Electronic Supplementary Information

A Universal Sensing Platform Based on the Repair Ligation-Mediated Light-Producing DNA Machine

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EXPERIMENTAL SECTION

Materials. Phospho(enol)pyruvic acid monosodium salt hydrate (PEP), nicotinamide adenine dinucleotide hydrate (NAD^+), adenylate kinase (AK) from chicken muscle, pyruvate kinase from rabbit muscle (PK), thrombin (lyophilized powder, 2000 units/mg), horseradish peroxidase (HRP), adenosine, guanosine, uridine, cytidine, calcium chloride ($CaCl_2$), sodium chloride (NaCl), potassium chloride (KCl), lithium chloride (LiCl), ammonium chloride (NH_4Cl) and human serum were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Hemoglobin was purchased from Worthington Biochemical Corporation (Lakewood, NJ, USA). Bovine serum albumin (BSA) was obtained from Shanghai Excell Biology, Inc. (Shanghai, China). The dCTP was purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). The *E.coli* ligase, restriction endonuclease (Nb.BbvCI, Nt.BstNBI, ApaI, BbvCI, and FokI), NEBuffer 2 (10×), NEBuffer 4 (10×) and the diluent buffer A were obtained from New England Biolabs (Ipswich, MA, USA). All oligonucleotides (Table S1) were synthesized and HPLC purified by Sangon Biotechnology Co.

Ltd. (Shanghai, China). The ATP determination kit consisting of D-luciferin, recombinant firefly luciferase and dithiothreitol was purchased from Invitrogen (Carlsbad, CA, USA). All solutions were prepared with Milli-Q water (Millipore, Bedford, MA, USA).

Instruments. Bioluminescence was measured with a Glomax 96-well luminometer (Promega, Madison, Wisconsin, USA). The homogeneous reactions and continuous measurements were performed on white 96-well microplates (Fisher Scientific, Pittsburgh, Pennsylvania, USA). The gel images were collected by Kodak 4000MM (Rochester, NY, USA). The temperature control was obtained by an Eppendorf thermocycler (Hamburg, Germany).

Gel Electrophoresis. To obtain the DNA duplexes, the chimeric probe and the assisted probe were mixed at the molar ratio of 1:1 in 1× NEBuffer 4 with a final concentration of 1.0 μM. The mixture was heated at 95°C for 5 min and then slowly cooled to 25°C. To investigate the cleavage/ligation motion of intramolecular DNA machine, 10 U/μL Nb.BbvCI (0.5 μL) was added to 2 μM DNA duplex, and incubated at 37°C for 30 min. The mixture was heated at 95°C for 5 min to inactivate the Nb.BbvCI endonuclease, followed by slowly cooling to 25°C for 1 h. With the addition of 10 U/μL ligase (0.5 μL), the solution was incubated at 37°C for 30 min, and then was inactivated by heating. The addition of Nb.BbvCI and ligase was repeated for three times. The reaction products (5.0 μL) were mixed with an equivalent volume of formamide (5.0 μL), loaded on a 14% denaturing gel (prepared with 8 M urea) for electrophoresis at a 110 V for 70 min, and finally screened with a standard silver-staining method.

Bioluminescence Monitoring of Intramolecular DNA Machine. In a typical intramolecular DNA machine, the repetitive DNA cleavage/ligation motion was performed in the presence of 10 U/μL Nb.BbvCI (0.5 μL), 10 U/μL *E.coli* ligase (0.5 μL), 1.0 μM DNA duplex (0.5 μL) and 30

μM NAD^+ (1.0 μL). The repetitive AMP pyrophosphorylation/ATP depyrophosphorylation motions were performed in the presence of the AMP-to-ATP conversion buffer (1.0 μL of 1U/μL AK, 1.0 μL of 1U/μL PK, 1.0 μL of 10 mM dCTP, and 1.0 μL of 4.8 mM PEP), and 0.5 μL of ATP detection buffer (5 mM D-luciferin, 12.5 μg/mL firefly luciferase, 25 mM Tricine buffer (pH 7.8), 5 mM $MgSO_4$, 100 μM EDTA and 10 mM DTT). These reagents were mixed in 60 μL of 1× NEBuffer 4, and the bioluminescence signals were recorded with a Glomax 96-well luminometer at room temperature.

