Published in final edited form as:

Biosens Bioelectron. 2015 April 15; 66: 520–526. doi:10.1016/j.bios.2014.11.035.

Highly sensitive DNA detection using cascade amplification strategy based on hybridization chain reaction and enzyme-induced metallization

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Abstract

A novel highly sensitive colorimetric assay for DNA detection using cascade amplification strategy based on hybridization chain reaction and enzyme-induced metallization was established. The DNA modified superparamagnetic beads were demonstrated to capture and enrich the target DNA in the hybridization buffer or human plasma. The hybridization chain reaction and enzyme-induced silver metallization on the gold nanoparticles were used as cascade signal amplification for the detection of target DNA. The metallization of silver on the gold nanoparticles induced a significant colour change from red to yellow until black depending on the concentration of the target DNA, which could be recognized by naked eyes. This method showed a good specificity for the target DNA detection, with the capability to discriminate single-base-pair mismatched DNA mutation (single nucleotide polymorphism). Meanwhile, this approach exhibited an excellent anti-interference capability with the convenience of the magnetic separation and washing, which enabled its usage in complex biological systems such as human blood plasma. As an added benefit, the utilization of hybridization chain reaction and enzyme-induced metallization improved detection sensitivity down to 10 pM, which is about 100-fold lower than that of traditional unamplified homogeneous assays.

Keywords

DNA detection; hybridization chain reaction; cascade amplification; metallization; Gold nanoparticles

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Introduction

In recent years, highly sensitive detection of specific DNA sequences associated with genetic and infectious diseases in complex media has become significantly important for early clinical diagnosis and gene therapy (Liu et al. 2013b; Ren et al. 2013). Due to the low abundance of disease specific DNA and the complexity of the biological samples, highly sensitive and selective approaches for DNA detection are required in clinical applications. To meet this challenge, many methods have been well developed, for example, electrochemical sensing (Kong et al. 2014; Liu et al. 2013a), fluorescence (Hu et al. 2013; Niu et al. 2010) and chemiluminescence detection (Li and He 2009; Wang et al. 2013c), and Förster resonance energy transfer (FRET) (Liu et al. 2013b; Su et al. 2014; Xing et al. 2013). To further boost the performance, signal amplification strategies with various enzymes are usually adopted, such as exonuclease III-assisted amplification (Gao and Li 2013; Luo et al. 2012), rolling circle amplification (Xu et al. 2012), and strand displacement amplification (Wang et al. 2011; Zhang et al. 2013). Among the various signal amplification strategies, the hybridization chain reaction (HCR) attracted particular attention because it doesn't need any enzymes or labelling processes (Dirks and Pierce 2004; Huang et al. 2011; Niu et al. 2010; Wang et al. 2013a). Huang et al. developed a DNA-amplified detection method, which combined the amplification of HCR and the fluorescence emission-switching property of the hairpin probes that were modified with the pyrene molecules. In their system, two hairpin probes H1* and H2* dual labelled with pyrene moieties at each end were used. Chain reactions of hybridization between alternating H1* and H2* can propagate along the target DNA to form nicked double-helix. In this case, a pyrene moiety on one probe was brought into close proximity to a pyrene moiety on the neighbouring probe, which could produce strong fluorescence.

The signal amplification based on metallization, especially silver metallization on the gold nanoparticles (AuNPs) have been used in the electrochemical biosensors and colorimetric detections (Lai et al. 2012; Nam et al. 2003; Xianyu et al. 2013). Mirkin and co-authors (Nam et al. 2003) developed a bio-barcode technology based on the polymerase chain reaction (PCR) amplification and silver metallization on the AuNPs for ultrasensitive detection of prostate-specific antigen (PSA). Jiang and co-authors (Xianyu et al. 2013) used glucose to reduce the growth of silver nanoparticles under the catalysis of the negatively charged AuNPs. Based on this, glucose detection in human plasma without any enzyme involvement was realized colorimetrically due to the generation of silver nanoparticles. Recently, a signal amplification strategy for highly sensitive colorimetric detection of avian influenza virus particles was reported (Zhou et al. 2014). The biometallization-based colorimetric assay was developed by combining the highly specific enzyme-induced silver metallization with the highly sensitive AuNPs induced silver deposition. The method was applied for the detection of alkaline phosphatase (ALP) and H₉N₂ avian influenza virus.

