Contribution of Gold Nanoparticles to the Catalytic DNA Strand Displacement in Leakage Reduction and Signal Amplification

Supplementary Information

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S1 Materials and Methods

Materials

All DNA oligonucleotides used in this study, including the fluorophore-labeled strands, were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences of these oligonucleotides are shown in Table S1. Ultrapure water with 18.2 MΩ·cm (Millipore simplicity, USA) was used in all experiments. In this study, all dry powders of the purchased DNA were dissolved in 1× PBS. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Alfa Aesar. Other chemicals were obtained from Sinopham Chemical Reagent Co., Ltd. (China).

Procedure of functionalization AuNPs with DNA

The size of AuNPs used in this study was 13 nm which was synthesized on the basis of previous literature.[1] The surface plasmon resonance maximum (λ_{max}) of obtained AuNPs was 520 nm and the concentration was measured by UV-Vis spectrophotometer. Thiol-modified DNAs were grafted onto AuNPs according to previous method.[2] 1 × PBS was used to dissolve AuNPs-DNA at the last step. And the AuNPs-DNA was stored at 4°C for further use.

Preparation of dsDNA on NP_Protector with Reporter by annealing

Reporter was mixed with NP_Protector at the ratio of 100:1, heated to 70°C for 10 min, and then allowed to cool slowly to room temperature. Then the mixture was kept at room temperature overnight for further incubation. In order to remove excess Reporter strands, the mixture was centrifuged twice at 11000-13000 rpm for 30 min and then resuspended in 1 × PBS.

Fluorescence measurement

The NP_Protector-Reporter complex was diluted to 1 nM in 1 × PBS buffer with certain amount of NP_Fuel. This solution was treated with Target strands for 10 h at 25°C. The fluorescence spectra were recorded on an F-7000 fluorometer (Hitachi) with 633 nm light excitation. The fluorescence intensity was normalized using the fluorescence from complete reaction. ([Target] =100 nM)

For kinetics study of this model, the fluorescence intensity was recorded at 25°C every 1 or 2 h. And the concentration of NP_Fuel is fourfold amount of NP_Protector-Reporter complex.

Investigation of the specificity of this model

The NP_Protector-Reporter complex was diluted to 1 nM in 1 × PBS buffer with fourfold amount of NP_Fuel. This solution was treated with fully matched Target and SNP targets for 10 h at 25°C. The fluorescence spectra were recorded and normalized using the fluorescence from complete reaction. ([Target] =100 nM)

Investigation of the comparison between the classical catalytic TMSDR in the solution and our model

Reporter was mixed with Protector (modified with BHQ2) at the ratio of 1:1.2, heated to 95°C for 5min, and then allowed to cool slowly to room temperature. The complex of Protector-Reporter was then diluted to 100nM in 1 × PBS buffer with or without fourfold amount of dissociative fuels. This solution was holding at 25°C for 10 h without Target strands. The fluorescence spectra were recorded on an F-7000 fluorometer (Hitachi) with 633 nm light excitation. The fluorescence intensity was normalized using the fluorescence from complete reaction. ([Target] =100 nM)

The NP_Protector-Reporter complex was diluted to 1 nM in 1 × PBS buffer with or without fourfold amount of NP_Fuel, as well as fourfold amount of dissociative fuels. This solution was holding at 25°C for 10 h. The fluorescence spectra were recorded and normalized using the fluorescence from complete reaction. ([Target] =100 nM)

For kinetics study of this experiment, the fluorescence intensity was recorded at 25°C for 2.5h.

