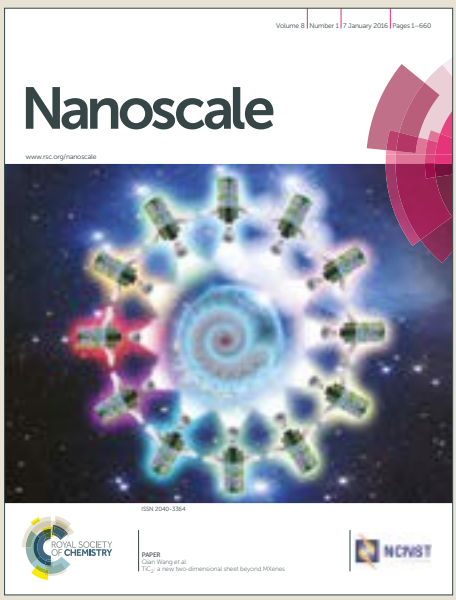
Nanoscale

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: J. Zheng, X. Ji, M. Du, S. Tian and Z. He, *Nanoscale*, 2018, DOI: 10.1039/C8NR05206A.



ROYAL SOCIETY OF CHEMISTRY

rsc.li/nanoscale

View Article Online
DOI: 10.1039/C8NR05206A

Journal Name

ARTICLE

Rational Construction of a DNA Nanomachine for HIV Nucleic Acids Ultrasensitive Sensing

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

DOI: 10.1039/x0xx00000x

www.rsc.org/

Jiao Zheng, Xinghu Ji, Mingyuan Du, Songbai Tian, Zhike He*

HIV nucleic acids, one kind of significant biomarker, play an inportant role in fundamental studies and clinical diagnosis. Importantly, the early accurate diagnosis for HIV nucleic acid at ultralow concentrations can potentially extend the life of patients. In current work, we developed a DNA nanomachine on gold nanoparticles (AuNPs) coupling rolling circle amplification and DNA walker cascade amplification for ultrasensitive detection of HIV nucleic acid. This DNA nanomachine sensing strategy exhibits a significantly low detection limit down to 1.46 fM. Furthermore, this DNA nanomachine biosensor is capable of detecting target DNA in real samples because of its high selectivity and sensitivity. Moreover, the DNA nanomachine biosensor is capable of discriminating single-based mismatch lower than 3.5 pM. The results showed that this DNA nanomachine biosensor has the potential for biomedical study and clinical application.

1. Introduction

DNA detection at trace level plays a crucial role in the fields ranging from virus detection to disease diagnosis.[1] Of late, a series of very powerful nucleic acid amplification techniques have been developed for sensitive detection of nucleic acid, such as polymerase chain reaction (PCR)[2], strand displacement amplification (SDA)[3], the hybridization chain reaction (HCR)[4], loop-mediated isothermal amplification of DNA[5](LAMP) and rolling circle amplification (RCA)[6]. Based on these amplification strategies, the trace level DNA detection has been widely developed.[7] Despite some advances in this field, but most of these methods suffer from inherent drawbacks. For example, in PCR amplification, special instrumentation is needed and it is difficult to achieve quantitative detection. Recently, DNA nanomachine technologies, which operate via the sequence-specific interaction that bind complementary oligonucleotides together in a double helix, have been used as simple, modular strategies for ultrasensitive DNA detection.

DNA nanomachines are viewed as fantastically sensitive biosensors that are capable of performing machine-like functions at the molecular level.[8] Most DNA nanomachines are based on the DNA self-assembly of well-defined Watson-Crick base pairing rule, including DNA walkers, DNA tweezers and DNA robots.[9] Due to DNA nanomachines can be simply and effectively operated to perform cascade amplification, exhibiting great potential in biosensors.[10] Of late, well-defined DNA nanomachine has attracted astonishing studies in realizing trace level biosensors in response to an external trigger.[11] DNA walker cascade amplification is an enzyme-free amplification strategy，which has been proven effective in amplifying and transducing signals for DNA nanomachine biosensors. Such DNA nanomachine biosensors work with negligible background and could operate in variety modalities, such as Fan et al. designed a stochastic DNA walker that autonomously moved on a spherical nucleic acid-based 3D track[7c] and Le et al. introduced an operation of a binding-induced DNA nanomachine that can be activated by proteins and nucleic acids.[8a] Both DNA nanomachines were based on DNA hybridization onto DNA-functionalized nanoparticle surfaces (e.g., in the form of a spherical nucleic acid (SNA)) which is known to be enhanced relative to hybridization free in solution.[12] This is the case for many of the SNA's uses as probes for high-sensitivity detection, where target concentration is relatively low with respect to probe.[12b] Thus, DNA walker cascade

