Electronic Supplementary Material (ESI) for Analyst.


Supporting Information for

# Fluorescent S1 nuclease assay utilizing exponential strand displacement amplification

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**Table S1** Comparison of this method with previous fluorescent methods for S1 nuclease.

| Key material/method | Detection limit (U/μL) | Limitations | Reference |
|---|---|---|---|
| AIE change of perylene derivative | $9.2 \times 10^{-5}$ | Synthesis of chemicals | 1 |
| AIE change of silole derivative | $7.5 \times 10^{-3}$ | Synthesis of chemicals | 2 |
| Molecular beacon labeled with fluorophore and quencher | $3.0 \times 10^{-3}$ | Labeling with fluorophore and quencher | 3 |
| FRET between fluorescent conjugated polymer and dsDNA-intercalating dye | $2.6 \times 10^{-6}$ | Synthesis of chemicals | 4 |
| DNA-AgNCs as an energy acceptor in FRET | $1.0 \times 10^{-3}$ | - Labeling with fluorophore<br>- Synthesis of nanomaterials | 5 |
| DNA-CuNPs | $3.0 \times 10^{-4}$ | Synthesis of nanomaterials | 6 |
| Fluorescence enhancement of DNA-AgNCs by G-rich sequence | $1.0 \times 10^{-5}$ | - Synthesis of nanomaterials<br>- Long assay time (120 min; 55 min for ours) | 7 |
| eSDA | $8.7 \times 10^{-5}$ | - | This work |

**Table S2** DNA sequences employed in this work.

| **Strand name** | **DNA sequence (5' → 3')[a,b]** |
|---|---|
| FP | AAA AAA AGG ATC GTG CGT CTC GGC TAG T |
| RP | AAA AAA AGG ATC GCG GTC GGA AGC TCC T |
| eTP | GCG GTC GGA AGC TCC TAT GAC AAT GCA CTA GCC GAG ACG CAC |

[a] The recognition sequence for Nt.AlwI is underlined.

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

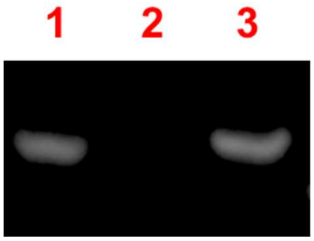


**Fig. S1** The confirmation of S1 nuclease-catalyzed degradation of eTP, where the protocols are the same with those described in 'Gel electrophoresis analysis', except that the gel was stained with GelRed. 1: w/o S1 nuclease, 2: w/ S1 nuclease, 3: w/ heat-inactivated S1 nuclease. The final concentrations of eTP and S1 nuclease are 100 nM and 2 U/μL, respectively, while the reaction time for S1 nuclease-catalyzed degradation is 30 min.

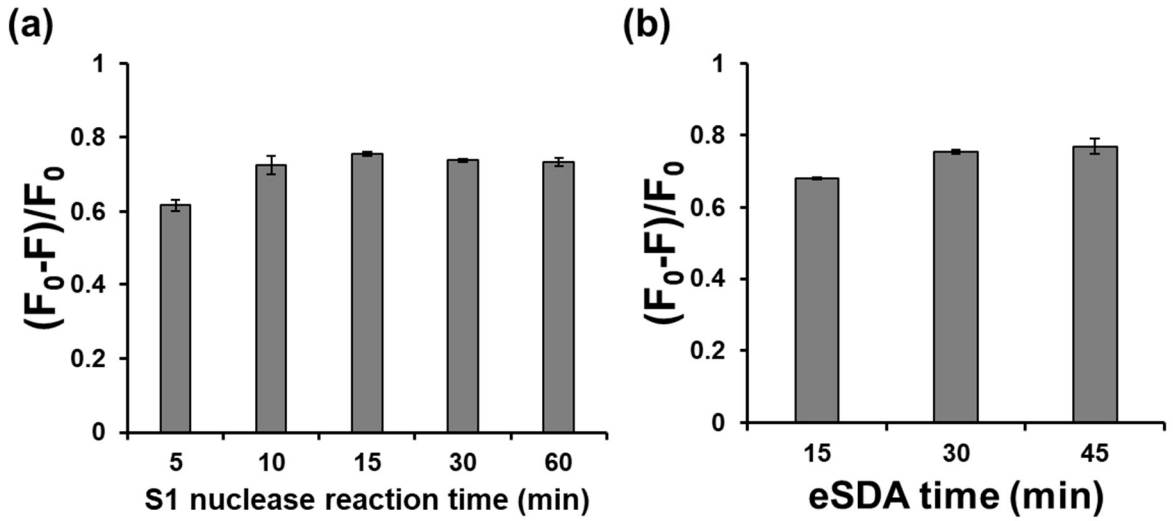


**Fig. S2** Optimization of the reaction times for S1 nuclease-catalyzed degradation and eSDA. (a) The degrees of signal change ($(F_0-F)/F_0$) at different times for S1 nuclease-catalyzed degradation, where $F_0$ and F are the fluorescence intensities at 527 nm from SG I in the absence and presence of S1 nuclease, respectively. The eSDA time is 30 min. (b) The degrees of signal change at different eSDA times. The S1 nuclease-catalyzed degradation time is 15 min. The final concentrations of eTP, primers, KF, Nt.AlwI, SG I, and S1 nuclease are 50 nM, 50 nM, 60 U/mL, 120 U/mL, 1X, and 1 U/μL, respectively.

