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Graphene oxide-based homogenous biosensing platform for ultrasensitive DNA detection based on chemiluminescence resonance energy transfer and exonuclease III-assisted target recycling amplification†

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We report an amplified chemiluminescence (CL) biosensing platform for ultrasensitive DNA detection. It is based on exonuclease III-assisted target recycling amplification and the super quenching efficiency of graphene oxide (GO). In the presence of target DNA, the target–probe hybrid forms a double-stranded structure, and exonuclease III catalyzes the stepwise removal of mononucleotides from the fluorescein-labeled probe DNA, resulting in the recycling of the target DNA and CL signal amplification of the luminol–H_2O_2–HRP–fluorescein chemiluminescence resonance energy transfer (CRET) system. The detection limit of target DNA was estimated to be as low as 9 fM, and the sensitivity was about 3 orders of magnitude better than that of GO-based fluorescence resonance energy transfer (FRET) sensors for DNA with exonuclease III-assisted amplification. This CL biosensor offers the advantages of being facile, sensitive, rapid and cost-effective.

Introduction

Detection of nucleic acids has great importance for a wide range of applications, including gene therapy, forensic investigations and clinical diagnostics. Seeking a simple and sensitive method for probing ultralow concentrations of specific single stranded DNA (ssDNA) has risen to become a great challenge in recent years. Among many methods developed for this purpose, nuclease-based amplification is one of the most significant ways. As the classical target amplification method, polymerase chain reaction (PCR) can amplify a oligonucleotide target, improving the signaling to the extent that the single-molecule detection limit is often realized.[1,2] However, PCR is a relatively complex multistep process, prone to false positives arising from artifactual amplification (*e.g.*, of primer dimers, *etc.*).[2,3] In response, the use of nicking endonucleases, that cleave only one strand of double-stranded DNA at specific recognition nucleotide sequences, has largely facilitated the development of signal amplification.[4,5] Unfortunately, nicking endonucleases are sequence-specific, and only 13 nicking endonucleases are commercially available, which severely restricts the application of nicking endonuclease-based technology.[5,6] Unlike sequence-specific nicking endonucleases, exonucleases do not require a specific recognition sequence, and so may be more beneficial in the development of universal signal amplification strategies for DNA detection. Exonuclease III (Exo III) is a kind of exonuclease that catalyzes the stepwise removal of mononucleotides from 3′-hydroxyl termini of double-stranded DNA, and is not active on 3′-overhang ends of double-stranded DNA or single-stranded DNA.[7,8] Thus, exonucleases provide a more versatile platform for amplification detection of DNA. Molecular beacons can rapidly and specifically report the presence of target DNA in homogenous solution without the need to remove unbound probes. Plaxco and co-workers[8] combined exonuclesae-based amplification with a stem-loop DNA molecular beacon to establish a fluorescence method for DNA detection. The approach achieved picomolar detection simply by mixing the molecular beacon, Exo III, and target DNA and incubating for 2 h at 37 °C. The method was simple, rapid and sensitive. However, in the method,[8] the molecular beacon was modified with a fluorophore at its 5′ terminus and a quencher at an internal position, thus suffering from problems associated with double labeling such as high cost, low yield and singly labeled impurities.[9] Furthermore, the fluorescence enhancement of molecular beacons is generally limited by background fluorescence, which comes from imperfect quenching of donors and conformational fluctuations of the hairpin structure.[10] Therefore, some strategies have been proposed to improve the performance of Exo III-based DNA detection.[11–20] Up until now, most of the reported methods using exonuclease-assisted amplification have been demonstrated with fluorescence resonance energy transfer (FRET). As with any fluorescence technique, however, photobleaching of fluorescence dyes,

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autofluorescence of biosystems, and excitation-induced cytotoxicity certainly limit the usefulness of exonuclease-assisted amplification.

