present method has promise in practical applications with great accuracy and reliability. A comparison between the current method and other reported methods was also made (Table S5). Our proposed method is one of the most sensitive assays for the environmental monitoring of Ag^+ with a background-free format.

Assays for Sequence-Specific DNA Using the Tb^{3+}/MB-$[G_3T]_5$ Probe. The development of rapid and simple methods for determining specific DNA sequences is of great importance in mutation identification, clinical diagnostics, forensic analysis, and other areas.[31] The search for a selective, sensitive, and low toxicity DNA probe is an active field of research. Molecular beacons (MBs) are dually labeled ssDNA that are internally quenched due to the neighborhood between a fluorophore and a quencher tagged at either end.[32] Traditional MBs have been used in various fields, but they have some limitations, such as a high cost, difficulty in selection of a dye-quencher pair in certain cases, and time-consuming dual labeling or chemical modification.[33] Therefore, it is highly desirable to exploit label-free or label-easy and simple MB-like probes.[34] In this part, we proposed a label-free MB-like probe for DNA sensing based on the luminescence of Tb^{3+}, in which a designed hairpin oligonucleotide (MB-$[G_3T]_5$) was used as a sensing element. As shown in Scheme 1c, the MB-$[G_3T]_5$ involves three regions, the loop region sequence complementary to the target DNA of interest, the $[G_3T]_5$ sequence for sensitizing the luminescence of Tb^{3+}, and the partial part of $c[G_3T]_5$ complementary to the $[G_3T]_5$ sequence. Recognition of target DNA via hybridization to MB-$[G_3T]_5$ can unfold the MB-$[G_3T]_5$ to release the $[G_3T]_5$ sequence for sensitizing the luminescence of Tb^{3+}, producing a detectable enhanced signal directly proportional to the amount of target DNA of interest (Scheme 1c). Herein, the BRCA1 breast cancer gene (BRCA1)

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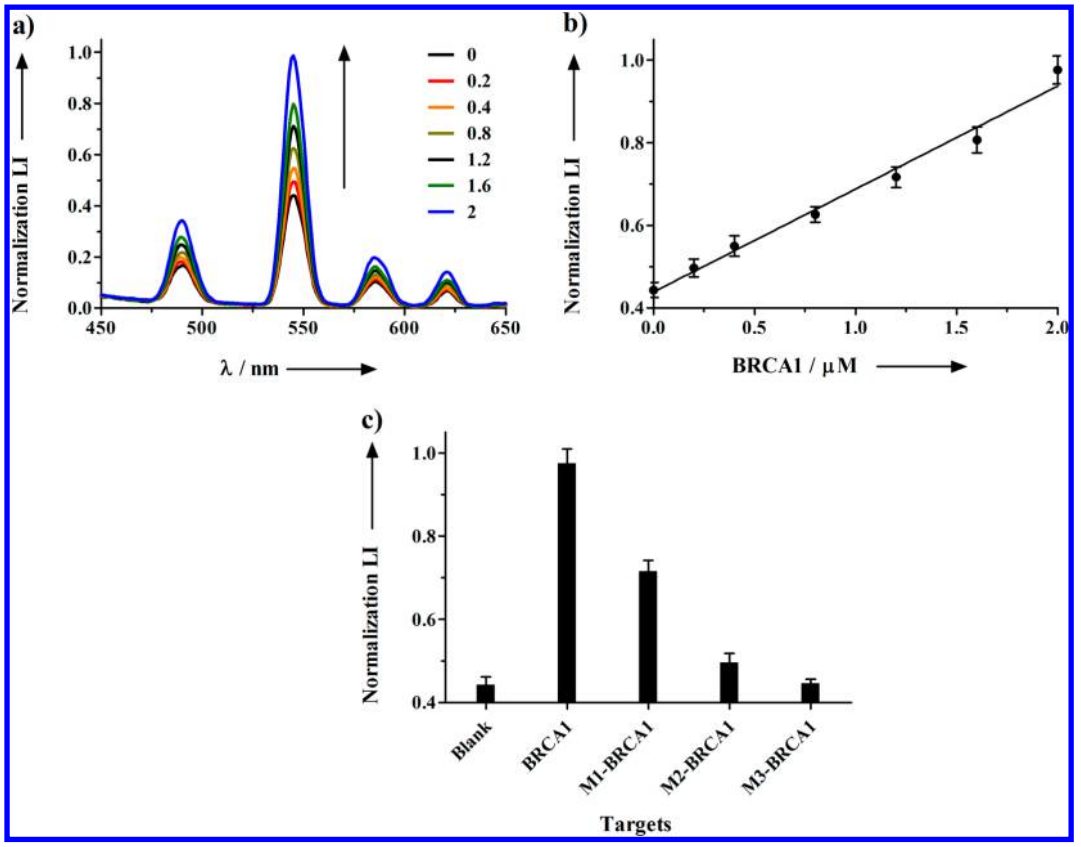

