Supporting information

Exonuclease III-powered self-propelled DNA machine for distinctly amplified detection of nucleic acid and protein

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Table S1. The DNA sequences used in current study [a]

Name	Sequence (5′ to 3′)
H1	CGCATCTCTT(FAM)ACACGTGATCGAATGTTATTAATACCG TAGTTGACCTATTCCCGACCCTAGAGATGCG
H2	CAGAGGGTCGGGAATAGGTCAACTACGGTATTAATAACAT TCGATCACGTGT(BHQ)AAGAGATGACCCTCTG
BHQ-free H2	CAGAGGGTCGGGAATAGGTCAACTACGGTATTAATAACAT TCGATCACGTGTAAGAGATGACCCTCTG
H3	ATCTCTT(FAM)ACACGTGATCGAATGTTATTAATACCGTAG TTGACCTATTCCCGACCCTAGAGATGCG
S1	AGGGTCGGGAATAGGTCAACTACGGTATTAATAACATTCG ATCACGTGT(BHQ)AAGAGATCTGGGACTG
H4	TTTTCGCATCTCTACCAACCACACCTTTTTTTTTTTTTTTTT TTTTGGTTGGTGTGGTTGGTAGAGATTTTTTT
Target DNA (TD1)	GACCACCGCATCTCTACATTCAA
1MT	GACCACCGCATATCTACATTCAA
2MT	GACCACCTCATCGCTACATTCAA
NC	GACCACATGGATCTAGTATTCAA
Target DNA (TD2)	CAAGATCAGAGGGTCACTTAGAA

[a] For H1, the green color represents the base sequences of domains c and c* in Scheme 1; the violet color represents the base sequences of domains of d and d*; the red color represents the base sequences of domain a; the blue color represents the base sequences of domain b. For H2, the dark yellow color represents the base sequences of domains e and e*; the red color represents the base sequences of domains of a and a*; the green color represents the base sequences of domain c; the blue color represents the base sequences of domain b*. For H3, S1 and H4, different domains are also indicated with different colors, which are related with the schemes in Figure 3A and 4A. The H1-H2 hybrid probe was obtained by annealing H1 with H2. The H3-S1 hybrid probe was obtained by annealing H3 with S1. The H4 is for thrombin aptamer switch. Target DNA (TD1) could recognize with the 3′-protruding domain of H1 in H1-H2 hybrid probe or the 3′-protruding domain of H3 in H3-S1 hybrid probe. The 1MT and 2MT represents the one-base and two-bases mismatched DNA. The mismatched bases in contrast with target DNA (TD1) have been indicated with red color. The target DNA (TD2) could be recognized by 3′-protruding domain of H2 in H1-H2 hybrid probe. The recognition regions for TD1 and TD2 were indicated with green color.

Table S2. Comparison of detection performance for target DNA by current sensing system with those reported methods

Ref.	Detection range	Detection limit	Strategy
[1]	1 fM-0.1 nM	1 fM	Target recycling and cascade circular exponential amplification
[2]	10 pM-0.15μM	10 pM	Palindromic molecular beacon-based intramolecular strand-displacement amplification
[3]	0.1 pM-200 nM	16 fM	Target self-amplification-based DNA machine
[4]	1 fM-100 fM	0.75 fM	Exonuclease III-induced isothermal amplification
[5]	100 fM-10 nM	21 fM	Exonuclease III-assisted recycling and DNAzyme
[6]	0.025 nM-500 nM	15 pM	Exonuclease III-assisted upconversion resonance energy transfer
[7]	10 fM-1 nM	3 fM	Exonuclease III-assisted multiple cycle amplification
This work	0.5 fM-1 pM	0.1 fM	Exonuclease III-powered self-propelled DNA amplification

Table S3. Comparison of detection performance for thrombin by current sensing system with those reported methods