Detection of Various Targets with the Self-Reporting DNA Machines. For DNA assay, 0.1 μM chimeric probe was incubated with 0.1 μM target DNA in 10 μL of 1× NEBuffer 4 at room temperature for 30 min. Then 0.2 μL of 20 U/ μL exonuclease I was added to the mixture (10 μL) and incubated at 25°C for 5 min, followed by adding 1.5 μL of 100 mM EDTA to stop the digestion. The reaction mixture (11.7 μL) further hybridized with 1 μM assisted probe (1.0 μL) to form the DNA duplex. The reaction product was analyzed by a 10% native polyacrylamide gel electrophoresis. For the bioluminescence measurement, the target DNA was incubated with 0.1 μM chimeric probe in 10 μL of 1× NEBuffer 4, then treated by 20 U/ μL exonuclease I (0.2 μL), and finally hybridized with the assisted probe. Aliquots of reaction solutions were heated at 95°C for 3 min to inactivate the exonuclease I, followed by gradual cooling to 25°C for 1 h. With the addition of 10 U/μL Nb.BbvCI (0.5 μL), 10 U/μL ligase (0.5 μL), 30 μM NAD^+ (1.0 μL), the AMP-to-ATP conversion buffer (4.0 μL) and the ATP detection buffer (0.5 μL), the DNA machine was activated, and the bioluminescence signals were recorded.

For thrombin assay, 1.0 μM chimeric probe was incubated with 4.4 μM thrombin in 10 μL of 1× NEBuffer 2 at 37°C for 30 min. Then 2.0 μL of 20 U/ μL exonuclease I was added to the

mixture and incubated at 25°C for 10 min, followed by the addition of 100 mM EDTA (1.5 μL) to stop the digestion. The reaction mixture hybridized with 10 μM assisted probe (1.0 μL) to form the DNA duplex. The following gel electrophoresis experiment and bioluminescence measurement were performed by using the same methods for DNA assay.

For adenosine assay, 1.0 μM chimeric probe was annealed in 10 mM Tris-HCl buffer (pH 8.0, 100 mM NaCl, and 5 mM $MgCl_2$) prior to the addition of adenosine. The chimeric probe (1.0 μM) was mixed with adenosine (5.0 mM) in 10 μL of annealing buffer and incubated at 37°C for 30 min. Then 20 U/ μL exonuclease I (2.0 μL) was added to the mixture and incubated at 25°C for 10 min, followed by the addition of 100 mM EDTA (1.5 μL) to stop the digestion. The reaction mixture hybridized with 1 μM assisted probe (1.0 μL) to form the DNA duplex. The following gel electrophoresis experiment and bioluminescence measurement were performed by using the same methods for DNA assay.

For potassium ion (K^+) assay, 1.0 μM chimeric probe was annealed in 10 mM Tris-HCl buffer (pH 8.0, 2 mM $MgCl_2$) prior to the addition of K^+. The chimeric probe (1.0 μM) was mixed with K^+ (10 mM) in 10 μL of annealing buffer and incubated at 37°C for 30 min. Then 20 U/ μL exonuclease I (1.0 μL) was added to the mixture and incubated at 25°C for 10 min, followed by the addition of 100 mM EDTA (1.5 μL) to stop the digestion. The reaction mixture hybridized with 1 μM assisted probe (1.0 μL) to form the DNA duplex. The following gel electrophoresis experiment and bioluminescence measurement were performed by using the same methods for DNA assay.

Bioluminescence Monitoring of Intermolecular DNA Machine. For the construction of intermolecular DNA machine, the restriction endonuclease of ApaI and its corresponding

recognition sequence were used to replace the nicking endonuclease of Nb.BbvCI and its recognition sequence in the intramolecular DNA machine. For DNA assay, the DNA duplex (Table S1) was mixed with 50 U/μL ApaI (1.0 μL), 10 U/μL ligase (0.5 μL), 30 μM NAD^+ (1.0 μL), the AMP-to-ATP conversion buffer (4.0 μL) and the ATP detection buffer (0.5 μL) in 60 μL of 1× NEBuffer 4, and incubated at 16°C for 2 h. For ApaI activity assay, ApaI (diluted with the diluent buffer A) was mixed with 1.0 μM DNA target (1.0 μL), 10 U/μL ligase (0.5 μL), 30 μM NAD^+ (1.0 μL), the AMP-to-ATP conversion buffer (4.0 μL) and the ATP detection buffer (0.5 μL) in 60 μL of 1× NEBuffer 4. The bioluminescence signal was recorded using the 96-well luminometer at room temperature in a real time.