Magnetic beads have been widely used in biological sample preparation and detection assays due to their benefits of large surface-to-volume ratio, easy manipulation, flexible functionalization, and excellent biocompatibility (Chen et al. 2010; Yu et al. 2011; Yu et al. 2014; Yu et al. 2013). Owing to the ease of separation and enrichment of the targets, magnetic beads were widely used to improve the selectivity and sensitivity of various

analytical methods. For example, they were successfully used in cell sorting (Adams et al. 2008), protein (Csordas et al. 2010) and virus detection (Ferguson et al. 2011; Zhao et al. 2012), and aptamer screening (He et al. 2014). Meanwhile, the magnetic beads modified with DNA could be used for detection of the complementary target DNA.

Inspired by these works, for the first time, we propose a highly sensitive and selective biosensor that combines HCR signal amplification with enzyme-induced metallization for colorimetric sequence-specific DNA detection. Superparamagnetic beads (SPMBs) were modified with a capture DNA and used as a solid carrier to catch the target DNA. The HCR was carried on with alternative DNA hybridizations of two biotin labelled stem-loop DNAs. The HCR and the following alkaline phosphatase induced silver metallization on the negatively charged gold nanoparticles constituted the cascade signal amplification for the target DNA detection. The metallization of the silver on the gold nanoparticles induced a significant colour change from red of pure gold nanoparticles to yellow and black of the silver-covered AuNPs, which could be easily recognized by the naked eyes or measured by a UV-Vis spectrometer. This novel approach could discriminate between single-base mismatched DNA and double-base mismatched DNA, which demonstrated a great specificity for target DNA detection. Furthermore, this biosensor showed a great anti-interference capability and could be used in complex biological samples without any sample pre-treatment. We believe this method for the target DNA detection is well suitable for the point-of-care diagnosis in future clinical trials.

2. Materials and methods

2.1 Chemical reagents

L-glutathione (GSH), hydrogen tetrachloroaurate (III) hydrate (HAuCl_4), silver nitrate (AgNO_3) and sodium tetrahydridoborate (NaBH_4) were purchased from Alfa Aesar. Superparamagnetic beads (SPMBs, 500 nm diameter) with core iron oxide nanoparticles (70% by weight) and carboxyl groups on the surface were obtained from Ademtech SA (Pessac, France). N-(3-dimethyl-aminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and diethanolamine (DEA) were purchased from Alfa Aesar. Target DNA was purchased from Life technologies. All the other DNA samples were obtained from Bio Basic Inc., Canada. Streptavidin-alkaline phosphatase conjugate (SA-ALP) was purchased from Vector Laboratories Inc. (Burlingame, CA, USA). 4-Aminophenyl phosphate-sodium salt (4-APP) was obtained from Enzo Life Sciences, Inc. (Farmingdale, NY, USA). Bovine serum albumin (BSA), Tween 20 and Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) and 2-(N-morpholino) ethanesulfonic acid hydrate (MES) were obtained from Sigma-Aldrich. The human blood samples were collected in EDTA tubes from healthy donors according to approved institutional review board (IRB) protocol (IRB protocol number: 31216). Plasma was obtained after removal of blood cells and stored at -20°C until use. All the other chemical reagents not mentioned here were obtained from VWR (Radnor, PA, USA) and used without further purification.

2.2 Conjugating capture DNA (C_{DNA}) to SPMBs

The SPMBs with carboxyl groups were functionalized with C_{DNA} according to our previous reports (He et al. 2014; Yu et al. 2011; Yu et al. 2014; Yu et al. 2013). Briefly, 40 μ L of 50.0 mg/mL SPMBs were first washed three times with MES buffer (50.0 mM MES, pH = 6.0). Then, 10 μ L of 100 μ M C_{DNA} was added and incubated for 30 min at room temperature. Afterwards, 60 μ L of 10 mg/mL EDC dissolved in cold MES buffer was added into the above mixture solution to the final volume of 460 μ L. The reaction of the carboxyl groups and the amine groups was allowed for 12 hours with slow tilted rotation of 120 rpm at room temperature. After that, the C_{DNA} modified SPMBs (C_{DNA} -SPMBs) were separated by a magnet (DynaMagTM-Spin, Life Technologies), and washed three times with DNA wash buffer (10 mM Tris-HCl, 0.2 M NaCl, 0.05% Tween 20, pH 8.0). The C_{DNA} -SPMBs were kept in 1 mL TE buffer (10 mM Tris-HCl, 1mM EDTA, 0.05% Tween 20, pH 7.5) at 4 °C until further experiments.