S2 Supplementary Tables

Table S1. DNA sequences used in the experiments

Name	Sequence	Modification
NP_Protector-15bp-spacer	5‘-$(T)_{15}$-AGTGATAGCTTATCAGACTGAT-3’	5‘-SH C6
NP_Protector-10bp-spacer	5‘-$(T)_{10}$-AGTGATAGCTTATCAGACTGAT-3’	5‘-SH C6
NP_Protector-m	5‘-$(T)_{10}$-AGTGTAGCTTATCAGACTG-3’	5‘-SH C6
NP_Fuel-5bp-spacer- (5-5)	5‘-AGTGATAGCTTATCAGACTGAT-$(T)_5$-3’	3‘-SH C6
NP_Fuel-10bp-spacer- (5-5)	5‘-AGTGATAGCTTATCAGACTGAT-$(T)_{10}$-3’	3‘-SH C6
NP_Fuel-5bp-spacer- (7-5)	5‘-GAAGTGATAGCTTATCAGACTGATGTATTTT-3’	3‘-SH C6
NP_Fuel-10bp-spacer- (6-4)	5‘-GAAGTGATAGCTTATCAGACTGATGTT-$(T)_{10}$-3’	3‘-SH C6
NP_Fuel-m	5‘-GAAGTGTAGCTTATCAGACTGATGTAA-$(T)_8$-3’	3‘-SH C6
Reporter-1	5‘-TCAACATCAGTCTGATAAGCTATCACT-$(T)_{10}$-3’	3‘-Cy5
Reporter-2	5‘-CATCTCAACATCAGTCTGATAAGCTATCACTTCTCTTTTTT-3’	3‘-Cy5
Reporter-m	5‘-TCAACATCAGTCTGATAAGCTACACTTCTTTTTTTT-3’	3‘-Cy5
Target- (5-5)	5‘-TAGCTTATCAGACTGATGTTGA-3’	
Target- (7-5)	5‘-TAGCTTATCAGACTGATGTTGAGA-3’	
Target- (7-4)	5‘-ATAGCTTATCAGACTGATGTTGAGA-3’	
Target-m	5‘-TAGCTTATCAGACTGATGTTGA-3’	
Target-m4	5‘-TAGCTTATCAGACTGATGCTGA-3’	
Target-m5	5‘-TAGCTTATCAGACTGATCTTGA-3’	
Target-m14	5‘-TAGCTTATGAGACTGATGTTGA-3’	
Target-m20	5‘-TACCTTATCAGACTGATGTTGA-3’	
Target-m20A	5‘-TAACTTATCAGACTGATGTTGA-3’	
Target-m20T	5‘-TATCTTATCAGACTGATGTTGA-3’	
Target-i5A	5‘-TAGCTTATCAGACTGATGATTGA-3’	
Target-i5T	5‘-TAGCTTATCAGACTGATGTTTGA-3’	
Target-i5C	5‘-TAGCTTATCAGACTGATGCTTGA-3’	
Target-i5G	5‘-TAGCTTATCAGACTGATGGTTGA-3’	
Target-i14A	5‘-TAGCTTATACAGACTGATGTTGA-3’	
Target-i14T	5‘-TAGCTTATTCAGACTGATGTTGA-3’	
Target-i14C	5‘-TAGCTTATCCAGACTGATGTTGA-3’	
Target-i14G	5‘-TAGCTTATGCAGACTGATGTTGA-3’	
Target-d5	5‘-TAGCTTATCAGACTGATTTGA-3’	
Target-d20	5‘-TAGCTTATAGACTGATGTTGA-3’	
Protector-contrast	5‘-AGTGTAGCTTATCAGACTG-3’	5‘-BHQ2
Reporter-contrast	5‘-TCAACATCAGTCTGATAAGCTACACTTC-3’	3‘-Cy5
Fuel-contrast	5‘-GAAGTGTAGCTTATCAGACTGATG-3’	

Table S2. Rough estimate for capacity of duplex on NP_Protector

Fluorescence Intensity	1350	1293	1172
Concentration (nM)	86.02	82.63	75.43
Capacity	83%	83%	85%