Chemiluminescence (CL) is known as a powerful and important analytical technique because of its extremely high sensitivity along with its other advantages, such as simple instrumentation, wide calibration ranges, and suitability for miniaturization in analytical chemistry.[21,22] Chemiluminescence resonance energy transfer (CRET) involves nonradioactive (dipole–dipole) energy transfer between a chemiluminescent donor and a fluorophore acceptor that are in close proximity (normally <10 nm). CRET occurs by oxidation of the chemiluminescent donor which then excites the fluorescent acceptor. Since no external light source is used for excitation in CRET approaches, nonspecific signals caused by external light excitation, as often observed in FRET measurements, can be minimized.[23] This makes CRET an attractive light measuring scheme in bioassays, as has been demonstrated in recent years.[23–26] Very recently, Bi and co-workers[25] used the fluorescein-labeled ssDNA as a recognition element, and developed a CRET platform for biomolecule detection based on a luminol–H_2O_2–HRP–fluorescein system. Zhao and co-workers[26] used *N*-(4-aminobutyl)-*N*-ethylisoluminol (as chemiluminescent donor) and fluorescein (as fluorescent acceptor) to label antigens and antibodies, respectively, and thus introduced CRET into immunoreactions to improve microfluidic immunoassay sensitivity.

Herein, we report an Exo III-amplified CRET biosensing platform for ultrasensitive DNA assay. In this CRET system, the luminol–H_2O_2–HRP CL reaction, which is one of the most sensitive and reliable CL reactions,[27] is used as the energy donor, and fluorescein, with its high fluorescent quantum yield is chosen as the energy acceptor. Meanwhile, we use the graphene oxide (GO) as a super quencher and Exo III as an amplifying biocatalyst to develop a facile, sensitive, and cost-effective method for DNA detection. The principle of the method is shown in Scheme 1. The probe DNA is labeled with fluorescein amidite (FAM). Due to the strong noncovalent binding between GO and ssDNA,[28] the FAM-labeled probe DNA is adsorbed onto the GO surface; because of the excellent quenching efficiency of GO,[29] the CRET-stimulated emission of FAM is significantly quenched by GO. However, in the presence of target DNA, the CRET signal could be observed, and the CRET sensing signal was amplified using an Exo III-based target recycling strategy. Therefore, due to both the high sensitivity of CRET and the signal amplification of Exo III, the biosensing platform for DNA detection achieved a detection limit of 9 fM in 2.5 h, with excellent selectivity that discriminates single nucleotide polymorphisms. This sensitive CL biosensor for direct DNA detection in homogeneous solution represents a promising path toward realizing DNA assay for practical applications.

Experimental

Chemicals and apparatus

All of the synthetic oligonucleotides were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Their base sequences were as follows: probe DNA: 5′-FAM-AGT CAG TGT GGA AAA TCT CTA GC-3′; perfectly complementary target sequence: 5′-GCT AGA GAT TTT CCA CAC TGA CTG AGA-3′; one-base mismatched sequence: 5′-GCT AGA GAT TT*C* CCA CAC TGA CTG AGA-3′; noncomplementary sequence: 5′-TGA GGT AGT AGA TTG TAT AGT TAG AGA-3′. The oligonucleotide stock solutions (25 μM) were prepared in 25 mM Tris–HCl buffer (pH 7.4) and kept frozen. Before use, the oligonucleotide solution was heated to 90 °C for 5 min and gradually cooled to room temperature to unwind the single-strand oligonucleotides. Exonuclease III (200 U μL^{-1}) was also purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Luminol and HRP were obtained from Sigma. A luminol stock solution (25 mM) was prepared by dissolving luminol (4.43 g) in 0.10 M NaOH (20 mL) and then diluting to 1 L with water. The luminol solution was stored in the dark for one week prior to use to ensure that the reagent had stabilized. Working solutions of H_2O_2 were freshly prepared daily from 30% (w/w) H_2O_2. Unless otherwise indicated, all reagents and solvents were purchased in their highest available purity and used without further purification. Millipore Milli-Q water (18 MΩ cm^{-1}) was used in all experiments.