2.3 Synthesis of GSH-capped AuNPs

GSH-capped AuNPs (GSH-AuNPs) was prepared according to the procedure described previously with some minor modification (Brinas et al. 2008; Chai et al. 2010; Kumar et al. 2013). The preparation scheme (Fig. S1) was shown in supplementary information S2. The as-prepared GSH-AuNPs was purified by Amicon Centrifugal Filter Unit (MWCO 30 kDa) to remove free agents and then dispersed in ultrapure water and stored at 4 °C until further experiments. The TEM characterization of the GSH-AuNPs was shown in Fig. S2 (Supplementary information S3). The size of the GSH-AuNPs was measured to be ~ 6 nm. The concentration of the GSH-AuNPs was determined by measuring the surface plasmon resonance (SPR) absorption peak at 520 nm as previously reported (Liu et al. 2007). The GSH-AuNPs were stable and could tolerate a high concentration of many metal ions except the Pb^{2+} (Chai et al. 2010).

2.4 Cascade amplification for detection of target DNA (T_{DNA})

A one-step hybridization reaction was performed by mixing the C_{DNA} -SPMBs modified SPMBs (C_{DNA} -SPMBs), the target DNA (T_{DNA}) and the Link DNA (L_{DNA}) together. In a typical experiment, 40 μ L of 2 mg/mL C_{DNA} -SPMBs were incubated in 1% BSA (0.01 M PBS, pH 7.4, 0.05% NaN_3) for 5 min in order to block the nonspecific adsorption. After rinsing, 50 μ L of T_{DNA} at various concentrations, 10 μ L of 10 μ M L_{DNA} and 340 μ L DNA hybridization buffer were added into the C_{DNA} -SPMBs and incubated for 30 minutes at room temperature with gentle shaking. The hybridization was carried out for 30 min at room temperature, which should be sufficient for hybridization between short complementary DNA (~30 base pairs) sequences (Chen et al. 2014; Hu et al. 2013; Liu et al. 2013b). Then, the hybrid-conjugated SPMBs were washed three times with the washing buffer (Tris-HCl 10 mM, Tween-20 0.01%, NaCl 100 mM, pH 7.5). Afterwards, the hybrid-conjugated SPMBs were immersed in 200 μ L of the HCR buffer (1 M NaCl, 50 mM Na_2HPO_4 , pH 7.5) with 1 μ M Biotin-H1 and 1 μ M Biotin-H2 and incubated at room temperature for 1 hour. After rinsed with 0.1 M phosphate buffer (PB, pH 7.4) containing 0.1% BSA and 0.05% Tween 20 for three times, 200 μ L of 5 μ g/mL SA-ALPs (0.1 M PB, 1 % BSA, pH 7.4) were added into the hybrid-conjugated SPMBs and incubated for 15 minutes and rinsed with 0.1

M PB (containing 0.1% BSA, 0.05% Tween 20, pH 7.4) for three times. Finally, 200 μ L of 8 mM 4-App (500 mM DEA buffer, pH 9.8) were added into the hybrid-conjugated SPMBs and incubated at 37 $^{\circ}$ C for 30 minutes. The hybrid-conjugated SPMB complex was separated by a magnet and the supernatant was collected into a new microcentrifuge tube. 5 μ L of GSH-AuNPs, 2 μ L of AgNO₃ were added into the supernatant and incubated 2 minutes for colorimetric detection by naked eye or ultraviolet–visible (UV-Vis) spectroscopy (SpectraMax M5 Microplate Reader, scan range 300–700 nm). The concentrations of the GSH-AuNPs and the AgNO₃ were kept at 15 nM and 2 mM, respectively. The whole detection process took about 2.5 hours.

