Supporting Information for

A simple and sensitive detection of small molecule-protein interaction based on terminal protection-mediated exponential strand displacement amplification

By Chang Yeol Lee,[a] Hyo Yong Kim,[a] Soeun Kim,[a] Ki Soo Park*[b] and Hyun Gyu Park*[a]

[a]Department of Chemical and Biomolecular Engineering (BK 21+ program),
KAIST, Daehak-ro 291, Yuseong-gu, Daejeon 34141, Republic of Korea
[b]Department of Biological Engineering, College of Engineering,
Konkuk University, Seoul 05029, Republic of Korea

* To whom correspondence should be addressed.
E-mail: hgpark@kaist.ac.kr (H.G. Park); Phone: +82-42-350-3932; Fax: +82-42-350-3910.
E-mail: kskonkuk@gmail.com (K.S. Park); Phone: +82-2-450-3742; Fax: +82-2-450-3742.

Table S1 Comparison of this method with previous terminal protection-based fluorescent methods.

Key material/method	Target	Limit of detection (pM)	Total detection time (hr)	Limitations	Reference
Quantum dot and ruthenium complex	Biotin-SA	40	1.2	- Synthesis of chemicals and nanomaterials - Low sensitivity	1
Poly T-templated copper nanoparticles	Biotin-SA	100	0.42	- Low sensitivity	2
MoS_2 nanosheet and Exo III-aided DNA recycling amplification	Biotin-SA	13	2	- Synthesis of nanomaterials - Modification with fluorophore	3
Catalytic hairpin assembly and Mg^{2+}-dependent DNAzyme	Biotin-SA	2	6.5	- Modification with fluorophore and quencher	4
Terminal protection of small molecule-linked loop DNA probe	Biotin-SA	400	2	- Low sensitivity	5
Fluorophore-labeled DNA covalently linked to graphene oxide	Biotin-SA	80	2	- Low sensitivity - Tedious preparation of nanocomposite - Modification with fluorophore	6
RCA combined with Exo III-aided signal amplification	Folate-FR	0.8	7 <	Modification with fluorophore and quencher	7
DNAzyme	Folate-FR	7	2.75	Modification with fluorophore and quencher	8
eSDA	Biotin-SA	16	2	-	This work

Table S2 DNA sequences employed in this work.

Strand name	DNA sequence (5' → 3')[a,b]
FP	CGA AGG ATC GCG GTC GGA AGC TC
RP	CGA AGG ATC TAG TGC GTC TCG G
TP-BT	GCG GTC GGA AGC TCC TAG AAT GCA CTG CCG AGA CGC ACT AGA TCC TTC G-biotin

[a] The recognition sequence for nicking endonuclease is underlined.

[b] The sequence in RP identical to TP-BT is highlighted in blue while the sequence in FP complementary to TP-BT is highlighted in red.

Conjugation of biotin to the 3'-end of DNA in TP-BT

Since the chemical DNA synthesis is carried out from the 3' to 5' direction. The first monomer at the 3'-end should be covalently attached to a solid support. Thus, the biotin-derivatized CPG support was used as a starting material for the synthesis of 3'-biotin-modified DNA (TP-BT), which was followed by the standard DNA synthesis steps consisting of repetitive cycles of detritylation, coupling, capping, and oxidation.[9, 10] Finally, the as-prepared TP-BT was purified by HPLC.

Fig. S1 Optimization of the reaction times for biotin-SA interaction, Exo I-catalyzed degradation, and eSDA. (a) Fluorescence signal-to-background ratios (F/F_0) at different biotin-SA interaction times. The reaction times for Exo I-catalyzed degradation and eSDA are 30 min and 30 min, respectively. (b) F/F_0 at different Exo I-catalyzed degradation times. The reaction times for biotin-SA interaction and eSDA are 30 min and 30 min, respectively. (c) F/F_0 at different eSDA reaction times. The reaction times for biotin-SA interaction and Exo I-catalyzed degradation are 30 min and 45 min, respectively. F_0 and F are the fluorescence intensities at 525 nm from SYBR green I in the absence and presence of SA, respectively. The final concentrations of KF, Nt.AlwI, SYBR green I, primers, Exo I, and SA are 60 U/mL, 120 U/mL, 1X, 100 nM, 0.5 U/μL, and 10 nM, respectively.

