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

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An enzyme-free stochastic DNA walker propelled by a single catalytic or double catalytic DNA assembly has been constructed. The application of the proposed DNA walking biosensor was successfully expanded to the detection of DNA and the enzymatic activity of T4 polynucleotide kinase.

For sensitive detection of DNA and proteins, many signal amplification strategies which are based on nuclease, such as the hybridization chain reaction (HCR), rolling circle amplification (RCA), isothermal strand-displacement polymerase reaction (ISDPR), and target DNA recycling amplification with endonuclease or exonuclease, have been combined with a plenty of materials like quantum dots[1,2] and employed in various biosensors.[3–6] Among them, catalyzed hairpin assembly (CHA), as a ripe nonenzymatic DNA hybridization strategy, has avoided the requirement of DNAzymes or protein enzymes.[7] In CHA, a single-stranded catalyst can trigger strand exchange reactions that lead to the formation of a double-stranded nucleic acid of two different hairpins and recycling of the catalyst.[8]

Molecular machines propelled by DNAzymes, protein enzymes and strand displacement usually depend on the above amplification strategies.[9–11] A major challenge in engineering molecular machines is to precisely make a nanoscale objective move along a designated path.[7] Due to the characteristics of DNAs that are robust and versatile building blocks for artificial machineries and the well-defined Watson–Crick base pairing rule,[12] numerous DNA walking devices, including DNA walkers, DNA motors and DNA tweezers, have been constructed.[13–15] Currently, almost all engineered DNA machines typically move along precisely defined one-dimensional (1-D) DNA footpaths or two-dimensional (2-D) DNA origami as tracks.[16,17] A few well-defined 3-D DNA tracks have been reported recently. For example, Ellington *et al.* have described a stochastic DNA walker that traverses a microparticle surface.[18] Compared with the existent 1-D or 2-D DNA nanomachines, 3-D DNA nanomachines have several advantages as a result of the high DNA loading density on the 3-D material.[8] The advantages of both CHA and 3D-DNA walker have attracted our interest.

Herein, a DNA walker biosensor has been constructed for the detection of DNA and T4 polynucleotide kinase (PNK) activity based on functionalized magnetic microparticles (MMPs) and CHA. As shown in Scheme 1A, a toehold of H1 conjugated with MMPs can interact with a single-stranded DNA catalyst to open the hairpin *via* toehold-mediated strand exchange. And then a toehold on biotin-labeled H2 would be hybridized by a newly exposed DNA segment in H1, and branch migration could be triggered to form a tripartite complex between H1, H2 and the catalyst. Through the strand displacement process, this complex would resolve into the most thermodynamically favorable configuration, the H1:H2 duplex, with displacement of the catalyst which can continue to attend the following reaction cycles. Finally, SA-HRP which could catalyze the CL system is loaded into the DNA walker products as a result of the interaction between SA and biotin. The principle in Scheme 1B is similar with 1A except the target which comprises a longer single-stranded DNA oligonucleotide that contains two catalyst domains separated by a linear, non-hybridizing spacer sequence. The double catalyst could catalyze proximal hairpin assembly reactions. As H2 displaces one "leg" of it, the other "leg" could still hybridize with neighboring H1, and the displaced one would be possible to induce more cycles. We have compared these two models and simply analyze the cause of this difference between them. In order to expand the application of the DNA walker, the detection of PNK activity has been explored. In Scheme 1C, in the presence of PNK, the hydroxyl group at the 5′-termini of PNK hairpin would be catalyzed through a phosphorylation reaction, and then immediately cleaved by λ exonuclease (λ exo) to release a catalytic DNA strand which could trigger the walk like in Scheme 1A and produce a strong CL signal.

