RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Journal Name

ARTICLE

Determination of binding constant of specific interactions and binding target concentration simultaneously using a general chemiluminescence method

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Lingfeng Gao, Li Ju, Hua Cui*

The measurement of binding constant of specific interactions and concentration of target is of considerable importance in clinical diagnosis, therapy, bioassays and drug designs. The development of methods combined high sensitivity with generalization and simplicity for measuring both of binding constant and target concentration are highly desired. Previously, we have developed a label-free chemiluminescence (CL) strategy for the measurement of target concentration and binding constant between DNA aptamer and target simultaneously based on that target could enhance the CL produced by the reaction of N-(4-Aminobutyl)-N-ethylisoluminol (ABEI) functionalized gold colloids with H_2O_2. In this work, the enhancement and inhibition effect of various targets on the CL reaction was studied. The generalization of the proposed CL strategy for various targets was explored. These results demonstrate that the proposed CL strategy is suitable for the targets that can cause a change in CL intensity, including enhancement and inhibition. It could be applied for measuring dissociation constant of aptamer-binding target, antibody-antigen complex, protein-binding small molecules and double-strand DNA hybrid from millimole to picomole level. It could be also used for the determination of target concentration sensitively, including 2,4,6-trinitrotoluene (TNT), dopamine, tetracycline, human IgG (hIgG), tuberculosis (TB) DNA and mannose, with the detection limit of 0.93 nM-4.1 fM. It is of great potential in fundamental research as well as in applications in life sciences.

Introduction

Studies on specific interactions such as aptamer with target, antibody with antigen, protein with small molecules and DNA hybridization is of considerable interests for clinical diagnosis and therapy, bioassays, drug designs, environmental monitoring and food safety assessment. The determination for binding constant of specific interactions and binding target concentration is very important in both fundamental research and applications in life sciences. Nowadays, various methods have been developed for measuring the binding constant of specific interactions.[1] Although these methods have been demonstrated to be efficient in measurement of binding constant, they mostly either required complicated operations[2-4], expensive instruments[5, 6] or time-consuming[7-9]. Some of methods are only suitable for the interactions either between small-molecule and macromolecule or between macromolecule and macromolecule.[10, 11] On the other hand, quantitative methods for target concentration based on specific interactions have been studied, including labeling and label-free methods. Labeling methods with high sensitivity involved in multi-step separations and purifications are laborious and time-consuming.[12, 13] Label-free methods are simple, fast and low-cost, but the sensitivity is not ideal.[14, 15] Usually, the methods for the measurement of binding constant are not suitable for the determination of target concentration due to their low sensitivity, whereas the methods for the determination of target concentration are not suitable for the measurement of binding constant since they are difficult to generate significant signal change at high target concentrations. Thus the methodologies for measuring both of binding constant and target concentration have rarely been reported. Hence, the development of methods combined high sensitivity with generalization and simplicity for measuring both of binding constant and target concentration are highly desired.

Recently, we have developed a novel chemiluminescence (CL) label-free method for measuring the DNA aptamer-target dissociation constant (the reciprocal of binding constant) and target concentration simultaneously.[16] The method was based on that DNA aptamer could capture corresponding target, which could enhance CL produced by the reaction of ABEI functionalized gold (ABEI-Au) colloids with H_2O_2. It is highly sensitive, selective, simple, fast and low-cost compared with previous methods. We considered that the proposed label-free CL method might be also suitable for targets that could cause a change in CL intensity from the reaction of ABEI-Au colloid with H_2O_2. In this work, the effect of various substances on the CL reaction between ABEI-Au colloid and H_2O_2 was studied, including bases, sugars, amino acids, proteins, anilines, phenols and nitrotoluenes. It was found that some substances could enhance or

CAS Key Laboratory of Soft Matter Chemistry,
Collaborative Innovation Center of Chemistry for Energy Materials,
Department of Chemistry, University of Science & Technology of China, Hefei, Anhui 230026, P. R. China.
E-mail: hcui@ustc.edu.cn; Fax: +86-551-63600730; Tel: +86-551-63600730
Electronic Supplementary Information (ESI) available: Fig. S1 and Table S1. See DOI: 10.1039/x0xx00000x

inhibit the CL reaction. On this basis, we further explored the applicability to measure binding constant between DNA aptamer and target which could inhibit CL signal as well as between peptide aptamer and target, antibody and antigen, protein and small molecules and DNA hybridization and to determine target concentration. The application scope of this CL strategy was greatly expanded.