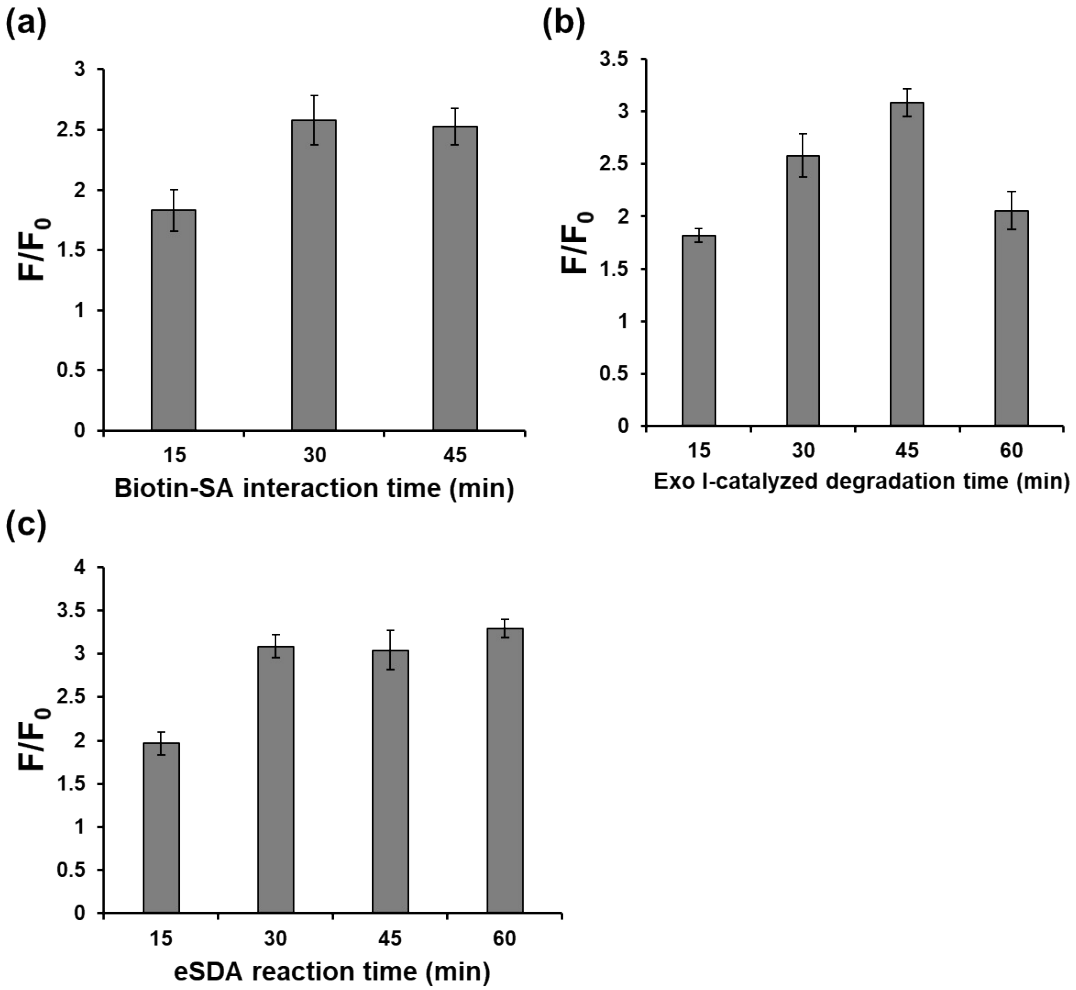


Fig. S2 Optimization of the reaction concentrations of KF, Nt.AlwI, SYBR green I, primers, and Exo I. (a) Fluorescence signal-to-background ratios (F/F_0) at different KF concentrations. The final concentrations of Nt.AlwI, SYBR green I, primers, and Exo I are 120 U/mL, 1X, 100 nM, and 0.5 U/μL, respectively. (b) F/F_0 at different Nt.AlwI concentrations. The final concentrations of KF, SYBR green I, primers, and Exo I are 60 U/mL, 1X, 100 nM, and 0.5 U/μL, respectively. (c) F/F_0 at different SYBR green I concentrations. The final concentrations of KF, Nt.AlwI, primers, and Exo I are 60 U/mL, 120 U/mL, 100 nM, and 0.5 U/μL, respectively. (d) F/F_0 at different primers concentrations. The final concentrations of KF, Nt.AlwI, SYBR green I, and Exo I are 60 U/mL, 120 U/mL, 0.5X, and 0.5 U/μL, respectively. (e) F/F_0 at different Exo I concentrations. The final concentrations of KF, Nt.AlwI, SYBR green I, and primers are 60 U/mL, 120 U/mL, 0.5X, and 10 nM, respectively. F_0 and F are the fluorescence intensities at 525 nm from SYBR green I in the absence and presence of SA, respectively. The final concentration of SA is 10 nM.

