Electronic Supplementary Information

Target-activated T7 transcription circuit-mediated multiple cycling signal amplification for monitoring of flap endonuclease 1 activity in cancer cells

Jin-zhi Zhang,[‡a] Ning-ning Zhao,[‡a] Zi-yue Wang,[‡a] Juan Hu,*[b] and Chun-yang Zhang*[a,b]

[a] College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan 250014, China.

[b] School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China.

* Corresponding authors.

‡ These authors contributed equally to this work.

E-mail: hujuan@seu.edu.cn (J. Hu), zhangcy@seu.edu.cn (C.-y. Zhang)

EXPERIMENTAL SECTION

Table S1. Sequences of the oligonucleotides

Names	Sequences (from 5' to 3')
Flapped dumbbell probe	TGG GTG TGG TTC TCT GGT AAT CGG GAA TTC CGC GAA GCG GAA TTC CCG ACG AGC TCC GGT TCG GAG CTC GT – NH_2
Synthesized 5′ flap	TGG GTG TGG TTC TCT GGT AAT
Template probe	TAA TAC GAC TCA CTA TAG GGC CCA TCT TTA GGA GAC AGT CTT AAT TAC CAG AGA ACC ACA CCC A
Molecular beacon	FAM – TAA GAC TGT CTC CTA AAG ATG G – BHQ1

SUPPLEMENTARY RESULTS

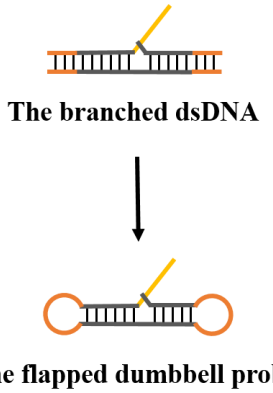

Figure S1. Design of the flapped dumbbell probe versus the branched dsDNA.

Optimization of the amount of DSN

In order to obtain the best assay performance, we optimized the experimental conditions including the amount of DSN, DSN incubation time, DSN reaction temperature, and the concentration of

molecular beacon. We investigated the influence of experimental parameters upon the value of (F – F_0) / F_0, where F and F_0 are the fluorescence intensity at 520 nm in the presence and absence of 0.01 U/μL FEN1, respectively.

In this assay, DSN is responsible for the cleavage of molecular beacon probes in ssRNA / molecular beacon heteroduplexes to dissociate FAM molecules and simultaneously release the ssRNA to initiate cyclic hybridization-cleavage-release reaction. Large amounts of DSN can cleave more molecular beacon probes to generate high fluorescence signal, but excess DSN may induce the increase of background signal due to non-specific cleavage of molecular beacons.[1] Therefore, the amount of DSN should be optimized. As shown in Figure S2, the value of ($F – F_0$) / F_0 improves with the increasing amounts of DSN from 0.15 to 0.25 U, followed by the decrease beyond the concentration of 0.25 U. Therefore, 0.25 U of DSN is used in the subsequent researches.

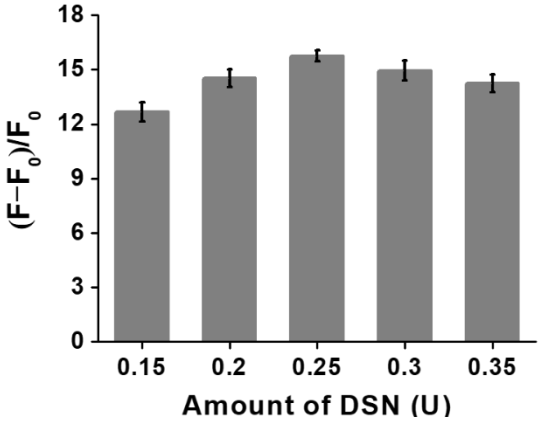

Figure S2. Variance of the (F – F_0) / F_0 value with different amounts of DSN. The FEN1 concentration is 0.01 U/μL. Error bars show the standard deviation of three independent experiments.

Optimization of the DSN reaction temperature and incubation time

Since higher temperature facilitates faster hybridization and plays a critical role in the subsequent DSN–mediated cyclic cleavage reaction, but it may also weaken the enzyme activity of DSN.[2] Thus,

the DSN reaction temperature should be optimized. As shown in Figure S3A, the value of ($F - F_0$) / F_0 enhances with the increase of reaction temperature from 40 to 55 ℃, followed by the decrease beyond 55 ℃. Therefore, 55 °C is used in the subsequent experiments. We further investigated the effect of DSN incubation time upon assay performance. As shown in Figure S3B, the value of ($F - F_0$) / F_0 enhances with the DSN reaction time from 10 to 40 min, followed by the decrease beyond 40 min. Thus, 40 min of DSN reaction time is selected in the following researches.

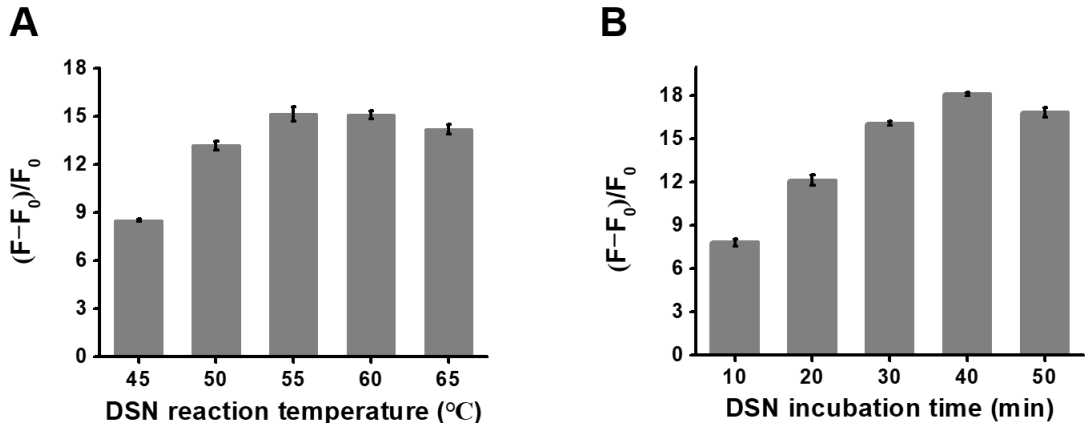

Figure S3. (A) Variance of the ($F - F_0$) / F_0 value with different DSN reaction temperatures. (B) Variance of the ($F - F_0$) / F_0 value with the DSN incubation time. The FEN1 concentration is 0.01 U/μL. Error bars show the standard deviation of three independent experiments.

