

Electronic Supplementary Material (ESI) for ChemComm.

This journal is © The Royal Society of Chemistry 2017

Supplementary Information

**An enzyme-free DNA walker that moves on the surface of
functionalized magnetic microparticles and its biosensing analysis**

Ningxing Li, Jiao Zheng, Chunrong Li, Xinxin Wang, Xinghu Ji, Zhike He*

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of
Education), College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072,
China

Corresponding author. Tel: +86 27-68756557; E-mail: zhkhe@whu.edu.cn (Z. He)

EXPERIMENTAL SECTION

Materials and Reagents

The carboxylated magnetic microparticles (1.02 μm and 2.70 μm , 10 mg mL⁻¹) were purchased from Invitrogen (Norway). The carboxylated MMPs (600 nm, 250 nm and 100 nm, 10 mg mL⁻¹) were gained from So-Fe Biomedicine Co., LTD (Shanghai, China). Trihydroxymethyl aminomethane (Tris), dithiothreitol (DTT), adenosine triphosphate (ATP), ammonium sulfate ((NH₄)₂SO₄), sodium phosphate dibasic (NaH₂PO₄), sodium phosphate monobasic dehydrate (Na₂HPO₄), potassium chloride (KCl), magnesium chloride (MgCl₂), sodium chloride (NaCl), Tween-20, ethylenediaminetetraacetic acid disodium salt (EDTA), 1-ethyl-3-(3-dimethylaminopro- pyl) carbodiimide (EDC), streptavidin- horseradish peroxidase (SA-HRP), bovine serum albumin (BSA), and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All above are of analytical-reagent grade or better. T4 polynucleotide kinase (10 units/ μL), exonuclease I (5 units/ μL) and exonuclease III were gained from Takara (Japan). λ exonuclease (5 units/ μL) and Nicking endonuclease (Nb.BbvCI) were obtained from New England BioLabs (Whitby, ON, Canada). Hydrochloric acid was purchased from Sinopharm Chemical Reagent Co.,Ltd. Thrombin was obtained from Shanghai Linc-Bio Co., Ltd.. And the HRP substrate kit was purchased from Millipore. Functionalized magnetic microparticles (MMPs) were stored in TE buffer (Tris 20 mM, EDTA 1 mM, pH 7.4). The composition of SA-HRP stock solution was 10 mM Na₂HPO₄, 10 mM NaH₂PO₄, and 15mM NaCl (pH 7.4). Then the washing buffer is 10 mM Na₂HPO₄ and 10 mM NaH₂PO₄ (0.1% (w/v) Tween-20, pH 7.4). All oligonucleotides with different sequences were synthesized and high-performance liquid chromatography (HPLC) purified by Sangon Biotechnology Co.Ltd. (Shanghai, China).

Table S1 Sequences of oligonucleotides used in this study

Name	Sequence (5'→3')
H1	5'-NH ₂ -GTC AGT GAG CTA GGT TAG ATG TCG CCA TGT GTA GAC GAC ATC TAA CCT AGC -3'
H2	5'-biotin-AGA TGT CGT CTA CAC ATG GCG ACA TCT AAC CTA GCC CAT GTG TAG A -3'
Catalyst	5'-CGA CAT CTA ACC TAG CTC ACT GAC -3'

Catalyst-biotin	5'-biotin-CGA CAT CTA ACC TAG CTC ACT GAC -3'
Double catalyst	5'-CGA CAT CTA ACC TAG CTC ACT GAC CAT ATC ACA TAC CTC TAC TCA TCC ACA CAT ATT ACA CAC ACC GAC ATC TAA CCT AGC TCA CTG AC-3'
MT-1	5'-CGA CAT CTA ACC TAG CTC AGT GAC -3'
MT-2	5'-CGA CAT CTA ACC TAG CTC TGT GAC -3'
MT-all	5'-GCT GTA GAT TGG ATC GAG TGA CTG -3'
HIV-H1	5'-NH ₂ -GTC ATG TTA TTC CAA ATA TCT TCT CCA TGT GTA GAA GAA GAT ATT TGG AAT-3'
HIV-H2	5'-biotin-TAT CTT CTT CTA CAC ATG GAG AAG ATA TTT GGA ATC CAT GTG TAG A-3'
HIV	5'-AGA AGA TAT TTG GAA TAA CAT GAC-3'
PNK hairpin	5'-CTG TAA GTA GTG CGA GTT AGT GAT GAA ACA CAC ACA CAC TTC ATC ACT AAC TCG CAC TAC TTA CAG-3'
PNK-H1	5'-CTA ACT CGC ACT ACT TAC GCT CAA CTT CAT CAC TGT AAG TAG TGC GAG TTA GTG ATG AA-NH ₂ -3'
PNK-H2	5'-ACG CTC AAC TTC ATC ACT AAC TCG CAC TAC TTA CAG TGA TGA AGT TGA GCG TAA GTA GT-biotin-3'