Experimental section

Materials and chemicals

Streptavidin (SA), bovine serum albumin (BSA), hIgG, goat-anti-human IgG, tetracycline HCl and amino acids were obtained from Solarbio (Beijing, China). Lysozyme, glucose oxidase (GOD), cytosine, adenosine triphosphate (ATP) and glutathione (GSH) were obtained from Sangon, Inc. (Shanghai, China). 2,4-Dinitrotoluene, 4-nitrotoluene, 2,4,6-trinitrotoluene (TNT) and D-ribose were purchased from Aladdin Reagent (Shanghai, China). Biotinylated concanavalin A was obtained from Sigma-Aldrich (USA). Dopamine was purchased from J&K Scientific Ltd. Guanine and thymine were obtained from Bio Basic Inc. Mannose, adenine, phenol, p-dihydroxybenzene, o-dihydroxybenzene, m-dihydroxybenzene, 1,2,3-Benzenetriol, tannic acid, gallic acid, aniline, o-phenylenediamine, m-phenylenediamine, p-phenylenediamine, 2,4-dihydroxybenzoic acid and 3,4-Dihydroxybenzoic acid were purchased from Shanghai Reagent Company (Shanghai, China). The sequence of TNT peptide aptamer is as follows: (N terminus) Trp-His-Trp-Gln-Arg-Pro-Leu-Met-Pro-Val-Ser-Ile-Lys-biotin (C terminus).[17] The TNT peptide aptamer was purchased from GL Biochem Co. Ltd. (Shanghai, China) and purified using high performance liquid chromatography. The dopamine aptamer (5′-GTC TCT GTG TGC GCC AGA GAA CAC TGG GGC AGA TAT GGG CCA GCA CAG AAT GAG GCC C-3′-spacer 93-biotin),[18] tetracycline aptamer (biotin-5′-TTT TTC GTA CGG AAT TCG CTA GCC CCC CGG CAG GCC ACG GCT TGG GTT GGT CCC ACT GCG CGT GGA TCC GAG CTC CAC GTG-3′)[19], TB capture DNA (biotin-5′- GGT GAC AAA GGC CAC GTA GGC GAA CCC TGC CCA GGT CGA CAC ATA GGT GAG GTC TGC TAC CCA CAG CCG ACC AGG TGC TGG -3′) and TB target DNA (5′-CCA GCA CCT AAC CGG CTG TGG GTA GCA GAC CTC ACC TAT GTG TCG ACC TGG GCA GGG TTC GCC TAC GTG GCC TTT GTC ACC-3′)[20] were synthesized by Sangon, Inc. (Shanghai, China) and purified by high performance liquid chromatography. A $HAuCl_4$ stock solution (2‰ w/w) was prepared by dissolving 1.0 g of $HAuCl_4{\cdot}4H_2O$ (Shanghai Reagent, China) in 412 mL of purified water and stored at 4 °C. ABEI-Au colloids used in this work were synthesized by a seed growth method as described previously.[21] The obtained ABEI-Au colloids were stored at 4 °C for further use. All other reagents were of analytical grade. Ultrapure water was prepared by a Milli-Q system (Millipore, France) and used throughout.

Measurement procedure

As shown in Fig. 1, a 100 µL portion of biotinylated aptamers, antibodies, DNAs or proteins in 0.02 M PBS (pH 7.4, containing NaCl 0.05 M) solution was injected into each SA-coated microwell.

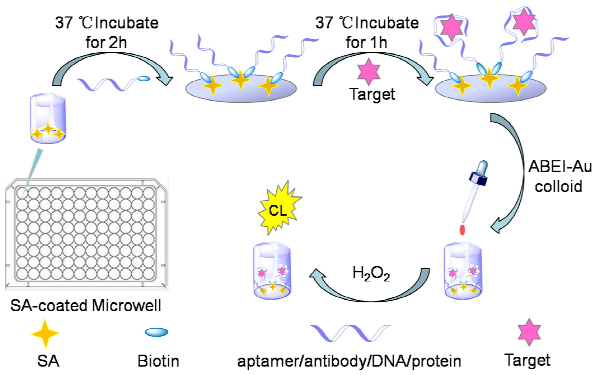


Fig. 1 Schematic illustration for detection of dissociation constant and target concentration

After incubated at 37 °C for 2 h, the unbounded aptamers, antibodies, DNAs or proteins were rinsed with 0.01 M Tris-HCl (pH 7.0, containing 0.05 M NaCl). To control the temperature of the reactions, an incubator (PST-60 HL plus Thermo Shaker, Biosan, Latvia) was used. Then a 100 µL portion of target solution in 0.02 M PBS (pH 7.4, containing NaCl 0.05 M) was added to each well and incubated at 37 °C for 1 h. Then rinse operation was repeated (0.01 M Tris-HCl, pH 7.0, containing 0.05 M NaCl) to remove unbound target molecules. Subsequently, a 100 µL portion of ABEI-Au colloid was added to each well and incubated at 37 °C for 10 min. When an aliquot of H_2O_2 (100 µL, 0.15 M H_2O_2 in 0.1 M NaOH) was injected into a well, the CL signal was recorded by a microplate luminometer (Centro LB 960, Berthold, Germany).

To explore the effect of various substances such as bases, sugars, amino acids, phenols, anilines, nitrotoluenes, peptides and proteins on the CL reaction between ABEI-Au colloid and H_2O_2, these substances were mixed separately with ABEI-Au colloid. The mixture was incubated at 37 °C for 10 min before testing.