Table S1. Sequences of Probes and Targets [α]

note	sequence
nicked DNA duplex (nonspecific)	5'-GGT TTG GCT TTC-3'
	5'-PO_4-TTG TTA GTC CGT-3'
	5'-ACG GAC TAA CAA GAA AGC CAA ACC-3'
nicked DNA duplex (specific)	5'-CTA AAC AAC TGC-3'
	5'-PO_4-TGA GGA TAA ACG-3'
	5'-CGT TTA TCC TCA GCA GTT GTT TAG-3'
intact DNA duplex (specific)	5'-CTA AAC AAC T**GC TGA GG**A TAA ACG-3'
	5'-CGT TTA T**CC TCA GC**A GTT GTT TAG-3'
assisted probe	5'-CGT TTA T**CC TCA GC**A GTT GTT TAG-3'
chimeric probe (DNA)	5'-CTA AAC AAC T**GC TGA GG**A TAA ACG TTA TTC CAA ATA TCT TCT-3'
target DNA	5'-AGA AGA TAT TTG GAA TAA-3'
target DNA (single-base mismatched)	5'-AGA AGA TAC TTG GAA TAA-3'
chimeric probe (thrombin)	5'-CTA AAC AAC T**GC TGA GG**A TAA ACG *AGT CCG TGG TAG GGC AGG TTG GGG TGA CT*-3'
chimeric probe (adenosine)	5'-CTA AAC AAC T**GC TGA GG**A TAA ACG *ACC TGG GGG AGT ATT GCG GAG GAA GGT*-3'
chimeric probe (K^+)	5'-CTA AAC AAC T**GC TGA GG**A TAA ACG *GGG TTA GGG TTA GGG TTA GGG*-3'
DNA duplex for ApaI (specific)	5'-CTA AAC AAC **GGG CCC** GGA TAA ACG-3'

	5’-CGT TTA TCC **<u>GGG CCC</u>** GTT GTT TAG-3’
DNA duplex for ApaI (nonspecific)	5’-GGT TTG GCT TTC TTG TTA GTC CGT-3’
	5’-ACG GAC TAA CAA GAA AGC CAA ACC-3’

[α]The boldface regions symbolize the recognition sequences of Nb.BbvCI endonuclease. The italic letters in the chimeric probe are the binding sequence of target DNA, thrombin, adenosine and K^+, respectively. The underlined bold letters symbolize the recognition sequences of ApaI.

SUPPLEMENTARY RESULTS

We used native PAGE to confirm the feasibility of DNA machine for DNA assay (Fig. S1a). In the absence of Exo I, the bands of chimeric probe (Fig. S1a, lane 1) and the hybridization products of chimeric probe with either the completely matched DNA (Fig. S1a, lane 2) or the single-base mismatched DNA (Fig. S1a, lane 3) are observed distinctly, with the band of chimeric probe (Fig. S1a, lane 1) migrating faster than those of the hybridization products of chimeric probe with the completely matched DNA (Fig. S1a, lane 2) and the single-base mismatched DNA (Fig. S1a, lane 3), suggesting that the target DNA can hybridize with the chimeric probe to form a dsDNA. While in the presence of Exo I, the bands of the chimeric probe (Fig. S1a, lane 5) and the control group with the nonspecific probe (Fig. S1a, lane 8) disappear due to the complete digestion of unfold ssDNA by Exo I. In contrast, the bands of the hybridization products of chimeric probe with the completely matched DNA (Fig. S1a, lane 6) and the single-base mismatched DNA (Fig. S1a, lane 7) are still observed, with a stronger band for the hybridization products of chimeric probe with the completely matched DNA (Fig. S1a, lane 6), suggesting that the hybridization product of chimeric probe with the completely matched DNA target is more resistant to the Exo I digestion.