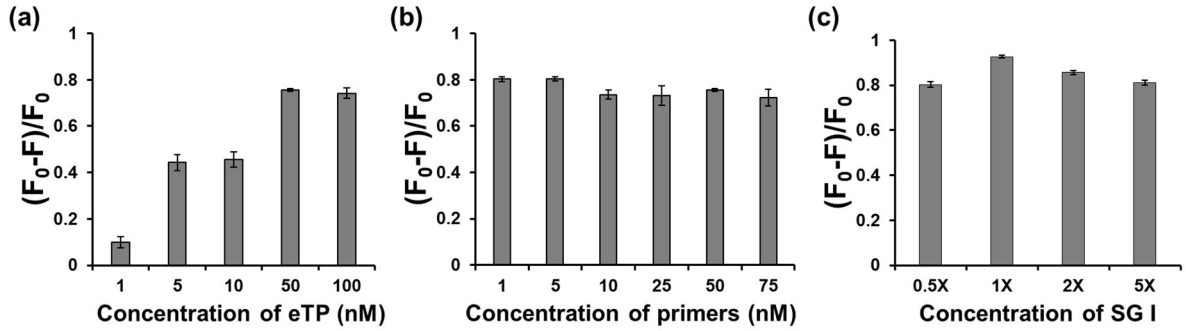


**Fig. S3** Optimization of the reaction concentrations of eTP, primers, and SG I. (a) The degrees of signal change ($(F_0-F)/F_0$) at different eTP concentrations, where $F_0$ and F are the fluorescence intensities at 527 nm from SG I in the absence and presence of S1 nuclease, respectively. The final concentrations of primers and SG I are 50 nM and 1X, respectively. (b) The degrees of signal change at different concentrations of primers (FP and RP). The final concentrations of eTP and SG I are 50 nM and 1X, respectively. (c) The degrees of signal change at different SG I concentrations. The final concentrations of eTP and primers are 50 nM and 5 nM, respectively. The final concentration of S1 nuclease is 1 U/μL.

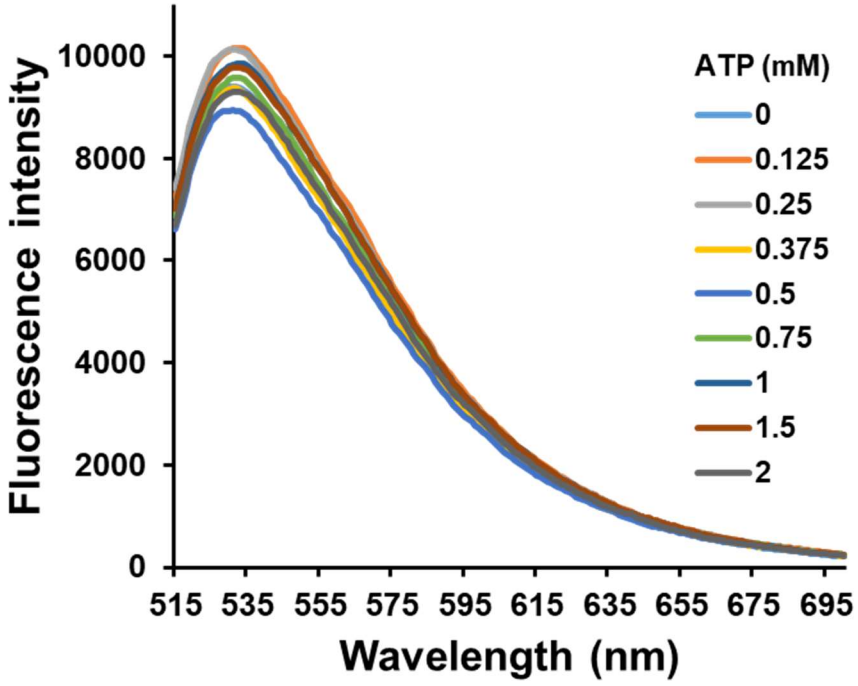


**Fig. S4** The direct effect of ATP on eSDA. The fluorescence emission spectra from SG I after eSDA executed in the presence of varying concentrations of ATP without S1 nuclease.

**References**


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