Result and discussion

Effect of various substances on CL reaction between ABEI-Au colloids and H_2O_2

In our previous work, it was found that the proposed label-free CL method for measuring dissociation constant of DNA aptamer-target and target concentration is suitable for targets that can enhance the CL produced by the reaction of ABEI-Au colloids with H_2O_2.[16] It was considered that the CL method might be generalized to the targets that could lead to a change in CL intensity from the reaction of ABEI-Au colloid with H_2O_2. Accordingly, the effect of various substances on the CL reaction between ABEI-Au colloid and H_2O_2 was studied by mixing tested substance solution with ABEI-Au colloid and then injecting H_2O_2 into the mixture. The tested substances include bases, sugars, amino acids, proteins, anilines, phenols and nitrotoluenes. Fig. 2 shows the effect of four bases, two sugars and ATP on the ABEI-Au colloid CL reaction. The results demonstrated that all of them could enhance CL intensity obviously. It was reported that the enhancement of ATP containing adenine and ribose on ABEI-Au colloids CL reaction was due to the fact that ATP promoted hydroxyl

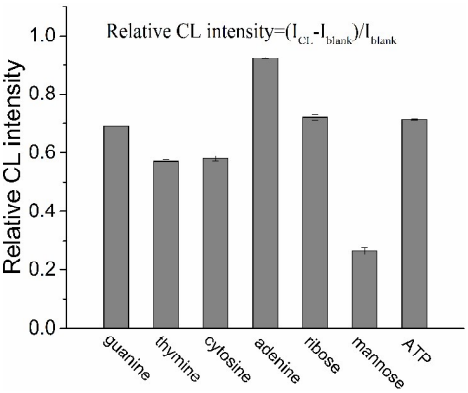


Fig. 2 Effect of bases, sugars and ATP on CL reaction between ABEI-Au colloid and H_2O_2. Reaction condition: 100 μL 0.15 M H_2O_2 (pH=13), 1 μM substance concentration

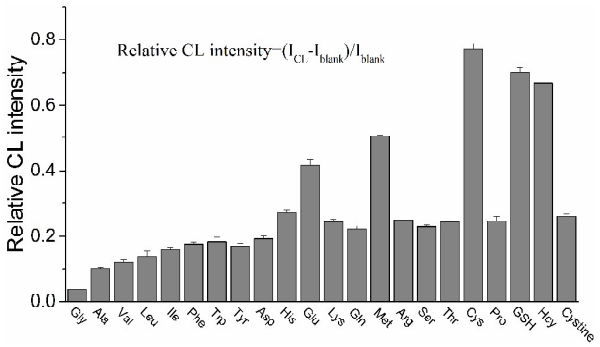


Fig. 3 Effect of amino acids on CL reaction between ABEI-Au colloid and H_2O_2. Reaction condition: 100 μL 0.15 M H_2O_2 (pH=13), 1 μM substance concentration

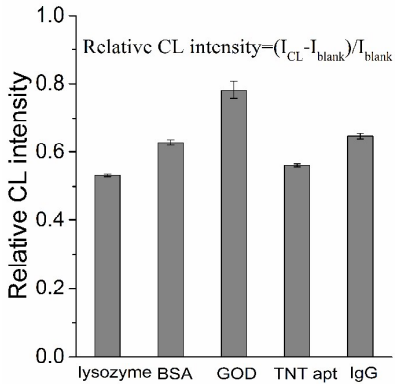


Fig. 4 Effect of proteins and peptide aptamer on CL reaction between ABEI-Au colloid and H_2O_2. Reaction condition: 100 μL 0.15 M H_2O_2 (pH=13), 1 μM substance concentration

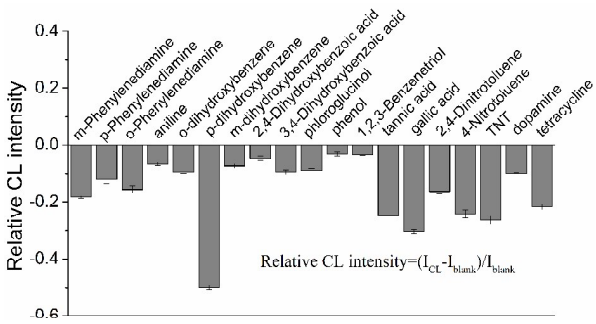


Fig. 5 Effect of phenols, anilines and nitrotoluenes on CL reaction between ABEI-Au colloid and H_2O_2. Reaction condition: 100 μL 0.15M H_2O_2 (pH=13), 1 μM substance concentration