2.5 Detection of the MT1_{DNA} and MT2_{DNA} in the hybridization buffer

In order to evaluate the selectivity of this method, we investigated two additional DNA sequences: a single-base-pair mismatched target (MT1_{DNA}) and a double-base mismatched target (MT2_{DNA}). The sequences of the MT1_{DNA} and MT2_{DNA} were listed in the supplementary information S1. MT1_{DNA} and MT2_{DNA} both at 1 nM were measured by the same method as described above and the results were compared with the result of the detection of the 1 nM T_{DNA} in the hybridization buffer.

2.6 Detection of T_{DNA} in human blood plasma sample

To assess the anti-interference capability of this method and thus its applicability in more complex biological systems, the analysis of the T_{DNA} in real human plasma was carried out. In this assay, T_{DNA} with different concentrations were spiked in to the human plasma, mimicking DNA detection in real biological samples. The results of detecting different concentrations of T_{DNA} in human plasma were compared with the performance of the T_{DNA} assay in the DNA hybridization buffer.

3. Results and discussion

3.1 Design principle of the cascade amplification approach for ultrasensitive target DNA detection

In this work, we chose a short DNA sequence related to human immunodeficiency virus (HIV) as the target (Hu et al. 2013). The principle of the cascade amplification approach is shown in Scheme 1. In this design, two complementary hairpins DNAs were labelled with biotin named biotin-H1 and biotin-H2, both of which have 18-base-pair stems, 6-base-pair loops and 6-base-pair sticky ends. The hairpins are stable and will not open or hybridize with each other at room temperature (Wang et al. 2013a). In the absence of T_{DNA}, the C_{DNA} on the C_{DNA}-SPMBs is unable to hybridize with the L_{DNA}, thus there is no specific sequence to open the stem structure of the biotin-H1 and biotin-H2. On the other hand, in the presence of the T_{DNA}, which can hybridize with part of the C_{DNA} on the C_{DNA}-SPMBs and part of the L_{DNA}, the L_{DNA} will be captured on the SPMBs. A specific sequence on the L_{DNA} can open the stem structure of the biotin-H1. The newly exposed sticky end of the biotin-H1 opens the hairpin of biotin-H2 to expose a newly sticky end on biotin-H2, which could in turn open the stem structure on another biotin-H1. In this way, each copy of the L_{DNA} can trigger the propagation of a chain reaction, where the hybridization events of alternating biotin-H1 and biotin-H2 form nicked double-helix (Scheme 1a). Since the two

hairpins, biotin-H1 and biotin-H2, are modified with biotin, SA-ALPs are then captured on the SPMBs through the high affinity between streptavidin and biotin. SA-ALPs triggers the conversion of inactive 4-APP to active 4-AP (Equation 1), which can induce the silver metallization on the negatively charged GSH-AuNPs (Equation 2). This metallization generates the colour change of the solution from red to yellow or black (Scheme 1b), which can be observed by naked eyes or measured by a spectrometer. It is worth to note that the presence of GSH-AuNPs can greatly enhance the enzyme-induced silver metallization reaction. The cascade amplification of HCR and enzyme-induced metallization results in a highly sensitive T_{DNA} detection. This method might be suitable for target DNA length from tens of base pairs to about a hundred base pairs.

