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Supporting Information

Photoregulated Split Aptaswitch for Small Molecules With Improved Sensitivity

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Supporting Information

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1 Materials and Instruments

Reagents and materials. All DNA oligonucleotides (HPLC purified) were purchased from Takara Biomedical Technology Co., Ltd. (Beijing, China). The names and sequences of oligonucleotides used in this work are listed in **Table S1**, where X represents an azobenzene (Azo) unit.

All reagents were used as received. Adenosine (Ade), Tris-HCl, sodium chloride (NaCl), and magnesium chloride ($MgCl_2$) were purchased from Sigma Aldrich. Molecular biology grade USP sterile purified water (Corning Cellgro, NY, USA) was used throughout the experiment.

Table S1 Oligonucleotide sequences used in this work

Name	Length	Sequence (5'-3')
wt-SA1	13	FAM-TGCGGAGGAAGGT
wt-SA2	13	ACCTGGGGGAGTA-BHQ1
M-F	19	FAM-CGCCTCACCTGGGGGAGTA
N-0X	19	TGCGGAGGAAGGTGAGGCG-BHQ1
N-1X	19	TGCGGAGGAAGGT**X**GAGGCG-BHQ1
N-2X	19	TGCGGAGGAAGGT**X**GAG**X**GCG-BHQ1
N-3X	19	TGCGGAGGAAGG**X**TG**X**AG**X**GCG-BHQ1
PSA1	17	CCTCACCTGGGGGAGTA-BHQ1
PSA2	16	FAM-TGCGGAGGAAG**X**GT**X**GA**X**GG

Instruments. All fluorescence (FL) spectra were obtained using an fluorescence spectrophotometer (F-7000 spectrophotometer, Hitachi, Japan). The temperature of cell holder is controlled using Quantum Northwest temperature controller (stirring speed 900 rpm). The fluorescence intensities of FAM-labeled oligonucleotide systems were recorded with an excitation wavelength of 495 nm. The UV irradiation is conducted using a portable UV flashlight (3W, 365 nm) in the dark environment. Visible light irradiation is conducted using a portable flashlight

(6W) with a 450 nm filter in the dark environment. The power supply of the flashlights are rechargeable 18650 batteries. A fully charged battery is used in each round of irradiation to eliminating errors caused by charge decay. Before each test, Azo-modified oligonucleotides were irradiated by visible light (450 nm) for 10 min.

2 NUPACK Simulation

NUPACK script for PD design. The simulation was run by the "Design" functional module of NUPACK online tool[1] based on the following script:

```
domain a = Nn (n =1 , 2 , 3… … )
domain b = ACCT
domain c = GGGGGAGTAT
domain d = TGCGGAGGA
material = dna
temperature[C] = 25.0 # optional units: C (default) or K
trials = 3
sodium[M] = 0.3       # optional units: M (default), mM, uM, nM, pM
magnesium[mM] = 5
dangles = some
# design Azo photoregulation cycle for Ade split aptamer
#
# target structures
structure output = D10(U10+U9)
domain a = NNNNNN
domain b = ACCT
domain c = GGGGGAGTAT
domain d = TGCGGAGGA
strand M = a b c # abc must be separated by blank
strand N = d b* a*
output.seq = M N
prevent = AAAA, CCCC, GGGG, UUUU, KKKKKK, MMMMMM, RRRRRR, SSSSSS, WWWWWW, YYYYYY
#
```

```
# specify stop conditions for normalized ensemble defect
# default: 1.0 percent for each target structure
#
output.stop[%] = 5.0   # optional units: % or frac
```

Equilibrium concentrations analysis by NUPACK. The simulation was run by the "Analysis" functional module of NUPACK online tool based on the following parameters: 100 nM Seq.M, 100 nM Seq.N, 250 mM Na^+, 5 mM Mg^{2+}. The Dangle treatment: Some. Nucleic acid type: DNA. Maximal complex size: 3 strands. Two rounds of simulations were run at 25 ^oC and 40 ^oC based on the sequences of M-F and N-0X, respectively. As a control, the equilibrium concentrations of wt-SA was analyzed at 25 ^oC using the same parameters.