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, Wuhan University, 430072, P. R. China. E-mail: zhkhe@whu.edu.cn
Electronic Supplementary Information (ESI) available: Experimental details and figures. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C8NR05206A

amplification on DNA-functionalized nanoparticle surfaces could be utilized as the versatile end-point transducer for the development of DNA nanomachines. Target recycling amplification strategy has attracted increasing interest in constructing DNA nanomachines for biomolecules high sensitive detection.[13] RCA is an isothermal nucleic acid amplification strategy, in which a long single-stranded DNA can be synthesized on a short circular single-stranded padlock DNA using DNA or RNA as primer.[14] The synthesized long single-stranded DNA consists of thousands of catalysts which can be used as triggers for initiating DNA nanomachines.[15] DNA walker cascade amplification reaction has previously provided a useful means for both amplifying and transuding signals from nucleic acid analytes.[16] Thus, the DNA nanomachine based on RCA and DNA cascade amplification could be used for ultrasensitive detection of specific nucleic acid. Inspired by these constructions, a simple DNA nanomachine was developed for sensitive and selective detection of human immune-deficiency virus (HIV) nucleic acid.

2. Experimental section

2.1 Materials

Solution of gold nanoparticles (28.3 nm in average diameter) was synthesized as previously reported.[17] Tris(hydroxy-methyl) aminomethane hydrochloride (Tris), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), sodium chloride (NaCl), potassium chloride (KCl) were purchased from Sigma-Aldrich (USA). T4 DNA Ligase (5 Weiss U/μL), phi29 DNA polymerase (10 U/μL) and 10 mM dNTP were purchased from Thermo (Dalian, China). Hydrochloric acid (HCl) was purchased from Sinopharm Chemical Reagent Co., Ltd. All of the other reagents were of analytical-reagent grade or better. Ultrapure water was produced by a Milli-Q Academic purification set (Millipore, Bedford, MA, USA).

All oligonucleotides with different sequences purified by HPLC were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of the oligonucleotide used in this work were showed as Table S1. Fluorescence spectra were recorded with a RF-5301PC spectro-photometer (Shimadzu, Japan) equipped with a 150W xenon lamp (Ushio Inc, Japan), and the fluorescence emission intensity was measured at 518 nm with an excitation wavelength of 480 nm. Gels were imaged by a ChemiDoc XRD system (Bio-Rad). Transmission electron microscopy (TEM) images were recorded on a JEOL Ltd. JEM 2100 electron microscope (Japan). The pH of solutions was measured by a pB-10 potentiometer (Sartorius).

2.2 The synthesis of gold nanoparticle and preparation of polyvalent oligonucleotides gold nanoparticle conjugates

The synthesis of 28.3 nm sized gold nanoparticle solution was as previously reported.[17] In brief, 12.5 mL of 40 mM sodium citrate was added to a boiling, vigorously stirred solution of $HAuCl_4$ (125 mL, 254 μM $HAuCl_4 \cdot 3H_2O$) at 125 – 150 °C. The boiling $HAuCl_4$ solution was resulted in a color change from pale yellow to dark red over several minutes. After 10 min persistent boiling of $HAuCl_4$ solution, the synthetic gold nanoparticles solution was removed from the heating silicone oil kettle. The solution was expected to keep stirring for another 15 min, and then cooled to room temperature. Thus prepared AuNPs were stored in brown reagent bottle at 4 °C.

AuNPs were modified with thiolated oligonucleotides (H1) based on the previously reported protocol.[17] 2 OD of H1 (100 μM) was added to 4.4 mL of AuNPs solution and then stirred overnight at room temperature. After this mixed solution incubation for 16 h at room temperature, phosphate buffer (0.15 M NaCl, pH 7.4) was added to the mixture for five times at 30 min interval to reach a final concentration of 0.1 M NaCl. Excess oligonucleotides were then removed via three successive rounds of centrifugation (15,000 rcf, 20 min) at 4°C. The precipitate was washed with PBS (100 mM NaCl, 10 mM phosphate, pH 7.4), with repetitive centrifugation and dispersion, the H1-AuNPs conjugates were finally resuspended in phosphate buffered saline (PBS) (100 mM NaCl, 10 mM phosphate, pH 7.4) and stored at 4 °C.