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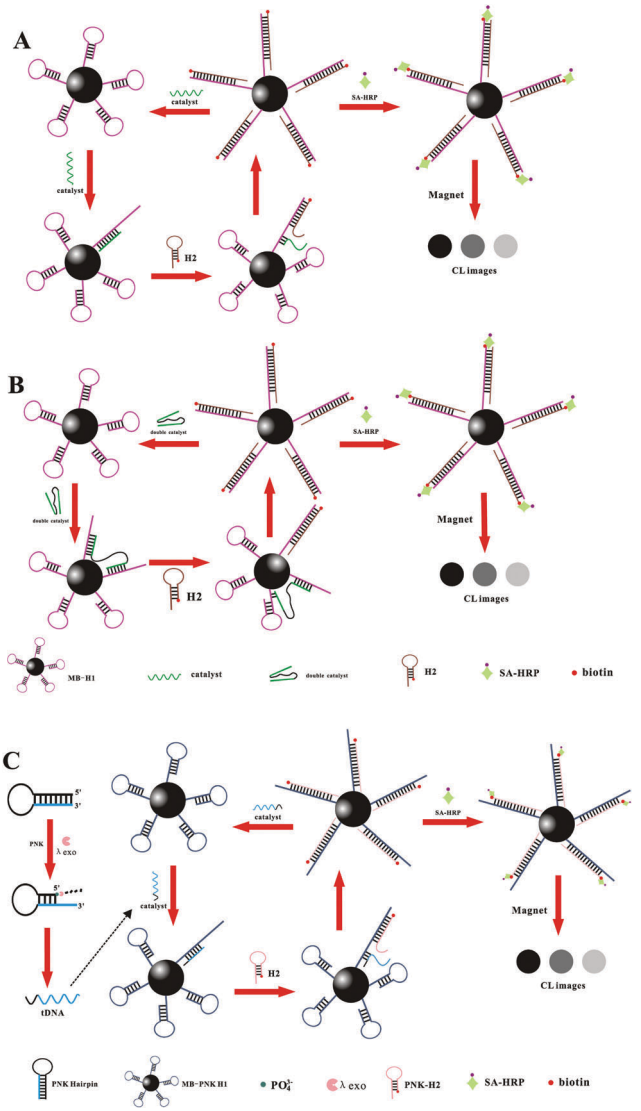

Scheme 1 Schematic illustration of the principle for the sensitive detection of DNA and PNK activity.

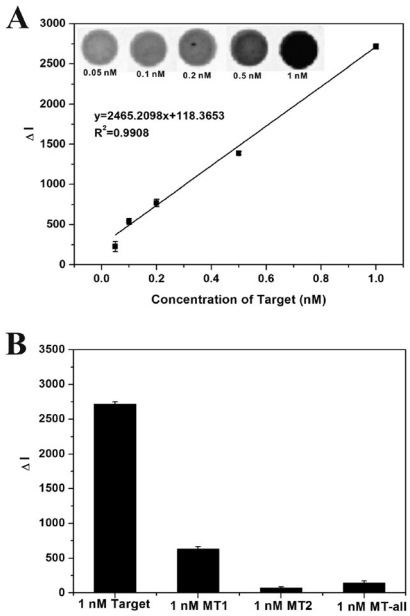

Fig. 1 (A) Relationship between the relative CL intensity and single catalyst concentration. Inset: CL images of single catalyst at different concentrations for Scheme 1A. (B) Specificity of the assay for single catalyst detection with different DNA sequences for Scheme 1A. Experimental conditions: 5 μg of MMPs, 50 nM H2, and 250 ng mL^{-1} SA-HRP.

Under the optimal conditions (Fig. S1–S3, ESI†), the sensitivity of the strategy has been investigated by performing in different models with a series of catalyst concentrations. In the figures, the relative CL intensity (ΔI) is defined as $\Delta I = I_0 - I$, where I_0 indicates the signal in the presence of target and I indicates the background signal. As presented in Fig. 1A, the relative CL intensity increases gradually with the increase of the concentration of catalyst, and there is a good linear relationship between them. In Fig. 1A, the regression equation in the linear range of 0.05–1 nM is $\Delta I = 2465.2098C + 118.3653$, where ΔI is the relative CL intensity and C is the concentration of catalyst ($R^2 = 0.9908$). According to the equation $C_{LOD} = 3S/k$, where C_{LOD} is the limit of detection, S is the standard deviation of the blank ($N = 5$), and k is the slope of the regression equation, the limit of detection (LOD) for the catalyst is 3.9 pM. Under the same conditions, we investigated the sensitivity of the strategy without H2. As shown in Fig. S6 (ESI†), the limit of detection (LOD) for the biotin-labeled catalyst is 55 pM. The result showed the model with the DNA walker is much more sensitive compared to that without it. Furthermore, the specificity of this method was evaluated using single-base mismatched target DNA (MT1), two-base mismatched target DNA (MT2) and all-mismatched target DNA (MT-all). As shown in Fig. 1B, the relative CL intensity of the catalyst is much higher than those of the others. This high selectivity is due to the conformational constraint of the stem-loop structure of H1. Moreover, the catalyst sequence could be designed for others such as a HIV sequence (Fig. S7, ESI†). Therefore, the proposed method provides an excellent capability for DNA detection.