Figure 4. (a) Luminescence responses of the Tb^{3+}/MB-$[G_3T]_5$ probe to target DNA (BRCA1). The luminescence emission spectra are shown for various BRCA1 concentrations of 0, 0.2, 0.4, 0.8, 1.2, 1.6, and 2 μM. (b) Plot of luminescence responses of the Tb^{3+}/MB-$[G_3T]_5$ probe to the various concentrations of BRCA1 indicated. (c) Bars represent luminescence responses of the Tb^{3+}/MB-$[G_3T]_5$ probe in the presence of different DNA inputs including perfect-matched (BRCA1), single-base mismatched (M1-BRCA1), two-base mismatched (M2-BRCA1), and three-base mismatched strands (M3-BRCA1), using the luminescence enhancement at 546 nm to monitor the responses.

is chosen as a model target, because its mutations may cause breast cancer.[35] To experimentally explore the potential of the proposed method for quantitative detection of target DNA, various concentrations of BRCA1 (0, 0.2, 0.4, 0.6, 1.2, 1.6, and 2 μM)were challenged by the Tb^{3+}/MB-$[G_3T]_5$ probe (Figure 4). As shown in Figure 4a, an obvious enhancement in the luminescence of Tb^{3+} was clearly detected with the addition of an increasing concentration of BRCA1 to the Tb^{3+}/MB-$[G_3T]_5$ probe. The sensitivity of the Tb^{3+}/MB-$[G_3T]_5$ probe for BRCA1 detection was investigated. From Figure 4b, it can be seen that the enhancing luminescence of Tb^{3+} is sensitive to the concentration of BRCA1, where the fitting range is from 0 to 2 μM with a linear equation. The limit of detection of BRCA1 based on 3σ was approximately 0.05 μM. The target-induced enhancing luminescence of Tb^{3+} can be finished within 60 min (Figure S9). In order to evaluate the feasibility of our proposed method in real samples, we studied the possible applicability of the Tb^{3+}/MB-$[G_3T]_5$ probe for the direct measuring of target DNA in urine and serum. Recovery of an added known amount of target DNA to the artificial urine and serum samples was in general larger than 95% (Table S6), which indicated that the present method has promise in practical applications with great accuracy and reliability. We also made a comparison between the current method and other reported methods (Table S7).

To investigate the sequence discrimination ability of the Tb^{3+}/MB-$[G_3T]_5$ probe, we prepared four different target DNA oligos: BRCA1 (perfectly match), M1-BRCA1 (single-base mismatch), M2-BRCA1 (two-base mismatch), and M3-BRCA1 (three-base mismatch). As shown in Figure 4c, the enhancing luminescences of Tb^{3+} for BRCA1, M1-BRCA1, M2-BRCA1, and M3-BRCA1 under the same conditions are appreciably discriminated using the Tb^{3+}/MB-$[G_3T]_5$ probe, demonstrating that the Tb^{3+}/MB-$[G_3T]_5$ probe can provide a powerful alternative to specifically detect target DNA and discriminate single-base mismatches with specific responses. The identification of single-base mismatches plays a vital role in single nucleotide polymorphism (SNP) genotyping and routine screening of genetic mutations and diseases. The results demonstrated that the Tb^{3+}/MB-$[G_3T]_5$ probe provides great potential for the quantification of nucleic acid as well as the detection of point mutation.