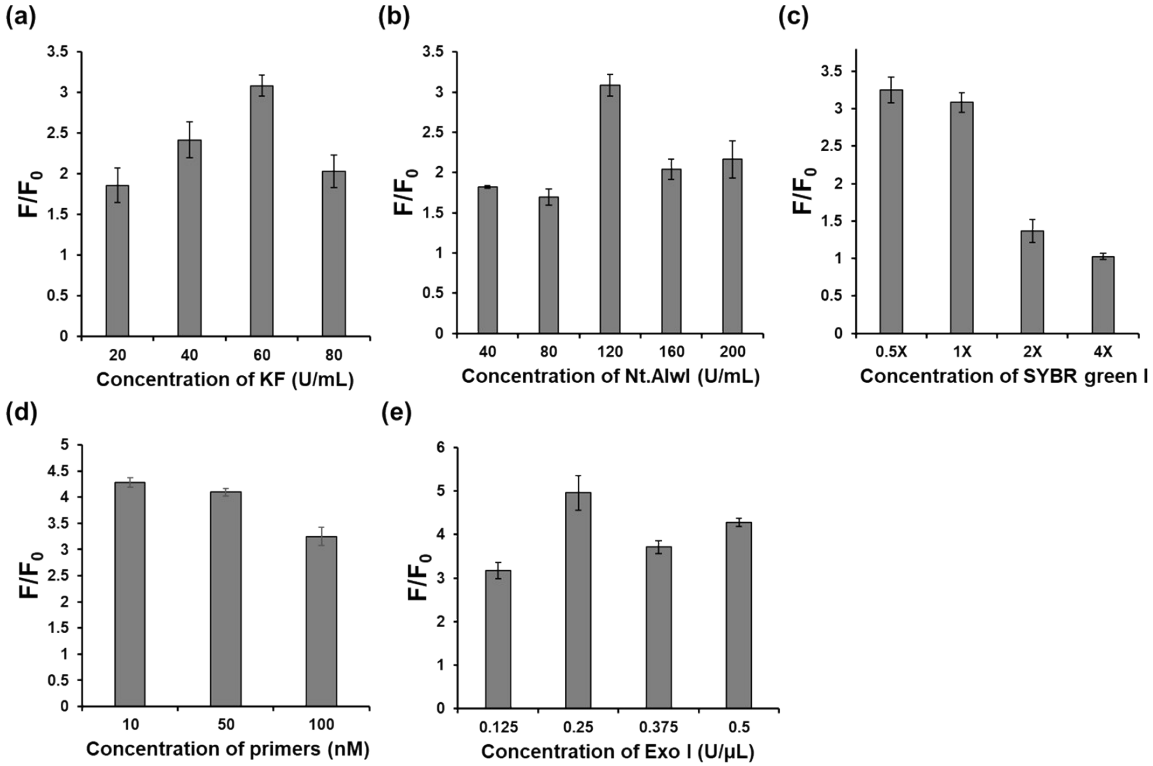
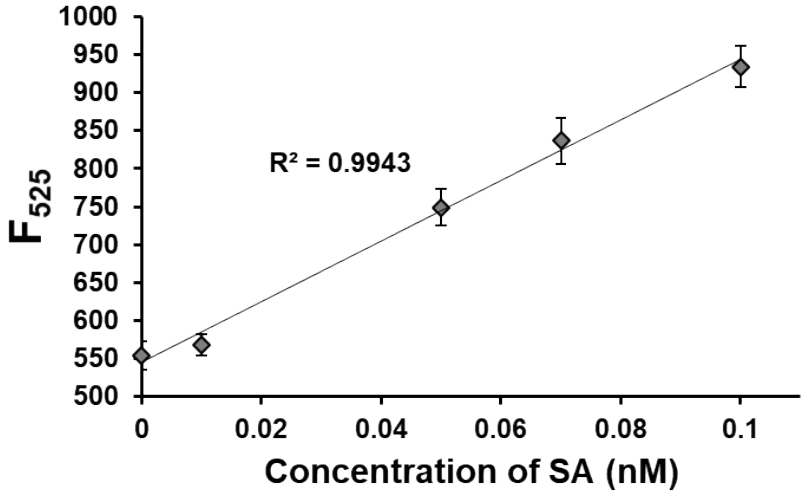
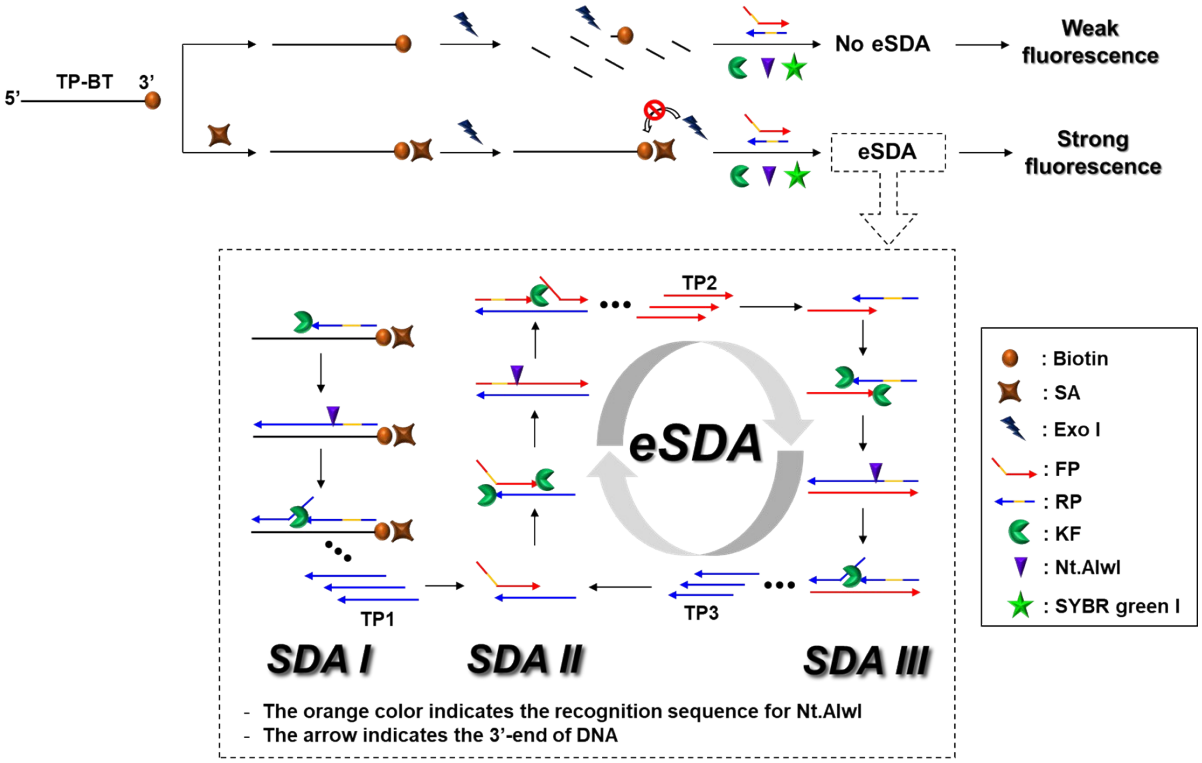


Fig. S3 The linear relationship between the fluorescence intensity at 525 nm (F_{525}) from SYBR green I and SA concentration spiked in diluted human serum (1%).