Ref.	Detection range	Detection limit	Strategy
[8]	0.28 nM-86 nM	30 pM	Exonuclease I assisted target recycling and SYBR Green I
[9]	0.1 pM-5 nM	23 fM	Binding induced 3D-bipedal DNA walker and catalytic hairpin assembly
[10]	0.01 nM-50 nM	6.9 pM	Aptamer structure switching and metal-ion dependent DNAzyme
[11]	20 pM-200 pM	9.2 pM	Hybridization chain reaction
[12]	5 pM-1 nM	1 pM	DNAzyme and entropy-driven amplification
[13]	0.1 pM-10 pM	56 fM	electrochemical ratiometric method and DNA walker strategy
[14]	7.5 fM-25 nM	2.2 fM	Dual-aptamer biorecognition and mesoporous silica encapsulated with iron porphyrin
[15]	20 pM-1 nM	8.3 pM	Proximity recognition-dependent strand translocation strategy with catalytic hairpin assembly
This work	5 fM-10 pM	5 fM	Exonuclease III-powered self-propelled DNA amplification

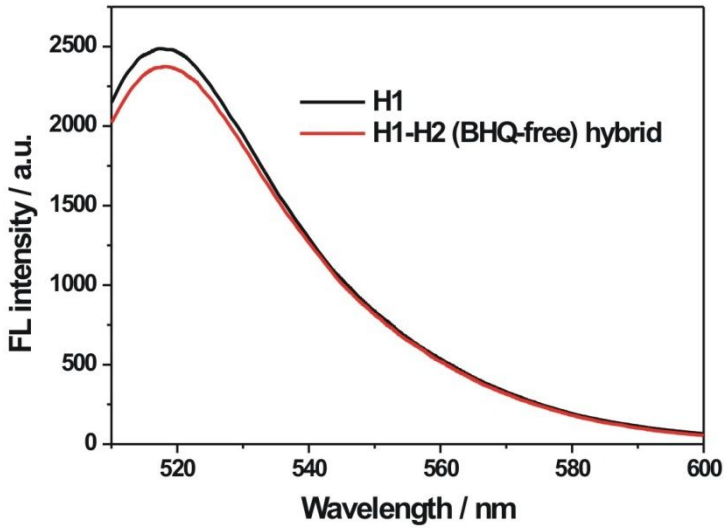

Figure S1. Fluorescence spectra recorded for H1 (200 nM) before and after annealing with 200 nM BHQ-free H2.

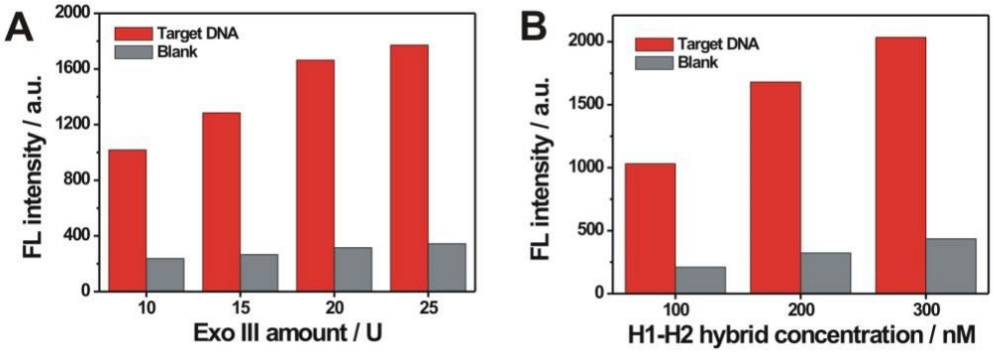

Figure S2. (A) Optimization of Exo III amount. Four different Exo III amounts (10, 15, 20 and 25 U) were used. (B) Optimization of H1-H2 hybrid concentration. Three different H1-H2 hybrid concentrations (100 nM, 200 nM and 300 nM) were studied. Target DNA concentration and reaction time was 0.1 pM and 60 min, respectively.