■ CONCLUSIONS

In this work, we first identified an optimum DNA antenna ligand ($[G_3T]_5$) for effective sensitizing of the luminescence of Tb^{3+}, and $[G_3T]_5$ improved the luminescence of Tb^{3+} by 3 orders of magnitude due to energy transfer from nucleic acids to Tb^{3+} (i.e., antenna effect). The long luminescence lifetime of the $[G_3T]_5$-sensitized Tb^{3+} probes readily enables time-resolving experiments. Label-free probes for the luminescent detection of biothiols, Ag^+, and sequence-specific DNA in an inexpensive, simple, and mix-and-read format are presented based on the guanine/thymine-rich DNA-sensitized luminescence of Tb^{3+} (GTSLT). Hg^{2+} can efficiently quench the luminescence of Tb^{3+} sensitized by $[G_3T]_5$ (Tb^{3+}/$[G_3T]_5$); however, biothiols are readily applicable to selectively grab

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Hg^{2+} for the restoration of luminescence of $Tb^{3+}/[G_3T]_5$, which can be used for "turn on" detection of biothiols. We further introduced a C-rich oligonucleotide $c[G_3T]_5$, which is complementary to $[G_3T]_5$, and the resultant $[G_3T]_5/c[G_3T]_5$ duplex does not sensitize the luminescence of Tb^{3+}. However, in the presence of Ag^+, the as-prepared $[G_3T]_5/c[G_3T]_5$ duplex can be split by the formation of $C-Ag^+-C$ complexes in $c[G_3T]_5$, and the released $[G_3T]_5$ acts as an antenna ligand for sensitizing the luminescence of Tb^{3+}, which can be applied to detect Ag^+ in a "turn on" format. Moreover, we proposed a label-free MB-like probe for DNA sensing based on the luminescence of Tb^{3+}, in which a designed hairpin oligonucleotide (MB-$[G_3T]_5$) is used as a sensing element. Recognition of target DNA via hybridization to MB-$[G_3T]_5$ can unfold the MB-$[G_3T]_5$ to release the $[G_3T]_5$ sequence for sensitizing the luminescence of Tb^{3+}, producing a detectable enhanced signal directly proportional to the amount of target DNA of interest. This allows the development of a fascinating label-free MB probe for DNA sensing based on the luminescence of Tb^{3+}. All in all, all of these proposed approaches need fewer types of reagents and simple rapid operation, and only label-free oligonucleotides are used during the whole procedure. This is a new concept for a label-free assay based on guanine/thymine-rich DNA-sensitized luminescence of Tb^{3+} and opens new opportunities for the design of more novel sensing strategies and expansion of its application in different fields. We believe this work will inspire the development of multifunctional Tb^{3+}-based biosensing platforms by sensitizing Tb^{3+} with many other biomolecular ligands.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Supporting Information

Time-Resolved Probes Based on Guanine/Thymine-Rich DNA-Sensitized Luminescence of Terbium (III)

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Table S1. Sequences of the Oligonucleotides.

Name	Sequence (5‘ to 3‘)
htel21	GGGTTAGGGTTAGGGTTAGGG
$[G_3T]_1$	GGGT
$[G_3T]_2$	GGGTGGGT
$[G_3T]_3$	GGGTGGGTGGGT
$[G_3T]_4$	GGGTGGGTGGGTGGGT
$[G_3T]_5$	GGGTGGGTGGGTGGGTGGGT
$[G_3T]_6$	GGGTGGGTGGGTGGGTGGGTGGGT
$[A_3T]_5$	AAATAAATAAATAAATAAAT
$[C_3T]_5$	CCCTCCCTCCCTCCCTCCCT
$[G_3A]_5$	GGGAGGGAGGGAGGGAGGGA
$[G_3C]_5$	GGGCGGGCGGGCGGGCGGGC
G_{25}	GGGGGGGGGGGGGGGGGGGGG
$[G_2T_2]_5$	GGTTGGTTGGTTGGTTGGTT
$[GT_3]_5$	GTTTGTTTGTTTGTTTGTTT
$[GT_3]_4T_4$	GTTTGTTTGTTTGTTTTTTT
$[GT_3]_3T_8$	GTTTGTTTGTTTTTTTTTTT
$[GT_3]_2T_{12}$	GTTTGTTTTTTTTTTTTTTT
$[GT_3]T_{16}$	GTTTTTTTTTTTTTTTTTTT
T_{25}	TTTTTTTTTTTTTTTTTTTTT
c$[G_3T]_5$	ACCCACCCACCCACCCACCC
MB-$[G_3T]_5$	GGGTGGGTGGGTGGGTGGGTGATTTTCTTCCTTTTGTTCACCCACCC
BRCA1	GAACAAAAGGAAGAAAATC
BRCA1-M1	GAACAAAAG<u>C</u>AAGAAAATC
BRCA1-M2	GAAC<u>T</u>AAAG<u>C</u>AAGAAAATC
BRCA1-M3	GAAC<u>T</u>AAAG<u>C</u>AAGA<u>C</u>AATC