radical production, accelerating the ABEI-Au colloid CL reaction.[16, 22] Both of bases and sugars are similar to some part of ATP structure, they might follow similar enhancement mechanism. Fig. 3 shows the effect of 22 amino acids on the CL intensity. All the amino acids exhibited the CL enhancement. For most of amino acids, the CL enhancement was weak. Glutamic acid (Glu), methionine (Met), cysteine (Cys), GSH and homocysteine (Hcy) show very strong enhancement effect. In earlier studies, the enhancement of amino acids on the luminol CL reaction has been proposed due to that the reaction of $-COO^-$ in their molecules with $O_2^{\bullet-}$ to form $-CO_4^{\bullet 2-}$, accelerating the CL reaction.[23] Hence, glutamic acid and GSH with two $-COO^-$ groups have strong enhancement. In addition, it was reported that sulfide and some thiol-containing compounds were ready to reduce the dissolved oxygen to form $O_2^{\bullet-}$, which would result in the CL enhancement.[24, 25] Thus methionine, cysteine, GSH and homocysteine with thiol or methylthiol group could strongly enhance the CL reaction. However, cystine formed by the oxidation of two cysteine molecules with a disulfide bond has weak enhancement effect. It might be due to that disulfide bond was hard to react with the dissolved oxygen to form $O_2^{\bullet-}$.[24] Fig. 4 shows the effect of various proteins and peptides on the CL reaction. Both proteins and peptides showed obvious CL enhancement because they are composed of various amino acids. Fig. 5 shows the effect of anilines, phenols and nitrotoluenes on the CL reaction. Most of them showed CL inhibition to some extent. Among them, TNT and its derivatives could inhibit the CL reaction obviously. The inhibition effect of aniline, phenol and their derivatives may be due to the competition of $-NH_2$ or $-OH$ groups in their molecules with luminol for $O_2^{\bullet-}$.[23] The different inhibition effect of aniline, phenol and their derivatives might due to their different reducing capability. TNT inhibition effect might be due to that TNT could interact with the luminophor excited-state N-(aminobutyl)-N-(ethylphthalate) formed by the CL reaction, leading to an energy loss and quenching of the CL emission.[26] In addition, TNT could consume •OH radical generated in the CL reaction, leading to a decrease in CL intensity. The derivatives of TNT might follow similar inhibition mechanism.[27]

The CL enhancement and inhibition by various organic compounds of the luminol CL reactions has been documented.[28-30] It has been known that the substances facilitating the formation of oxygen-related radicals such as $OH^{\bullet}$, $O_2^{\bullet-}$, $CO_3^{\bullet-}$, $CO_4^{\bullet-}$ would lead to the CL enhancement, while the substances eliminating these radicals

would result in the CL inhibition. In present work, some of tested substances could cause CL enhancement and others CL inhibition, which follows the similar roles to earlier studies.[22-24, 26] We considered that the proposed label-free CL method might be applicable to measure the binding constant of specific interactions and the concentration of target leading to a change in CL intensity, which was explored in following work.

Measurement of dissociation constant of DNA aptamer-binding target and concentration of target inhibiting CL signal

In order to investigate whether or not the CL strategy could be applicable for measuring aptamer-target binding and target concentration simultaneously when targets would inhibit CL intensity, three kinds of model targets TNT, dopamine and tetracycline and corresponding aptamers were tested.

As shown in Fig. 6, the CL signal intensity decreased linearly with the logarithm of TNT concentration at lower TNT concentrations over the range from 1.0×10^{-10} M to 1.0×10^{-8} M. The linear response of the logarithm of TNT concentration could be fitted by the equation $I_{CL} = 1.4840\times10^{6} - 4.2454\times10^{4}\log C$ with a correlation coefficient of R = 0.9964, where I_{CL} was the CL integrated intensity in 10 s, and C was the concentration of target TNT. The limit of detection (LOD), at a signal to noise ratio of three (S/N = 3), was 0.080 nM. The relative standard deviation (RSD) of seven replicate detections of TNT at 1.0×10^{-9} M (n = 7) was 0.11%, indicating good repeatability of the proposed strategy. When TNT concentration was over 1.0×10^{-8} M, the CL intensity deviated from the linear relationship. The binding ratio of aptamer for TNT was estimated to be less than 2.3% and could be ignored when TNT concentration was below 7.0 nM (supporting information section S1). Based on the principle used in measuring dissociation constant,[31-35] the sigmoid fitted curve from 1.0×10^{-8} to 7.0×10^{-7} M was achieved as shown in Fig. 6 (upper inset). From the sigmoid fitted curve, a dissociation constant of 0.142 μM for aptamer binding TNT was obtained, which

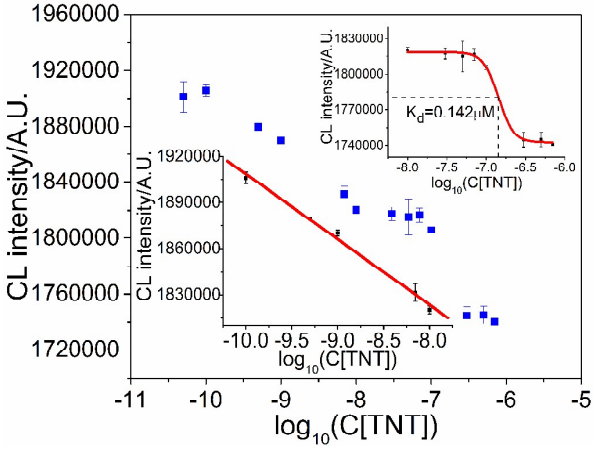


Fig. 6 Measurement of aptamer-TNT dissociation constant and TNT concentration. Upper inset: measurement of aptamer-TNT dissociation constant. Lower inset: working curve for determination of TNT concentration.