We used native PAGE to confirm the feasibility of DNA machine for thrombin assay (Fig. S1b). In the absence of Exo I, the bands of chimeric probe (Fig. S1b, lane 1) and the mixture of chimeric probe with either thrombin (Fig. S1b, lane 2) or two control groups including an irrelevant protein (bovine serum albumin, BSA) (Fig. S1b, lane 3) and a nonspecific aptamer of thrombin (Fig. S1b, lane 4) are observed distinctly, with a larger band for thrombin-chimeric probe complexes (Fig. S1b, lane 2) due to the slower migration of ssDNA-protein complexes than

that of free ssDNA. While in the presence of Exo I, the bands of chimeric probe (Fig. S1b, lane 5) and the mixture of chimeric probe with two control groups (Fig. S1b, lanes 7 and 8) disappear due to the complete digestion of unfold ssDNA by Exo I. In contrast, an obvious band (Fig. S1b, lane 6) is still observed for the mixture of chimeric probe and thrombin, suggesting that the G-quadruplex structure formed by the interaction of chimeric probe with thrombin is resistant to the Exo I digestion. It should be noted that the observed band (Fig. S1b, lane 6) results from the chimeric probes rather than the chimeric probe-thrombin complexes due to the disassociation of aptamer-thrombin complexes in the process of electrophoresis.[1]

We used native PAGE to confirm the feasibility of DNA machine for adenosine assay (Fig. S1c). In the absence of Exo I, the bands of chimeric probes (Fig. S1c, lane 1) and the mixture of chimeric probe with either adenosine (Fig. S1c, lane 2) or two control groups including guanosine (Fig. S1c, lane 3) and the nonspecific aptamer of adenosine (Fig. S1c, lane 4) are observed distinctly. It should be noted that no difference is observed between the band of chimeric probe-adenosine complexes (Fig. S1c, lane 2) and that of chimeric probes alone (Fig. S1c, lane 1) due to the disassociation of chimeric probe-adenosine complexes in the process of electrophoresis and the weak affinity between adenosine and the chimeric probe.[1] While in the presence of Exo I, the bands of chimeric probe (Fig. S1c, lane 5) and the mixture of chimeric probe with two control groups (Fig. S1c, lanes 7 and 8) disappear due to the complete digestion of unfold ssDNA by Exo I. In contrast, a distinct band (Fig. S1c, lane 6) is still observed for the chimeric probe-adenosine complexes, suggesting that the hairpin structure formed by the interaction of chimeric probe with adenosine is resistant to the Exo I digestion.

We used native PAGE to confirm the feasibility of DNA machine for K^+ assay (Fig. S1d). In the absence of Exo I, the bands of chimeric probe (Fig. S1d, lane 1) and the mixture of chimeric probe with either K^+ (Fig. S1d, lane 2) or two control groups including Na^+ (Fig. S1d, lane 3) and a nonspecific aptamer of K^+ (Fig. S1d, lane 4) are observed distinctly, with no difference being observed between the band of chimeric probe-potassium complexes (Fig. S1d, lane 2) and that of chimeric probes alone (Fig. S1d, lane 1) due to the disassociation of chimeric probe-potassium complexes in the process of electrophoresis.[1] While in the presence of Exo I, the bands of chimeric probe (Fig. S1d, lane 5) and the mixture of chimeric probe with two control groups (Fig. S1d, lanes 7 and 8) disappear due to the complete digestion of unfold ssDNA by Exo I. In contrast, an obvious band (Fig. S1d, lane 6) is still observed for the chimeric probe-potassium complexes, suggesting that the G-quadruplex structure formed by the interaction of chimeric probe with K^+ is resistant to the Exo I digestion.

