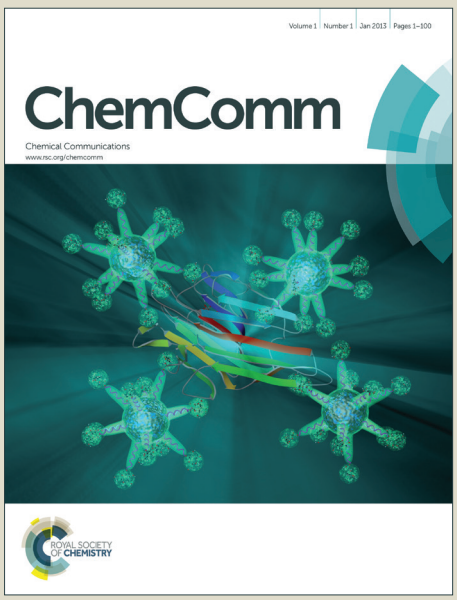
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

RSCPublishing

COMMUNICATION

DNA-based sensitization of Tb^{3+} luminescence regulated by Ag^{+} and cysteine: use as a logic gate and a H_2O_2 sensor

hCite this: DOI: 10.1039/x0xx00000x

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

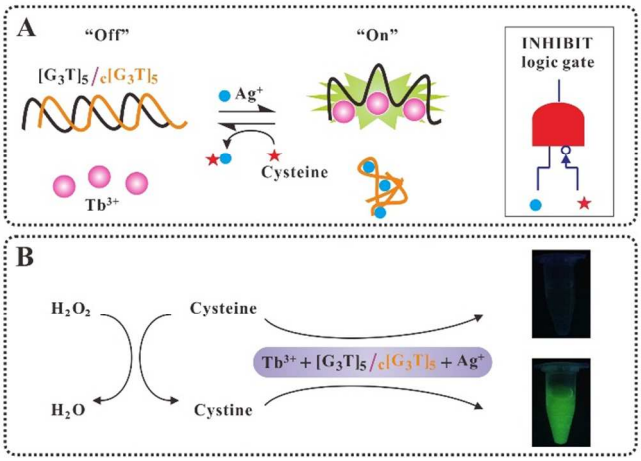
www.rsc.org/

Min Zhang,[a] Zhi-bei Qu,[a] Hai-Yan Ma,[a] Tianshu Zhou,[b] Guoyue Shi*[a]

A simple and facile strategy was developed for regulating the luminescence of Tb^{3+} sensitized by DNA, in which Ag^{+} and cysteine (Cys) act as activators. The Ag^{+}/Cys-mediated reversible luminescence changes in the Tb^{3+}-DNA sensing system enabled the design of a DNA INHIBIT logic gate and a H_2O_2 sensor in a time-resolved luminescence format.

Trivalent lanthanide ions (Ln^{3+}) have remarkable luminescent properties due to their unique 4f orbitals. Ln^{3+} are very poor at absorbing light directly because their f−f transitions are Laporte-forbidden. Typically, direct excitation of Ln^{3+} is difficult to generate efficient luminescence, which can be settled by Ln^{3+} chelated with certain adjacent strongly absorbing ligands in the ultraviolet (UV) region for sensitization of Ln^{3+} luminescence.[1] Upon UV light irradiation, the central Ln^{3+} displays the characteristic luminescence resulting from the efficient intermolecular energy transfer through the excited triplet state of the antenna ligand to the emitting electronic level of Ln^{3+} (the so-called "antenna effect").[2] Among them, ligand-sensitized, luminescent terbium ion (Tb^{3+}) complexes are highly favourable because their unique photophysical capabilities (large Stokes' shifts, sharply spiked emission bands, and long lifetimes) make them well suited as luminescent probes in time-resolved luminescence bioassays.[3] Time-resolved luminescence bioassays could efficiently avoid background interference. There is increasing interest in time-resolved luminescence probes.[4]

Previous studies reveal that single-stranded DNA (ssDNA) can greatly enhance the Tb^{3+} emission, but double-stranded DNA (dsDNA) cannot.[5] On the basis of these facts, in our recent work, an optimum ssDNA antenna ligand ($[G_3T]_5$) was identified 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).[6] Such Tb^{3+}/DNA complexes have been utilized for the development of luminescent probes utilizing both the physicochemical properties of Tb^{3+} and the structure-switching of DNA. Herein, we describe our ongoing efforts to develop a simple and facile strategy for regulating the luminescence of Tb^{3+} sensitized by DNA, in which Ag^{+} and cysteine (Cys) act as activators. Moreover, the proposed Ag^{+}/Cys-mediated reversible luminescence changes in the Tb^{3+}-DNA sensing system enabled the design of a DNA INHIBIT logic gate (Scheme 1A) and a H_2O_2 sensor (Scheme 1B).