3.2 Signal amplification by the HCR reaction

In order to confirm the signal amplification by the HCR reaction, we detected the 1 nM T_{DNA} with different HCR reaction conditions, while the other reaction conditions were kept the same. In the absence of the Biotin-H1 and Biotin-H2, 1 nM T_{DNA} showed no visible colour change (data not shown). The UV-Vis absorbance spectrum (Fig. 2, curve black) was similar to that of the absorbance of the GSH-AuNPs and there was no significant absorbance peak at 370 nm, which indicated that no silver nanoparticles were generated. Meanwhile, it showed that the GSH-AuNPs were stable without obvious aggregation even at a high concentration of the Ag^+ (2 mM), which would be suitable for the colorimetric detection. Also shown in Fig. 2, in the presence of 1 μ M Biotin-H1 or 1 μ M Biotin-H2 only, there were obvious UV absorbance peaks of the silver nanoparticles at 370 nm with the value of 1.36 and 1.44 respectively, which indicated that the SA-ALPs were conjugated to the SPMBs and the 4-AP induced the silver metallization on the surface of the GSH-AuNPs (Equations (1) and (2)). The biotin-H1 and biotin-H2 share an 18 base pair sequence that can open and hybridize to the L_{DNA} . Thus they can obtain a similar UV absorbance signal for the T_{DNA} detection (Fig. 2 blue and red curves). On the other hand, in the presence of both 1 μ M Biotin-H1 and 1 μ M Biotin-H2, the UV absorbance peak at 370 nm of the silver metallization on the gold nanoparticles reached to 2.62 (curve green), significantly higher than those with 1 μ M Biotin-H1 or 1 μ M Biotin-H2 alone.

3.3 Optimization of the HCR reaction time

The hybridization time of the biotin-H1 and biotin-H2 could influence the first stage signal amplification by the HCR reaction (Liu et al. 2013b). So the hybridization time of the HCR reaction needs to be optimized. In order to obtain high hybridization efficiency in the HCR reaction, we chose a relatively high concentration of the biotin-H1 and biotin-H2 for the HCR reaction. At the target DNA concentration of 1 nM in the presence of 1 μ M biotin-H1 and biotin-H2, the UV absorbance signal at 370 nm increased with the hybridization time from 10 minutes to 60 minutes (Fig. 3). However, it didn't change significantly when the

reaction time of the HCR was increased from 60 minutes to 120 minutes. This suggested that the signal by the HCR reached to a plateau around 60 minutes. Thus, 60 minutes was used as the optimal hybridization time for the target DNA detection.

3.4 Characterization of silver metallization on the AuNPs

The silver metallization on the AuNPs could be observed in the TEM images (Supplementary information S4, Fig. S3 and Fig. S4). In order to prove that the silver metallization on AuNPs, an Energy Dispersive X-ray spectrometer (EDX) experiment was carried out further on the same nanoparticles shown in Fig. S3c. From the result of the EDX (Fig. S5), there were strong characteristic peaks of the silver element at 3 KeV, 22 KeV and 25 KeV and those of the gold element (2 KeV, 12 KeV and 14 KeV). Fig. S6 showed the time-dependent UV absorption curves of the silver metallization on the GSH-AuNPs at different T_{DNA} concentrations monitored at 370 nm. In general, the UV absorbance increased with the reaction time. 2 minutes incubation time was adopted throughout our experiment for good signal magnitude and relatively short detection time, which is preferred for real world applications.

3.5 Analytical performance of the biosensor for target DNA detection

The cascade signal amplification by the HCR and enzyme-induced silver metallization on the gold nanoparticles were applied for quantitative detection of the T_{DNA} at the optimal experimental conditions determined above. Fig. 4a displayed the colour change of the solution when detecting different concentrations of the T_{DNA} . The significant colour change by the silver metallization on the GSH-AuNPs allowed identification of the T_{DNA} at very low concentration of 10 pM. Fig. 4b showed the UV-Vis absorbance spectra corresponding to the different concentrations of T_{DNA} (0 pM, 10 pM, 100 pM, 300 pM, 500 pM, 800 pM, 1 nM and 2 nM). The UV absorbance intensity at 370 nm increased as the concentration of the T_{DNA} increased from 10 pM to 2 nM. When the concentration of the T_{DNA} was higher than 2 nM, the UV absorbance intensity at 370 nm would exceed the detection range of the microplate reader. Meanwhile, a significant absorbance peak could be observed at 600 nm when the concentration of the T_{DNA} reached 2 nM. This is because the silver nanoparticles in the bulk grew and aggregated. Moreover, the linear range of target DNA was from 10 pM to 1 nM with coefficient of determination $R^2 = 0.998$ (Fig. 4c). Experiment at each concentration was repeated at least three times. The relative standard deviation (RSD) was 2.65% at the T_{DNA} concentration of 1 nM ($n = 8$), suggesting that this approach had good reproducibility. For target DNA detection, if there is no amplification strategy involved in the assay, the detection sensitivity of the method is commonly low, at nM or sub-nM level (Wang et al. 2013b; Zhu et al. 2013a; Zhu et al. 2013b). Using the cascade signal amplification strategy of the HCR and the enzyme-induced metallization improved the detection sensitivity down to 10 pM, which is about 100-fold lower than that of the conventional unamplified homogeneous assays (Hu et al. 2014). The detection sensitivity of this method could be compared to other amplification strategies (Gao and Li 2013; Kong et al. 2014; Luo et al. 2012; Zhang et al. 2013), in which the detection limits were reported commonly in the range of fM to nM. For our method, when the concentration of the T_{DNA} reached down to the 0.1–1 pM range, it was difficult to distinguish the colour change by the naked eyes. The UV absorbance intensities at 370 nm corresponding to the T_{DNA}