Note: the mutation base is indicated in underlined portion.

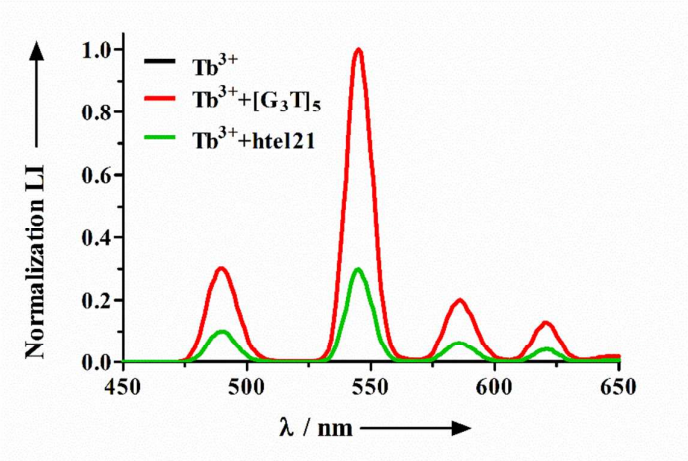

Figure S1 A comparison of our screened DNA ligand ($[G_3T]_5$) and reported ligand (htel21) for the sensitizing responses of luminescence of Tb^{3+} in the same condition.

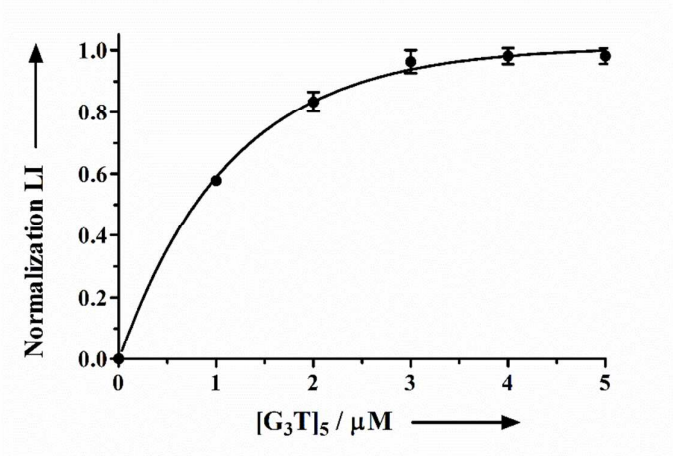

Figure S2 Investigation of stoichiometry of binding of Tb^{3+} to $[G_3T]_5$, determined by titrating various concentrations of $[G_3T]_5$ with 2 μM Tb^{3+}.