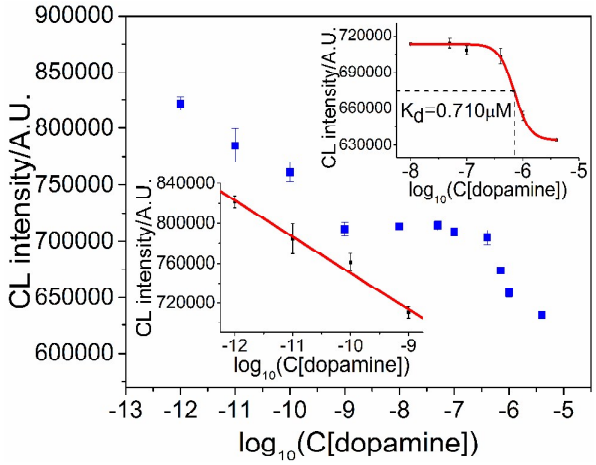


Fig. 7 Measurement of aptamer-dopamine dissociation constant and dopamine concentration. Upper inset: measurement of aptamer-dopamine dissociation constant. Lower inset: working curve for determination of dopamine concentration.

was comparable with 0.0710 μM from literatures.[17, 36, 37]

The result for measuring aptamer-dopamine dissociation constant and dopamine concentration is shown in Fig. 7. The regression equation was $I_{CL} = 3.8602\times10^{5} - 3.6418\times10^{4}\log C$, where I_{CL} was the CL integrated intensity in 10 s, and C was the concentration of target dopamine, with a correlation coefficient of R = 0.9945. The linear range was 1.0×10^{-12} - 1.0×10^{-9} M, LOD was 0.90 pM (S/N = 3) and RSD at 1.0×10^{-10} M dopamine (n = 7) was 2.52 %. The CL intensity was deviated from linearity when dopamine concentration was over 1.0×10^{-9} M. The curve from 1.0×10^{-9} M to 4.0×10^{-6} M was applied for sigmoid fitting and dissociation constant was calculated to be 0.710 μM (the constant measured by literatures was 0.700 μM[18, 38]).

The result for measuring aptamer-tetracycline dissociation constant and tetracycline concentration is shown in Fig. 8. The CL intensity

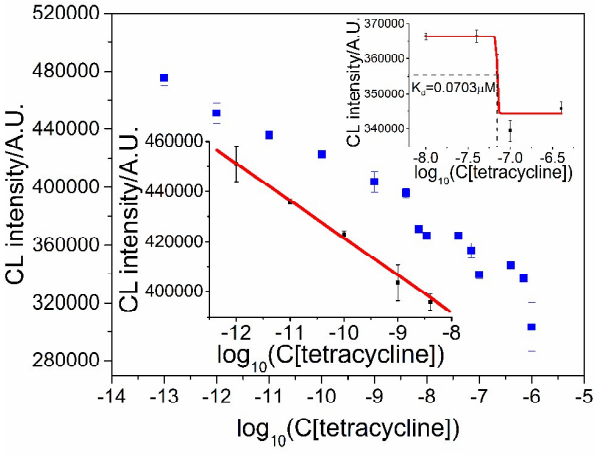


Fig. 8 Measurement of aptamer-tetracycline dissociation constant and tetracycline concentration. Upper inset: measurement of aptamer-tetracycline dissociation constant. Lower inset: working curve for determination of tetracycline concentration.

was a linear function of the logarithm of tetracycline concentration in the range of 1.0×10^{-12} M to 4.0×10^{-9} M with regression equation $I_{CL}= 2.6581\times10^{5}-1.5547\times10^{4}$logC (R = 0.9925), as shown in Fig. 8 (lower inset). The detection limit was (S/N = 3) 0.20 pM, and RSD at 1.0×10^{-10} M tetracycline was 0.25 % (n = 7). The dissociation constant of tetracycline-aptamer was calculated to be 0.0703 μM, which was comparable to 0.0636 μM measured by Javed and coworkers.[4, 19, 39]

These results demonstrated that the proposed CL strategy could be suitable for multiple types of targets which could inhibit ABEI-Au colloid initiated CL.

Measurement of dissociation constant of antibody-antigen complex and concentration of antigen

In order to investigate whether or not the CL strategy could be applicable for the measurement of dissociation constant of antibody-antigen complex and antigen concentration in single detection procedure, goat-anti-human IgG and hIgG were chosen as a model. As shown in Fig. 9, the linear response of the logarithm of hIgG concentration could be fitted by the equation I_{CL} = $1.5440\times10^{6}+4.7244\times10^{4}$logC in the range from 1.0×10^{-13} M to 5.0×10^{-9} M (R = 0.9976). The detection limit was (S/N = 3) 0.092 pM and RSD at 1.0×10^{-11} M hIgG was 2.9 % (n = 7). The curve from 1.0×10^{-8} M to 1.0×10^{-6} M was applied for sigmoid fitting and dissociation constant was calculated to be 0.0589 μM. The results demonstrate that the proposed CL strategy is also suitable for the measurement of binding constant between antigen and antibody and concentration of antigen simultaneously.