Scheme 1. Schematic representation of the DNA-based sensitization of Tb^{3+} luminescence regulated by Ag^{+} and cysteine: use as (A) an INHIBIT logic gate and (B) a H_2O_2 sensor. The photos were taken under a 254 nm UV lamp excitation using a digital camera.

Scheme 1A shows a schematic representation of this novel Ag^{+}/Cys-mediated reversible luminescence changes in the Tb^{3+}-DNA sensing system. Cytosine (C) has been demonstrated to be one of the most specific ligands for Ag^{+}, forming a C−Ag^{+}−C base pair complex with high selectivity and strong affinity.[7] It was found that Cys captures Ag^{+} through the interaction between thiol groups and Ag^{+}, which can disrupt the base pair complex of C−Ag^{+}−C.[7a,b] In this respect, facile and label-free luminescence assays for Ag^{+} and Cys can be constructed based on a 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 ($c[G_3T]_5$: C-rich oligonucleotide complementary to $[G_3T]_5$) (Scheme 1A). In the presence of Ag^{+}, the as-prepared $[G_3T]_5/c[G_3T]_5$ duplex can be opened by the formation of C−Ag^{+}−C base pair complex in $c[G_3T]_5$, and the released $[G_3T]_5$ acts as an antenna ligand for sensitizing the luminescence of Tb^{3+} and leading to the Tb^{3+}

luminescence "On" state. However, by continuing to add Cys, the C−Ag^+−C structure is disrupted by the interaction between thiol groups and Ag^+ and then c$[G_3T]_5$ hybridize with $[G_3T]_5$ anew, switching the system to the Tb^{3+} luminescence "Off" state again.

Figure 1A shows that the presence of increasing concentrations of Ag^+ to the Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe leads to the gradual luminescent enhancement of Tb^{3+}, which owes to the formation of a Ag^+-mediated C−Ag^+−C complex resulting in the split of $[G_3T]_5$/c$[G_3T]_5$ duplex that facilitates the released $[G_3T]_5$ sensitizes the luminescence of Tb^{3+}. Also, the increasing luminescence of Tb^{3+} was sensitive to Ag^+ in a concentration-dependant manner (Figure 1A inset). To validate the selectivity of Ag^+-stimulated luminescent enhancement of the Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe, competing metal ions including 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+} and Co^{2+} were tested under the same conditions as for Ag^+. Figure 1B clearly shows that significant luminescent enhancement was only observed in the case of Ag^+, while there was a nearly negligible luminescence change when adding the competing metal ions.

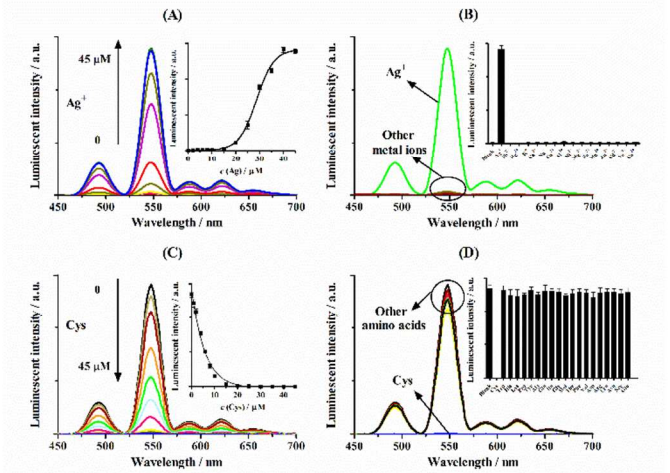
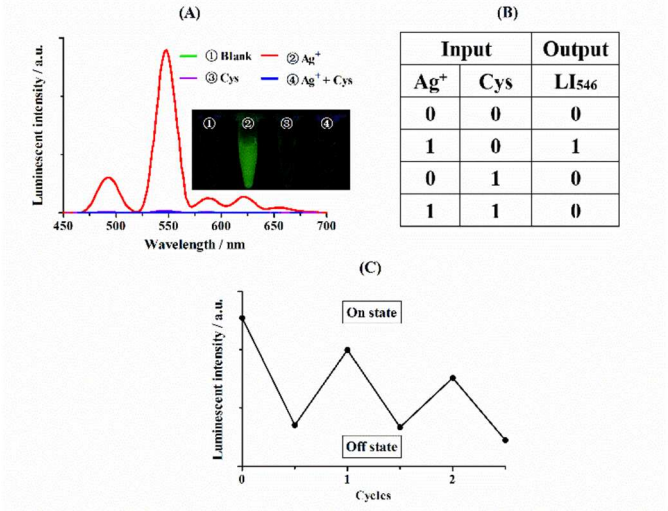


