

## Electronic Supplementary Information

# Ultrasensitive Detection of Small Molecule-Protein Interaction *via* Terminal Protection of Small Molecule Linked DNA and Exo III-Aided DNA Recycling Amplification

Guohua Zhou, Xian Zhang, Xihu Ji\* and 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*

## 1. EXPERIMENTAL SECTION

### Chemicals and Materials.

All oligonucleotides with different sequences were synthesized and purified with HPLC by Sangon Biotechnology Co., Ltd (Shanghai, China). The sequences of the oligonucleotide used in this work are as follows:

- (1) 5'-ROX-AGGAAGACGTACGTATCTTCCT-(BHQ-2)-TTTGTTC-3' (Molecular Beacon, MB);
- (2) 5'-GAACAAAAGGAAGATACGTACAAAATC -3' (Trigger strand in "turn-off" strategy);
- (3) 5'-GTACGTATCTTCCTTTTGTTC-Biotin-3' (Antisense strand of trigger strand in "turn-off" strategy);
- (4) 5'-GAACAAAAGGAAGATACGTACAAAATC-Biotin-3' (SA binding and trigger strand in "turn-on" strategy);
- (5) 5'-GATTTTGTACGTATCTTCCTTTTGTTC-3' (Antisense strand of trigger strand in "turn-on" strategy).

Exonuclease III was purchased from Takara Biotechnology (Dalian) Co., Ltd. Streptavidin (SA) was purchased from Amresco (USA). Anti-biotin antibody was purchased from Jackson ImmunoResearch Laboratories, Inc. (USA). The other reagents were obtained from Sigma-Aldrich (USA). All pure water with a resistivity of 18.2 MΩ cm was purified by Milli-Q Academic purification set from Millipore (USA).

### Agarose-gel electrophoresis

All of the samples were incubated at 37 °C for 1 hour, and then 10 μL of samples were mixed with SYBR Green I fluorescent dye and incubated at room temperature for 10 min before loading to 2% agarose gel. The electrophoresis was run with a voltage of 10 V/cm for 1 hour.

### General determination of biotin-SA interaction with "turn-off" and "turn-on" strategies.

If not specifically defined, the SA binding was determined using 100 nM MBs, 10 nM probes and 50 U Exo III. For both strategies, different concentrations of SA were mixed with probes in 270 μL Tris-HCl buffer (50 mM Tris, 300 mM NaCl, 5 mM Mg<sup>2+</sup>, pH = 8.0), and then the mixture was incubated at 37 °C for 30 min. After sufficient binding of the SA, MBs and Exo III were added, and incubated for another one hour to generate the signal. The fluorescence of solution was measured with RF-5301pc spectrofluorophotometer, Shimadzu (Japan).

### Ultrasensitive determination of biotin-SA interaction.

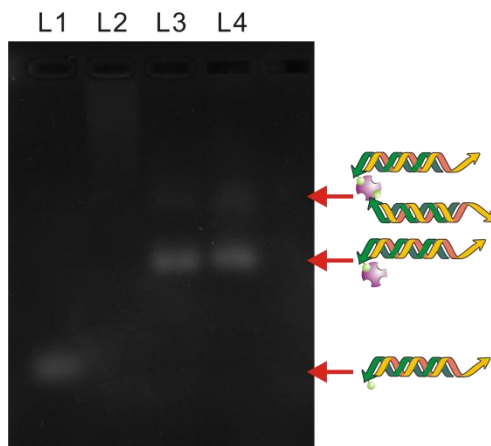
For the ultrasensitive determination of biotin-SA interaction, different concentration of SA were mixed with 100 nM probes first in 20 μL Tris-HCl buffer, and incubated at 37 °C for 30 min. Subsequently, 1 μM MBs and 400 U Exo III were added, and the mixtures were kept at 4 °C for 24 hours. After incubation, the fluorescence of solution was measured with a Square Micro Fluorometer Cell (3 × 3 mm, Starna Cells, USA).

### Determination of biotin-antibody interaction.

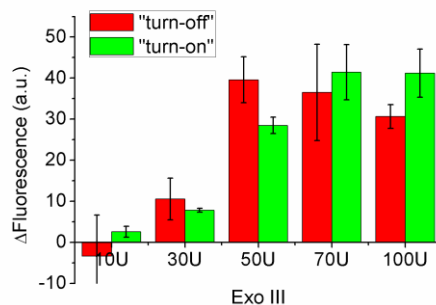
To determine the interaction of biotin-antibody with "turn-off" strategy, different concentrations of antibody bond with 10 nM probes firstly in 20 μL buffer solutions at 37 °C for 30 min. Then 0.5 μM MBs and 5 U Exo III were added, and then incubated for another one hour at 37 °C. Before measurement of fluorescence, 250 μL buffer was added.