Ultrapure water was produced by a Milli-Q Academic purification set (Millipore, Bedford, MA, USA). Polystyrene microplates (Costar) were used as carriers of CL imaging. The CL images were recorded by a ChemiDoc XRD system (Bio-Rad). The pH of solutions was measured by a pB-10 potentiometer (Sartorius).

Modification of MMPs

The carboxyl modified MMPs were conjugated with H1 or PNK-H1 according to the improved protocol suggested by the manufacturer, respectively. Briefly, 400 μL of 10 mg mL^{-1} carboxyl-modified MMPs suspension were washed three times with the same volume PBS buffer (Na_2HPO_4 10 mM, NaH_2PO_4 10 mM, NaCl 15mM, pH 7.4). A certain amount of DNA was dissolved in 340 μL of PBS buffer, and mixed with the washed MMPs. After incubated at room temperature under stirring for 15 min, 20 μL of 0.1 M EDC and 40 μL of 0.05 M NHS was added into the MMPs suspension, and then continued stirring for over night. The conjugated MMPs were

washed three times with Tris-Tween buffer (Tris 0.25 M, Tween-20 0.01% (w/v), and pH 8.0) for 30 min to remove the unreacted activated carboxylic acid groups at room temperature under shaking. Later, the MMPs closed by 1% BSA were dispersed in TE buffer and stored at 4 °C.

Procedures for DNA detection

First of all, 5 µL of H1 modified MMPs, 10 µL of H2 and 10 µL of target DNA were added into 75 µL of CHA buffer solution (NaCl 300 mM), followed by incubating for 60 min at 37 °C under stirring. After washing MMPs for three times, 50 µL of SA-HRP was added into PE pipe included the walker products, and incubated for 30 min. After the complexes were rinsed three times to remove the redundant SA-HRP, the SA-HRP-MMPs were transferred into the polystyrene microplates. Then, 50 µL of CL reaction solution, was added into each well of the microplate, and commixed. The CL imaging detection was implemented by the ChemiDoc XRD system and the obtained images were processed by the Quantity One analysis software. The Chemi Hi Sensitivity application model was used to perform this experiment. The CL imaging signal of the polystyrene microplate was accurately analyzed and the intensity was recorded.

DNA detection in complex biological environment

The detection of target DNA in human serum was realized. First, different concentrations of DNA and 10 µL of H2 were added into 85 µL buffer (NaCl 300 mM, human serum 20 % (v/v)) including with 5 µg of MMPs, and then the mixture was shaking for 1 hour. After washing the complexes three times, 50 µL of SA-HRP was mixed with them. The SA-HRP-MMPs which rinsed three times to remove the redundant SA-HRP were removed into the polystyrene microplates. Finally, 50 µL of CL reaction solution was added into each well of the microplate to mix with the complexes. The CL signal of each well was individually analyzed and the intensity was recorded.

Procedures for T4 polynucleotide kinase activity detection

In this assay, 10 nM PNK hairpin, 0.5 mM ATP, 10 units of λ exo, and a certain amount of PNK were mixed in a 100 µL PNK reaction buffer solution (70 mM Tris-HCl, 10 mM MgCl₂, 5 mM DTT, pH 8). After 180 minutes incubation of PNK-catalyzed phosphorylation at 37 °C, the solution was heated to 90 °C for 10 min, and then kept at room temperature for 2 h. 5 µL of PNK-H1 modified MMPs and 10 µL of PNK-H2 were added into the above phosphorylation solution and kept at 37 °C for 2 h under stirring. After washing MMPs for three times, 50 µL of SA-HRP

was added into PE pipe, and incubated for 30 min. Through three times washing to remove redundant SA-HRP, the SA-HRP-MMPs were transferred into the polystyrene microplates. Finally, 50 μ L of CL reaction solution, was added into each well of the microplate, and commixed. The CL imaging signal of the polystyrene microplate was recorded.

