Electronic Supplementary Information

Self-assembled conformational switch: host-guest stabilized triple stem molecular beacon *via* photoactivated and thermal regeneration mode

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Experimental details

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1. Materials and Apparatus

All oligonucleotides except CD-DNA in this work were custom-designed and then synthesized by Takara Bio Inc. Sequences of the oligonucleotides were listed in Table S1. The oligonucleotides concentrations were accurately identified according to UV absorption at 260 nm. Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) was provided by China National Medicines Co. Ltd. (Beijing, China). Azide-β-cyclodextrin was synthesized by Shandong Binzhou Zhiyuan Bio-Technology Co.,Ltd. Tris(benzyltriazolylmethylamine) (TBTA), tris(carboxyethyl) phosphine (TCEP) and the other reagents used in this work were purchased from Aldrich (Milwaukee, WI, USA) without further purification. 20 mM Tris-HCl buffer (pH 7.4, 140 mM NaCl, 5 mM $MgCl_2$) as the work solution was prepared with ultrapure Milli-Q water (resistance >18.2 MΩ cm^{-1}).

UV-vis spectra were collected by UV-1601 spectrophotometer (Shimadzu, Japan). All fluorescence spectra were recorded on an F-7000 spectrophotometer (Hitachi, Japan). β-cyclodextrin modified oligos were purified by an Agilent (USA) 1200 series HPLC system on a reverse-phase C18 column. The UV irradiation experiment was performed using a 6 W hand-held UV lamp (365 nm) for 30 min at 25°C. The probe regeneration experiment was performed on a Thermomixer comfort (eppendorf, Germany). The calculated Tm of stem2 and stem3 (63.9/67°C, respectively) in the TMB1 by the M-fold program (http://mfold.rna.albany.edu/?q=DINAMelt/Two-state -melting) was agreement with the reported melting point of them (R. Nutiu, Y. Li,

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Table S1 The name and sequence of the oligonucleotides used in this work[a]

Name	**Sequences**	**MW (**Da**)**
Hairpin DNA[b]	5'-CCTGCCACGCTCCGCT^{BHQ1}**GCGAGC***CACCAAATAT* *GATAT***GCTCGC**T^{FAM}CTCGCACCGTCCACC -3'	19040
Az-DNA	5'- Azobenzene -TGGT GGA CGG TGC GAG -3'	5388
CD-DNA	5'- GCG GAG CGT GGC AGG -β-cyclodextrin-3'	6222
Ak-DNA	5'- GCG GAG CGT GGC AGG-Alkyne -3'	5042
Target DNA	5'- TACTCTT*ATATCATATTTGGTG*TTTGCTTT -3'	9149

[a] TMB1 consists of Hairpin DNA, Az-DNA and CD-DNA. As a control, TMB2 consists of hairpin DNA, Az-DNA and Ak-DNA, which could not be in locked state. The molecular weight (MW) characterization data of the Hairpin DNA, Az-DNA and Target DNA were provided by Takara Bio Inc. The MW of CD-DNA and Ak-DNA were identified by MALDI-TOF mass spectrometry (as shown in Fig. S1).

[b] For Hairpin DNA, the middle domain complementary to Target DNA is presented in italic, and the bold domain could form an intramolecular stem by self-complement, designated as stem-1; the 3′ underlined domain is complement to Az-DNA, designated as stem-2; the 5′ underlined domain is complement to CD-DNA or Ak-DNA, designated as stem-3.

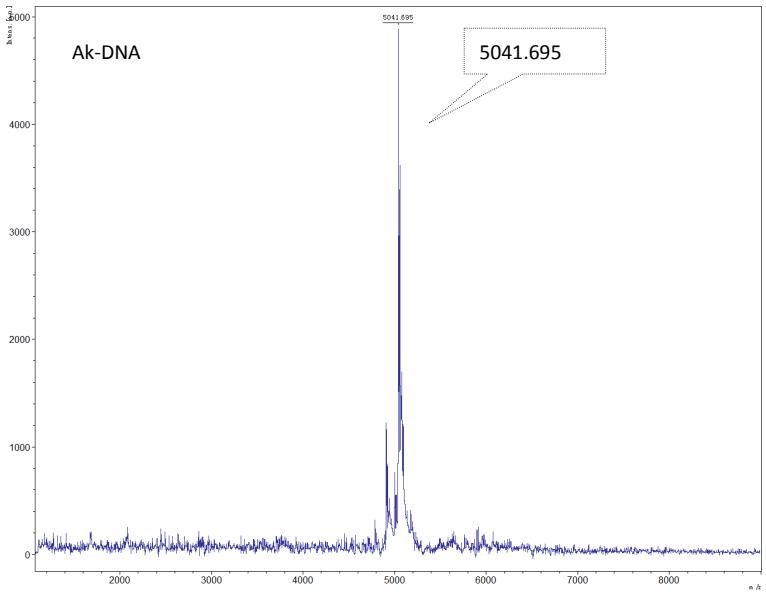
2. Synthesis and characterization of β-CD Modified DNA (CD-DNA)