2.3 Assay Protocol for Target HIV nucleic acid

The ligation reaction was carried out with a 10 μL reaction mixture containing 1 × ligation buffer [40 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 0.5 mM ATP (pH 7.8)], 20 U of T4 DNA ligase, 20 μL the padlock (100 nM) and 20 μL target HIV DNA with various concentrations. Before adding T4 DNA ligase and ligation buffer, the oligonucleotide mixture was denatured at 90 °C for 10 min, and cooled slowly to room temperature over a 10-min period. After annealing, T4 DNA ligase and ligation buffer were added to the mixture and incubated at 37 °C for 2 h. During this process, the padlock probe was ligated in the presence of primer

View Article Online
DOI: 10.1039/C8NR05206A

and ligase (Scheme 1A). Afterward, the added T4 DNA ligase was denatured by heating at 90 °C for 10 min.

Next, the resulting product was used for the rolling circle amplification reaction. Initially, the above-prepared mixture was diluted to 200 μL with 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 10 mM $(NH4)_2SO_4$, 1 mM each dNTP and 10 U Phi29 DNA polymerase. Then the RCA reaction was conducted at 37 °C for 3 h, and terminated by heating at 90 °C for 10 min. During this process, the RCA product autonomously replicated a multiple machinery cutter cycle and generated accumulated amount of products (Scheme 1). The procedures of Nb.BtsI assay were almost similar to that of RCA reaction mentioned above, except that the digestion reaction was produced in the buffer solution of Nb.BtsI containing 50 mM Potassium Acetate, 20 mM Tris-acetate, 10 mM Magnesium Acetate, 100 μg/ml BSA (pH = 7.9). After that, 30 μL H1 conjugated AuNPs solution and 33.3 nM H2 were simultaneously injected into the mixture, and incubated for 200 min at 37 °C. Following that, the resulting solution was removed into a quartz cuvette recorded with a RF-5301PC spectrophotometer (Shimadzu, Japan) equipped with a 150W xenon lamp (Ushio Inc, Japan), and the fluorescence emission intensity was measured at 518 nm with an excitation wavelength of 480 nm.

2.4 Agarose gel electrophoresis

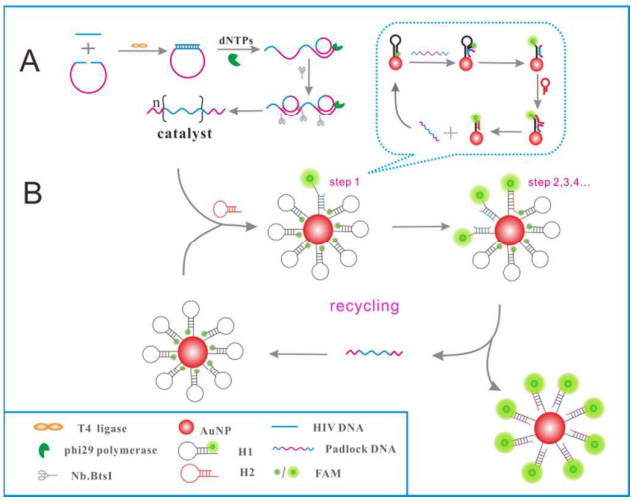
In a typical procedure, the padlock and primer DNA was diluted with 20 mM Tris-HCl buffer solution (140 mM NaCl, 5 mM KCl, pH = 7.4) and denatured at 90 °C for 10 min followed with a slow annealing treatment to room temperature. The gel electrophoresis were registered by adding 5 μL of different DNA solution (final concentration was 10 μM) in the mixture of loading buffer and 3.0 μL of bromophenol blue, respectively. The mixture was stained with 100×SYBR Green I (1 μL). 3% agarose gel was prepared by using 1×TAE buffer (40 mM Tris, AcOH, 2 mM Na_2EDTA, pH 8.5). The electrophoresis was carried out at 100 V for approximately 40 min in 1×TAE buffer. Then the gel was imaged by a ChemiDoc XRD system (Bio-Rad).

2.5 In vitro detection of target HIV nucleic acid

The performance of the DNA nanomachine responding to HIV DNA was investigated by the fluorescence intensity of the dye recovered from AuNPs surface. Unless otherwise stated, sample solutions contained 30 μL DNA-functionalized AuNPs, 33.3 nM H2, different concentrations of HIV DNA (primer) in 20 mM Tris-HCl (pH 7.4) buffer containing 140 mM NaCl, 5 mM KCl. After that, the stock solution of DNA walker cascade amplification reactions was incubated for 200 min at 37 °C. The fluorescence intensity was measured by using 480 nm for excitation and 518 nm for emission wavelength, respectively.

3.Results and discussion

3.1 The working principle of DNA nanomachine



Scheme 1 Schematic illustration of the principle for the sensitive detection of HIV DNA by DNA nanomachine (inset: the DNA walker cascade amplification process on AuNP surface).