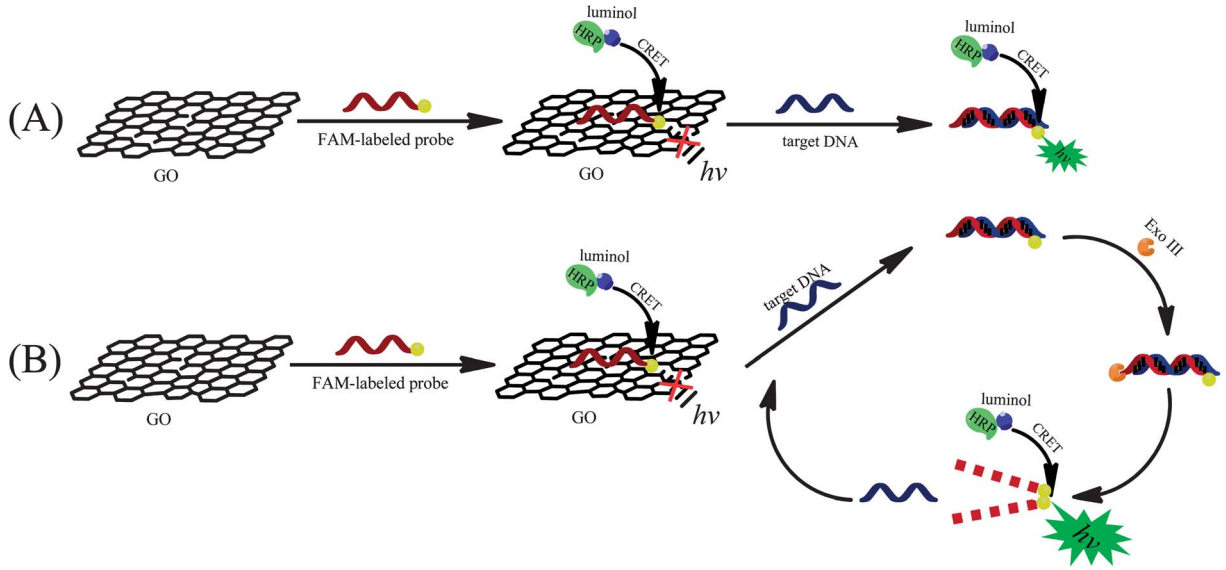
The CL intensity was measured and recorded with a BPCL ultraweak chemiluminescence analyzer (Institute of Biophysics, Chinese Academy of Sciences, Beijing, China). The CL spectra and fluorescence spectra were recorded with a model F-4600 fluorescence spectrophotometer (Hitachi, Japan). An HH-1 thermostatic water bath (Beijing Kewen Instrumental Factory, Beijing, China) was used to control the temperature at 0.1 °C intervals.

Preparation of GO solution

First, graphite oxide powders were produced from graphite using a modified Hummer's method.[30] Graphite oxide powder (50 mg) was dissolved in 100 mL water and then sonicated strongly for 1 h (300 W). Subsequently, the resulting GO solution (0.5 mg mL^{-1}) was stored at 4 °C.

Procedure for the CL detection of DNA

A typical CL analysis was realized by following the procedure given in Scheme 1. The whole procedure could be divided into two stages: DNA hybridization and CL detection. First, 50 μL GO solution (80 μg mL^{-1}) was mixed with 100 μL FAM-labeled DNA probe (1.0×10^{-7} M) for 30 min at room temperature, followed by the addition of 10 μL Exo III (2 U μL^{-1}) and 100 μL target DNA with different concentrations. After allowing this mixture to hybridize for 2 h at 37 °C, 200 μL mixed solution containing H_2O_2 (1.0×10^{-3} M) and HRP (2.0×10^{-6} g mL^{-1}) was added. Next, 100 μL of the resulting solution was decanted into a quartz cuvette (used as the CL reactor), and the CL reaction was triggered by injecting 100 μL of luminol (5.0×10^{-4} M). The CL signal was measured and recorded using the BPCL ultraweak chemiluminescence analyzer. The concentration of target DNA was quantified by the CL peak intensity. The CL experiments in this work were not consecutive measurements.



Scheme 1 Schematic illustration of the sensing principle with the CRET platform for DNA detection. (A) Traditional binding strategy that one target can release only one probe from the GO surface. (B) Amplification *via* Exo III digestion of the probe to release the target DNA for more reaction cycles.