Figure 1. (A) Time-resolved luminescence spectra of the Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe to various Ag^+ concentrations of 0, 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40 and 45 μM; inset: plot of luminescence responses of the Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe to the various concentrations of Ag^+ indicated. (B) Time-resolved luminescence spectra of the Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe to various cations with concentrations of 40 μM; inset: 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. (C) Time-resolved luminescence spectra of Tb^{3+}+$[G_3T]_5$/c$[G_3T]_5$+Ag^+ sensing system (40 μM Ag^+ added) to various Cys concentrations of 0, 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40 and 45 μM; inset: the plot of the luminescence intensities *vs.* the increasing concentrations of Cys of the same data. (D) Selectivity analysis for Cys detection. Time-resolved luminescence spectra of Tb^{3+}+$[G_3T]_5$/c$[G_3T]_5$+Ag^+ sensing system (40 μM Ag^+ added) for all tested amino acids with concentrations of 40 μM; inset: bars represent the luminescence responses to all tested amino acids, using the luminescence enhancement at 546 nm to monitor the responses.

In the absence of Ag^+, the Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe is in the close state with Tb^{3+} luminescence "Off". However, upon challenged with Ag^+, c$[G_3T]_5$ undergoes a conformational alteration due to the formation of C−Ag^+−C complex and $[G_3T]_5$ is liberated from the $[G_3T]_5$/c$[G_3T]_5$ duplex, leading to Tb^{3+} luminescence "On". Further addition of Cys to the above system, Ag^+ is grabbed from the C−Ag^+−C complex and then the c$[G_3T]_5$ re-hybridize with $[G_3T]_5$ to turn off the Tb^{3+} luminescence again. Thus, we further examined the feasibility of the quantitative determine of Cys based on the above Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe containing Ag^+. A solution of Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe containing Ag^+ (Tb^{3+}+$[G_3T]_5$/c$[G_3T]_5$+Ag^+ sensing system) was prepared (see Supplementary Information). Upon addition of Cys with increasing concentrations, a gradual luminescence decrease in the Tb^{3+}+$[G_3T]_5$/c$[G_3T]_5$+Ag^+ sensing system was observed (Figure 1C). Figure 1C, inset, depicts a dependence of luminescent decrease on Cys concentration. The specific response of the probe to Cys was investigated by examining the luminescence signal of the Tb^{3+}+$[G_3T]_5$/c$[G_3T]_5$+Ag^+ sensing system in the presence of various amino acids under the same conditions. It was found that none of these amino acids, except for Cys, could result in an obvious luminescence decrease, indicating the Tb^{3+}+$[G_3T]_5$/c$[G_3T]_5$+Ag^+ sensing system is suitable for specific Cys detection against other amino acids (Figure 1D).

Molecular logic systems, performing mechanical functions using functional biomolecules, have attracted significant recent interest.[8] In recent years, a series of DNA logic gates have been reported, which are recognized as a fascinating generation of molecular logic systems.[9] In this work, the Ag^+/Cys-mediated reversible luminescence changes in the Tb^{3+}-$[G_3T]_5$/c$[G_3T]_5$ system provides a potential for the design of a DNA INHIBIT logic system based on Boolean logic (Figure 2). For input, the presence of Ag^+ or Cys was defined as 1 and their absence as 0. Luminescence intensity at a wavelength of 546 nm (LI_{546}) was defined as the output (1 or 0) for the logic gate. With no input, or with Cys input alone, the output was 0 (Figure 2A, green line and violet line). With Ag^+ input alone, the Ag^+-mediated the split of $[G_3T]_5$/c$[G_3T]_5$ duplex that facilitates the released $[G_3T]_5$ sensitizes the luminescence of Tb^{3+} and the LI_{546} of the Tb^{3+}-$[G_3T]_5$/c$[G_3T]_5$ sensing system sharply increased, giving an output signal of 1 (Figure 2A, red line). When the system was subjected to the two inputs together, the luminescence output was 0 (Figure 2A, blue line). The four possible input combinations were (0, 0), (1, 0), (0, 1) and (1, 1) listed in the truth table (Figure 2B). Moreover, the reversibility of this logic gate operation was also studied with alternating addition of Ag^+ and Cys (Figure 2C). The luminescence intensity change of the Tb^{3+}-$[G_3T]_5$/c$[G_3T]_5$ system gradually decreased after cyclc treatment with Ag^+ and Cys, which can be possibly attributed to the dilution effect, because of the increase in the solution volume with the alternating addition of Ag^+ and Cys.