Gel electrophoresis

Different CHA sequence was added into polyethylene pipe respectively to hybrid for 1 h. After this, bromophenol blue and SYBR Green I were mixed with the above products for 15 min. In the end, a 3% agarose gel electrophoresis analysis of the products via the CHA was carried out in 1 \times Tris-borate-EDTA (pH 8.3) at 110 V constant voltages for about 30 min, and imaged by the ChemiDoc XRD system (Fig. S5).

RESULTS

Optimization of detection conditions

Many parameters of DNA detection in Scheme 1A influence CL intensity so that we have investigated them. As presented in Fig. S1A, 5 different amounts of H1 in this work are chosen to get the optimal one for conjugating with magnet beads. With the increase of the amount of H1, the relative CL intensity rises rapidly, and then it is gradually stable after reaching the maximum. Therefore, 1.8 nmol of H1 is chosen for the assay. Fig. S1B presents the relationship between the relative CL intensity and the concentration of H2. When the concentration of H2 increases, the relative CL intensity increases quickly at first, and decreases after the peak. So the concentration of 50 nM for H2 is selected. The reason of appearing a peak may be that the relative CL intensity increases at first due to the faster hybridization between H1 and H2 with the increasing of the concentration for H2; however, too high concentration of H2 leads increase of background more than signal's so that the CL intensity decreases. The relationship between the relative CL intensity and the amount of MMPs in the range from 2 to 20 μ g is similar with Fig. S1C. Thus, 5 μ g of MMPs is used for the study. The concentration of SA-HRP is examined from 50 to 300 ng/mL, and the results are shown in Fig. S1D. The relative CL intensity increases at first with the variation of the concentration, and reaches a plateau after 250 ng/mL. So, 250 ng/mL of SA-HRP is selected as the best one. Besides, similar to Fig. S1, Fig. S2 and S3 present that the optimization for the size of MMPs and the concentrations of EDC and NHS, and 1 μ m MMPs, 0.05 M of EDC and 0.025 M of NHS are chosen in this study.

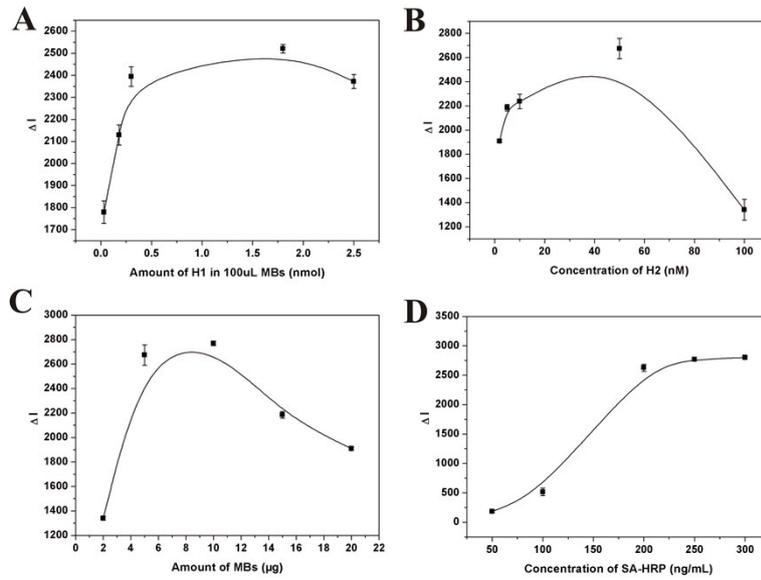


Fig. S1. Effects of (A) the amount of H1, (B) the concentration of H2, (C) the amount of MMPs (D) the concentration of SA-HRP on the relative CL intensity of the sensing system.