Optimization of the concentration of molecular beacon

In this assay, the molecular beacon can hybridize with the ssRNA generated from the T7-based transcription amplification reactions to form the ssRNA/molecular beacon duplex, inducing DSN–directed cyclic digestion of molecular beacons in heteroduplexes to liberate abundant FAM fluorophores. Thus, the concentration of molecular beacon is closely associated with the detection sensitivity of the proposed method and should be optimized. As shown in Figure S4, the value of ($F - F_0$) / F_0 improves with the increasing concentration of molecular beacon from 100 to 600 nM,

followed by decrease beyond the concentration of 600 nM. Thus, 600 nM molecular beacon is used in the subsequent research.

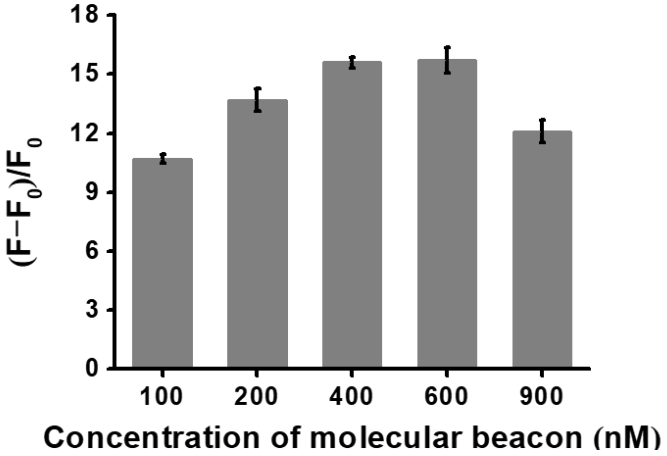

Figure S4. Variance of the $(F - F_0) / F_0$ value with different concentrations of molecular beacon. The FEN1 concentration is 0.01 U/μL. Error bars show the standard deviation of three independent experiments.

We investigated the effect of ATA upon Klenow fragment (KF) DNA polymerase, T7 RNA polymerase, and DSN. The synthesized 5′ flap probe is used to mimic the FEN1–mediated cleavage product. The 1 μM of synthesized flap was subjected to the polymerization reaction and T7 RNA polymerase–mediated transcription amplification. As shown in Figure S5, the fluorescence signal in the presence of ATA shows no significant change compared with that in the absence of ATA, suggesting that ATA has no obvious effect upon the activity of KF DNA polymerase, T7 RNA polymerase, and DSN.

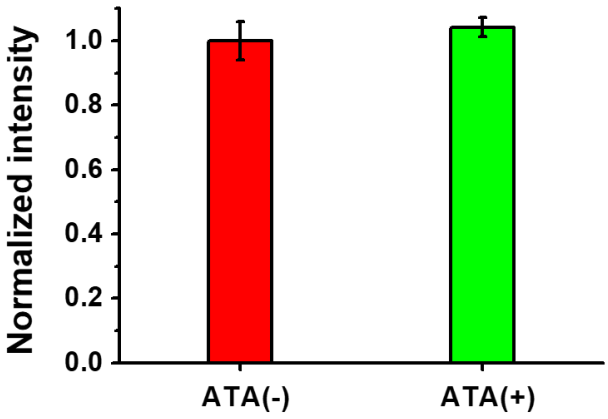

Figure S5. Effect of ATA upon KF DNA polymerase, T7 RNA polymerase, and DSN in the absence (red column) and presence of ATA (green column). 2 μM ATA was used in the experiments. Error bars are standard deviations across three repetitive experiments.

Table S2. Comparison of the proposed method with the reported methods for FEN1 assay.

Strategy	Signal model	Linear range	LOD	Ref.
Poly dA_{20}-based nanoprobe	Fluorescence	0.05 – 2 U	0.007 U	[3]
Fluorescent nanoprobe based on DNA-silver nanoclusters	Fluorescence	0.8 – 40 U	0.4 U	[4]
Graphene oxide-based fluorescent assay	Fluorescence	0.008 – 4 U	0.015 U	[5]
Nt.BstNBI-induced tandem signal amplification	Colorimetric	0.03 – 1.5 U	0.01 U	[6]
Nicking enzyme-assisted signal amplification based on ZIF-8	Fluorescence	0.05 – 2 U	0.007 U	[7]

Gold nanostar-based fluorescent assay	Fluorescence	0 – 3 U	0.016 U	[8]
Hyperbranched rolling circle amplification	ECL	6.5×10^{-2} – 6.5×10^{3} U/L	2.2×10^{-2} U/L	[9]
Magnetic separation-assisted cascade hybridization chain reaction amplification	Fluorescence	0.002 – 0.25 U	1.80×10^{-3} U	[10]
T7 transcription circuit-mediated multiple cycling signal amplification	Fluorescence	1.0×10^{-4} – 1.0×10^{-2} U	1.75×10^{-5} U	This work

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