To 80 μL of 0.17 mM Ak-DNA solution (13.6 nmol) in water, 40 μL of azide-β-cyclodextrin solution (20 mM) and 20 μL of solution containing $CuSO_4$ and TBTA Ligand in a 1:1 ratio in 4:3:1 H_2O/DMSO/*t*-BuOH (tert-Butanol) was added. The mixture was vortexed and as the last component 10 μL of a freshly prepared TCEP solution in water was added (80 mM). The mixtures were shaken at 15°C overnight, and then centrifuged at 8000 rpm for 5 min to obtain the colourless supernatants. The impure oligonucleotides were desalted and concentrated. The above process was repeated three times in order to obtain high yields of β-CD modified DNA. Finally, the mixtures were used for HPLC without further purification to accomplish the Ak-DNA modified with β-CD (named as CD-DNA).

The CD-DNA was twice purified using gradient elution RP-HPLC on a C-18 column with acetonitrile (buffer A) and 0.05 M triethylammonium acetate aqueous solution containing 5% acetonitrile (buffer B) as mobile phases (flow rate: 1 mL/min). The collected DNA products were desalted and dried. Then the purified DNAs were again quantified by determining the UV absorption at 260 nm using a UV-vis spectrometer.

The purified CD-DNAs were identified by MALDI-TOF mass spectrometry. Briefly, 1 μL of matrix 3-hydroxypicolinic acid (3-HPA) was first pipetted onto the plate, and then 1 μL of samples were pipetted and mixed thoroughly with the matrix. After the solvent evaporated, the plate was sent for MS analysis. The measured molecular weight (MW) of Ak-DNA was to be 5042 Da, which then modified with

azide-β-cyclodextrin (MW=1182 Da). So the calculated MW of CD-DNA should be 6224 Da, and the measured MW (CD-DNA) was 6222 Da in Fig. S1 where the MW (6245 Da) was a adduct peak $[CD\text{-}DNA+Na]^+$. Mass spectroscopy results confirmed that β-CD modified DNA (CD-DNA) had been successfully synthesized through click reaction (J. Gierlich, G. A. Burley, P. M. E. Gramlich, D. M. Hammond and T. Carell, *Org. Lett.* 2006, **8**, 3639-3642).



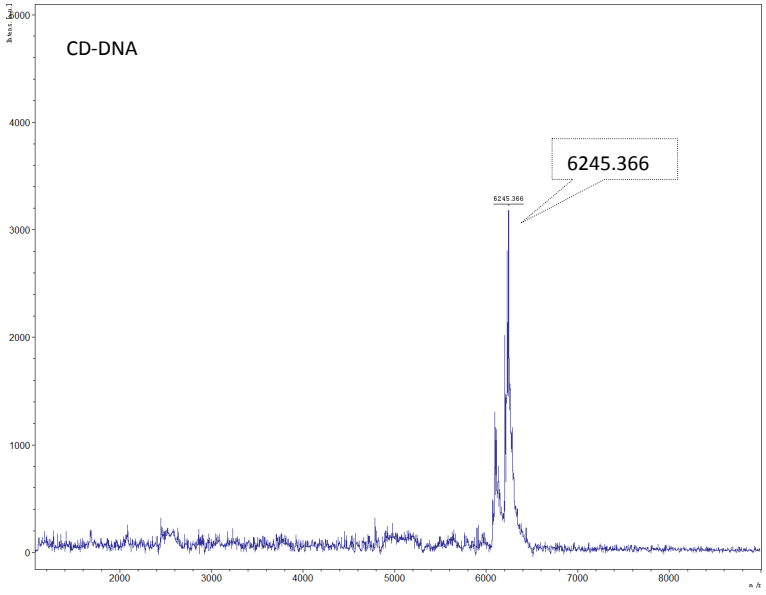


Fig. S1 MALDI-MS characterization of Ak-DNA(up) and CD-DNA(down).