3 Experimental Methods

Hybridization of photoregulated domain (PD). 100 nM M-F and 100 nM N-3X were incubated together in 1 mL buffer (10 mM Tris, 1 mM $MgCl_2$, pH 7.6) at 25 ^oC or 40 ^oC for different times. After incubation, the fluorescence intensities at different times (F) were measured by a spectrometer, as shown in **Fig. S3**, where F0 represents fluorescence intensity of 100 nM M-F without N-3X addition. The fluorescence variation of the solution containing 100 nM wt-SA1 and 100 nM wt-SA2 against time was measured as a control group (**Fig. S4**).

Fluorescence recovery upon UV irradiation. As abovementioned, 100 nM M-F and 100 nM N-3X were hybridized in 1 mL buffer (10 mM Tris, 1 mM $MgCl_2$, pH 7.6). Next, the samples were irradiated by UV for different times at 25 ^oC or 40 ^oC. Under UV irradiation, the conformation of Azo units inserted in N-3X changed from to *trans*-Azo to *cis*-Azo, which can lead to the dissociation of M-F and 100 N-3X. Therefore a gradual recovery of fluorescence can be observed. The fluorescence intensities after different irradiation times (F) were measured by a spectrometer, as shown in **Fig. S5**, where F0 represents fluorescence intensity of 100 nM M-F without N-3X addition.

Optimizations of pH and salt concentrations. The optimal reaction pH, Na^+ concentration and Mg^{2+} concentration were investigated using M-F and N-3X system at 40 ^oC. For pH optimization, 100 nM M-F and 100 nM N-3X were hybridized in 1 mL buffer (10 mM Tris, 1 mM $MgCl_2$) of different pH values. For Na^+ concentration optimization, 100 nM M-F and 100 nM N-3X were hybridized in 1

mL buffer (10 mM Tris, 1 mM $MgCl_2$, pH 7.6) containing different concentrations of NaCl. For Mg^{2+} concentration optimization, 100 nM M-F and 100 nM N-3X were hybridized in 1 mL buffer (10 mM Tris, 50 mM NaCl, pH 7.6) containing different concentrations of $MgCl_2$. After hybridization, the samples were treated by UV irradiation for 10 min. The photoregulation performances under different reaction conditions were compared using photoregulation efficiency (PE), which is calculated based on the following equation:

$$PE = \frac{F_{UV} - F_2}{F_1 - F_2}$$

where F_1 and F_2 represent the fluorescence intensities measured before and after the hybridization of M-F and N-3X. F_1 was measured immediately after the addition of N-3X; and F_2 was measured 20 min after the addition of N-3X. F_{uv} represents the FL intensity measured after UV 10-min irradiation.

Optimizations of the numbers of Azo units. Under the optimal condition obtained, numbers of Azo units was optimized using M-F and N-3X system at 40 ^oC. 100 nM M-F solutions are incubated with 100 nM Seq.N containing different numbers of Azo units (N-0X, N-1X, N-2X and N-3X) in 1 mL buffer (10 mM Tris, 50 mM NaCl, 1 mM $MgCl_2$, pH 7.6). After hybridization, the samples were treated by UV irradiation for 10 min. The PEs of different samples were compared.

Dynamic biorecognition of small-molecule target using PSA. In testing the reaction rate of PSA system, equal concentrations of PSA1, PSA2 (100 nM) were mixed in 990 μL Tris buffer (10 mM Tris, 50 mM NaCl, 1 mM $MgCl_2$, pH 7.6), then 10 μL of 100 mM adenosine was added into the PSA mixture, and the fluorescence intensity was observed at 25 ^oC for around 900 s (**Fig. S9**). In this system, normalized FL (equation (5) in the maintext) can reflect the content of reactant (1-*x*/*a*). Thus the reaction rate constant *k* can be calculated by correlating ln(1-*x*/a) with *t* (**Fig. 3**B in the maintext).

