Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

The fast detection of streptavidin based on initial reaction rate of the binding-induced DNA strand-displacement reaction

Chenxi Li,[ab] Ruoyun Lin,[a] Tian Li,[a] Feng Liu,[a] Na Li*[a]

[a]*Beijing National Laboratory for Molecular Sciences (BNLMS), Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, Institute of Analytical Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing, 100871, China.*
[b]*Luoyang R&D Center of Technology, SINOPEC Engineering (Group) Co., Ltd., Luoyang, Henan, 471000, China.*

*E-mail address: lina@pku.edu.cn

Materials and reagents

Streptavidin and 100×Tris-EDTA (TE, pH 7.4) buffer were purchased from Sigma-Aldrich (St. Louis, MO). Tween 20 was purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All other reagents used were of analytical grade and purchased from Beijing Chemical Works (Beijing, China). Wahaha® purified water was used for all experiments. All oligonucleotides listed in Table S1 were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China) without further purification. All oligonucleotide stock solutions were TE-Mg buffer (1×Tris-EDTA, 10 mM $MgCl_2$, 0.05% Tween 20) and stored in dark at 4 ^{o}C.

General procedures for fluorescence measurements

Fluorescence emission at 539 nm with excitation at 522 nm was measured with a F-7000 spectrofluorometer (Hitachi, Japan). The temperature as indicated was maintained with a water-bath circulator.

Double-stranded probes were prepared by mixing the complementary single strands at 1:1 ratio in TE-Mg buffer, heated to 90 ^{o}C for 5 min and cooled to room temperature slowly. In a typical experiment, the fluorescence intensity of 20 nM double-stranded probes was recorded as the reagent blank signal. Then 20 nM single-stranded DNA probe and streptavidin with concentrations as indicated were added to the solution and mixed, and the time-dependent fluorescence of the mixture was recorded every 5 s.

Data analysis

The fluorescence intensities were all normalized as the ratio of the fluorescence to the reagent blank. For the initial reaction rate method, the fluorescence change rate of the first 5 min of the reaction, which is linearly proportional to the reaction rate, was used in the experiment instead of the reaction rate for convenience.

Table S1 Sequences of oligonucleotides used in this work

Name	**Sequences (5' to 3')**
O_n	ATAGATCCTCATAGCGAGACCTAGCAA
T14n	TTTTTTTTTTTTTTTTTGCTAGGTCTCGC
C-4n	GCGAGACCTAGCAAAAAA
C-5n	GCGAGACCTAGCAAAAAAA
T14	Biotin-TTTTTTTTTTTTTTTTTGCTAGGTCTCGC
C16	TAGCGAGACCTAGCAATTTTTTTTTTTTTTT-biotin
F	FAM-ATAGATCCTCATAGCGAGAC
Q	TTGCTAGGTCTCGCTATGAGGATCTAT-Dabcyl
O	TET-ATAGATCCTCATAGCGAGACCTAGCAA-TAMRA
T20	Biotin-TTTTTTTTTTTTTTTTTGCTAGGTCTCGCTATGAG
T26	Biotin-TTTTTTTTTTTTTTTTTGCTAGGTCTCGCTATGAGGATCTA
C8	CCTAGCAATTTTTTTTTTTTTT-biotin
C10	GACCTAGCAATTTTTTTTTTTTTT-biotin
C12	GAGACCTAGCAATTTTTTTTTTTTTT-biotin
C14	GCGAGACCTAGCAATTTTTTTTTTTTTT-biotin
C16	TAGCGAGACCTAGCAATTTTTTTTTTTTTT-biotin
C18	CATAGCGAGACCTAGCAATTTTTTTTTTTTTT-biotin
C20	CTCATAGCGAGACCTAGCAATTTTTTTTTTTTTT-biotin
C22	TCCTCATAGCGAGACCTAGCAATTTTTTTTTTTTTT-biotin
C24	GATCCTCATAGCGAGACCTAGCAATTTTTTTTTTTTTT-biotin
C26	TAGATCCTCATAGCGAGACCTAGCAATTTTTTTTTTTTTT-biotin
C28	GATAGATCCTCATAGCGAGACCTAGCAATTTTTTTTTTTTTT-biotin

The numbers after the names represent the length (measured as the number of the nucleotides, nt) of the oligonucleotide except for the 15-nt spacer in the sequence of

the strand and they were used as the lengths of strand T and C in the main text.

Table S2 The kinetic constants of streptavidin-biotin binding reactions and toehold mediated DNA strand displacement reactions with 4 nt and 5nt toehold[1-4]

Reactions	***k*($M^{-1}s^{-1}$)**
Streptavidin-biotin binding	10^6~10^7
DNA strand displacement reaction with 4 nt toehold	5×10^3
DNA strand displacement reaction with 5 nt toehold	5×10^4

Table S3 Analytical performance of the initial reaction rate method and the fluorescence intensity method

Detection method	Initial reaction rate		Fluorescence intensity
Detection time	5 min		1 h
Temperature (℃)	25	37	25
LOD (nM)	0.4	0.1	1
Linear range (nM)	1-10	0.5-10	1-10

References

1 D. Y. Zhang and E. Winfree, *J. Am. Chem. Soc.*, 2009, **131**, 17303-17314.

2 M. Srisa-Art, E. C. Dyson, A. J. deMello and J. B. Edel, *Anal. Chem.*, 2008, **80**, 7063-7067.

3 T. Buranda, G. M. Jones, J. P. Nolan, J. Keij, G. P. Lopez and L. A. Sklar, *J. Phys. Chem. B*, 1999, **103**, 3399-3410.

4 C. E. Chivers, E. Crozat, C. Chu, V. T. Moy, D. J. Sherratt and M. Howarth, *Nat. Methods*, 2010, **7**, 391-U376.