Since the antibody is more specific to conjugated biotin (cBiotin), we employed biotinamidocaproyl hydrazide as the target to investigate the performance of proposed method for competitive detection of small molecule. Different concentrations of cBiotin were mixed with 1 μM antibody and 10 nM probes, and incubated at 37 °C for 1 hour. Subsequently, the signal generation and measurement were as the same as that of determination of biotin-antibody interaction.

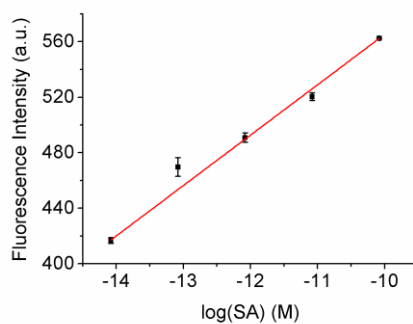
## 2. SUPPLEMENTARY FIGURES AND TABLE



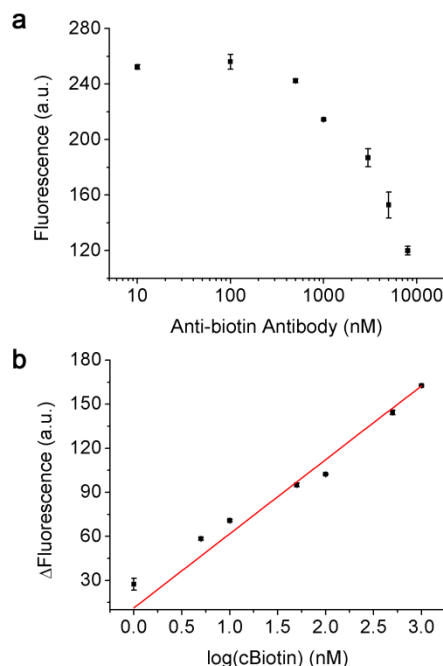
**Figure S1.** Agarose-gel electrophoresis result showing the binding of SA to biotin-labeled DNA and the protection of probes from degradation by Exo III. The double strand biotin-labeled DNA probe shows a clear fluorescent band in the presence of SYBR Green I fluorescent dye (L1). After digestion by Exo III, the band of probe disappears, due to the antisense strand was degraded and the double strand structure of probe was lost (L2). In the presence of SA, the migration of fluorescent band is less, resulting in the formation of large SA-DNA complex (L3). These bands of SA-DNA complex still exist after incubated with Exo III, indicating the protection of DNA from degradation (L4).



**Figure S2.** Fluorescence change of different strategies in the presence of SA with different Exo III. In the “turn-off” strategy, fluorescence change increased with increasing of Exo III at first, and then decreased with further increase of Exo III. Differently, the fluorescence change was increased with the increasing of Exo III in the “turn-on” strategy. The concentration of SA was 1.2 nM in the assays.



**Figure S3.** Ultrasensitive determination of SA-biotin interaction with “turn-on” strategy by directly measuring the fluorescence of 20  $\mu$ L reacting solution.



**Figure S4.** (a) Binding of anti-biotin antibody to biotin-labeled DNA determined by “turn-off” strategy. (b) Fluorescence change plotted versus the concentration of conjugated biotin. A linear response in the range of 1 to 1000 nM was obtained, showing the potential of proposed method for competitive immunoassay of small molecules.

**Table S1** Comparison of proposed method with other methods that are capable for small molecule-protein interaction analysis

Target	Amplification method	signal	Response range <sup>a</sup>	Ref.
abscisic acid	Multi-loaded enzyme on quantum dots	Chemiluminescence	1 pM–10 nM	1
p53 protein	Multi-loaded enzyme on gold nanoparticles	Chemiluminescence	0.1 pM–5.7 nM	2
PSA protein	Rolling circle amplification (RCA)	Fluorescent imaging	3.5 fM–350 fM	3
IL-9 protein	Hybridization Chain Reaction (HCR)	Fluorescent imaging	1.3 fM–0.13 nM	4
ETS1 protein	polymerase chain reaction (PCR)	Fluorescent imaging	0.019 fM–19.2 $\mu$ M	5
PSA protein	Multi-loaded DNA on gold nanoparticles coupled with PCR	Light scattering	3 aM–300 fM	6
human IgG	Multi-loaded DNA on magnetic particles coupled with RCA	Fluorescence	10 aM–1 pM	7
Human IgG	Multi-loaded DNA on gold nanoparticles coupled with HCR	Electrochemical response	0.7 aM–0.7 nM	8
SA binding	Exonuclease III aided target recycling	Fluorescence	8.3 fM–83.3 pM	This work

<sup>a</sup>The concentrations were calculated based on the molecular weight of proteins obtained from Protein Knowledgebase (UniProtKB): p53, 43653 Da; PSA, 28741 Da; IL-9, 15909 Da; ETS1, 50408 Da.

### Reference for the Supplementary Information:

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