Electronic Supplementary Information

Application of DNA machine in amplified DNA detection

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Experimental section

Materials. The synthesized oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). NMM was purchased from Porphyrin Products (Logan, UT). All the other chemicals were purchased from Aladin Ltd. (Shanghai, China) and used as received without further purification. The stock solution of NMM (1 mM) was prepared in dimethyl sulfoxide (DMSO) and stored in darkness at −20 ºC. The water used throughout all experiments was purified through a Millipore system. The sequence of the used oligonucleotide is listed as follows:

H1: 5’-GCT AGA GAT TTT CCA CAC TGA CTT CTC TAG CGG GTT TTG GGT TTT AGT CAG TGT GGA AAA-3’

H2: 5’-CTG ACT AAA ACC CAA AAC CCG CTA GAG AAG TCA GTG TGG AAA ATC TCT AGC GGG TTT TGG GTT TTG GGT TTT GGG-3’

Complementary target (THIV): 5’-AGT CAG TGT GGA AAA TCT CTA GC-3’

Target with base mismatch underlined:

T1AA: 5’-AGT CAG TGT GGA AAA ACT CTA GC-3’

T1AC: 5’-AGT CAG TGT GGA AAA CCT CTA GC-3’
T_{1AG}: 5’-AGT CAG TGT GGA AAA GCT CTA GC-3’
T_{1B}: 5’-AGT CAG TGT GCA AAA TCT CTA GC-3’
T_{1C}: 5’-AGT CAA TGT GGA AAA TCT CTA GC-3’
T_{2AB}: 5’-AGT CAG TGT GCA AAA ACT CTA GC-3’
T_{2AC}: 5’-AGT CAA TGT GCA AAA ACT CTA GC-3’
T_{2BC}: 5’-AGT CAA TGT GCA AAA ACT CTA GC-3’
T_{3}: 5’-AGT CAA TGT GCA AAA ACT CTA GC-3’

Longer target (T_L): 5’-TTTT AGT CAG TGT GGA AAA TCT CTA GC TTTT-3’ as a model DNA.

T_L with one-base mismatch (T_{L1}): 5’-TTTT AGT CAG TGT GCA AAA TCT CTA GC TTTT-3’

**Instruments.** Cary 500 Scan UV/Vis Spectrophotometer (Varian, USA) was used to quantify the oligonucleotides. DNA concentration was estimated by measuring the absorbance at 260 nm. Fluorescence intensities were recorded on a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., France). The emission spectra were recorded within the range of 540–720 nm upon excitation at 399 nm. All the experiments were carried out at room temperature (about 25 ºC) if not specified.

**DNA Detection.** The desired concentration of NMM or DNA was achieved by diluting the corresponding stock solution with 10 mM Tris-HCl buffer containing 5 mM MgCl₂ and 15 mM KCl (pH: 8.0), respectively. The final volume of each sample for fluorescence measurement is 500 µL 10 mM Tris-HCl buffer containing 5 mM MgCl₂ and 15 mM KCl (pH: 8.0). Excitation was at 399 nm, and the emission was monitored at 608 nm. Slit widths for both of the excitation and emission were set at 5 nm. To achieve high sensitivity, the assay mixture was pre-incubated for 12 h, allowing to reach efficient reaction before NMM was added.

**Native Polyacrylamide Gel Electrophoresis.** Polyacrylamide gel (12%) was prepared with 1×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3). 10 µL of each sample was mixed with 2 µL of Gel-Dye Super Buffer Mix before loading into the gels. The gel was run under a constant voltage of 110 V over a period
of about 2 h. Then the gel was photographed under UV light using a fluorescence imaging system (Vilber Lourmat, Marne laVallee, France).

Table S1. Comparison of the linear range and detection limit of various amplified DNA sensors.