Scheme 1 (Enlarged version) Schematic illustration of the detection strategy for biotin-SA interaction based on terminal protection-mediated eSDA.

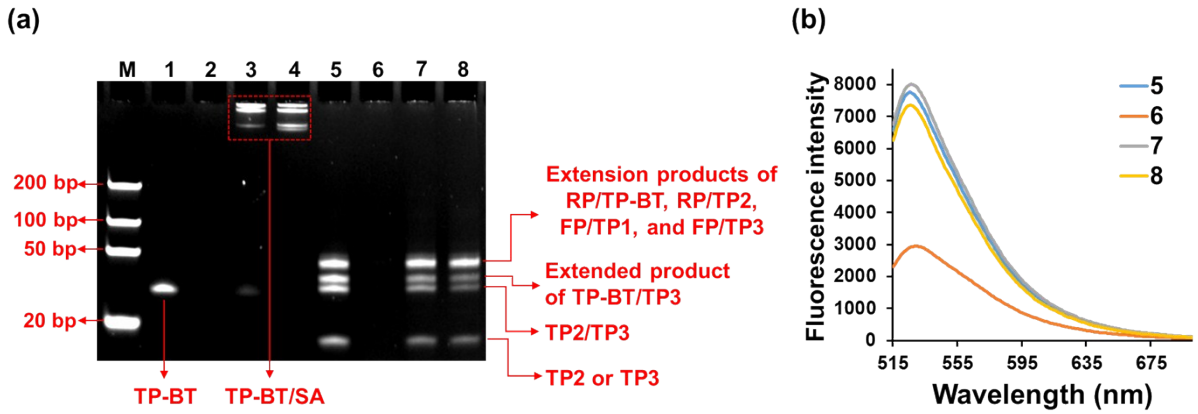


Fig. 1 (Enlarged version) Feasibility of the detection strategy for biotin-SA interaction. (a) Polyacrylamide gel electrophoresis image of the products obtained under different conditions. (b) Fluorescence emission spectra from SYBR green I under different conditions. M: DNA ladder, 1: TP-BT, 2: TP-BT + Exo I, 3: TP-BT + SA, 4: TP-BT + SA + Exo I, 5: TP-BT + eSDA components including KF, Nt.AlwI, and primers, 6: TP-BT + Exo I + eSDA components, 7: TP-BT + SA + eSDA components, and 8: TP-BT + SA + Exo I + eSDA components. For 1-4, the final concentrations of TP-BT, Exo I, and SA are 200 nM, 1 U/μL, and 200 nM, respectively. For 5-8, the final concentrations of KF, Nt.AlwI, SYBR green I, TP-BT, primers, Exo I, and SA are 60 U/mL, 120 U/mL, 1X, 10 nM, 100 nM, 0.5 U/μL, and 10 nM, respectively. For 1-8, the reaction times for biotin-SA interaction, Exo I-catalyzed degradation, and eSDA are 30 min, 30 min, and 30 min, respectively.