Similarly, the sensitivity of the strategy for the model in Scheme 1B is also evaluated. As shown in Fig. 2, the linear range is from 0.05 to 1 nM, and a good linear relationship is between the relative CL intensity and the concentrations of the double catalyst ($\Delta I = 1433.5425C + 11.6358$, $R^2 = 0.9997$). The limit of detection (LOD) for the double catalyst is 6.5 pM.

The LOD for the double catalyst is lower than the single catalyst's, so we have preliminarily discussed the reason which may be the spaces steric hindrance causes the phenomenon. In order to prove the hypothesis, the MMPs coupling with H1 of two different amounts have been constructed. In Fig. 3, the relative CL intensity of the single catalyst is a little higher than that of the double catalyst in MMPs of high density DNA, while the result becomes opposite in MMPs of low density DNA. The consequence could attest our assumption effectively.

In order to expand the application of the DNA walker, the detection of PNK activity has been explored. The sensitivity of the strategy has been investigated with a series of PNK concentrations.

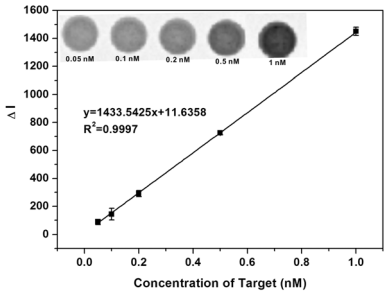

Fig. 2 Relationship between the relative CL intensity and double catalyst concentration. Inset: CL images of double catalyst at different concentrations for Scheme 1B. Experimental conditions: 5 μg of MMPs, 50 nM H2, and 250 ng mL^{-1} SA-HRP.

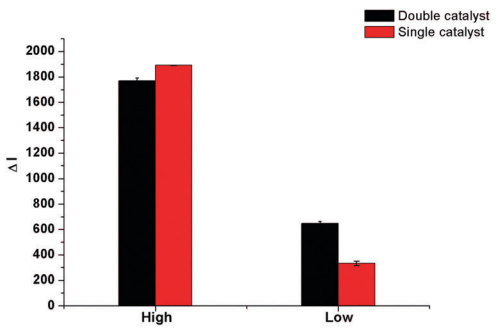

Fig. 3 The relative CL intensities of single catalyst and double catalyst in different density-DNA MMPs. Experimental conditions: 10 nM single catalyst or double catalyst, 5 μg of MMPs, 100 nM H2, and 250 ng mL^{-1} SA-HRP.

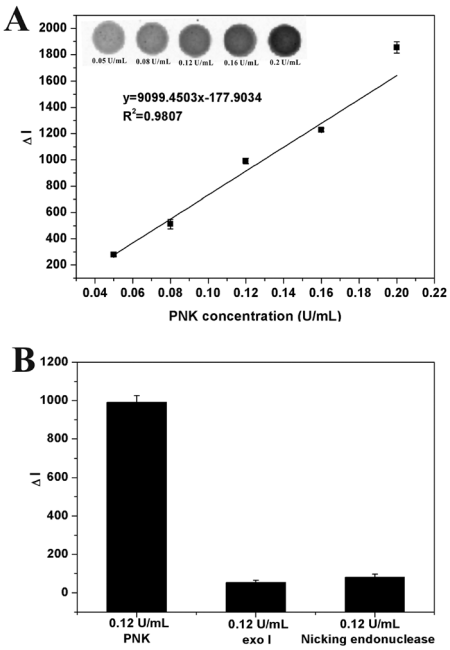

Fig. 4 (A) Relationship between the relative CL intensity and PNK concentration. Inset: CL images of PNK at different concentrations for Scheme 1C. (B) Specificity of the assay for PNK with different enzymes for Scheme 1C. Experimental conditions: 5 μg of MMPs, 50 nM PNK-H2, and 250 ng mL^{-1} SA-HRP.