Input		Output
Ag^+	Cys	LI_{546}
0	0	0
1	0	1
0	1	0
1	1	0

Figure 2. An INHIBIT logic gate system consisting of the Tb^{3+}-$[G_3T]_5$/c$[G_3T]_5$ system using both Ag^+ and Cys as inputs. (A) Time-resolved luminescence spectra of the Tb^{3+}-$[G_3T]_5$/c$[G_3T]_5$ system in the presence of different inputs: ①Ag^+ (0 μM) + Cys (0 μM) (green line), ②Ag^+ (40 μM) + Cys (0 μM) (red line), ③Ag^+ (0 μM) + Cys (20 μM) (violet line) and ④Ag^+ (40 μM) + Cys (20 μM) (blue line); inset: visual representation of the INHIBIT logic gate based on our proposed method, and the picture was taken under a 254 nm UV lamp excitation using a digital camera. (B) Truth table for the INHIBIT logic gate. (C) Reversible switching of the described logic system between the on and off states through the addition of Ag^+ and Cys.

Hydrogen peroxide (H_2O_2) is the simplest peroxide, which has been broadly used in a wealth of applications, e.g. in water treatment, industrial applications, therapeutic use, etc.[10] It is reported that high concentrations of H_2O_2 can induce various cellular damage. Therefore, the sensitive and specific recognition of H_2O_2 to detect and control its concentration is important before and after its exposure to the environment. The oxidation of Cys to cystine by H_2O_2 was previously reported.[11] Enlightened by this fact, it can be envisioned that Tb^{3+}/DNA complex-based luminescent probe can be devised for the detection of H_2O_2 utilizing both Cys-mediated luminescence changes in the Tb^{3+}+$[G_3T]_5$/c$[G_3T]_5$+Ag^+ sensing system and the oxidation of Cys to cystine by H_2O_2. Scheme 1B illustrates a schematic representation of this novel Tb^{3+}/DNA complex-based luminescent probe for H_2O_2 sensing. The oxidation of Cys by H_2O_2 yields cystine, and the resulting disulfide reverses the Cys-mediated luminescence decrease in the Tb^{3+}+$[G_3T]_5$/c$[G_3T]_5$+Ag^+ sensing system, which can be applied to the development of a "turn on" luminescent assay for H_2O_2. Figure 3 shows the luminescence responses of the Tb^{3+}+$[G_3T]$/c$[G_3T]$+Ag^+ sensing system to 40 μM Cys treated with various H_2O_2 concentrations from 0 to 400 μM. The results reveal that after adding the equal amount of Cys treated with increasing concentrations of H_2O_2, a gradual luminescence increase in the Tb^{3+}+$[G_3T]$/c$[G_3T]$+Ag^+ sensing system was observed. From Figure 3, inset, it can be seen that the luminescence increase in the Tb^{3+}+$[G_3T]$/c$[G_3T]$+Ag^+ sensing system is sensitive to the oxidation of Cys by H_2O_2 with increasing concentrations, which could ultimately obtain a novel detection system for H_2O_2.

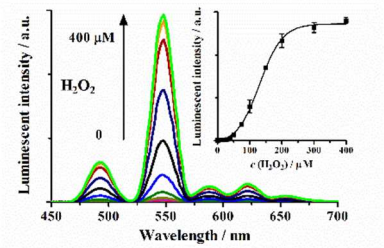


Figure 3. The determination of H_2O_2 via reversing the Cys-mediated luminescence changes in the Tb^{3+}+$[G_3T]_5$/c$[G_3T]_5$+Ag^+ sensing system (40 μM Cys used). Luminescence responses of the Tb^{3+}+$[G_3T]$/c$[G_3T]$+Ag^+ sensing system to 40 μM Cys treated with various H_2O_2 concentrations of 0, 0.1, 1, 2, 5, 10, 12.5, 15, 20, 25, 30, 40, 50, 75, 100, 150, 200, 300 and 400 μM; inset: plot of luminescence responses of the Tb^{3+}+$[G_3T]$/c$[G_3T]$+Ag^+ sensing system to 40 μM Cys treated with the various concentrations of H_2O_2 indicated.