Measurement of dissociation constant of double-strand DNA hybrid and concentration of specific DNA sequence

The DNA hybridization reaction was also explored by taking DNA from TB and its complementary chain as an example. As shown in Fig.10, the linear response of the logarithm of DNA concentration could be fitted by the equation I_{CL} = $1.4878\times10^{6}+4.1982\times10^{4}$logC in

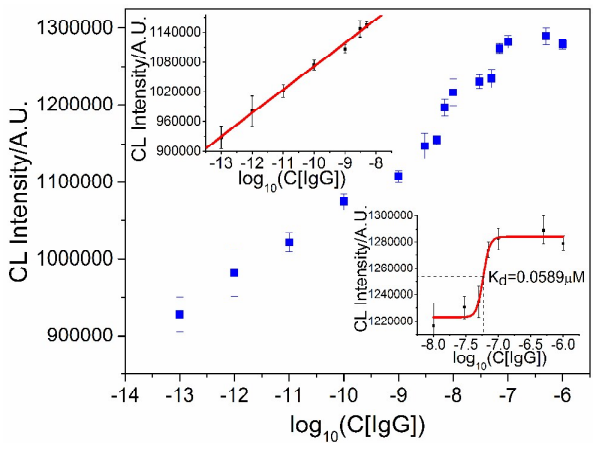


Fig. 9 Measurement of goat-anti-human IgG-hIgG dissociation constant and hIgG concentration. Upper inset: working curve for determination of hIgG concentration. Lower inset: measurement of goat-anti-human IgG-hIgG dissociation constant.

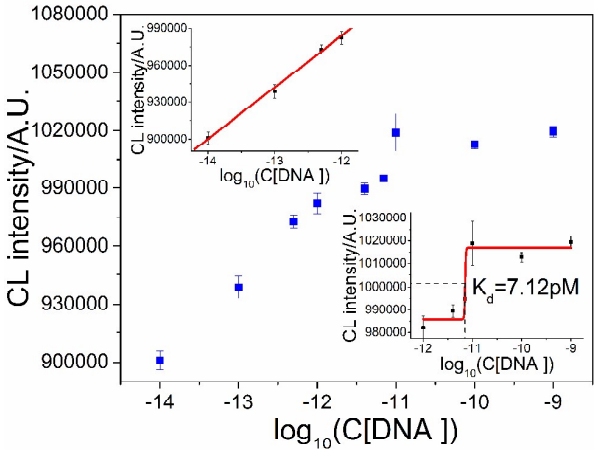


Fig. 10 Measurement of double-strand DNA hybrid dissociation constant and TB DNA concentration. Upper inset: working curve for determination of DNA concentration. Lower inset: measurement of DNA hybrid dissociation constant.

the range from 1.0×10^{-14} M to 1.0×10^{-12} M (R = 0.9981). The detection limit was (S/N = 3) 4.1 fM and RSD at 1.0×10^{-12} M DNA was 2.6 % (n = 7). The dissociation constant between TB DNA and its complementary sequence was 7.12 pM as shown in Fig. 10 (lower inset). The result demonstrated that the interaction between DNA sequences could also be studied by this method. In addition, it also demonstrated that this CL method could be applicable for measuring a wide range of dissociation constant.

Measurement of dissociation constant of protein-binding small molecules and concentration of small molecule

The applicability of this method for measuring dissociation constant of protein-binding small molecule was examined by taking concanavalin A and mannose as a model. The interaction between

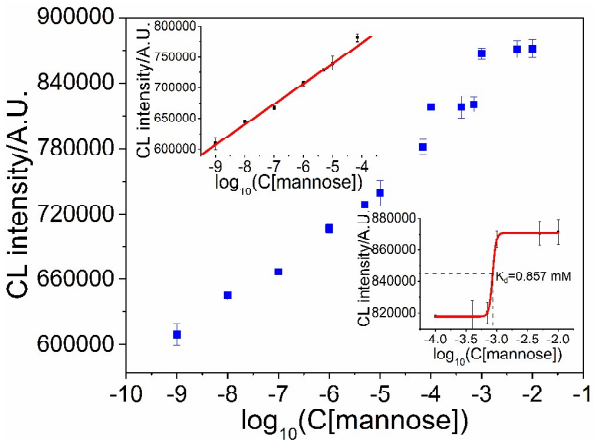


Fig. 11 Measurement of concanavalin A-mannose dissociation constant and mannose concentration. Upper inset: working curve for determination of mannose concentration. Lower inset: measurement of concanavalin A-mannose dissociation constant.

concanavalin A and mannose is much weaker than those we studied above. Surprisingly, this CL method was still valid. As shown in Fig. 11 (upper inset), the CL intensity was a linear function of the logarithm of mannose concentration in the range from 1.0×10^{-9} M to 1.0×10^{-4} M with regression equation $I_{CL}= 9.0548\times10^{5} + 3.3181\times10^{4}$logC (R = 0.9918). The detection limit was (S/N = 3) 0.93 nM and RSD at 1.0×10^{-6} M mannose was 0.52% (n=7). As shown in Fig. 11 (lower inset), the dissociation constant of concanavalin A-mannose was 0.857 mM, which was comparable to that measured by literatures (0.450 mM).[40-43]