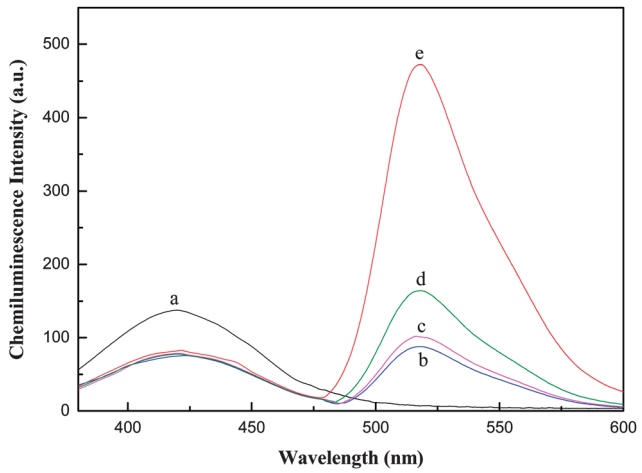


Fig. 1 CL spectra obtained under different conditions (a) luminol–H_2O_2–HRP; (b) luminol–H_2O_2–HRP + GO + probe (1.0×10^{-7} M); (c) luminol–H_2O_2–HRP + GO + probe (1.0×10^{-7} M) + target (1.0×10^{-13} M); (d) luminol–H_2O_2–HRP + GO + probe (1.0×10^{-7} M) + target (1.0×10^{-13} M) + Exo III (20 U); (e) luminol–H_2O_2–HRP + probe (1.0×10^{-7} M).

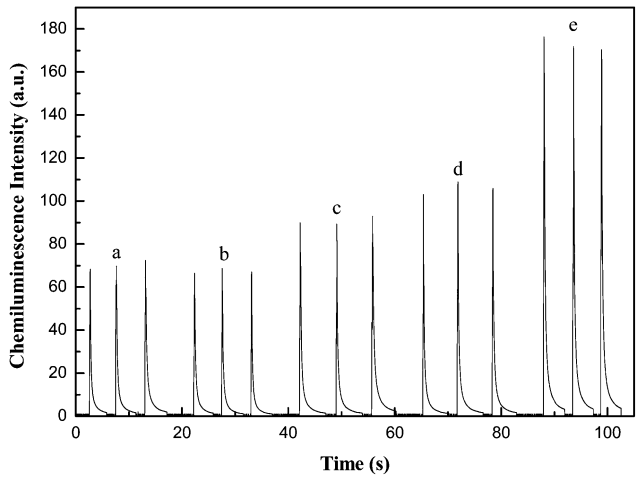


Fig. 2 Measurement results of the sensing system under different conditions. (a) Luminol–H_2O_2–HRP; (b) luminol–H_2O_2–HRP + GO; (c) luminol–H_2O_2–HRP + GO + probe (1.0×10^{-7} M); (d) luminol–H_2O_2–HRP + GO + probe (1.0×10^{-7} M) + target (1.0×10^{-13} M); (e) luminol–H_2O_2–HRP + GO + probe (1.0×10^{-7} M) + target (1.0×10^{-13} M) + Exo III (20 U).

Results and discussion

Principle and design of CRET for DNA detection

The luminol–H_2O_2 reaction is a popular CL reaction. In capillary electrophoresis with luminol–H_2O_2 CL detection, the detection limit of HRP was below 10^{-19} mol in the presence of *para*-iodophenol as an enhancer.[31] The luminol–H_2O_2–HRP CL reaction is one of the most sensitive CL reactions. Therefore, we chose the luminol–H_2O_2–HRP CL reaction as the energy donor in the CRET system. Fig. 1 depicts the CL spectra obtained under different conditions. As can be seen, only the CL emission with maximal wavelength at *ca.* 420 nm, which is ascribed to the emission of 3-aminophthalate (an oxidation product of luminol), was observed when the luminol–H_2O_2–HRP system was tested. Upon addition of FAM-labeled DNA to the luminol–H_2O_2–HRP system, the CL emission at 420 nm decreased, and a new emission at *ca.* 510 nm, corresponding to FAM was obtained. This indicates the occurrence of CRET from luminol (donor) to FAM (acceptor). Furthermore, the CL intensity of the luminol–H_2O_2–HRP CL reaction was increased in the presence of FAM-labeled DNA. So, fluorescein acts as both an enhancer and an acceptor in this proposed CRET system.

GO is a single-atom-thick two-dimensional carbon material. GO exhibits a highly planar surface (*ca.* 2620 $m^2\ g^{-1}$, theoretical