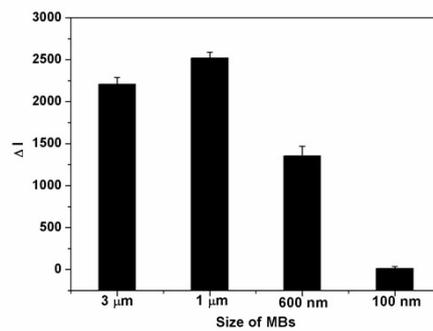


Fig. S2. Effects of the size of MMPs on the relative CL intensity of the sensing system. Experimental conditions: 5 μ g of MMPs, 50 nM H2, and 200 ng/mL SA-HRP.

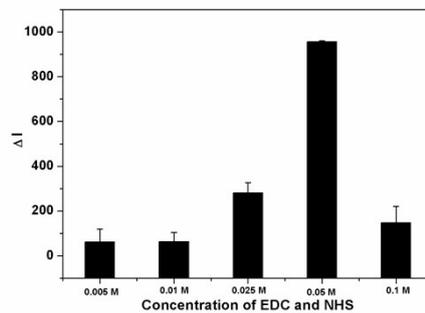


Fig. S3. Effects of the concentrations of EDC and NHS on the relative CL intensity of the sensing system. Experimental conditions: 5 μ g of MMPs, 5 nM H2, and 200 ng/mL SA-HRP.

DNA detection in complex biological environment

In order to introduce the method in complex environment, the proposed method has been developed to analyze DNA in 20 % human serum. As Fig. S4 described, there are slightly differences of the relative CL intensities between the diluted human serum and buffer, and the relative standard error of CL intensities detected between in buffer and in human serum is acceptable. Therefore, it proves that the method is potential for DNA detection in complex biological environment.

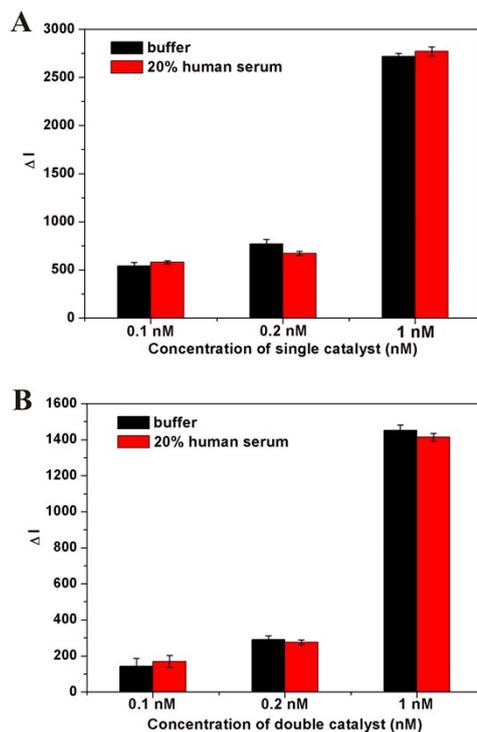


Fig. S4. The comparisons of the sensing system for DNA detection (A) single catalyst, (B) double catalyst in buffer solution and 20% diluted human serum samples, respectively.

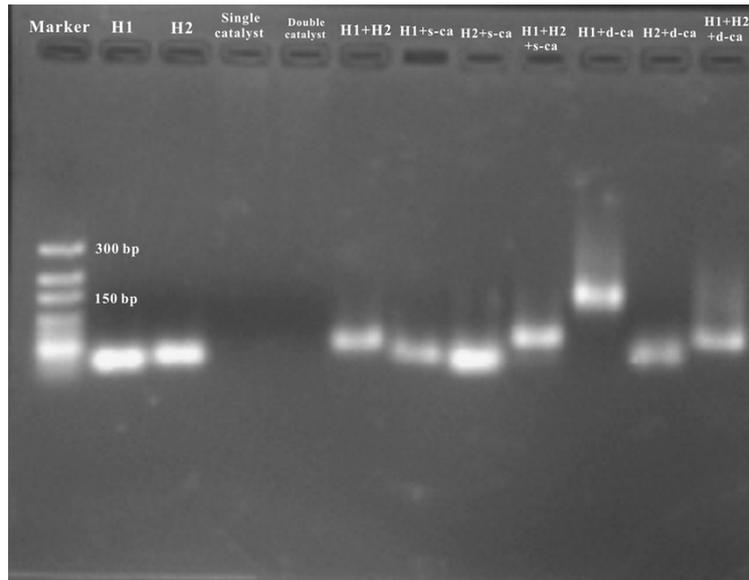


Fig. S5. The result of gel electrophoresis for DNA walker products.