Conclusion

In this work, it has been found that some substances including bases, sugars, amino acids and proteins could enhance the CL reaction between ABEI-Au colloid and H_2O_2 whereas other substances including anilines, phenols and nitrotoluenes could inhibit the CL reaction. This work has also demonstrated that the proposed label-free CL strategy could be used to determine binding constant between DNA aptamer and target and concentration of target which could not only enhance but also inhibit CL signal. Moreover, the CL strategy is also applicable for measuring binding constant between peptide aptamer and target, antibody and antigen, protein and small molecules and DNA hybridization and target concentration. The dissociation constant could be measured from millimole to picomole level and concentration of target, including TNT, dopamine, tetracycline, hIgG, TB DNA and mannose, could be detected sensitively with the detection limit of 0.93 nM-4.1 fM. Compared with previously reported label-free methods based on specific interactions, LOD for TNT is comparable with that in literatures. LOD for dopamine, tetracycline and TB DNA is 1-3 orders of magnitude lower than references except that LOD for hIgG is higher than that in literatures (Table S1). Therefore, the CL strategy is a general and sensitive strategy for measuring the binding constant of specific interactions and the concentration of target that could lead to a change in CL intensity from the reaction of ABEI-Au colloid with H_2O_2. This provides a rapid, simple and effective method for studying specific interactions between aptamer and target, antibody and antigen, protein and small molecules and DNA hybridization as well as target concentration, showing great potential in fundamental research as well as in applications such as clinical diagnosis, therapy, drug design, bioassays, environmental monitoring and food safety assessment.

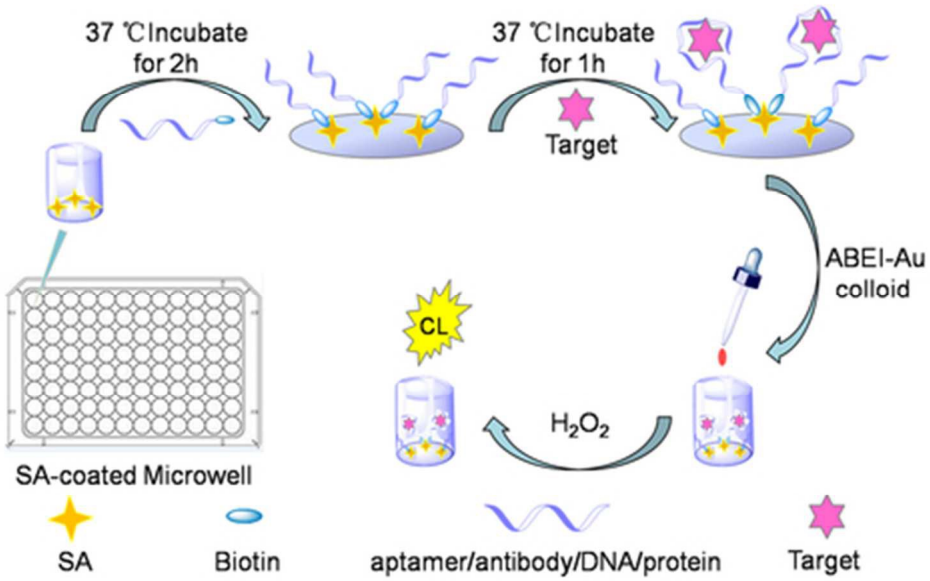
Acknowledgments

The support of this research by the National Natural Science Foundation of P. R. China (Grant Nos. 21173201, 21075115, 20625517 and 20573101), and the Fundamental Research Funds for the Central Universities (Grant No.WK2060190007) is gratefully acknowledged.