Conclusions

In summary, we have successfully demonstrated a sample and facile strategy to regulate the luminescence of Tb^{3+} sensitized by DNA ($[G_3T]_5$), in which Ag^+ and cysteine (Cys) act as activators. Moreover, the proposed Ag^+/Cys-mediated reversible luminescence changes in the Tb^{3+}/$[G_3T]_5$/c$[G_3T]_5$ probe enabled the design of a DNA INHIBIT logic gate. This is a new concept for performance of logic systems proved with a Tb^{3+}-DNA-based Ag^+/Cys-driven luminescent DNA INHIBIT logic gate. Moreover, a novel time-resolved luminescence detection system has been devised for the detection of H_2O_2 utilizing both Cys-mediated luminescence changes in the Tb^{3+}+$[G_3T]_5$/c$[G_3T]_5$+Ag^+ sensing system and the oxidation of Cys to cystine by H_2O_2. We believe this work will inspire the development of multifunctional Tb^{3+}-based biosensing platforms for various applications.

This work is supported by the National Natural Science Foundation of China (21275055, 21277048). This work is supported by the Natural Science Foundation of Shanghai (11ZR1410700). We also greatly thank the Research Fund for the Doctoral Program of Higher Education (20100076110002).

Notes and references

[a] Department of Chemistry, East China Normal University, 500 Dongchuan Road, Shanghai 200241, China. Email: gyshi@chem.ecnu.edu.cn; Tel&Fax: +86-21-54340043.
[b] Department of Environmental Science, East China Normal University, 500 Dongchuan Road, Shanghai 200241, China.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

1 X. M. Guo, H. D. Guo, L. S. Fu, L. D. Carlos, R. A. S. Ferreira, L. N. Sun, R. P. Deng and H. J. Zhang, *J. Phys. Chem. C*, 2009, **113**, 12538.
2 T. Wu, B. Fang, L. Chang, M. Liu and F. Chen, *Luminescence*, 2013, **28**, 894.
3 E. G. Moore, A. P. Samuel and K. N. Raymond, *Acc. Chem. Res.*, 2009, **42**, 542.
4 D. Zhu, Y. Chen, L. Jiang, J. Geng, J. Zhang, J. J. Zhu, *Anal. Chem.*, 2011, **83**, 9076.
5 P. K. L. Fu and C. Turro, *J. Am. Chem. Soc.*, 1999, **121**,1.
6 M. Zhang, H. N. Le, X. Q. Jiang, B. C. Yin and B. C. Ye, *Anal. Chem.*, 2013, **85**, 11665.
7 a) M. Zhang, H. N. Le, P. Wang and B. C. Ye, *Chem. Commun.*, 2012, **48**, 10004; b) M. Zhang, B. C. Yin, W. Tan and B. C. Ye, *Biosens. Bioelectron.*, 2011, **26**, 3260; c) G. D. Huy, M. Zhang, P. Zuo and B. C. Ye, *Analyst*, 2011, **136**, 3289.
8 a) M. Zhou and J. Wang, *Electroanalysis*, 2012, **24**, 197; b) M. Zhou and S. Dong, *Acc. Chem. Res.*, 2011, **44**, 1232; c) J. Elbaz, O. Lioubashevski, F. Wang, F. Remacle, R. D. Levine and I. Willner, *Nat. Nanotechnol.*, 2010, **5**, 417.
9 a) M. Zhou, Y. Du, C. Chen, B. Li, D. Wen, S. Dong and E. Wang, J. Am. Chem. Soc., 2010, 132, 2172; b) A. Saghatelian, N. H. Volcker, K. M. Guckian, V. S. Lin and M. Z. Ghadiri, *J. Am. Chem. Soc.*, 2003, 125, 346; c) T. Li, E. Wang and S. Dong, *J. Am. Chem. Soc.*, 2009, 131, 15082; d) S. Bi, B. Ji, Z. Zhang and J. J. Zhu, *Chem. Sci.*, 2013, **4**, 1858.
10 W. Lei, A. Dürkop, Z. Lin, M. Wu and O. S. Wolfbeis, *Microchim. Acta*, 2003, **143**, 269.
11 F. Wang, X. Liu, C. H. Lu and I. Willner, *ACS Nano*, 2013, 7, 7278.