As described in Fig. 4A, there is a linear relationship between the relative CL intensity and PNK concentrations. In the linear range of 0.05–0.2 U mL^{-1}, the regression equation is $\Delta I = 9099.4503C - 177.9034$, where ΔI is the relative CL intensity and C is the PNK concentration ($R^2 = 0.9807$). Besides, the limit of detection (LOD) for PNK is 0.003 U mL^{-1}. Furthermore, the inhibition effect on PNK was also investigated by using ammonium sulfate ($(NH_4)_2SO_4$) (Fig. S8, ESI†). It could be seen that the addition of approximately 10 mM $(NH_4)_2SO_4$ could result in about 50% decrease of DNA phosphorylation. And the comparison of this strategy with other methods reported is presented in Table S2 (ESI†). The LOD of this method reaches the same level as those of some previously reported methods. In this work, the specificity for PNK has been evaluated as Fig. 4B presented. The relative CL intensity of PNK is much higher than the others. Hence, the proposed method provides a pretty good selectivity for PNK activity detection.

In conclusion, a sensitive DNA walker biosensor for DNA and PNK activity was constructed based on the functionalized MMPs and CHA. The high sensitivity and selectivity for DNA and PNK activity have been proved. Also, the comparison between a single catalyst and a double catalyst allow us to do further exploration in the future. Moreover, the strategy was applied to detect DNA in human serum with good results. Hence, a broad platform will be provided by this sensitive biosensor to detect various enzymes and DNA.

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Notes and references

1 J. Zhou, Y. Yang and C. Y. Zhang, *Chem. Rev.*, 2015, **115**, 11669.
2 F. Ma, Y. Li, B. Tang and C. Y. Zhang, *Acc. Chem. Res.*, 2016, **49**, 1722.
3 C. C. Wu, S. Cansiz, L. Q. Zhang, I. T. Teng, L. P. Qiu, J. Li, Y. Liu, C. S. Zhou, R. Hu, T. Zhang, C. Cui, L. Cui and W. H. Tan, *J. Am. Chem. Soc.*, 2015, **137**, 4900.
4 Y. Li, L. Liang and C. Y. Zhang, *Anal. Chem.*, 2013, **85**, 11174.
5 M. Luo, N. X. Li, Y. F. Liu, C. H. Chen, X. Xiang, X. H. Ji and Z. K. He, *Biosens. Bioelectron.*, 2014, **55**, 318.
6 X. C. Lin, T. Zhang, L. Liu, H. Tang, R. Q. Yu and J. H. Jiang, *Anal. Chem.*, 2016, **88**, 1083.
7 J. Pan, F. Li, T. G. Cha, H. Chen and J. H. Choi, *Curr. Opin. Biotechnol.*, 2015, **34**, 56.
8 X. Y. Jiang, H. J. Wang, H. J. Wang, Y. Zhuo, R. Yuan and Y. Q. Chai, *Anal. Chem.*, 2017, **89**, 4280.
9 P. Yin, H. Yan, X. G. Daniell, A. J. Turberfield and J. H. Reif, *Angew. Chem., Int. Ed.*, 2004, **43**, 4906.
10 J. Bath, S. J. Green and A. J. Turberfield, *Angew. Chem., Int. Ed.*, 2005, **117**, 4432.
11 T. Omabegho, R. Sha and N. C. Seeman, *Science*, 2009, **324**, 67.
12 X. L. Yang, Y. N. Tang, S. D. Mason, J. B. Chen and F. Li, *ACS Nano*, 2016, **10**, 2324.
13 C. Zhou, X. Duan and N. Liu, *Nat. Commun.*, 2015, **6**, 8102.
14 T. G. Cha, J. Pan, H. R. Chen, J. Salgado, X. Li, C. D. Mao and J. H. Choi, *Nat. Nanotechnol.*, 2014, **9**, 39.
15 M. H. Liu, J. L. Fu, C. Hejesen, Y. H. Yang, N. W. Woodbury, K. Gothelf, L. Yan and H. Yan, *Nat. Commun.*, 2013, **4**, 2127.
16 M. You, Y. Chen, X. Zhang, H. Liu, R. Wang, K. Wang, K. R. Williams and W. Tan, *Angew. Chem., Int. Ed.*, 2012, **51**, 2457.
17 S. F. J. Wickham, J. Bath, Y. Katsuda, M. Endo, K. Hidaka, H. Sugiyama and A. J. Turberfield, *Nat. Nanotechnol.*, 2012, **7**, 169.
18 C. Jung, P. B. Allen and A. D. Ellington, *Nat. Nanotechnol.*, 2015, **11**, 157.