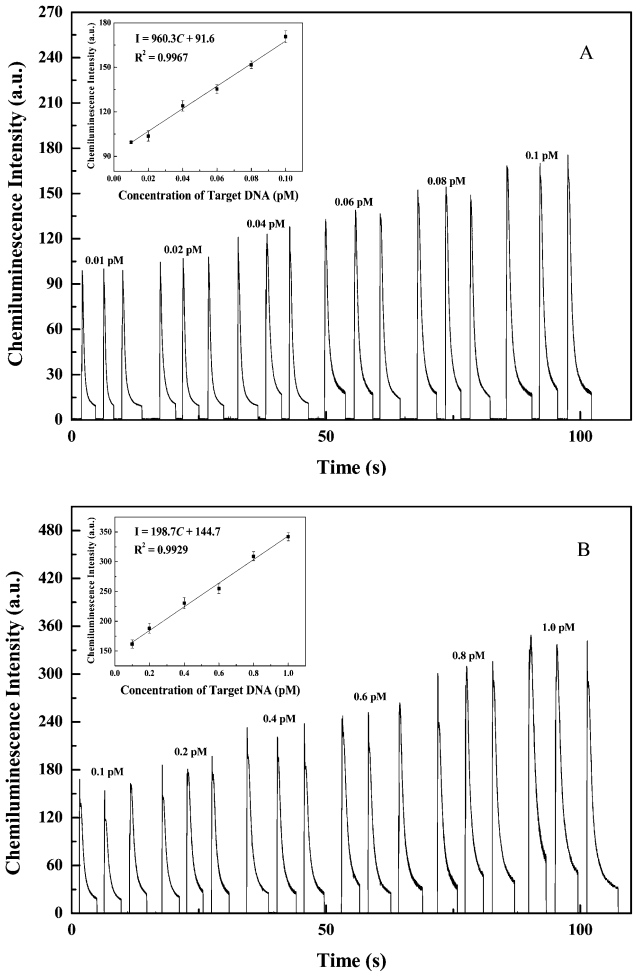


Fig. 3 Representative recorder outputs of the CRET system in the presence of different concentrations of target DNA under the optimized experimental conditions. The inset shows a linear relationship between the CL intensity (*I*) and target DNA concentration (*C*) from 0.01 pM to 1.0 pM. Error bars represent the standard deviations of three independent measurements.

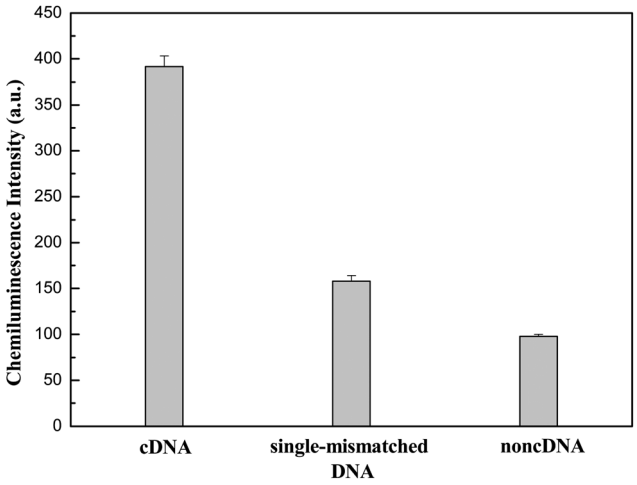


Fig. 4 Specificity of the assay for DNA detection by hybridizing probe DNA with different target DNA: completely complementary target DNA (cDNA), single-base mismatched DNA and noncomplementary DNA (noncDNA), respectively. Probe DNA concentration is 1.0×10^{-7} M, and each target DNA is 1.0×10^{-12} M. Error bars are the standard deviation of three repetitive measurements.

specific surface area for completely exfoliated and isolated graphene),[32] which enables an increased number of FRET acceptor molecules to be present. In a FRET system, the fraction of photons absorbed by the donor that are transferred to the acceptor is generally enhanced by increasing the number of acceptor molecules. In this regard, GO is suggested to be an optimal quencher as a lattice of acceptor molecules is exposed on the surface in a two-dimensional structure; in fact, experimental results have demonstrated that GO is the best FRET acceptor when compared to other carbon structures such as graphite, carbon nanotubes and carbon nanofibers.[33] Hence, GO can be proposed as a universal and highly efficient long-range quencher. We anticipated that GO could serve as a very good acceptor in the CRET system. On the other hand, GO sheets can strongly adsorb ssDNA, as a result of π-stacking interactions between the ring structures in the nucleobases and the hexagonal cells of GO, and hydrogen bonding interactions between –OH or –COOH of GO and –OH or $–NH_2$ of ssDNA.[34] However, GO scarcely interacts with the rigid structure of double stranded DNA (dsDNA), likely due to the efficient shielding of the nucleobases within the negatively charged phosphate backbone of dsDNA.[35]