concentrations from 0.1 pM to 10 pM were shown in supplementary information S6 (Fig. S7). The detection of the T_{DNA} at this concentration range has a smaller sensitivity compared to that of the 10 pM to 1 nM concentration range, which suggests it might be difficult for quantitative detection of the T_{DNA} at this lower concentration range. However it was still useful for qualitative DNA detection in this low target DNA range of 0.1–10 pM, since the signals were significantly higher than the blank (0 pM T_{DNA}) plus three times of the standard deviation (SD) of the noise measured by the negative controls.

3.6 Detection specificity of the DNA biosensor

In order to evaluate the selectivity of this method, we investigated three different DNA sequences including the perfectly complementary target DNA (T_{DNA}), single-base mismatched target DNA ($MT1_{DNA}$) and double-base mismatched target DNA ($MT2_{DNA}$) at the same concentration. With a high stringency washing, the selectivity of this method mainly depends on the DNA hybridization. 1 nM of the $MT1_{DNA}$ and $MT2_{DNA}$ were measured by the same method and the results were compared with the result of the 1 nM T_{DNA} in the hybridization buffer. As shown in Fig. 5, the response of the $MT1_{DNA}$ was 56 % of that of the perfectly complementary target T_{DNA} due to the partially hybridization between the T_{DNA} and the L_{DNA} . At this signal level, with the excellent reproducibility of the method, the sensor exhibited good selectivity to discriminate the perfectly complementary target T_{DNA} and single-base-pair mismatched mutation $MT1_{DNA}$. Meanwhile, the UV absorbance of the 1 nM double-base-pair mismatched $MT2_{DNA}$ was only 0.63, which was lower than that generated by 10 pM of the perfectly complementary target. The high selectivity of the proposed colorimetric DNA sensing strategy can be contributed to the efficient separation of the magnetic beads and washing process as well as the strong affinity provided by the DNA hybridization.

3.7 Excellent anti-interference capability of this approach

An approach with excellent anti-interference capability for the target DNA detection is highly desirable for real-world samples such as serum, plasma, whole blood or urine, because these samples can potentially have ingredients comprising over 1000 different proteins with wide concentration ranges (Yu et al. 2013), DNA and RNA species with various length, ions and metabolites, all of which might interfere with the detection of the target DNA. To investigate the anti-interference capability of this method, we performed spiking experiments with human plasma, one of the most complicated body fluid samples. In this assay, the hybridization buffer and the plasma without T_{DNA} spiked-in were used as negative controls. From the results (Fig. 6), the UV absorbance signals at 370 nm of the negative controls were both lower than the expected signal from the established detection limit, suggesting blood plasma itself didn't generate much additional signal beyond the background noise in the hybridization buffer. Then, 1 nM target DNA was spiked into the human plasma samples. As shown in Fig. 6, there was no significant difference between the T_{DNA} detection in the hybridization buffer and that in the blood plasma. Again, this excellent anti-interference capability could be attributed to the heterogeneity and the high specificity of the DNA hybridization. The heterogeneous DNA hybridization was taken place on the surfaces of the magnetic beads. The unreacted substances and interfering materials could be easily removed from the colorimetric detection system. This suggests that

the DNA biosensor system can be used for complex real-world samples without complicated sample pre-treatment and expensive instruments, which is well suitable for point-of-care diagnostic applications.