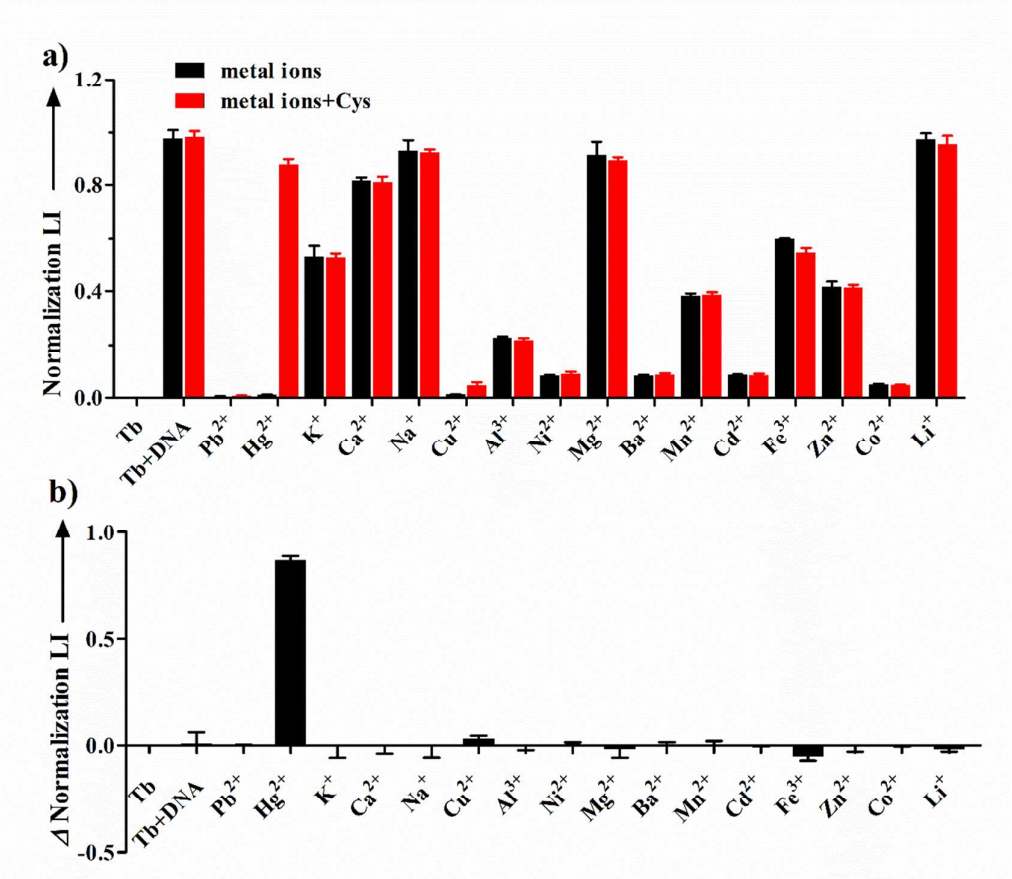

Figure S3 a) The luminescence response investigations of $Tb^{3+}/[G_3T]_5$ to various metal ions and the luminescence responses of $Tb^{3+}/[G_3T]_5$ with those metal ions towards cysteine (Cys); b) Bars represent the luminescence responses of $Tb^{3+}/[G_3T]_5$ to various metal ions subtracted from the luminescence responses of $Tb^{3+}/[G_3T]_5$ with those metal ions towards Cys.

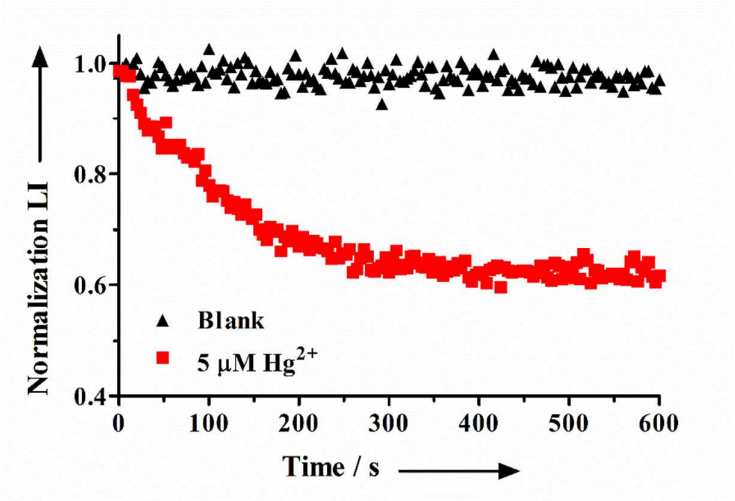

Figure S4 Kinetics investigation of luminescence response of $Tb^{3+}/[G_3T]_5$ to Hg^{2+}.