As shown by Figure 1, we first constructed the duplex on AuNPs by attaching the Reporter to NP_Protector via hybridization. Given that the payload on AuNPs considerably affect the efficiency of reaction and steric effect, adding excessive Reporter at 1:100 in the process of annealing is essential. Complexes of Reporter and NP_Protector were purified by high-speed centrifugation to control the fluorescence background from redundant Reporter strands. The fluorescence intensity of supernatant from

first centrifugation was measured to quantitatively characterize the grafting ratio of duplex on AuNPs. The grafting ratio was calculated by a standard curve method (Figure. S3), which showed that NP_Protector could bind up to 80% of Reporter (Table S2). The fluorescence intensity was measured by supernatant after first centrifugation. And the concentration was calculated by using the following equation from standard curve. [Supplementary Equation (1)]:

$$\text{Fluorescence Intensity} = 16.82 \times \text{Concentration} - 96.84 \tag{1}$$

The initial concentration of Reporter strands is 500 nM. It could be assumed that the volume of the solution would not change after centrifugation. And then the capacity of duplex on NP_Protector was calculated by using the equation [Supplementary Equation (2)]:

$$\text{Capacity} = (500 \times \text{V} - \text{Concentration} \times \text{V}) / (500 \times \text{V}) \times 100\% \tag{2}$$

Table S3. Limit of Detection (LOD) in classical AuNPs-DNA system without fuels and our model with fourfold amount of NP_Fuel

Type	LOD (nM)
Classical AuNPs-DNA	1.45
Our model	0.258

The LOD was calculated by using the following equation [Supplementary Equation (3)]:

$$\mathrm{LOD} = 3 \times \sigma \,/\, \mathrm{slope} \tag{3}$$

Where the slope could be got from the linear simulation of concentration dependent experiments. The value of σ denotes the variation of fluorescence intensity without Target. It would be calculated respectively for different types of the reaction.

For classical AuNPs-DNA system without fuels, the variation of fluorescence intensity without Target might come from the machine error or the artificial error, and could be calculated by standard deviation from the fluorescence intensity without Target.

Then we had [Supplementary Equation (4)]:

$$\mathrm{LOD}_C = 3 \times \mathrm{SD}_C \,/\, \mathrm{slope}_C \tag{4}$$

Where $\mathrm{SD}_C = 0.0084$ and $\mathrm{slope}_C = 0.01737$ in this case.

For our model with fourfold amount of NP_Fuel, the reaction trend of concentration dependent experiment is polynomial, while it is almost linear under lower concentration (Figure 3, insert). The variation of fluorescence intensity from this system are the leakage with NP_Fuel relative to no NP_Fuel when there is no Target.

Then we had [Supplementary Equation (5)]:

$$\mathrm{LOD}_O = 3 \times \mathrm{SD}_O \,/\, \mathrm{slope}_O \tag{5}$$

Where SD_O could be derived as [Supplementary Equation (6)]:

$$\mathrm{SD}_O = |\, \mathrm{F}_{4xNP\_Fuel} - \mathrm{F}_{0xNP\_Fuel} \,| \tag{6}$$

F is the fluorescence intensity. Herein, $\mathrm{SD}_O = 0.004886$ and $\mathrm{slope}_O = 0.05691$ (Figure 3, insert).

Table S4. Selection factor of our model for SNP targets

SNP	m4	m5	m14	m20	i5A	i5T	i5C	i5G	i14A	i14T	i14C	i14G	d5	d14
Selection factor	52.9	17.31	29.7	1.66	95.9	20.64	43.85	16.31	7.75	2.26	3.46	20.18	73.15	40.10

The selection factor could be derived as [Supplementary Equation (7)[3]]:

$$\text{Selection factor} = (F_{[Target]} - F_{[Control]}) \,/\, |\, F_{[Mismatch]} - F_{[Control]} \,| \tag{7}$$