As shown in Scheme 1, the proposed strategy for DNA nanomachine biosensor assay involves two steps: (A) RCA reaction and (B) catalytic recycling for DNA walker cascade amplification on AuNPs surface. The HIV specific nucleic acid (target) was chosen as a primer in RCA reaction, which consists of a hybridization sequence with padlock DNA at the 5' and 3' ends. The bases of padlock DNA both ends are specifically ligated and circularized with primer DNA as a template by the T4 DNA ligase. In this case, the RCA reaction was driven by the addition of phi29 DNA polymerase. The RCA product contained hundreds of repetitive catalytic sequences for the DNA walker cascade amplification reaction (designed as catalyst for the trigger of DNA walker cascade amplification). Then, the extended strand contained a recognition sequence (3'CGTCACNN5', N is any nucleotide) that was nicked by the nicking enzyme Nb.BtsI, which produced hundreds of catalyst for the trigger of DNA walker

View Article Online
DOI: 10.1039/C8NR05206A

and the catalyst as a initiating agent could transfer to next round. In detail, the catalysts as initiators used to initiate hairpin DNA (H1) conjugated on 28.3 nm gold nanoparticles (AuNP) surface (Fig. 1A, S1, ESI†) and H2 assembly to produce numerous H1/H2 duplexes, which resulted in liberating the FAM (5-carboxyfluorescein)-labeled DNA payload and turning on the fluorescence. Meanwhile, the duplexes could release the catalyst, thereby promoting the DNA walker cascade amplification and the operation of the DNA nanomachine biosensors. In addition, gold nanoparticles, as a platform, constructed a three-dimensional spherical nucleic acid (SNA) track which made the operation locally ordered on nanoparticle surface and the corresponding increases in local effective concentrations of H1 on AuNP surface also accelerated the catalytic efficiency just because DNA hybridization on the SNA differs significantly from hybridization free in solution and which resulted in highly sensitive detection[12b]. Therefore, the DNA nanomachine biosensor could realize HIV specific nucleic acid highly sensitive detection.

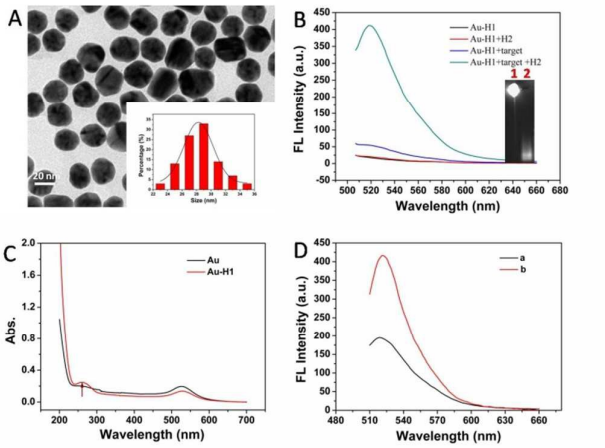


Fig. 1 (A) TEM images of the synthesis of AuNPs. The inset is the size distribution of AuNPs. (B) Fluorescence intensity of amplification products in the absence (red curve) and presence (green curve) of target DNA (3.3 pM), and RCA products hybridize with Au-H1 in the absence of H2 (blue curve). Inset: agarose gel electrophoresis (3%) of amplification products by the RCA reaction in the presence (1) and absence (2) of Phi29 DNA polymerase. (C) UV-vis absorption spectra of different solutions: AuNPs (black curve), AuNPs conjugated with H1 (red curve). (D) The fluorescence intensity of H1 conjugated on AuNPs in the absence (a) or presence (b) of nicking enzyme at the same concentration of target HIV DNA (3.3 pM).