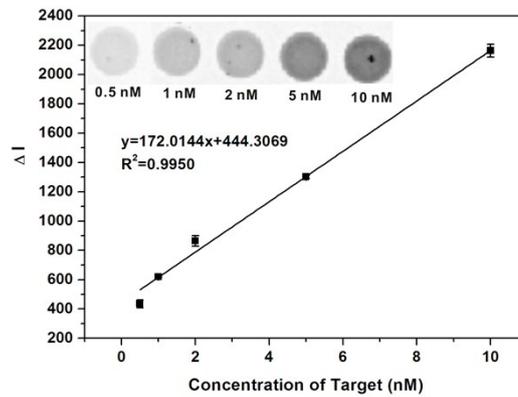


Fig. S6. Relationship between the relative CL intensity and catalyst concentration without H2. Inset: CL images of catalyst at different concentrations. Experimental conditions: 5 μ g of MMPs and 250 ng/mL SA-HRP.

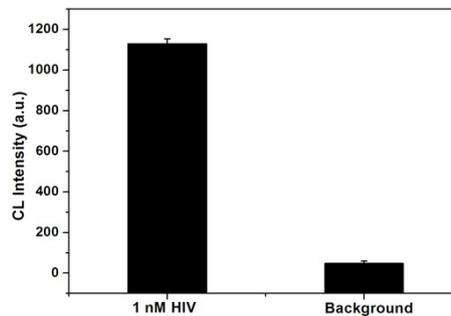


Fig. S7. The feasibility for the detection of HIV sequence. Experimental conditions: 5 μ g of MMPs, 50 nM HIV-H2, and 250 ng/mL SA-HRP.

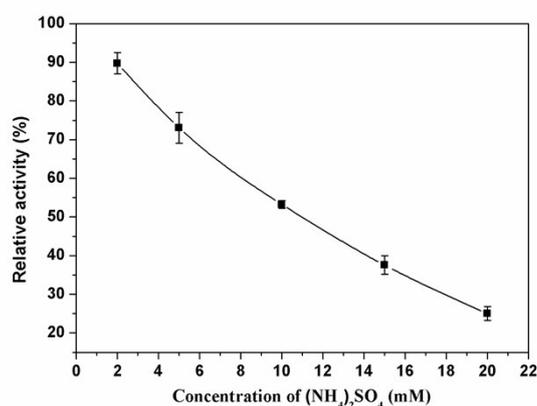


Fig. S8. Inhibition effects of (NH₄)₂SO₄ on phosphorylation. Experimental conditions: 0.12 U/mL PNK, 5 μg MMPs, 50 nM PNK-H2, and 250 ng/mL SA-HRP.

Table S2. The limits of PNK activity detection in recent references.

Strategy	LOD	Ref.
Bioluminescence	0.004 U/mL	1
Electroanalysis	0.0018 U/mL	2
Fluorescence	0.001 U/mL	3
Fluorescence	0.0000977U/mL	4
Chemiluminescence	0.003 U/mL	This method

Reference

1. J. Du, Q. F. Xu, X. Q. Lu and C. Y. Zhang, *Anal. Chem.*, **2014**, 86, 8481.
2. Q. M. Zhang, Z. Li, Y. L. Zhou, X. Li, B. C. Li, H. S. Yin and S. Y. Ai, *Sensors and Actuators B.*, **2016**, 225, 151.
3. T. Hou, X. Z. Wang, X. J. Liu, T. T. Lu, S. F. Liu and F. Li, *Anal. Chem.*, **2014**, 86, 884.
4. L. J. Wang, Q. Y. Zhang, B. Tang and C. Y. Zhang, *Anal. Chem.*, **2017**, DOI: 10.1021/acs.analchem.7b01783.