<table>
<thead>
<tr>
<th>Method and Reference</th>
<th>Amplified DNA detection</th>
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<tbody>
<tr>
<td></td>
<td>Linear range</td>
</tr>
<tr>
<td>Molecular beacon in combination with DNAzyme</td>
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<tr>
<td>Label-free colorimetric detection of DNA based on DNAzyme</td>
<td>0.01–0.3 µM</td>
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<tr>
<td>Amplified fluorescence DNA detection using bimolecular beacons</td>
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<td>DNAzyme-based amplified detection of DNA</td>
<td>100 pM–10 nM</td>
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<td>G-quadruplex based hybridization chain reaction</td>
<td>20–120 nM</td>
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<tr>
<td>Autonomous assembly of polymers consisting of DNAzyme wires</td>
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<td>Detection method combining rolling circle amplification and surface-enhanced raman scattering spectroscopy</td>
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<td>PCR-based SERS method</td>
<td>Not given</td>
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<tr>
<td>RCA-based electrochemical method</td>
<td>1–600 nM</td>
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<td>Amplified fluorescence DNA detection based on Exonuclease III-aided target recycling</td>
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<td>An enzyme-amplified amperometric DNA hybridization assay</td>
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<td>Conjugated polyelectrolyte amplified thiazole orange emission for label free DNA detection</td>
<td>Not given</td>
</tr>
<tr>
<td>Our method</td>
<td>0.2 nM–100 nM</td>
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</table>

Reference

**Fig. S1.** Native polyacrylamide gel analysis of the catalytic circuit after 14 h reaction.
Lane 1: \(H1\); Lane 2: \(H2\); Lane 3: \(H1 + H2\); Lane 4: \(H1 + H2 + 200 \text{ nM } T_{HIV}\); Lane 5: \(H1 + H2 + 500 \text{ nM } T_{HIV}\); Lane 6: \(H1 + H2 + 1 \mu \text{M } T_{HIV}\); Lane 7: \(H1 + H2\) annealed. \([H1]=[H2]=1 \mu \text{M}\).

Discussion of Fig. S1: By comparing the position of DNA belts, it can be concluded that the hybridization between \(H1\) and \(H2\) occurred in the presence of initiator \(T_{HIV}\) (Lane 3 and 4). With the increment of \(T_{HIV}\) concentration, more \(H1\) and \(H2\) were consumed and their respective belt fade accordingly (From Lane 4 to 6).
**Fig. S2.** Fluorescence spectra of the system after mixing for 5 h under different conditions: (a) NMM; (b) NMM + H1; (c) NMM + H2; (d) NMM + H1 + H2; (e) NMM + H1 + H2 + T_{HIV}. \([T_{HIV}]=100\) nM, \([H1]=100\) nM, \([H2]=100\) nM, \([\text{NMM}]=400\) nM. All measurements were carried out in 10 mM Tris-HCl buffer containing 5 mM MgCl₂ and 15 mM KCl (pH: 8.0).

**Discussion of Fig. S2:** The fluorescence response of the system under different conditions was firstly compared, as shown in Fig. 1. The presence of only \(H1\) or \(H2\) enhanced the fluorescence intensity of NMM slightly. The mixture of \(H1\) and \(H2\) did not enhance the fluorescence intensity evidently either, indicating that both of the hairpins are stable enough to coexist in solution. However, upon the presence of target \(T_{HIV}\), the fluorescence intensity was greatly enhanced, confirming the feasibility of the suggested approach.
**Fig. S3.** The fluorescence intensity changes \((F_{(H1+H2+T_{HIV}+NMM)} - F_{NMM})\) using different concentrations of NMM: 50, 100, 200, 300, 400 nM. \(F_{(H1+H2+T_{HIV}+NMM)}\) and \(F_{NMM}\) are the fluorescence intensities of the system containing different components as the subscript indicates and the concentration of each DNA sequence is fixed at 100 nM. All measurements were carried out in 10 mM Tris-HCl buffer containing 5 mM MgCl\(_2\) and 15 mM KCl (pH: 8.0).