Detection of small-molecule target using PSA, CT1 and CT2 systems. In detecting adenosine (Ade) using PSA system, 100 nM PSA (PSA1 and PSA2) were hybridized in 1 mL Tris buffer (10 mM Tris, 50 mM NaCl, 1 mM $MgCl_2$, pH 7.6) for 20 min at 25 ^oC. After hybridization, the fluorescence intensity of PSA system was recorded as *F*1. Next, small volumes of different

concentrations of Ade were added into 1 mL ph-SA system for 20 min, the obtained fluorescence intensity was recorded as *F*2. Then the samples were treated with UV irradiation for 10 min, the obtained fluorescence intensity was recorded as *F*3. The fluorescence recovery ratios for calibration curve plotting can be calculated based on *F*1, *F*2 and *F*3.

As a control group without prehybridization (CT1), 100 nM wt-SA (wt-SA1 and wt-SA2) and different concentrations of Ade was mixted in 1 mL Ade binding buffer (10 mM Tris, 250 mM NaCl, 5 mM $MgCl_2$, pH 7.6) for 20 min at 25 ^oC, then the fluorescence intensities of obtained solutions were measured to plot the calibration curve (**Fig. 3**E). As a control group without photoregulation (CT2), 100 nM PSA (PSA1 and PSA2) are hybridized in 1 mL Tris buffer (10 mM Tris, 50 mM NaCl, 1 mM $MgCl_2$, pH 7.6) for 20 min at 25 ^oC to form prehybridized SA (ph-SA). The fluorescence intensity of ph-SA system was recorded as *F*1. Next, small volumes of different concentrations of Ade were added into 1 mL ph-SA system for 20 min, the obtained fluorescence intensity was recorded as *F*2. The decreased fluorescence (*F*2 – *F*1) was calculated to plot the calibration curve (**Fig. 3**F).

4 Supplementary Figures

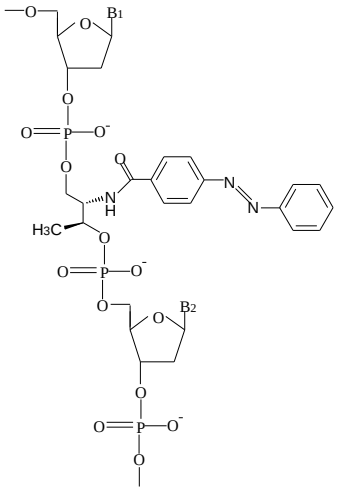

Fig. S1 Chemical structure of an azobenzene (Azo) unit inserted in the oligonucleotide. B_1 and B_2 represent two bases.

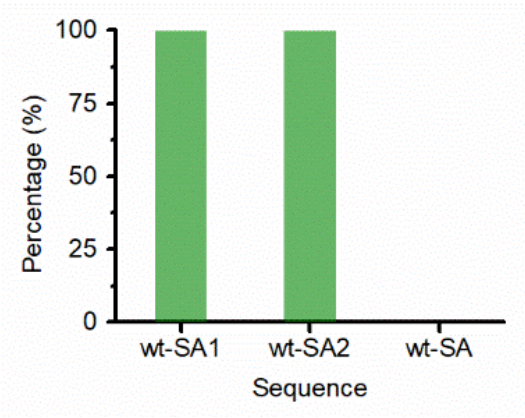

Fig. S2 Percentages of different wt-SA1, wt-SA2 components at equilibrium state predicted by NUPACK online tool.

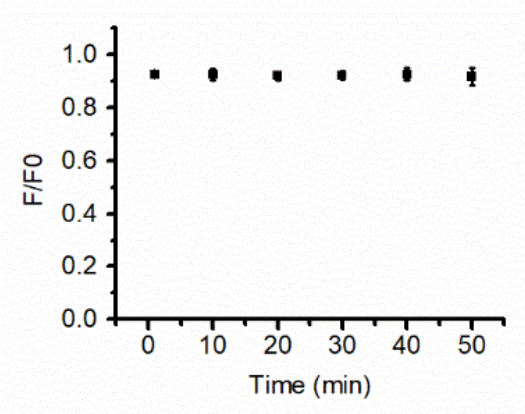

Fig. S3 F/F0 against time at 25 ºC (100 nM wt-SA1 and 100 nM wt-SA2, 10 mM Tris, 1 mM Mg^{2+}).