3. Gel Electrophoresis

A 3% agarose gel was prepared using a 1 × TAE buffer (40 mM Tris AcOH, 2.0 mM Na_2EDTA, pH 8.5). The SYBR Gold was used as an oligonucleotide dye and mixed with samples. The gel was run at 100 V for 30 min in a 1 × TAE buffer and was then scanned using the gel image analysis system (Tanon 2500R, Shanghai, China).

The constructs (TMB1 and TMB2) were examined by agarose gel electrophoresis (Fig. S2). By mixing three DNA strands (Hairpin DNA, Az-DNA, CD-DNA), a new bright band, which migrated slower than the Hairpin DNA, Az-DNA, and CD-DNA, was observed. The new bright band indicated that the TMB1 indeed consisted of three DNA strands (Hairpin DNA/Az-DNA/CD-DNA). The similar tendency of gel electrophoresis was observed for the TMB2, indicating that the TMB2 also consisted of three DNA strands (Hairpin DNA/Az-DNA/Ak-DNA).

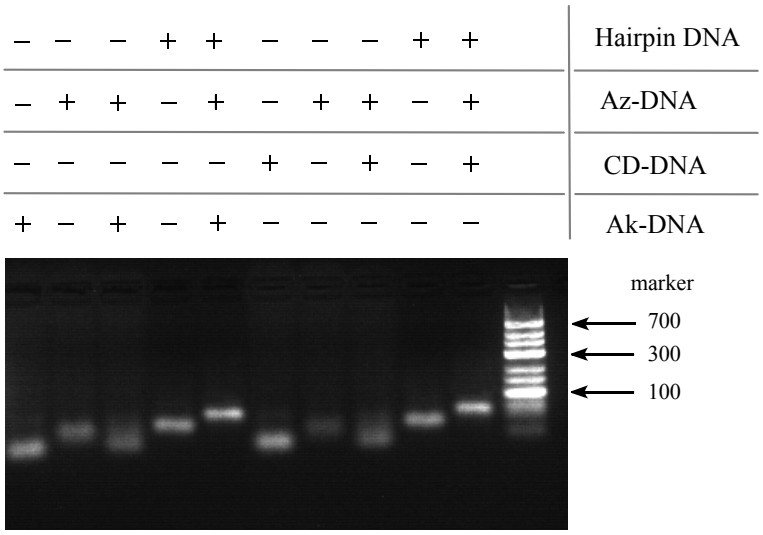


Fig. S2 Agarose gel (3%) electrophoresis images of various conditions. The concentration of Hairpin DNA, Az-DNA, CD-DNA and Ak-DNA were all 1 μM.

4. Photoactive assay for nucleic acid hybridization

For the triple-stem molecular beacon photoactive hybridization assay, 20 μL 2 μM of the hairpin DNA, 6 μL 20 μM CD-DNA (or 6 μL 20 μM Ak-DNA), 6 μL 20 μM Az-DNA and 8 μL 20 μM of target DNA (or blank buffer solution) were mixed and diluted to 400 μL with Tris-HCl buffer. After irradiation with UV light (365 nm) and stirring for 30 min at room temperature, the fluorescence emission spectra (Fig. S2) were recorded by exciting the samples at 488 nm and scanning the emission from 500 to 600 nm at 1 nm intervals. In time scanning mode, excitation and emission wavelengths were set at 488 and 520 nm, respectively, with the bandwidth of 5 nm.

By setting the fluorescence intensity when the locked TMB1 solution without target DNA was on close state as a baseline (0%) and the intensity after addition of excess amount of cDNA (4-fold) to the irradiation-treated TMB2 solution as 100%, we could estimate the percentage of open state under various conditions. In our case, we were able to define a fluorescence recovery parameter as recovery (%), which was based on the following three states in order to evaluate the percentage of open state.

$$\textbf{recovery (\%)} = (I_{vc} - I_0)/(I_1 - I_0) \qquad \textbf{Formula.1}$$

I_{vc} is the fluorescence intensity of the TMB1 solution under various conditions, I_0 is the fluorescence intensity when the locked TMB1 solution was on close state, I_1 is the fluorescence intensity of TMB2 solution when 4-fold excess of cDNA was added after UV irradiation. As such, we found only 7.9% of recovery for the TMB1 solution which was hybridized with target DNA without UV irradiation, but approximately 74% for the above solution after UV irradiation for 30 min.