**Discussion of Fig. S3:** In order to achieve the best fluorescence enhancement of NMM, the fluorescence intensity changes \((F_{(H1+H2+T_{HIV}+NMM)} - F_{NMM})\) were used to evaluate detailed conditions achieve a good signal-to-background ratio, high sensitivity, and wide linear range, where \(F_{(H1+H2+T_{HIV}+NMM)}\) and \(F_{NMM}\) are the fluorescence intensities of the system containing different components as the subscript indicates. With fixed concentration of \(H1\), \(H2\) and \(T_{HIV}\) (each is 100 nM), the fluorescence difference \((F_{(H1+H2+T_{HIV}+NMM)} - F_{NMM})\) reached maximum when NMM concentration is 300 nM. Therefore, this condition was used in the following experiments for the purpose to achieve a good signal-to-background ratio, high sensitivity, and wide linear range.
Fig. S4. (A) The ratio of \( F(\text{H1+H2+Different targets+NMM}) - F(\text{H1+H2+NMM}) \) in diluted urine under different conditions, where \( F(\text{H1+H2+Different targets+NMM}) \) and \( F(\text{H1+H2+THIV+NMM}) \) are the fluorescence intensities of the system containing different components as the subscript indicates. (B) The fluorescence spectra of (a) \( \text{H1} + \text{H2} \), (b) \( \text{H1} + \text{H2} + T_L \), (c) \( \text{H1} + \text{H2} + T_{LI} \). Inset is the corresponding fluorescence intensity histograms with error bar. \([\text{H1}]=100 \text{ nM}, [\text{H2}]=100 \text{ nM}, [\text{NMM}]=300 \text{ nM}, [\text{THIV}]=100 \text{ nM}, [\text{Different Targets}]=100 \text{ nM}, [T_L]=100 \text{ nM}, [T_{LI}]=100 \text{ nM}. \) Urine was diluted 50-fold with 10 mM Tris-HCl buffer containing 5 mM MgCl₂ and 15 mM KCl (pH: 8.0). The error bar represents the standard deviation of three measurements.

Discussion of Fig. S4: We performed DNA detection in diluted urine as an example of physiological fluid to mimic real samples, where urine was diluted 50-fold with Tris-HCl buffer. The corresponding results are demonstrated in Fig. S4. The fluorescence difference \( F(\text{H1+H2+Different targets+NMM}) - F(\text{H1+H2+NMM}) \) of the system in the presence of one-base-mismatched targets \( T_{1AA}, T_{1AC}, T_{1AG}, T_{1B}, T_{1C} \), two-base-mismatched targets \( T_{2AB}, T_{2AC}, T_{2BC} \), and three-base-mismatched target \( T_3 \) corresponds to 63%, 66%, 67%, 58%, 60%, 9%, 4%, 2% and 1% of the value in the presence of perfect complementary target \( T_{\text{HIV}} \), respectively (shown in Fig. S4A). From these results, it can be concluded that the present system can withstand the interference from urine, exhibiting high selectivity down to single-base mismatch and great potential in SNPs detection. Considering that the target sequence is usually
embedded in a longer sequence, the potential application of the present approach was further evaluated by challenging the sensor with longer DNA strand $T_L$, the middle part of which is the same to $T_{HIV}$. Furthermore, it can discriminate single-base-mismatched target sequence embedded in longer sequence ($T_{L1}$). Fig. S4B shows the fluorescence responses of the system under different conditions. Upon the presence of $T_L$, the fluorescence intensity of the system was significantly enhanced, indicating great potential of the suggested approach to detect target sequence embedded in longer DNA strand. The ratio

\[ \frac{(F(H1+H2+T_L+NMM)-F(H1+H2+NMM))}{(F(H1+H2+T_L+NMM)-F(H1+H2+NMM))} \]

was calculated to be about 78.5%, indicating that the ability to discriminate single-base mismatch is weakened when target sequences embedded in longer strands, however, an obvious difference still can be observed in comparison with the embedded complementary target. The inset in Fig. 4B shows the corresponding fluorescence intensity histograms with error bar, validating the reliability of the developed method. From the performance of DNA detection in the presence of urine as well as when target sequence was embedded in longer strands, the present method exhibits great promise for practical application upon further development.