S3 Supplementary Figures

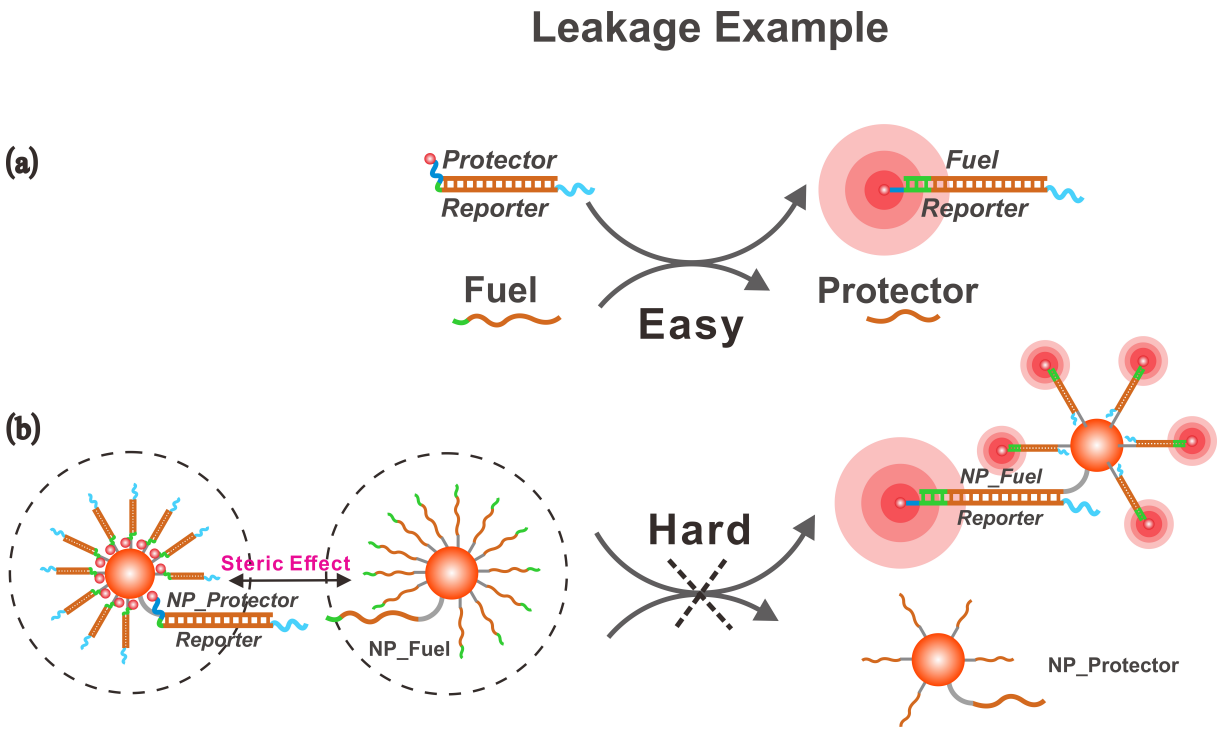

Fig. S1. Graphical representation of the possible leak reaction due to Fuel strand. (**a**) Classical catalytic TMSDR in the solution. (**b**) our model. The steric effect between AuNPs may come from high payload DNAs and help to make a crowded environment. It may make NP_Fuel hard to approach the toehold which is near the surface of NP_Protector -Reporter complex.