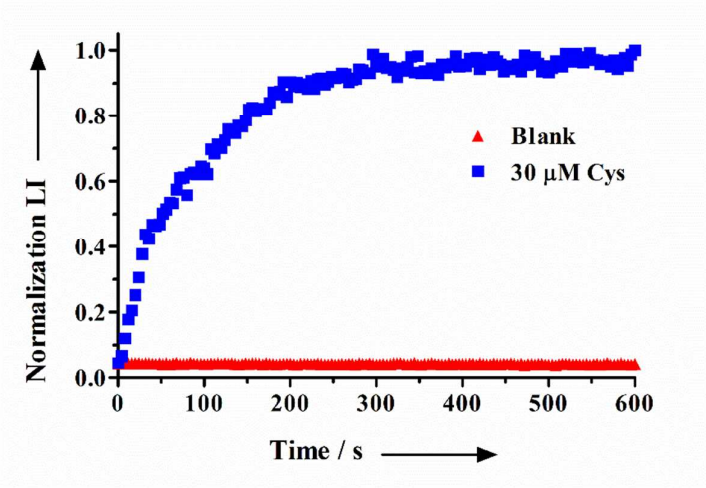

Figure S5 Kinetics investigation of luminescence response of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe to Cys. 20 μM Hg^{2+} was used.