3.2 Characterizations of Au, Au-H1 and feasibility of this DNA nanomachine

The biosensor of DNA nanomachine was triggered by the nicked RCA product (catalyst), and the fluorescence signal was positive correlation with the concentration of target HIV specific nucleic acid. As shown in Fig. 1B, in the absence of target HIV DNA, the RCA reaction could not happen and the DNA walker could not be powered, which resulted in the quenched fluorescence of FAM (red curve) could not be restored. On the contrary, in the presence of target HIV DNA, the RCA reaction could happen successfully and the DNA walker could be triggered, which resulted in a strong increase of the fluorescence intensity (green curve). These results demonstrated that the RCA product was significant for DNA nanomachine biosensor to obtain strong fluorescence signal. The image of gel electrophoresis was also provided to clarify the RCA product could be formed in the presence and absence of specific HIV DNA (Fig. 1B inset). In lane 2, no RCA product was appeared and a distinct band of padlock/specific HIV DNA complex was obtained in the absence of phi29 DNA polymerase. Upon the phi29 DNA polymerase, target HIV DNA and primer DNA introduction, the RCA reaction could be triggered and a long single-strand DNA was produced. The RCA product aggregated at the beginning of lane 1 and can't migrate, thereby a strong spot at the beginning of lane 1 was observed. For comparison, the DNA walker cascade amplification was investigated based on the fluorescence intensity recovery of fluorophores which tagged on H1 on AuNP surface with presence or absence of H2 (Fig. 1B, green curve and blue curve, respectively). Due to catalyst hybridize with H1 in an equivalent reaction ratio (1:1)，the catalyst can't walk to the next round and the DNA walker cascade amplification can't be triggered in the absence of H2. Proven that catalyst could be exchanged by H2 and which would resolve into the most thermodynamically favorable configuration H1-H2, the H1-H2 duplex and target is able to yield multiple signal outputs (1:m). Then with displacement of the walking catalyst which could generate a 2–3 orders of magnitude higher fluorescence signal than that of no DNA walker cascade amplification experiments. Based on these results, a DNA nanomachine biosensor could be proposed for HIV DNA detection. As shown in Fig. 1C, the characteristic absorption peak of nucleic acid can be seen in purified Au-H1 solutions.

View Article Online
DOI: 10.1039/C8NR05206A

To prove the significant role of the nicking enzyme (Nb.BtsI) in this DNA nanomachine biosensor, the long RCA product carried with the catalytic sequence strand were directly hybridized with the hairpin H1 modified on AuNP surface. In the absence of nicking enzyme, compared with the results of the introduction of nicking enzyme, the fluorescence intensity was lower even in the same concentration of target HIV DNA (Fig. 1D). The reason was probably that the excess H1 on AuNP surface resulted in a strong steric inhibition. In addition, the amplification efficiency was decreased by the long RCA product carried with the catalytic sequence strand. In other words, the nicking enzyme could greatly improve the sensitivity of the proposed method for low abundance of target HIV DNA detection.

3.3 Optimization of detection conditions

To achieve an optimal analytical performance for the DNA nanomachine biosensor，the concentration of T4 DNA ligase, phi29 DNA polymerase and the length of padlock DNA should be investigated in detail by using 3.0 pM HIV DNA as an example. The optimal hybridization time for the DNA walker of constructed RCA-based DNA nanomachine was studied to meet the goal of efficient payload release. Due to different enzymes have different reactivity in the same buffer solution, then according to an investigation of the limited influence (Fig. 2A, B, C, S2, ESI†) 20 U T4 DNA ligase, 15 U Nb. BtsI, 10 U phi29 DNA polymerase condition and P3 were chosen for the RCA reaction. On the other hand, hybridization time for the DNA Walker Cascade Amplification on AuNPs nanoparticle surface was investigated toward efficient payload release. Fig. 2D revealed the fluorescence signals corresponding to different time periods. The fluorescence intensity of FAM dye (5-carboxyfluorescein) was very slight at first and then increased gradually before reaching a platform. 200 min was selected as the optimum reaction time of DNA nanomachine.

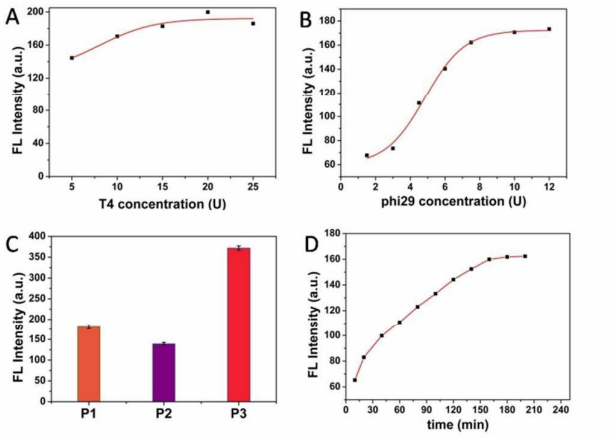


Fig. 2 The effects of (A) the concentration of T4 DNA ligase, (B) the concentration of phi29 DNA polymerase, (C) the length of padlock for the RCA-based DNA machine, and (D) hybridization time for the DNA Walker Cascade Amplification on AuNPs nanoparticle surface. Padlock-3: 33.3 nM, HIV DNA: 3.0 pM, RCA reaction: 37 °C for 3 h. Rolling circle amplification-assisted target recycling and DNA walker cascade amplification reaction: 37 °C for 200 min. The error bar represents the standard deviation of three measurements.