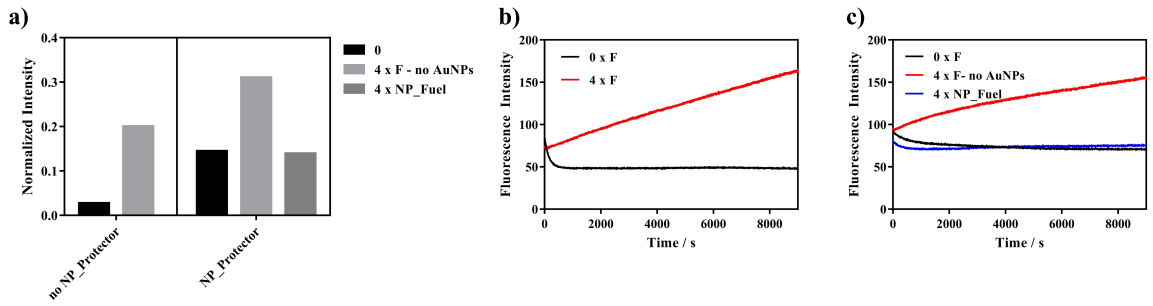

Fig. S2. Experimental data of comparison between the classical catalytic TMSDR in the solution and our model. (**a**) The comparison of the reaction with and without fuels. When there was fourfold amount of dissociative fuels (compared to Protector-Reporter complex), the leakage under no targets was extremely obvious. Nonetheless our model had better performance on leakage reduction. When our model was treated with fourfold amount of NP_Fuel or without fuels, the intensity was almost same after 10 h, however, the intensity increased a lot when the model was treated with fourfold amount of dissociative fuels. (**b**) Kinetics study of the classical catalytic TMSDR in the solution. The fluorescence intensity increased within 2.5 h in the reaction with dissociative strands. (**c**) Kinetics study of our model. The fluorescence intensity increased within 2.5 h in the reaction with fourfold amount of dissociative fuels, while the intensity of the reaction with fourfold amount of NP_Fuel still held in.

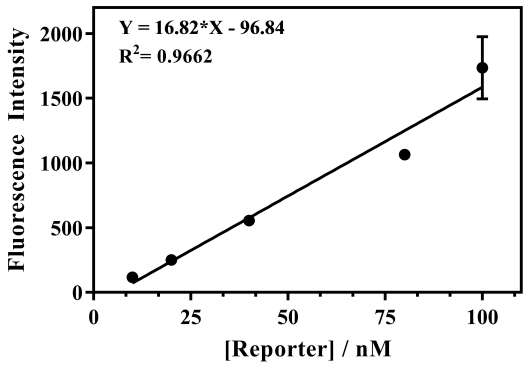

Fig. S3. Standard curve of the fluorescence intensity from Reporter strands to its concentration. Error bars indicate standard deviation (s.d.); N=3.

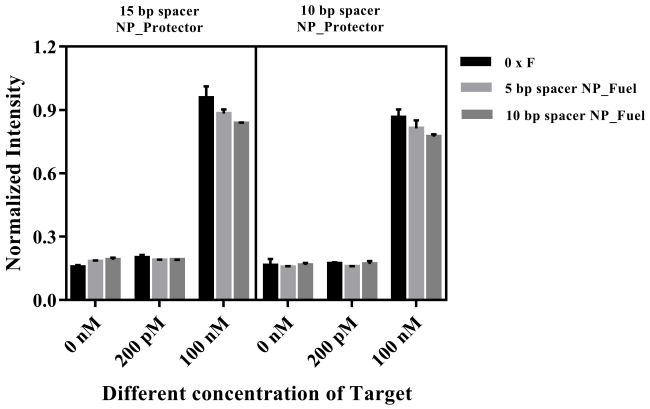

Fig. S4. Effects of spacer on NP_Protector and NP_Fuel in catalytic TMSDR with different concentrations of Target. The concentration of NP_Fuel was twice that of the NP_Protector-Reporter complex. Error bars indicate standard deviation (s.d.); N=3.

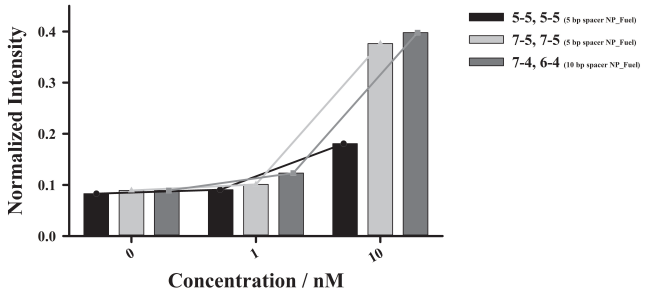

Fig. S5. Performance of the proposed model with different strategies of toehold under different concentrations of Target. Toehold (5-5, 5-5) and Toehold (7-5, 7-5) were applied to the model of NP_Fuel with 5 bp spacer. Toehold (7-4,6-4) was set for the model of NP_Fuel with 10 bp spacer. The concentration of NP_Fuel was twice that of the NP_Protector-Reporter complex.