References

1. M. Jing and M. T. Bowser, *Anal. Chim. Acta*, 2011, **686**, 9-18.
2. Y. Li, H. J. Lee and R. M. Corn, *Nucleic Acids Res.*, 2006, **34**, 6416-6424.
3. S. Jaouen, L. de Koning, C. Gaillard, E. Muselíková-Polanská, M. Štros and F. Strauss, *J. Mol. Biol.*, 2005, **353**, 822-837.
4. J. H. Niazi, S. J. Lee and M. B. Gu, *Bioorg. Med. Chem.*, 2008, **16**, 7245-7253.
5. D. S. Hage and S. A. Tweed, *Journal of Chromatography B: Biomedical Sciences and Applications*, 1997, **699**, 499-525.
6. D. E. Huizenga and J. W. Szostak, *Biochemistry*, 1995, **34**, 656-665.
7. M. Müller, J. E. Weigand, O. Weichenrieder and B. Suess, *Nucleic Acids Res.*, 2006, **34**, 2607-2617.
8. J. A. Cruz-Aguado and G. Penner, *J. Agric. Food Chem.*, 2008, **56**, 10456-10461.
9. D. K. Mandal, N. Kishore and C. F. Brewer, *Biochemistry*, 1994, **33**, 1149-1156.
10. L. G. Fägerstam, Å. Frostell-Karlsson, R. Karlsson, B. Persson and I. Rönnberg, *J. Chromatogr. A*, 1992, **597**, 397-410.
11. V. C. Ozalp, *Analyst*, 2011, **136**, 5046-5050.
12. Y. Chai, D. Tian and H. Cui, *Anal. Chim. Acta*, 2012, **715**, 86-92.
13. X. Yu, Y. Chai, J. Jiang and H. Cui, *J. Photochem. Photobiol. A Chem.*, 2012, **241**, 45-51.
14. F. Li and H. Cui, *Biosens. Bioelectron.*, 2013, **39**, 261-267.
15. F. Li, Y. Yu, H. Cui, D. Yang and Z. Bian, *Analyst*, 2013, **138**, 1844-1850.
16. S. Li, D. Chen, Q. Zhou, W. Wang, L. Gao, J. Jiang, H. Liang, Y. Liu, G. Liang and H. Cui, *Anal. Chem.*, 2014, **86**, 5559-5566.
17. Y. Yu, Q. Cao, M. Zhou and H. Cui, *Biosens. Bioelectron.*, 2013, **43**, 137-142.
18. R. Walsh and M. C. DeRosa, *Biochem. Biophys. Res. Commun.*, 2009, **388**, 732-735.
19. Y. J. Kim, Y. S. Kim, J. H. Niazi and M. B. Gu, *Bioprocess Biosyst. Eng.*, 2010, **33**, 31-37.
20. J. Jiang, Y. Chai and H. Cui, *RSC Advances*, 2011, **1**, 247-254.
21. D. Tian, H. Zhang, Y. Chai and H. Cui, *Chem. Commun.*, 2011, **47**, 4959-4961.
22. S. Aoyagi, M. Yamazaki, T. Miyasaka and K. Sakai, *J. Chem. Eng. Jpn.*, 2001, **34**, 956-959.
23. H. Cui, M. Shi, R. Meng, J. Zhou, C. Lai and X. Lin, *Photochem. Photobiol.*, 2004, **79**, 233-241.
24. J. Du, Y. Li and J. Lu, *Anal. Chim. Acta*, 2001, **448**, 79-83.
25. E. de Lamirande and C. Gagnon, *Free Radic. Biol. Med.*, 1998, **25**, 803-817.
26. Y. Jiang, H. Zhao, N. Zhu, Y. Lin, P. Yu and L. Mao, *Angew. Chem.*, 2008, **120**, 8729-8732.
27. H. Yu, Y. He, W. Li and T. Duan, *Sens. Actuators, B*, 2015, **220**, 516-521.
28. A. Economou, D. G. Themelis, G. Theodoridis and P. D. Tzanavaras, *Anal. Chim. Acta*, 2002, **463**, 249-255.
29. J. Wang, H. Ye, Z. Jiang, N. Chen and J. Huang, *Anal. Chim. Acta*, 2004, **508**, 171-176.
30. J. Zhou, H. Xu, G. Wan, C. Duan and H. Cui, *Talanta*, 2004, **64**, 467-477.
31. T. H. Nguyen, L. J. Steinbock, H. J. Butt, M. Helm and R. Berger, *J. Am. Chem. Soc.*, 2011, **133**, 2025-2027.
32. D. Shangguan, Y. Li, Z. Tang, Z. C. Cao, H. W. Chen, P. Mallikaratchy, K. Sefah, C. J. Yang and W. Tan, *Proceedings of the National Academy of Sciences*, 2006, **103**, 11838-11843.
33. K. Yamana, Y. Ohtani, H. Nakano and I. Saito, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 3429-3431.
34. T. Endoh, H. Funabashi, M. Mie and E. Kobatake, *Anal. Chem.*, 2005, **77**, 4308-4314.

35. W. Lee, A. Obubuafo, Y. I. Lee, L. M. Davis and S. A. Soper, *J. Fluoresc.*, 2010, **20**, 203-213.
36. J. W. Jaworski, D. Raorane, J. H. Huh, A. Majumdar and S.-W. Lee, *Langmuir*, 2008, **24**, 4938-4943.
37. D. L. Roy, W. Yang, X. Yin, R. Y. Lai, S. H. Liou and D. J. Sellmyer, *J. Appl. Phys.*, 2011, **109**, 07E532.
38. H. Park and I. R. Paeng, *Anal. Chim. Acta*, 2011, **685**, 65-73.
39. C. Berens, A. Thain and R. Schroeder, *Bioorg. Med. Chem.*, 2001, **9**, 2549-2556.
40. S. Takahashi, K. Sato and J. I. Anzai, *Anal. Bioanal. Chem.*, 2012, **402**, 1749-1758.
41. M. Farooqi, M. Saleemuddin, R. Ulber, P. Sosnitza and T. Scheper, *J. Biotechnol.*, 1997, **55**, 171-179.
42. Y. Lvov, K. Ariga, I. Ichinose and T. Kunitake, *J. Chem. Soc., Chem. Commun.*, 1995, **22**, 2313-2314.
43. S. Z. Zhang, F. L. Zhao, K. A. Li and S. Y. Tong, *Talanta*, 2001, **54**, 333-342.



39x25mm (300 x 300 DPI)