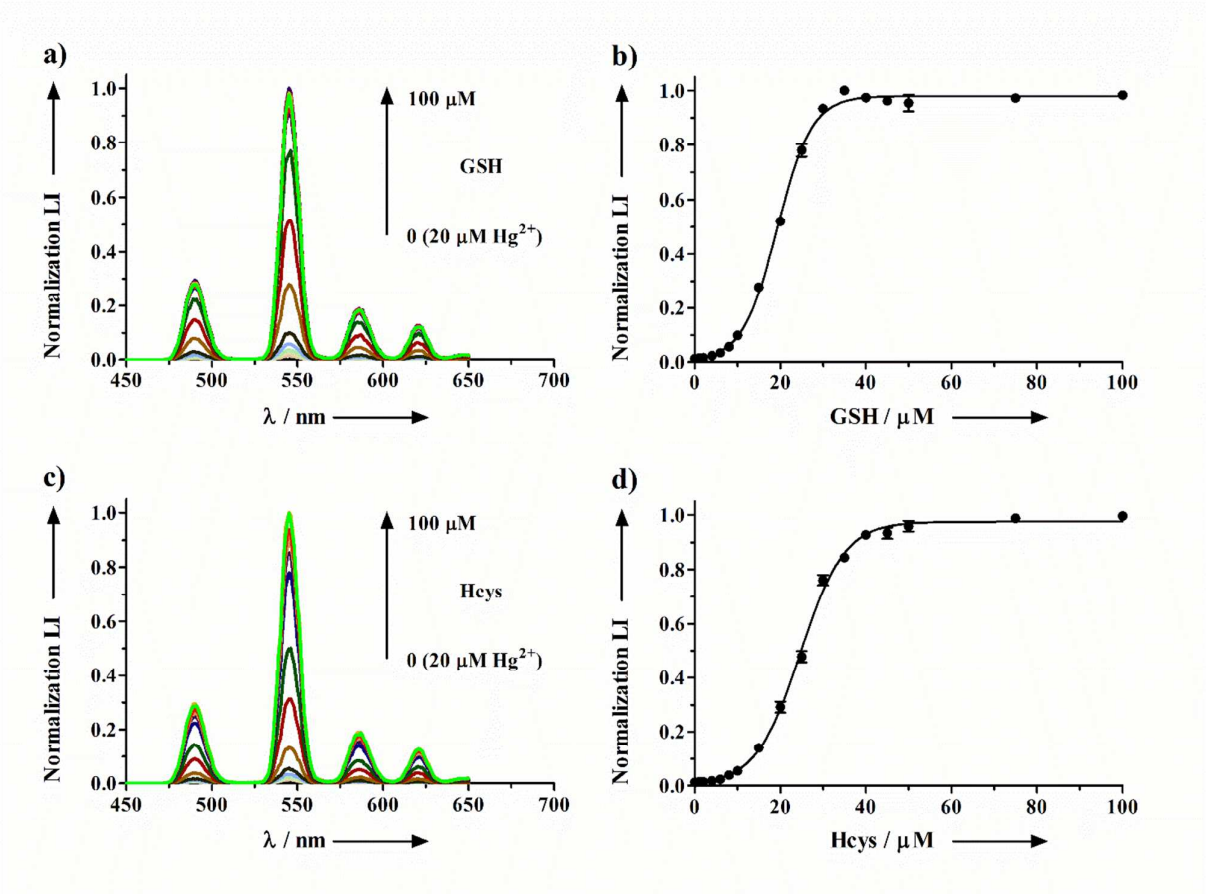

Figure S6 (a) Luminescence responses of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe to GSH. The luminescence emission spectra are shown for various GSH concentrations of 0, 0.1, 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75 and 100 μM; (b) Plot of luminescence responses of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe to the various concentrations of GSH indicated; (c) Luminescence responses of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe to Hcys. The luminescence emission spectra are shown for various Hcys concentrations of 0, 0.1, 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75 and 100 μM; (d) Plot of luminescence responses of the $Tb^{3+}/[G_3T]_5$-Hg^{2+} probe to the various concentrations of Hcys indicated.

Table S2. Determination of Cys in real samples using the Tb^{3+}/$[G_3T]_5$-Hg^{2+} probe.

*Sample	**Detected (μM)	Added (μM)	Found (μM)	Recovery (%)
Urine (2%)	Not found	28.00	28.13	100.47
		33.00	32.93	99.80
Serum (2%)	Not found	28.00	28.15	100.52
		33.00	32.90	99.71

*High concentration of urine and serum is unsuitable for the presented sensor to follow the spiked amount of Cys; **Before spiking Cys in real samples, no luminescence response of the Tb^{3+}/$[G_3T]_5$-Hg^{2+} probe was observed in the real samples, indicating Cys in these real samples was not detected (below the limit of detection of concentration in the proposed method). The final concentration of Tb^{3+}, $[G_3T]_5$ and Hg^{2+} was 4 μM, 12 μM and 20 μM, respectively.

Table S3. A comparison of probes for the detection of biothiols.

Probes	Detection limit (μM)			Chemical modification	Real samples	Background removal	Refs
	Cys	GSH	Hcys				
Fluorescein derivative	0.05	0.053	0.1	No	No	No	1
CdTe/CdSe Quantum dots	0.131	0.02	0.026	Yes	Urine and plasma	Yes	2
Terbium chelate	0.4	0.08	0.1	No	Urine and plasma	Yes	3
Proposed method	0.5	0.2	0.34	No	Urine and serum	Yes	

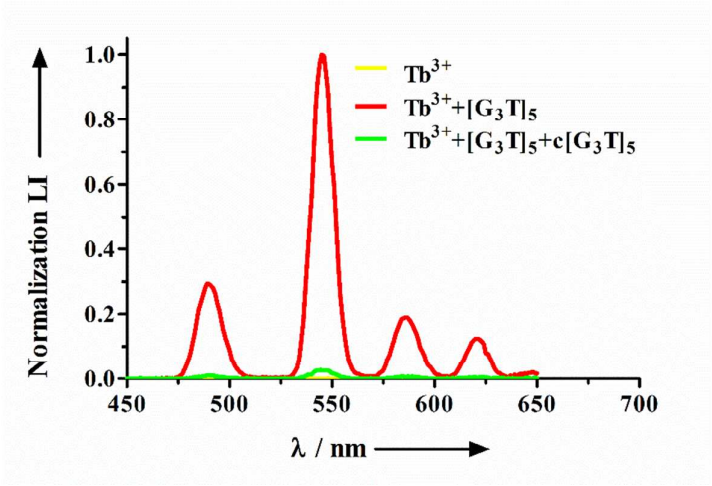

Figure S7 Luminescence responses of Tb^{3+} in the presence of $[G_3T]_5$ and $[G_3T]_5$/c$[G_3T]_5$ duplex ($[G_3T]_5$+c$[G_3T]_5$), respectively.

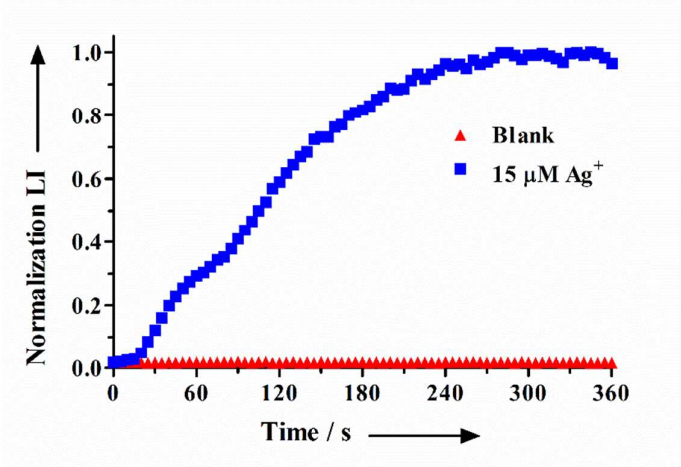

Figure S8 Kinetics investigation of luminescence response of the Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe to Ag^+.