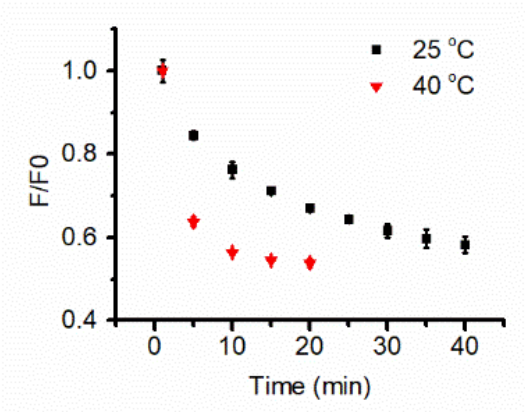

Fig. S4 F/F0 against time at 25 ºC or 40 ºC (100 nM M-F and 100 nM N-3X, 10 mM Tris, 1 mM Mg^{2+}).

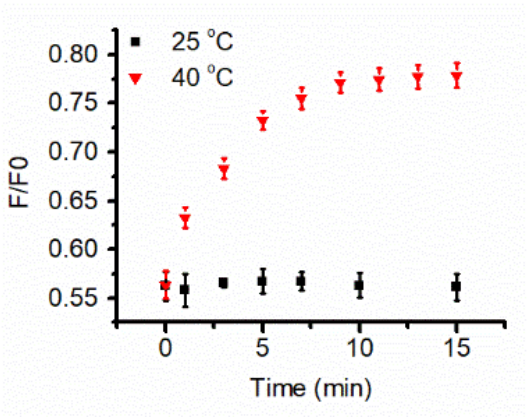

Fig. S5 UV irradiation induced FL recovery against time at 25 ºC or 40 ºC (100 nM M-F and 100 nM N-3X, 10 mM Tris, 1 mM Mg^{2+}).

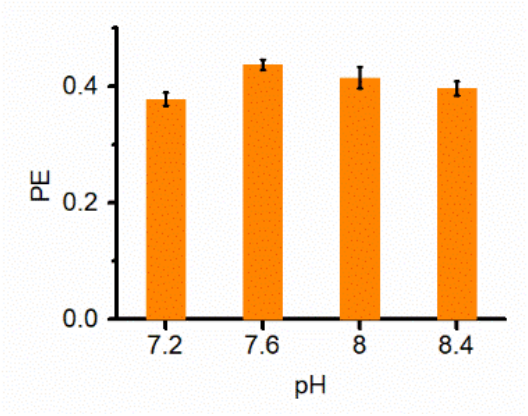

Fig. S6 Photoregulation efficiencies (PEs) in solutions of different pH values.

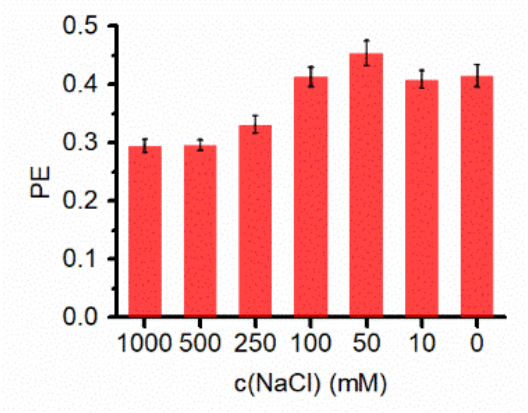

Fig. S7 Photoregulation efficiencies (PEs) in solutions containing different concentrations of NaCl.