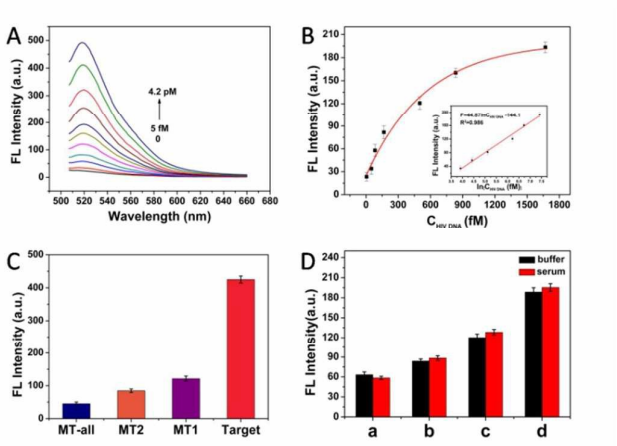


Fig. 3 (A) Fluorescence emission spectra of various concentration of target HIV DNA. (B) Changes of HIV DNA concentration. Insert is the linear relationship between the fluorescence intensity and the logarithm of target HIV DNA concentration. (C) The specificity of the RCA-based DNA machine in the same concentration (3.5 pM). (D) Comparison of the assay results toward target HIV DNA into buffer and serum (a, b, c, d represent the different concentration of specific HIV DNA).

3.4 Performance of DNA nanomachine for HIV nucleic acid

To achieve the best biosensing performance for RCA and DNA walker cascade amplification-based DNA nanomachine, some experimental conditions, e.g. the concentration of Phi29 DNA polymerase, T4 ligase and nicking enzyme Nb.BtsI; the operation time for DNA nanomachine biosensor were investigated in detail by using 3.0 pM HIV DNA as an example . Under the optimal conditions, the sensitivity and dynamic range of the DNA nanomachine biosensor were evaluated toward HIV DNA with various concentrations by using the designed method. As shown in Fig. 3A,

View Article Online
DOI: 10.1039/C8NR05206A

with the increasing of the concentration of target HIV DNA, large numbers of the catalyst are produced for hairpin DNA cascade amplification on AuNPs surface to restore the fluorescence of FAM-labeled H1, resulting in the increase of fluorescence intensity correspondingly. A linear relationship between the fluorescence intensity and the logarithm of HIV DNA concentration was obtained in the range from 5 fM to 1.67 pM with a detection limit of 1.46 fM estimated at the 3 times the average standard deviation of the blank response. The regression equation is F= 44.87lnCHIV DNA - 144.1 with a correlation coefficient of 0.986, where F and CHIV DNA represent the fluorescence intensity and the concentration of target HIV DNA, respectively. As can be seen from table 1, compared with reported methods, this proposed assay has achieved one of the most sensitivity for HIV DNA detection.

The specificity of the proposed DNA nanomachine biosensor was investigated by four kinds of DNA sequences,[18] including perfect catalytic target DNA (target), single-base mismatched DNA (MT1), two-base mismatched DNA (MT2) and arbitrary sequence DNA (MT-all) at the same concentration. As shown in Fig. 3C , only the target DNA could cause the strong signal and the mismatched DNA (MT1, MT2, MT-all) cause low fluorescence intensity. It was due to the high hybridization efficiency of the target DNA resulting from the RCA product could power the DNA nanomachine. All these observations validate the excellent selectivity of the present DNA nanomachine biosensor. Hence, this strategy exhibited good performance to distinguish the target DNA and the base mismatched targets.

Table 1 Comparison of the reported chemosensors for nucleic acid.

Detection method	Dynamic range	Limit of detection	Refs.
Fluorescence detection	0.2 nM-200 nM	0.2 nM	19
Electronic monitoring	5 fM-10 pM	2.56 fM	20
Electrochemical detection	1 pM-100 pM	1 pM	21
Fluorescence detection	Not mentioned	0.2 pM	22
Enzyme linked immunosorbent detection	5 fM-5 nM	5 fM	23
Lateral flow detection	0.1 pM-250 nM	0.01 pM	24
Colorimetric detection	10 pM-10 nM	2.5 pM	25
Fluorescence detection	5 fM-1.67 pM	1.46 fM	This method

In order to evaluate the applicability of the proposed biosensor, the DNA nanomachine biosensor was challenged for the determination of HIV specific nucleic acid in a complicated sample matrix by dispensing different concentrations (85 fM, 170 fM, 510 fM and 1.6 pM) of target HIV DNA with 20 mM Tris-HCl buffer solution (pH 7.4) and bovine serum via a standard addition method. As shown in Fig. 3D, the recovery (the ratio between the found concentration and added concentration) was calculated to be 92.53, 105.4, 106.8, and 103.6%, respectively, with a satisfactory RSD from 2.92% to 4.04 %. The results indicated that this proposed biosensor had a potential application in clinical determination of the HIV DNA.