As a proof-of-concept, we demonstrated that the intermolecular DNA machine can be used for sensitive and selective detection of restriction endonuclease ApaI. As shown in Fig. 3c, no significant bioluminescence signal is observed in the absence of ApaI endonuclease. While in the presence of ApaI endonuclease, a significant bioluminescence enhancement is observed due to the activation of the intermolecular DNA machine. A linear correlation is obtained between the logarithm of bioluminescence intensity and the logarithm of ApaI concentration over a range of 3 orders of magnitude from 0.033 U/μL to 1.67 U/μL (Fig. 3d). The detection limit is calculated to be 0.01 U/μL, which is comparable with that of the fluorescence-labeled method (0.05 U/μL).[2] Moreover, this intermolecular DNA machine is very selective with no significant bioluminescence signal being observed in response to the nonspecific restriction endonucleases such as FokI,

BbvCI and Nt.BstNBI (inset of Fig. 3d).

Since human serum is the most widely used biological samples for biomarker detection and clinical diagnostics, we tested the feasibility of our DNA machine for practical applications using human serum. Even though the reported ATP concentration is up to 1000 nM in human plasma,[3] the actual concentration of AMP/ADP/ATP is negligible for bioluminescence measurement due to their rapid degradation by some components in serum [4, 5] as well as the long clot time required for serum collection from the whole blood (usually 30-60 min at room temperature).[6] We have tested the application of this DNA machine in real biological samples using human serum (Figure S3). As shown in Figure S3a, no obvious bioluminescence background signal is observed in the presence of human serum. In contrast, a significant bioluminescence enhancement is observed when adding AMP into the human serum. This result is consistent with the previous report that no distinct bioluminescence signal is observed in the presence of fetal bovine serum,[7] suggesting the feasibility of this DNA machine for practical applications.

We further detected target DNA (Fig. S3b) and thrombin (Fig. S3c) in human serum samples using the proposed sensing platform. As shown in Fig. S3 b-c, no significant bioluminescence enhancement is observed in the absence of target DNA and thrombin, but a significant bioluminescence enhancement is observed in response to target DNA and thrombin, further confirming that the target-dependent activation of DNA machine can be applied for the analysis of complex biological samples.

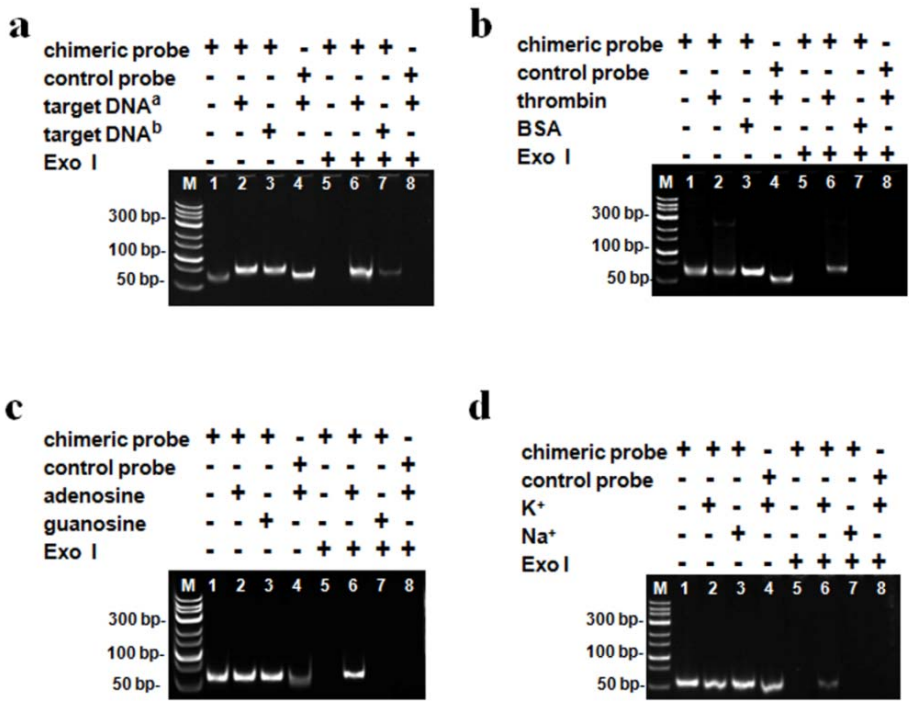

Fig. S1 Native gel electrophoresis analysis of selective digestion capability of exonuclease I towards target-binding chimeric probe under different conditions. The target is DNA (a), thrombin (b), adenosine (c), and K^+ (d), respectively.