Therefore, we took full advantage of the performance of luminol–H_2O_2–HRP–fluorescein CRET and the properties of GO to design this simple strategy for DNA detection. The strategy behind our method is depicted schematically in Scheme 1. The 5′ end of the probe DNA is labeled with fluorescein (FAM). The FAM-labeled probe DNA first noncovalently assembles on the GO, resulting in resonance energy transfer from FAM to GO. Once target DNA is introduced, the conformation of the probe DNA on GO is changed due to binding between the probe DNA and the target DNA, resulting in a disturbance of the interaction between the probe DNA and GO, and the release of the probe DNA from the GO surface. The increasing probe DNA–GO distance and the weakened probe DNA–GO interaction thus restore the CRET from luminol–H_2O_2–HRP to FAM. In the amplification strategy, to ensure that the Exo III can cleave the fluorescently labeled DNA, an additional four bases were overhung at the 3′ termini of the target DNA. Exo III can catalyze the stepwise removal of mononucleotides from the blunt 3′ termini of FAM-labeled probe DNA, which results in the release of the target and the fluorophore. The released target DNA can hybridize with another probe DNA and then initiate the next round of cleavage. The numbers of releasing fluorophore are positively related to the CL intensity of the CRET system, which leads to significant amplification of the signal.

The CL spectra measurements (Fig. 1) were carried out to serve as a proof of the concept and to test the principle of our design. Compared with the spectrum (curve e), upon the addition of GO into the CRET system the emission of FAM at ~510 nm was significantly quenched (curve b). This observation indicates the strong adsorption of ssDNA on GO and the high CL quenching efficiency of GO on FAM. Upon the introduction

of target DNA, the emission of FAM at 510 nm was weakly enhanced. In the presence of Exo III, the emission at 510 nm enhanced largely (curve d). The Exo III-assisted signal amplification was proved. Meanwhile, the emission intensity of luminol–H_2O_2–HRP at 420 nm was almost unchanged regardless of whether GO and Exo III were present. This could be explained as in the CRET system the energy of the donor at 420 nm has been transferred to the acceptor at 510 nm.

In order to further evaluate the amplification function of the proposed sensing system, the target-induced CL signal in the presence and absence of Exo III were recorded using a CL analyzer, which collected the total luminescence intensity. As shown in Fig. 2, an obvious signal enhancement was observed due to Exo III catalyzing target DNA recycling upon the addition of 0.1 pM target DNA (curve e). In contrast, only a weak signal enhancement was observed in the absence of Exo III (curve d). The amplification provided by Exo III led to a *ca.* 8-fold increase in the final CL intensity upon addition of 0.1 pM target DNA. The control experiments showed that GO hardly affected the CL of luminol–H_2O_2–HRP (curve a and curve b), which suggested that the energy of luminol–H_2O_2–HRP could not directly transfer to GO because of the greater distance between luminol and GO in this system. These results indicated that Exo III-catalyzed amplified target DNA detection has been successfully achieved.

Optimization of assay conditions

Carbon nanotubes are also a good class of energy acceptors with long-range energy transfer properties. Therefore, we compared the quenching efficiency of GO with single-walled carbon nanotubes (SWCNT) in this CRET system. The experimental results (Fig. S1†) showed that the degree of CL quenching reached approximately 84% with 80 μg mL^{-1} GO, and the quenching efficiency of 80 μg mL^{-1} SWCNT was only 61%, which indicates that GO is a more suitable acceptor for the CRET than SWNT. Furthermore, the GO amount influenced the sensitivity of the CL biosensing for DNA. When the GO concentration was too low, the background was rather high, possibly due to the incomplete quenching of GO on luminescence of FAM-labeled probe DNA. When the GO concentration was too high, the signal of small quantities of target DNA was small, which might be ascribed to the adsorption of the target DNA itself onto the excess GO. The highest signal-to-noise ratio was obtained when 80 μg mL^{-1} GO was used, so we chose 80 μg mL^{-1} GO as the optimum assay condition.