4. Conclusion

In summary, a convenient and feasible DNA nanomachine biosensor with high sensitivity and selectivity was successfully designed. The RCA-assisted signal amplification could produce a large number of catalytic strands through nicking enzyme Nb.BtsI to initiate DNA walker cascade amplification for realizing HIV DNA ultrasensitive detection. Significantly, the developed DNA nanomachine can be easily extended for use with several various biomolecules and represents a versatile detection method.

5. Acknowledgements

This work was supported by National Natural Science Foundation of China (21675119) and National Major Science and Technology Projects (2018ZX10301405).

Conflicts of interest

There are no conflicts to declare.

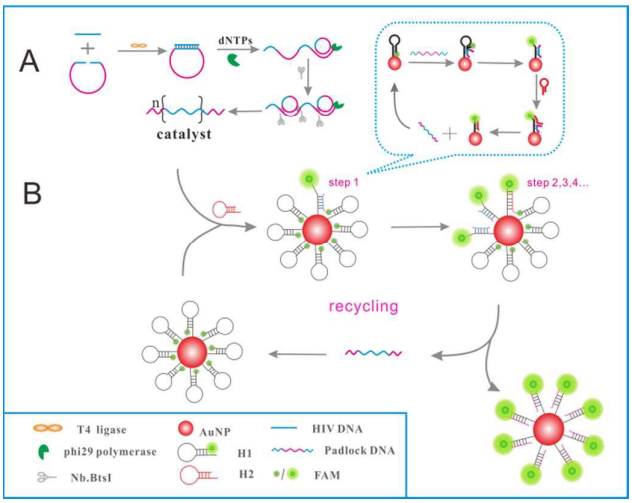
Notes and references

1. (a) C. P. Liang, P. Q. Ma, H. Liu, X. G. Guo, B. C. Yin, B. C. Ye, *Angew. Chem. Int. Ed.,* 2017, **56**, 9077; (b) S. P. Li, Q. Jiang, S. L. Liu, Y. L. Zhang, Y. H. Tian, C. Song, J. Wang, Y. G. Zou, G. J. Anderson, J. Y. Han, Y. Chang, Y. Liu, C. Zhang, L. Chen, G. B. Zhou, G. G. Nie, H. Yan, B. Q. Ding and Y. L. Zhao, *Nat. Biotechnol.,* 2018, **36**, 258.
2. G. L. Wang and C. Y. Zhang, *Anal. Chem.,* 2012, **84**, 7037.
3. F. Li, Y. N. Tang, S. M. Traynor, X. F. Li and X. C. Le, *Anal. Chem.*, 2016, **88**, 8152.
4. G. B. Zhou, M. H. Lin, P. Song, X. Q. Chen, J. Chao, L. H. Wang, Q. Huang, W. Huang, C. H. Fan, X. L. Zuo, *Anal. Chem.,* 2014, **86**, 7843.
5. J. K. F. Wong, S. P. Yip and T. M. H. Lee, *Small* 2014, **10**, 1495.