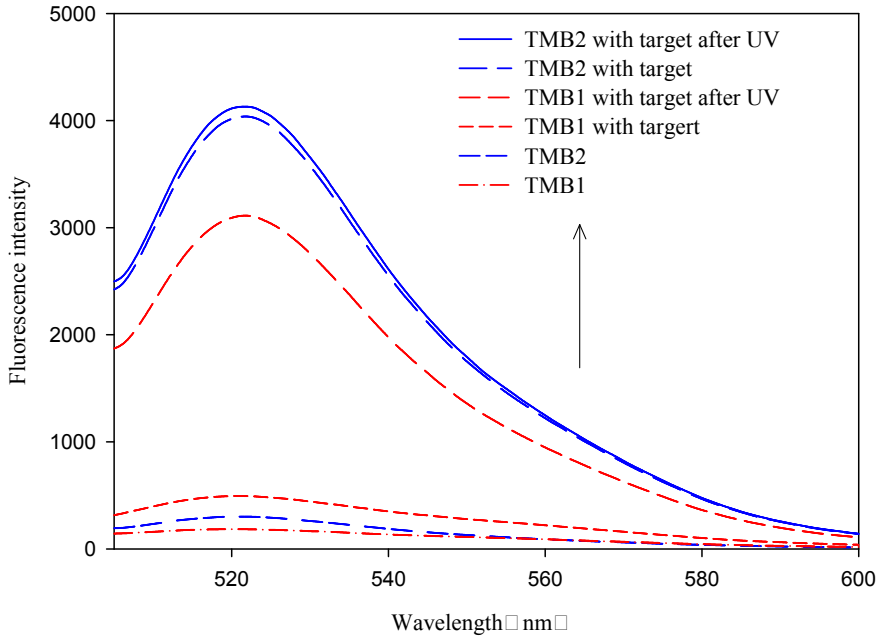


Fig. S3 Fluorescence emission spectra of TMB1 and TMB2 under different conditions (λ_{ex}=488 nm). 100 nM TMB1 or TMB2, 400 nM target DNA in 20 mM Tris-HCl buffer (pH 7.4, 140 mM NaCl, 5 mM $MgCl_2$).

5. Regulation of the TMB1 by adding competitive guest molecules

20 μL 2 μM of the hairpin DNA, 6 μL 20 μM CD-DNA (or 6 μL 20 μM Ak-DNA), 6 μL 20 μM Az-DNA and 8 μL 20 μM of target DNA (or water) were mixed and diluted to 396 μL with Tris-HCl buffer. After stirring for 30 min at room temperature, addition of 4 μL 30 mM amantadine hydrochloride (Ad-HCl) or water was used to monitor fluorescence recovery of this system. The fluorescence emission spectra (Fig. 3) were recorded by exciting the samples at 488 nm and scanning the emission from 500 to 600 nm at 1 nm intervals. In time scanning mode, excitation and emission wavelengths were set at 488 and 520 nm, respectively, with the bandwidth of 5 nm.