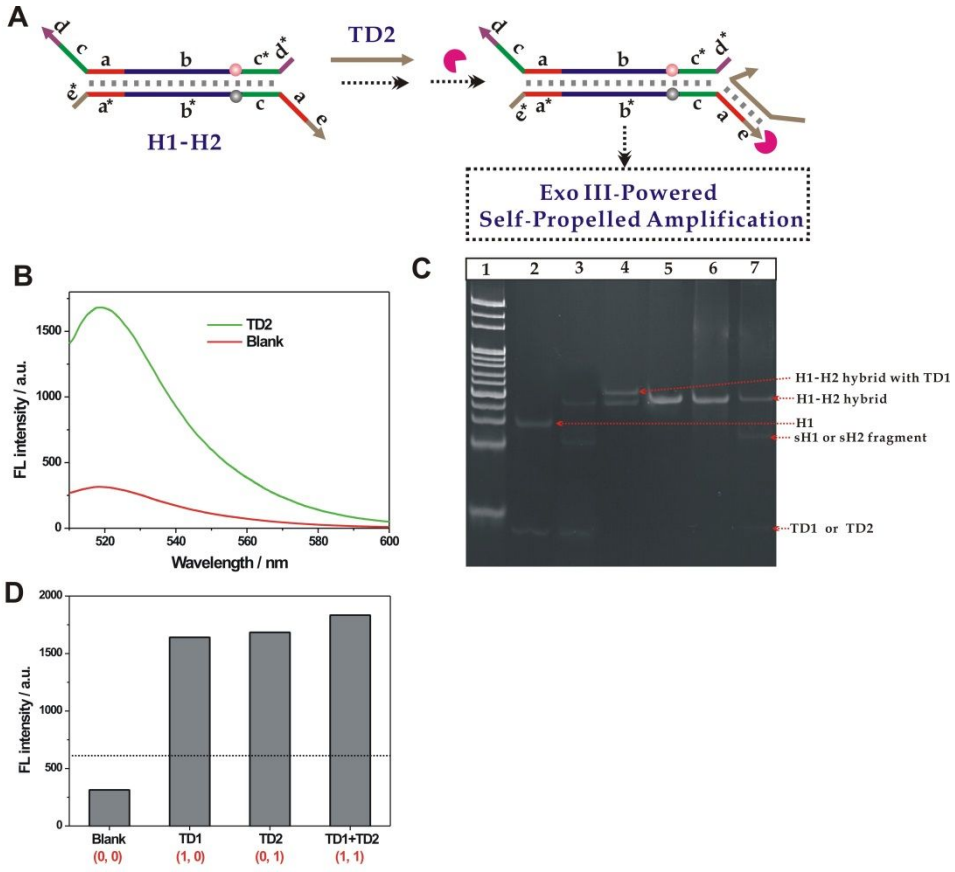

Figure S3. (A) Schematic illustration of the H1-H2 hybrid probe for the sensing of target DNA 2 (TD2). TD2 was recognized by the protruding 3′-domain of H2 in H1-H2 hybrid to trigger the successive Exo III-powered self-propelled DNA amplification process. (B) Fluorescence spectra of the sensing system in the presence and absence of 0.1 pM TD2. (C) Gel electrophoresis images: lane 1, DNA maker; lane 2, TD1 + H1; lane 3, TD1 + H1-H2 hybrid + Exo III; lane 4, TD1 + H1-H2 hybrid; lane 5, H1-H2 hybrid; lane 6, H1-H2 hybrid + Exo III; lane 7, TD2 + H1-H2 hybrid + Exo III. (D) Fluorescence responses of the sensing system by H1-H2 hybrid toward different inputs including no TD1 and TD2, TD1 only, TD2 only, and both TD1 and TD2. TD1 represents target DNA that could recognize with the protruding 3′-domain of H1 in H1-H2 hybrid. TD2 could recognize with the protruding 3′-domain of H2 in H1-H2 hybrid. The concentrations of TD1 and TD2 were both 0.1 pM.