Table S4. Determination of Ag^+ in real water samples using the Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe.

Sample	*Detected (μM)	Added (μM)	Found (μM)	Recovery (%)
Tap water	Not found	18.00	17.92	99.57
		22.00	21.90	99.56
#River water	Not found	18.00	17.81	98.92
		22.00	21.82	99.19

*Before spiking Ag^+ in real samples, no luminescence response of the Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe was observed in the real samples, indicating Ag^+ in these real samples was not detected (below the limit of detection of concentration in the proposed method); #The water sample was collected from the Qin Chun River in the campus of East China University of Science and Technology. The sample was first filtered through a 0.22 μM membrane to remove soil and other particles, and then tested by the proposed method. The final concentration of Tb^{3+}, $[G_3T]_5$ and c$[G_3T]_5$ was 2 μM, 3 μM and 3 μM, respectively.

Table S5. A comparison of probes for the detection of Ag^+.

Probes	Detection limit (μM)	Chemical modification	Real samples	Background removal	Refs
FAM-labeled ssDNA/GO	0.02	Yes	River water	No	4
FITC-labeled ssDNA	0.05	Yes	Tap and river water	No	5
Terbium coordination	0.06	No	Tap and river water	Yes	6
Proposed method	0.01	No	Tap and river water	Yes	

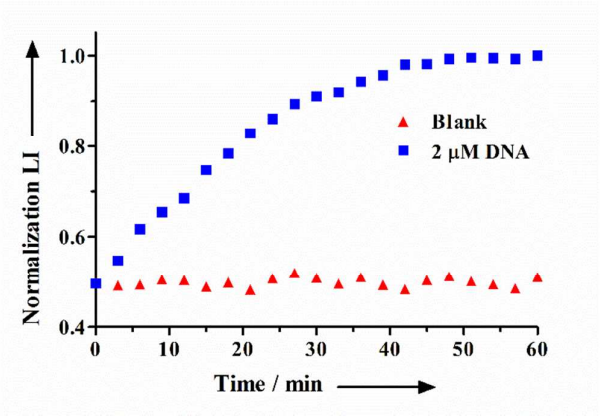

Figure S9 Kinetics investigation of luminescence response of the Tb^{3+}/MB-$[G_3T]_5$ probe to target DNA (BRCA1).

Table S6. Determination of sequence-specific DNA in real samples using the Tb^{3+}/MB-$[G_3T]_5$ probe.

*Sample	**Detected (μM)	Added (μM)	Found (μM)	Recovery (%)
Urine (2%)	Not found	0.50	0.51	101.39
		1.00	1.04	103.97
		1.50	1.49	99.04
Serum (2%)	Not found	0.50	0.51	102.27
		1.00	1.04	104.41
		1.50	1.48	98.52

*High concentration of urine and serum is unsuitable for the presented sensor to follow the spiked amount of sequence-specific DNA; **Before spiking sequence-specific DNA in real samples, no luminescence response of the Tb^{3+}/MB-$[G_3T]_5$ probe was observed in the real samples, indicating sequence-specific DNA in these real samples was not detected (below the limit of detection of concentration in the proposed method). The final concentration of Tb^{3+} and MB-$[G_3T]_5$ was 8 μM and 12 μM, respectively.

Table S7. A comparison of probes for the detection of sequence-specific DNA.

Probes	Detection limit (μM)	Chemical modification	Real samples	Background removal	Refs
DNA-hosted Hoechst Dyes	0.002	No	/	No	7
FITC-labeled ssDNA/GO	0.001	Yes	/	No	8
LFG4-MB	0.0023	No	/	No	9
Proposed method	0.05	No	Urine and serum	Yes	

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(9) D. Hu et al., *Chem. Eur. J.* **2011**, *17*, 1635-1641.