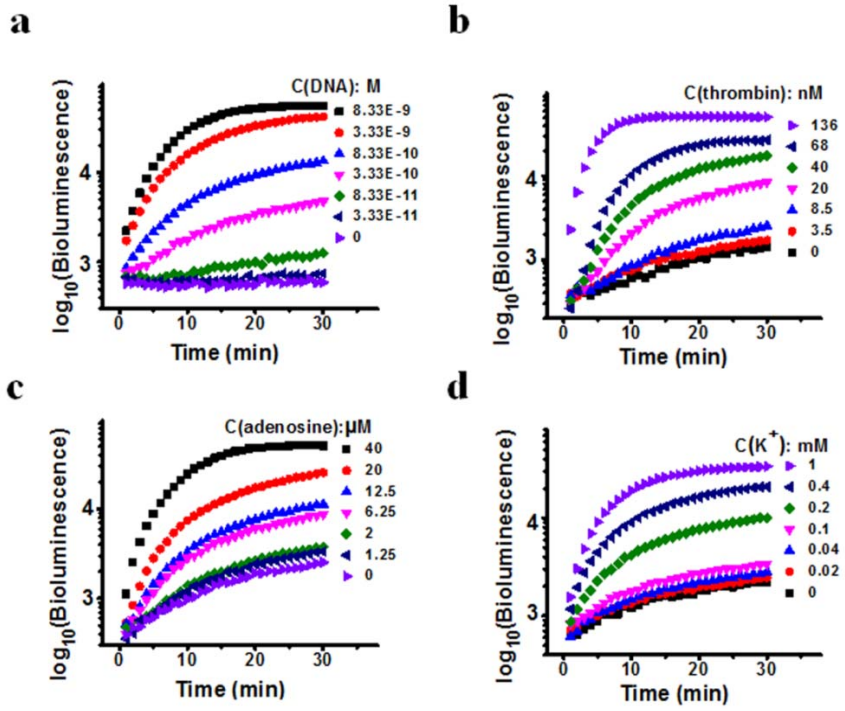

Fig. S2 Real-time monitoring of the variance of bioluminescence signal with the concentration of target DNA (a), thrombin (b), adenosine (c), and K^+ (d), respectively.

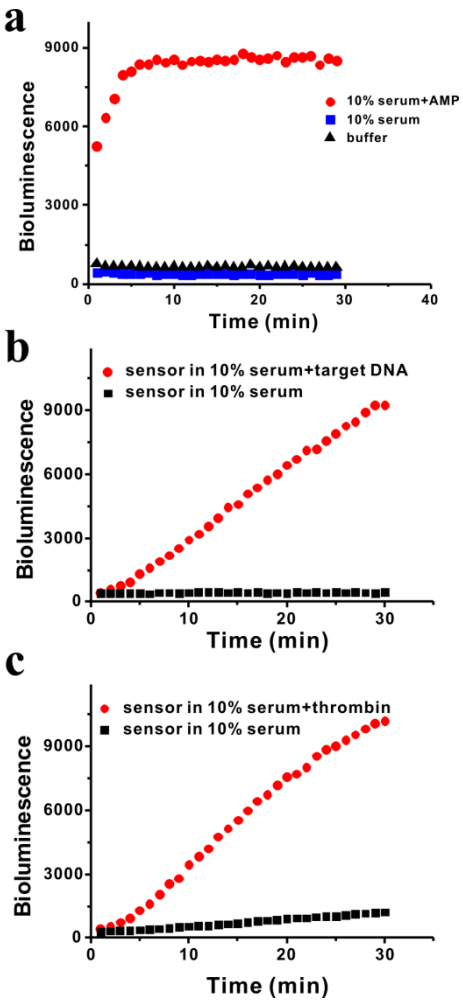

Fig. S3 (a) No obvious bioluminescence signal is observed (blue and black color) in the presence of 10% human serum, and only the addition of AMP can induce the bioluminescence enhancement (red color). (b-c) A significant bioluminescence enhancement is observed in response to target DNA (b, red color) and thrombin (c, red color) in the presence of 10% human serum. The concentration of AMP, target DNA and thrombin is 10 nM, 0.8 mM and 40 nM, respectively.

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