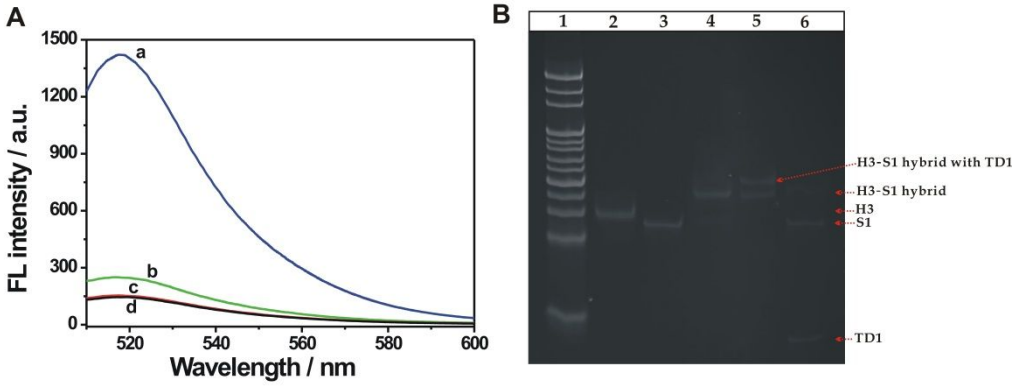

Figure S4. (A) Fluorescence spectra of the sensing system containing 200 nM H3-S1 hybrid probe and 20 U Exo III in the absence (b) and presence (a) of 0.1 nM target DNA. Control experiments are also conducted in the case of no Exo III (c), and no Exo III and target DNA (d), respectively. (B) Gel electrophoresis images: lane 1, DNA maker; lane 2, H3; lane 3, S1; lane 4, H3-S1 hybrid; lane 5, H3-S1 hybrid + TD1; lane 6, H3-S1 hybrid + TD1 + Exo III.

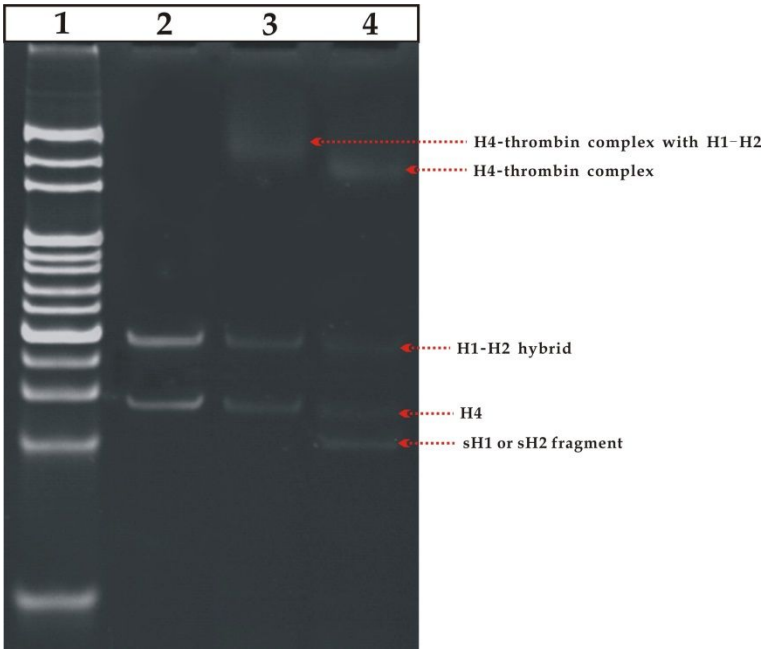

Figure S5. Gel electrophoresis images: lane 1, DNA maker; lane 2, H4 + H1-H2 hybrid; lane 3, H4 + H1-H2 hybrid + thrombin; lane 4, H4 + H1-H2 hybrid + thrombin + Exo III.