Conclusions

In summary, a highly sensitive and selective DNA biosensor using a novel cascade amplification strategy based on hybridization chain reaction and enzyme-induced metallization was established. The hybridization chain reaction magnifies the detection signal by propagation of a chain reaction to form nicked double-helix with biotin labeled hairpin-DNAs. The streptavidin conjugated enzyme induces silver metallization on the negatively charged gold nanoparticles, which results in the colour change of the solution from red to yellow or black depending on the concentration of the target DNA. Both steps improved the detection limit while the end colorimetric assay could be easily visualized by naked eyes, a highly favorable feature for point-of-care applications. Linear quantitative detection range of the target DNA was determined to be 10 pM to 1 nM, while the target DNA concentration down to 0.1 pM could be detected qualitatively. In addition to high sensitivity, our method showed a great selectivity for the target DNA detection by discriminating single-base-pair and double-base-pair mismatched DNA mutations from the target DNA. This method was also shown to have an excellent anti-interference capability, and thus could be used for target DNA detection in complex media, such as human plasma, without any sample pre-treatment and expensive instruments. Furthermore, this universal method can potentially be applied in the detection of protein, bacteria, cell or virus, if the target DNA and the link DNA are replaced by these targets and their corresponding aptamers (Fig. S8). We believe this biosensor platform may find important point-of-care applications in clinical diagnosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Penn State Materials Research Institute and Huck Institute of Life Sciences for their support. This work is partially supported by the Pennsylvania State University start-up fund to S.-Y. Zheng and the National Institutes of Health grant under award number DP2CA174508. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at
doi:xxxx.xxxx

Highlights

1. A highly sensitive DNA detection using a cascade amplification strategy based on hybridization chain reaction and enzyme-induced metallization was established.
2. The metalization of silver on the gold nanoparticles induces a significant color change from red to yellow or black, which can be recognized by naked eyes.
3. The DNA sensor has been demonstrated with great selectivity and excellent anti-inference capability.
4. This universal cascade amplification strategy method could be applied in the detection of many other biological targets.

Fig. 1. Schematic illustration of the DNA biosensor based on cascade amplification of two stages: hybridization chain reaction (a) and enzyme-induced silver metallization on the AuNPs (b).

Fig. 2.

UV-Vis spectra responding to 1 nM T_{DNA} in the absence of Biotin-H1 and Biotin-H2 (curve black), in the presence of 1 μM Biotin-H1 only (curve blue), 1 μM Biotin-H2 only (curve red), and 1 μM Biotin-H1 and 1 μM Biotin-H2 both (curve green).

Fig. 3. UV-Vis spectra responding to 1 nM T_{DNA} under different reaction time of the HCR from 10 minutes to 120 minutes. Experimental conditions were SPMBs- C_{DNA} , 40 μ L; 200 μ L 1 μ M Biotin-H1 and 1 μ M Biotin-H2 at room temperature; SA-ALP, 5 μ g/mL; 4-App, 8 mM; GSH-AuNPs, 15 nM; $AgNO_3$, 2 mM.

Fig. 4.

Detection of the T_{DNA} at different concentrations. (A) Photos showing colorimetric changes when the T_{DNA} concentration increased from 0 pM to 2 mM. (B) UV-Vis absorbance spectra for detection of 0 pM, 10 pM, 100 pM, 300 pM, 500 pM, 800 pM, 1 nM and 2 nM T_{DNA}. (C) The UV absorbance different concentrations of the T_{DNA}. The linear fitting of the UV absorbance at 370 nm corresponding to the concentration of the T_{DNA} from 10 pM to 1 nM.

Fig. 5.

UV absorbance at 370 nm for the detection of different DNA sequences: no target DNA (labelled as 0), 1 nM T_{DNA}, 1 nM single-base mismatched target DNA (MT1_{DNA}) and 1 nM double-base mismatched target DNA (MT2_{DNA}).

Fig. 6.

UV absorbance at 370 nm corresponding to 0 nM T_{DNA} and 1nM T_{DNA} detection in hybridization buffer and human plasma.