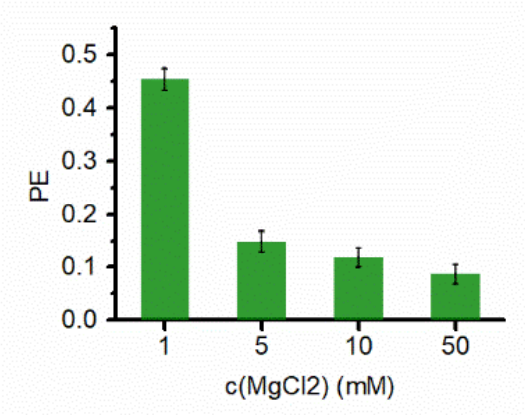

Fig. S8 Photoregulation efficiencies (PEs) in solutions containing different concentrations of $MgCl_2$.

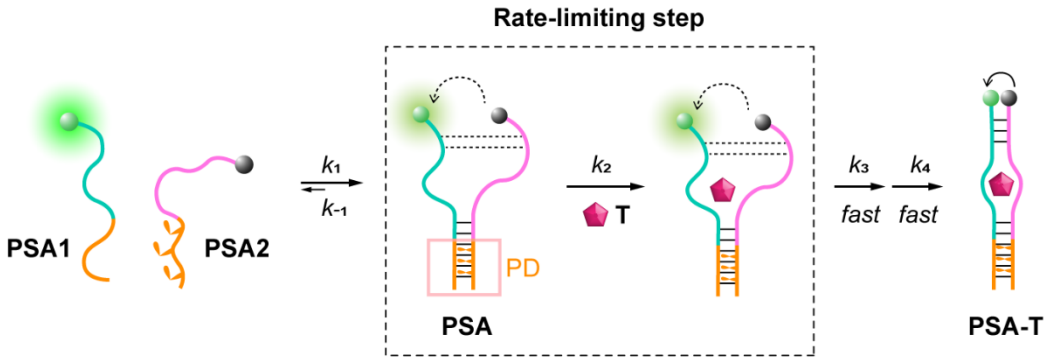

Fig. S9 Schematic illustration of the "bimolecular" reaction pathway of PSA system.

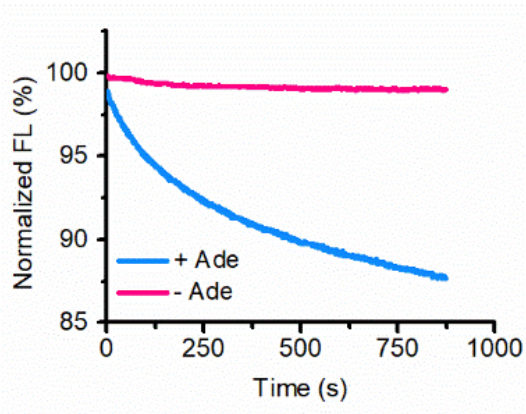

Fig. S10 Time scan fluorescence spectra of PSA system with or without Ade addition (10 mM Tris, 1 mM $MgCl_2$, 100 nM PSA, 100 nM N-3X).

Table S2 Several adenosine biosensing assays using split aptamers

Entry	Method	LOD /μM	Detection time [a] /min	Reference
1	Gold nanoparticles based colorimetric biosensor	250	30	[2]
2	One-step dynamic light scattering method	0.007	30	[3]
3	Split aptazyme-based catalytic molecular beacons	1	150	[4]
4	Adenosine-dependent assembly of aptazyme-functionalized gold nanoparticles	100	30	[5]
5	Fluorescence resonance energy transfer-based DNA nanoprism	30	60	[6]
6	Electrochemiluminescence aptasensor based on target-induced linkage of split aptamer fragments	0.64	/	[7]
7	Split aptamer-based dipstick strip	0.5	20	[8]
8	Luminescent aptamer sensor attached to a terbium complex	60	70	[9]
9	Photoregulated split aptaswitch	0.3	50	This work

[a] Please note that the preparation of functional nanomaterials and the formation of nucleic aicd structures take extra time in entries 1-8, which are not inclueded in the detection time. For example, the time required for the preparation of AuNPs-DNA is about 57h.

5 References

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