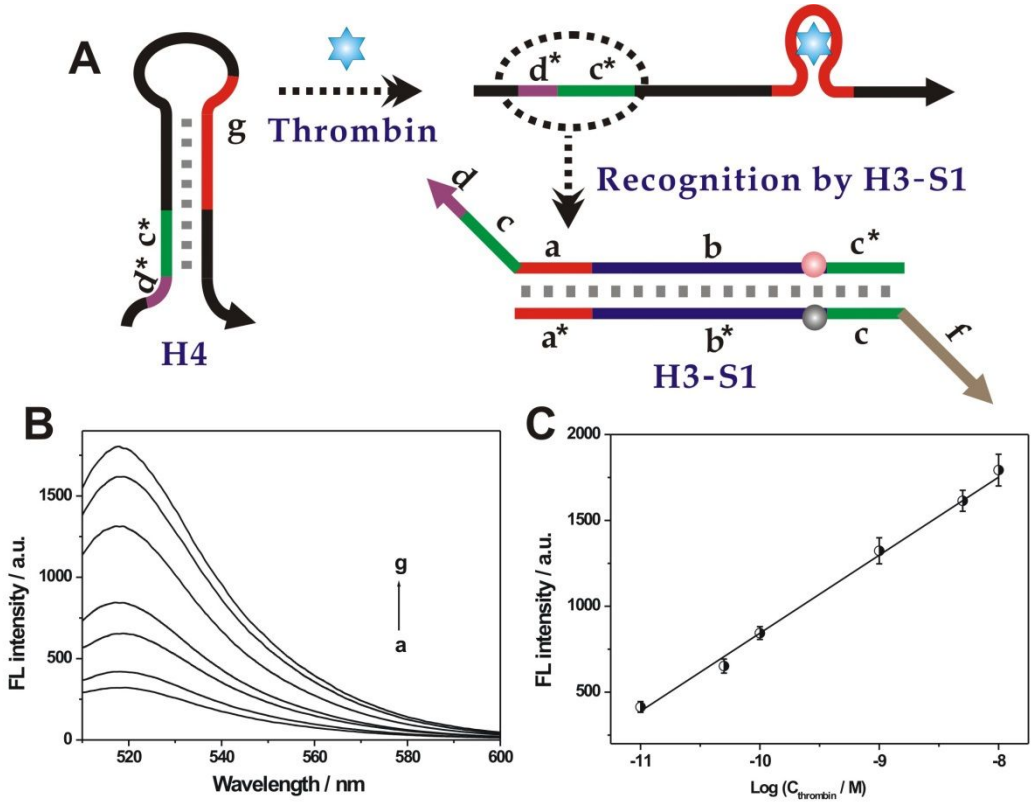

Figure S6. (A) Schematic illustration of thrombin detection by the sensing system of H3-S1 hybrid based on a typical Exo III-powered target recycling strategy. (B) Fluorescence spectra recorded at different concentrations of thrombin (from curves a to g: 0, 10 pM, 50 pM, 100 pM, 1 nM, 5 nM, and 10 nM, respectively). (C) Calibration curve for the fluorescence intensity vs thrombin concentration.

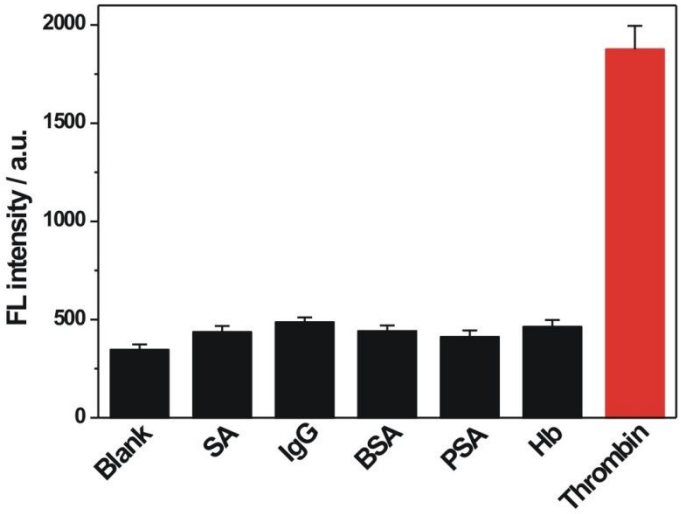

Figure S7. The selectivity of the sensing system (H1-H2 hybrid) toward thrombin and other non-specific proteins including streptavidin (SA), normal mouse immunoglobulin G (IgG), bovine serum albumin (BSA), prostate specific antigen (PSA), and Hemoglobin (Hb). These proteins have a same concentration of 10 pM.

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