In this proposed assay, the sensitivity could be dramatically increased using Exo III as a biocatalyst. As such, the Exo III concentration is also a key factor in this system. The experimental results showed that the sensitivity for detecting target DNA increased with increasing Exo III concentration in the range of 0–30 U. Taking into account the consumption of enzyme, 20 U Exo III was used in the final solution. The effect of the incubation time of Exo III and DNA was investigated. When the assay was conducted at 37 °C, the CL signal increased with increasing incubation time before reaching saturation after 2 h (Fig. S2†). The continuously increasing signal indicates that the designed Exo III-assisted target recycling was indeed taking place. Thus, the incubation time was fixed at 2 h.

With the fixed concentrations of GO and Exo III, the performance of the developed CL biosensing for DNA was still strongly influenced by the CL conditions, such as luminol concentration, H_2O_2 concentration, HRP concentration and media pH. Different assay conditions were investigated by fixing the concentrations at 100 nM probe and 0.01 nM target DNA. The experimental results showed that the optimal concentrations of luminol, H_2O_2 and HRP were 5.0×10^{-4} M, 1.0×10^{-3} M and 2.0×10^{-6} g mL^{-1}, respectively. The optimal pH for this CRET system was 11.

Analytical performance of the CRET biosensing for DNA

Under the optimized conditions, experiments were carried out adding increasing amounts of target DNA into the CRET system to examine whether the CL change could be used for DNA quantification. As shown in Fig. 3, the CL intensity increased with increasing target DNA concentration, to reveal a linear relationship in the target DNA concentration range from 0.01 pM to 1.0 pM. Control experiments showed that only slight CL changes occurred in the absence of Exo III (Fig. S3, ESI†). The detection limit (taken to be 3 times the standard deviation in the blank solution) was 9 fM, which was about 11-fold lower than the previously reported GO-based CRET system for DNA detection without Exo III amplification.[25] Furthermore, for comparison, we also measured the fluorescence signal of a GO-based FRET system with Exo III-assisted target recycling amplification in the presence of target DNA at concentration from 0.01 to 1.2 pM. The results (Fig. S4, ESI†) showed that the fluorescence intensity was almost unchanged, whereas the CL increased gradually with increasing the target DNA concentration. The sensitivity was about 3 orders of magnitude better than that of GO-based FRET sensor for DNA with Exo III-assisted amplification developed in 2012 by Zhao and co-workers.[36] As far as we known, the present assay is far more sensitive than most of the assays reported so far whose detection limits were at the pM level.[17]

The sequence specificity of the present CRET strategy for DNA detection was also investigated by testing the CL response to other DNA molecules, including a single-base mismatched DNA and a noncomplementary DNA (noncDNA). Fig. 4 shows the CL intensity changes with target DNA and other mismatched DNA strands. The completely complementary target DNA (cDNA), single-base mismatched DNA and noncDNA were distinctly discriminated under the same detection conditions. These results indicate the high specificity of the proposed CRET strategy for DNA detection.

Conclusion

In summary, a simple CRET biosensing platform for ultrasensitive DNA assay is proposed in this study. By taking advantage of the super quenching efficiency of GO and Exo III-assisted target recycling amplification, this proposed method exhibited high sensitivity towards target DNA with a detection limit of

9 fM, which is far more sensitive than most of the DNA assays reported so far. This assay is homogenous and occurs in the liquid phase, which makes it easy to automate by standard robotic manipulation of microwell plates. The assay avoids separation and wash steps, and the whole detection can be completed in 2.5 h. Furthermore, combined with aptamer (specific single-stranded oligonucleotides) recognition, the GO-based CRET system can be extended to sensitively detect non-nucleic acid targets (such as small molecules or protein).