View Article Online
DOI: 10.1039/C8NR05206A

6. (a) Y. Q. Cheng, X. Zhang, Z. P. Li, X. X. Jiao, Y. C. Wang, Y. L. Zhang, *Angew. Chem. Int. Ed.,* 2009, **48**, 3268; (b) H. Y. Liu, L. Li, L. L. Du, X. Wang, Y. X. Xie, L. L. Tong, Q. Wang, B. Tang, *Anal. Chem.*, 2013, **85**, 7941; (c) Y.X. Zhao, Q. Lin, F. Chen, Y. H. Dong, Y. Kong, Y. Y. Wu, C. H. Fan, *Chem. Commun.,*2012. **48**, 3354.
7. (a) C. Feng, Z. H. Wang, T. S. Chen, X. X. Chen, D. S. Mao, J. Zhao and G. X. Li , *Anal. Chem.,* 2018, **90**, 2810; (b) Z. K. Wu, H. H. Fan, N. S. R. Satyavolu, W. J. Wang, R. Lake, J. H. Jiang and Y. Lu, *Angew. Chem. Int. Ed.***,** 2017, **56**, 8721; (c) X. M. Qu, D. Zhu, G. B. Yao, S. Su, J. Chao, H. J. Liu, X. L. Zuo, L. H. Wang, J. Y. Shi, L. H. Wang, W. Huang, H. Pei and C. H. Fan, *Angew. Chem. Int. Ed.,* 2017, **56**, 1855.
8. (a) H. Q. Zhang, M. D. Lai, A. Zuehlke, H. Y. Peng, X. F. Li and X. C. Le, *Angew. Chem. Int. Ed.,* 2015, **54**, 14326; (b) J. Zheng, N. X. Li, C. R. Li, X. X. Wang, Y. C. Liu, G. B. Mao, X. H. Ji and Z. K. He, *Biosens. Bioelectron.,* 2018, **107**, 40.
9. (a) W. Li, L. Wang and W. Jiang, *Chem. Commun.,* 2017, **53**, 5527; (b) P. Zhang, Z. Y. Li, H. J. Wang, Y. Zhuo, R. Yuan and Y. Q. Chai, *Nanoscale* 2017, **9**, 2310; M. Luo, Y. Z. Feng, T. W. Wang, J. G. Guan, *Adv. Funct. Mater.,2018, 1706110.*
10. X. Y. Jiang, H. J. Wang, H. J. Wang, Y. Zhuo, R. Yuan and Y. Q. Chai, *Anal. Chem.,* 2017, **89**, 4280; (b) C. Jung, P. B. Allen and A. D. Ellington, *Nat. Nanotech.,* 2016, **11**, 157.
11. D. X. Li, W. J. Zhou, R. Yuan and Y. Xiang, *Anal. Chem.,* 2017, **89**, 9934.
12.(a) K. Skakuj, S. Wang, L. Qin, A. Lee, B. Zhang, C. A. Mirkin, *J. Am. Chem. Soc.* 2018, **140**,1227; (b) L. K. Fong, Z.W. Wang, G. C. Schatz, E. Luijten, C. A. Mirkin, *J. Am. Chem. Soc.*, 2018, **140**, 6226-6230.
13. M. Q. He, K. Wang, W. J. Wang, Y. L. Yu, J. H. Wang, *Anal. Chem.,* 2017, **89**, 9292.
14. (a) Y. Y. Long, C. S. Zhou, C. M. Wang, H. L. Cai, C. Y. Yin, Q. F. Yang, and D. Xiao, *Sci. Rep.,* 2016, **6**, 23949; (b) R. J. Deng, L. H. Tang, Q. Q. Tian, Y. Wang, L. Lin, and J. H. Li, *Angew. Chem. Int. Ed.,* 2014, **53**, 2389.
15. J. Y. Zhuang, W. Q. Lai, G. N. Chen, D. P. Tang, *Chem. Commun.* 2014, **40**, 2935.
16. Y. Jiang, B. L. Li, J. N. Milligan, S. Bhadra, A. D. Ellington, *J. Am. Chem. Soc.* 2013, **135**, 7430.
17. W. G. Haiss, N. T. K. Thanh, J. Aveyard, D. G. Fernig, *Anal. Chem.* 2007, **79**, 4215.
18. (a) W. Y. Zhong, W. Y. Tang, J. Fan, J. M. Zhang, X. C. Zhou, Y. Z. Liu, *Chem. Commun.* 2018, **54**, 1311; (b) S. X. Chen, G. Seelig, *J. Am. Chem. Soc.* 2016, **138**, 5076.
19. H. L. Li, J. T. Ren, Y. Q. Liu, E. K. Wang, Chem. Commun., 2014, **50** (6), 704.
20. J. Y. Zhuang, D. P. Tang, W. Q. Lai, G. N. Chen, H. H. Yang, *Anal. Chem.*,2014, **86** (16), 8400.
21. S. F. Liu, T. Liu, L. Wang, *Chem. Commun.*,2015, **51**, 176.
22. B. J. Zou, Q. X. Song, J. P. Wang, Y. L. Liu, G. H. Zhou, *Chem. Commun.* 2014, **50**, 13722.
23. J. L. Wen, J. H. Chen, L. Zhuang, S. L. Zhou, *Biosens. Bioelectron.*, 2017, **90**, 481.
24. Y. Gao, X. L. Deng, W. Wen, X. H. Zhang, S. F. Wang, *Biosens. Bioelectron.*, 2017, **92**, 529.
25. J. Nie, D. W. Zhang, C. Tie, Y. L. Zhou, X. X. Zhang, Biosens. Bioelectron., 2014, **56**, 237.

Entry for the table of contents



A rational DNA Nanomachine was constructed for HIV Nucleic Acids Ultrasensitive Sensing.