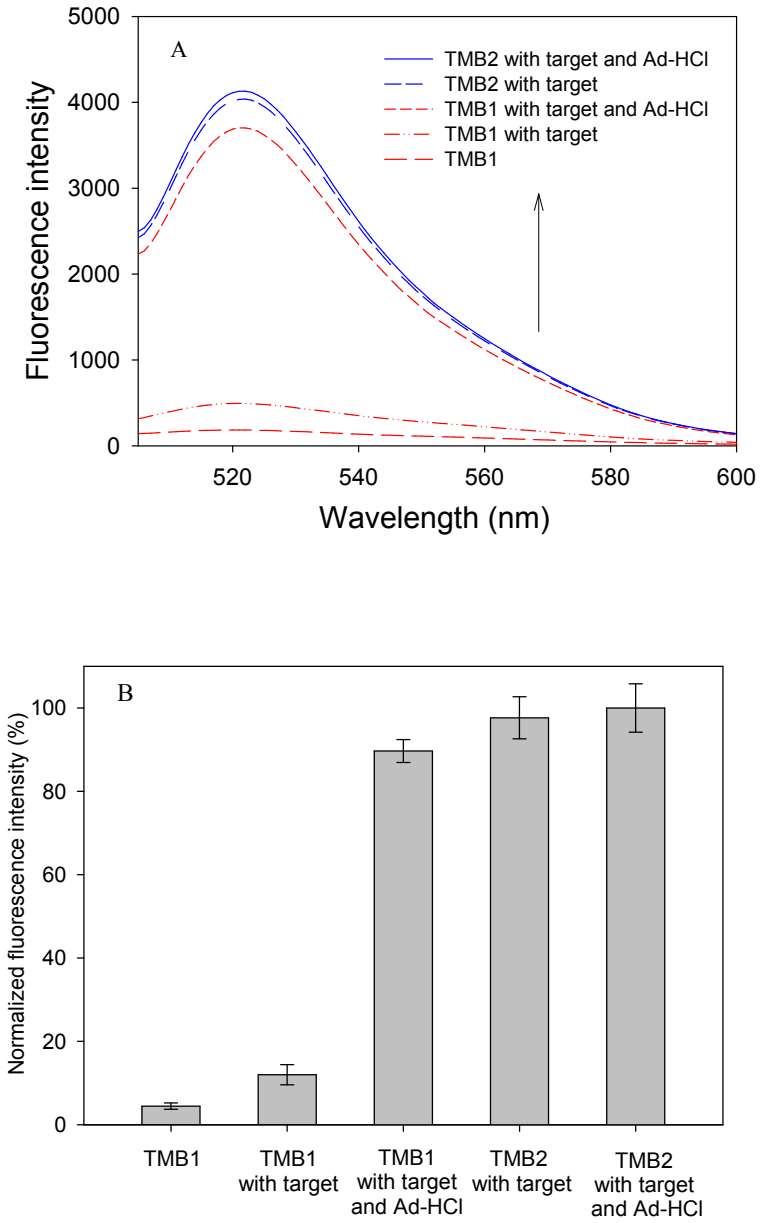


Fig. S4 Fluorescence response of the host-guest stabilized triple stem molecular beacon in the presence of amantadine hydrochloride (Ad-HCl). 100 nM TMB1 or TMB2, 400 nM target DNA in 20 mM Tris-HCl buffer (pH 7.4, 140 mM NaCl, 5 mM $MgCl_2$).

A. Fluorescence emission spectra of TMB1 and TMB2 under different conditions (λ_{ex} = 488 nm).

B. The corresponding fluorescence response histogram ($\lambda_{ex/em}$ = 488/520 nm).

Fluorescence intensity was normalized to a percentage of the maximum signal coming from 100 nM TMB2 hybridized with 400 nM target DNA in the presence of 0.3 mM amantadine hydrochloride (Ad-HCl).

6. Kinetics study of the TMB1

To study kinetics and efficiency of the conformation switch, the fluorescence of the TMB1 with targets were monitored under various conditions. As shown in Fig.S4A, with increasing UV illumination time at 25°C, the photoactive significant signal changes (75% of the fluorescence intensity at 30 min) of the probe could be observed in 5 min and the signal tended to a stable value in 10 min. Similarly, the tendency of thermal regeneration was observed (as shown in Fig.S4B).

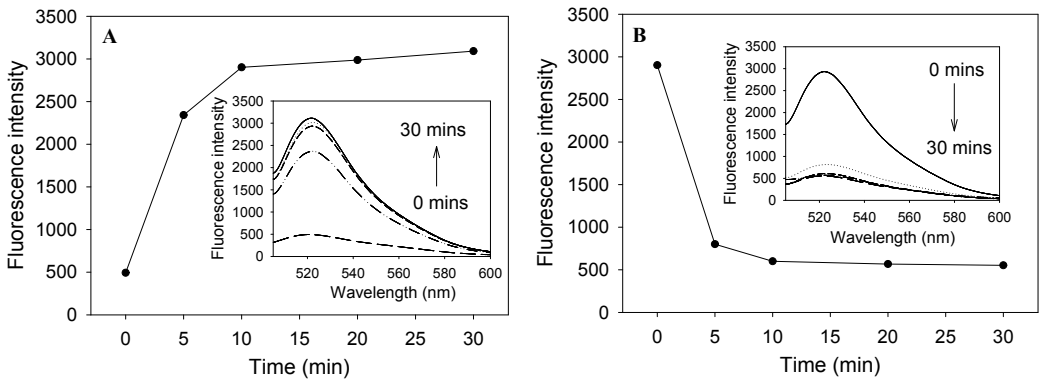


Fig. S5 Kinetics study of the TMB1 in the presence of target DNA under different conditions: A) UV illumination at 25°C, B) heating up to 55°C for different times (0, 5, 10, 20, 30 min). Inset shows the corresponding fluorescent spectra.