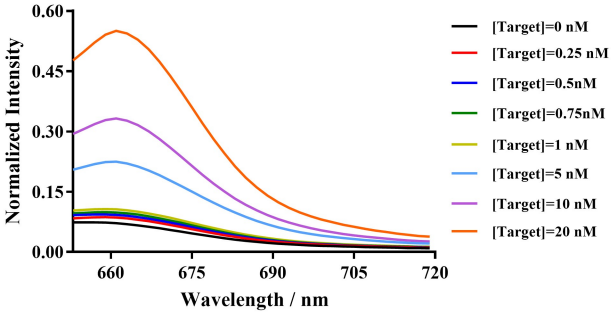

Fig. S6. The fluorescence spectra got from concentration dependent experiments. [Target] varies from 0 nM to 20 nM. The fluorescence intensity began to rise from 0.25 nM target.

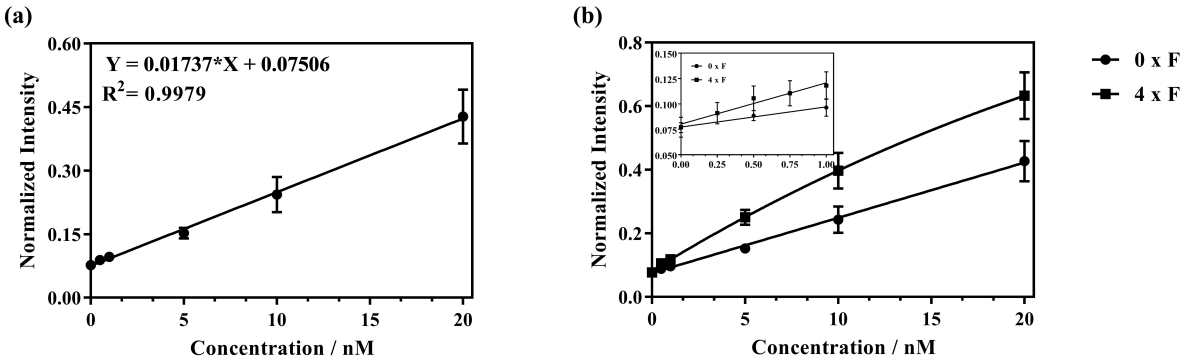

Fig. S7. Quantification of Target using fluorescence of Reporter measured after 10h. (**a**) Classical AuNPs-DNA system without NP_Fuel. [Target] varies from 0 nM to 20 nM. Error bars indicate standard deviation (s.d.); N=3. LOD of classical AuNPs-DNA system is 1.45 nM. Compared with it, our model had improved the efficiency with signal amplification. (**b**) The comparison of reaction trend between classical AuNPs-system without fuels and our model with fourfold amount of NP_Fuel. The inner image represents the quantification under lower concentration from 0 nM to 1 nM. Compared with it, our model had higher intensity after 0.25 nM and no obvious leak signal at the beginning without Target.

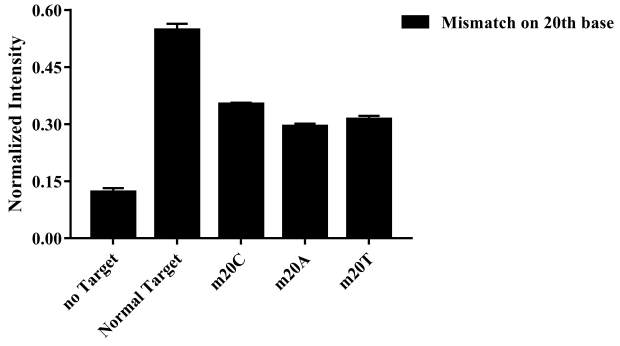

Fig. S8. Comparison of Normal Target and mismatches with different kinds of bases on 20th site.

References

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[2] R. C. Jin, G. S. Wu, Z. Li, C.A. Mirkin and G.C. Schatz, *J. Am. Chem. Soc.*, 2003, **125**, 1643–1654

[3] H. Y. Peng, X.-F. Li, H. Q. Zhang and X. C. Le, *Nat. Commun.*, 2017, **8**, 14378 EP–