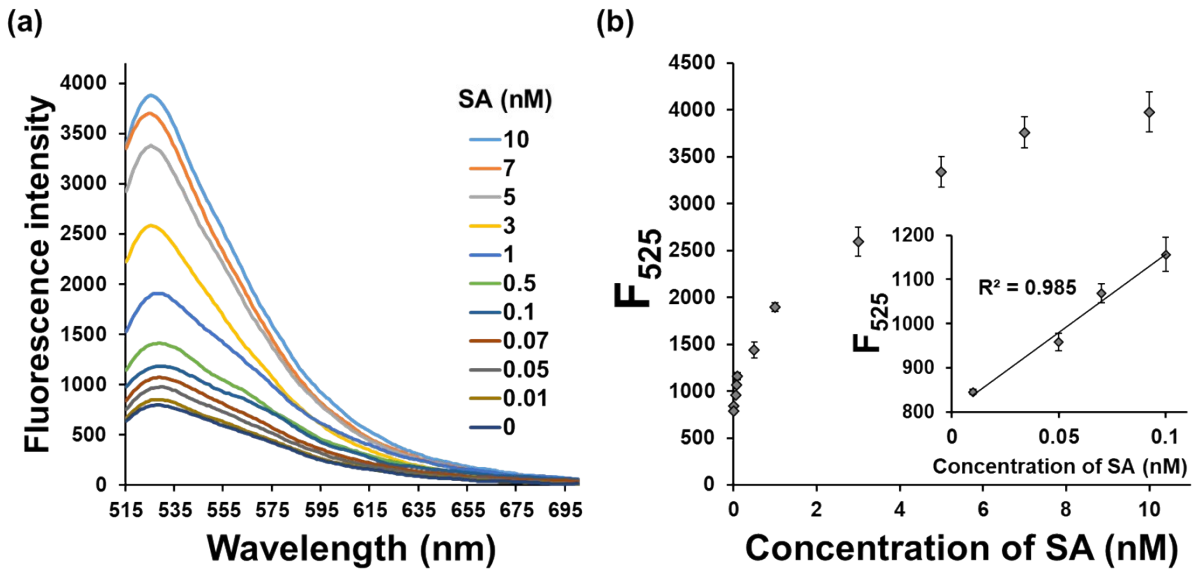


Fig. 2 (Enlarged version) Sensitivity of the detection strategy for biotin-SA interaction. (a) Fluorescence emission spectra and (b) Fluorescence intensities at 525 nm (F_{525}) from SYBR green I in the presence of SA at varying concentrations. Inset in (b): linear relationship between F_{525} and SA concentration (0.01-0.1 nM).

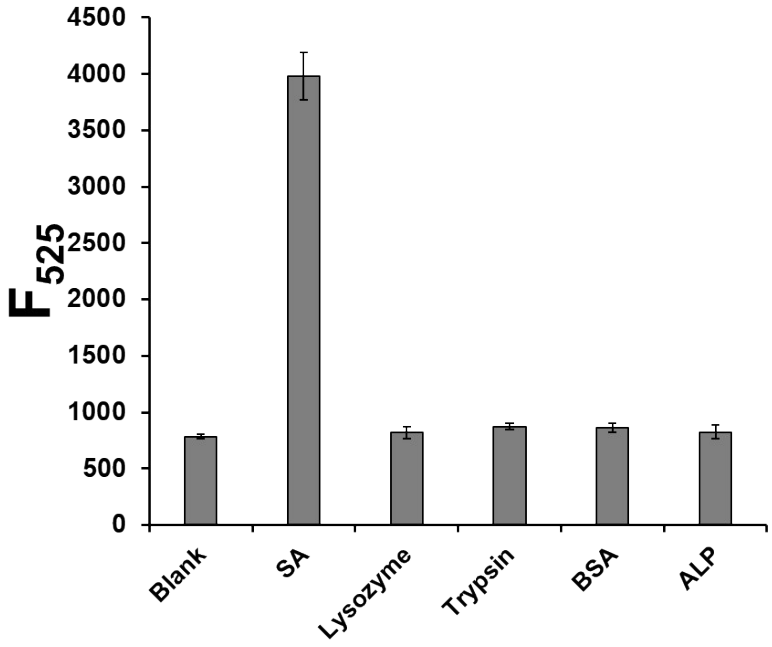


Fig. 3 (Enlarged version) Specificity of the detection strategy for biotin-SA interaction. Fluorescence intensities at 525 nm (F_{525}) from SYBR green I in the presence of SA (10 nM) or other proteins (100 nM).

References

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