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References

1 C. A. Heid, J. Stevens, K. J. Livak and P. M. Williams, *Genome Res.*, 1996, **6**, 986–994.
2 Y. S. Lie and C. J. Petropoulos, *Curr. Opin. Biotechnol.*, 1998, **9**, 43–48.
3 S. Song, Z. Liang, J. Zhang, L. Wang, G. Li and C. Fan, *Angew. Chem., Int. Ed.*, 2009, **48**, 8670–8674.
4 E. Tan, B. Erwin, S. Dames, K. Voelkerding and A. Niemz, *Clin. Chem.*, 2007, **53**, 2017–2020.
5 W. Xu, X. Xue, T. Li, H. Zeng and X. Liu, *Angew. Chem., Int. Ed.*, 2009, **48**, 6849–6852.
6 J. Zhao, T. Liu, Q. Fan and G. Li, *Chem. Commun.*, 2011, **47**, 5262–5264.
7 K. Okano and H. Kambara, *Anal. Biochem.*, 1995, **228**, 101–108.
8 X. Zuo, F. Xia, Y. Xiao and K. W. Plaxco, *J. Am. Chem. Soc.*, 2010, **132**, 1816–1818.
9 H. C. Yeh, J. J. Sharma, J. J. Han, S. J. Martinez and H. Werner, *Nano Lett.*, 2010, **10**, 3106–3110.
10 G. Bonnet, O. Krichevsky and A. Libchaber, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 8602–8606.
11 S. Bi, L. Li and Y. Cui, *Chem. Commun.*, 2012, **48**, 1018–1020.
12 Q. Fan, J. Zhao, H. Li, L. Zhu and G. Li, *Biosens. Bioelectron.*, 2012, **33**, 211–215.
13 H. Chen, J. Wang, G. Liang, P. Zhang and J. Kong, *Chem. Commun.*, 2012, **48**, 269–271.
14 Z. Zhou, Y. Du, L. Zhang and S. Dong, *Biosens. Bioelectron.*, 2012, **34**, 100–105.
15 Q. Xu, A. Cao, L. Zhang and C. Zhang, *Anal. Chem.*, 2012, **84**, 10845–10851.
16 F. Xuan, X. Luo and I. M. Hsing, *Anal. Chem.*, 2012, **84**, 5216–5220.
17 Y. Huang, S. Zhao, Y. Liu, J. Chen, Z. Chen, M. Shi and H. Liang, *Chem. Commun.*, 2012, **48**, 9400–9402.
18 L. Cui, G. Ke, C. Wang and C. J. Yang, *Analyst*, 2010, **135**, 2069–2073.
19 C. J. Yang, L. Cui, J. Huang, L. Yan, X. Lin, C. Wang, W. Zhang and H. Kang, *Biosens. Bioelectron.*, 2011, **27**, 119–124.
20 M. Zhang, Y. M. Guan and B. C. Ye, *Chem. Commun.*, 2011, **47**, 3478–3480.
21 H. Chen, H. Li and J. Lin, *Anal. Chem.*, 2012, **84**, 8871–8879.
22 Y. Qi and B. Li, *Chem.–Eur. J.*, 2011, **17**, 1642–1648.
23 J. S. Lee, H. A. Joung, M. G. Kim and C. B. Park, *ACS Nano*, 2012, **6**, 2978–2983.
24 X. Huang and J. Ren, *TrAC,Trends Anal. Chem.*, 2012, **40**, 77–89.
25 S. Bi, T. Zhao and B. Luo, *Chem. Commun.*, 2012, **48**, 106–108.
26 S. Zhao, J. Liu, Y. Huang and Y. Liu, *Chem. Commun.*, 2012, **48**, 699–701.
27 X. Huang and J. Ren, *Anal. Chim. Acta*, 2011, **686**, 115–120.
28 C.-H. Lu, H.-H. Yang, C.-L. Zhu, X. Chen and G.-N. Chen, *Angew. Chem.*, 2009, **121**, 4879–4881.
29 S. He, B. Song, D. Li, C. Zhu, W. Qi, Y. Wen, L. Wang, S. Song, H. Fang and C. Fan, *Adv. Funct. Mater.*, 2010, **20**, 453–459.
30 W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339.
31 J. Wang and J. Ren, *Electrophoresis*, 2005, **26**, 2402–2408.
32 S. Stankovich, D. A. Dikin, G. H. B. Dommett, K. M. Kohlhaas, E. J. Zimney, E. A. Stach, R. D. Piner, S. T. Nguyen and R. S. Ruoff, *Nature*, 2006, **442**, 282–286.
33 E. Morales-Narváez, B. Pérez-López, L. B. Pires and A. Merkoçi, *Carbon*, 2012, **50**, 2987–2993.
34 N. Varghese, U. Mogera, A. Govindaraj, A. Das, P. K. Maiti, A. K. Sood and C. N. R. Rao, *ChemPhysChem*, 2009, **10**, 206–210.
35 Y. Wang, Z. Li, D. Hu, C. Lin, J. Li and Y. Lin, *J. Am. Chem. Soc.*, 2010, **132**, 9274–9276.
36 X. Zhao, Q. Ma, X. Wu and X. Zhu, *Anal. Chim. Acta*, 2012, **727**, 67–70.