Electronic Supplementary Information

Label-free fluorescence detection of protein-ligand interaction based on binding-induced enzymatic cleavage protection

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Table S1. Sequences of used oligonucleotides

Name	Sequence	Length
ssDNA	5′-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3′	30 nt
dsDNA	5′-TATATATATATATATATATA-3′ 3′-ATATATATATATATATATAT-5′	20 bp
hpDNA′	5′-GATCTACTATGTACAGTTTTCTGTACATAGTAGATC-3′	26 nt
hpDNA	5′-GATCTACTATGTACAGTTTTCTGTACATAGTAGATC-Biotin	36 nt

Table S2. Comparison of different fluorescent strategy for SA detection

Detection methods	Detection limit (pM)	Detection time	Limitations	Ref.
Quantum dot and ruthenium complex	40 pM (2.11 ng/mL)	1.2 h	Synthesis of chemicals and nanomaterials	1
Copper nanoclusters	100	25 min	Synthesis of nanomaterial; Low sensitivity	2
MoS_2 nanosheet and Exo III	13	2 h	Synthesis of nanomaterials; Modification with fluorophore	3
Catalytic hairpin assembly and DNAzyme	2 pM	6.5 h	Modification with fluorophore and quencher; Time-consuming	4
Loop DNA probe and SYBR Green I	400	2 h	Low sensitivity	5
Fluorophore-labeled DNA and graphene oxide	80	2 h	Tedious preparation of nanocomposite; Modification with fluorophore	6
RCA combined with Exo III-aided signal amplification	0.8	>7 h	Modification with fluorophore and quencher; Time-consuming	7
Exonuclease I and DNAzyme	7	135 min	Modification with fluorophore and quencher	8
Fluorophore-labeled duplex DNA probe	2930	10 min	Modification with Fluorophore; Low sensitivity	9
dsDNA-lighted	450	30 min	Tedious preparation of	10

fluorophore			fluorescent molecule	
SYBR Green I and Exo III	~19 (1 ng/mL)	20 min	/	This work

References

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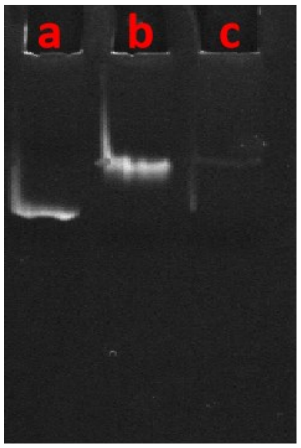

Fig. S1 Electrophoresis characterization of binding-induced enzymatic cleavage protection. lane a: hpDNA; lane b: hpDNA + SA + Exo III; lane c, hpDNA + Exo III. The final concentration of Exo III, SA, hpDNA, was 1 unit μL^{-1}, 10 $\mu g\ mL^{-1}$, 200 nM, respectively.

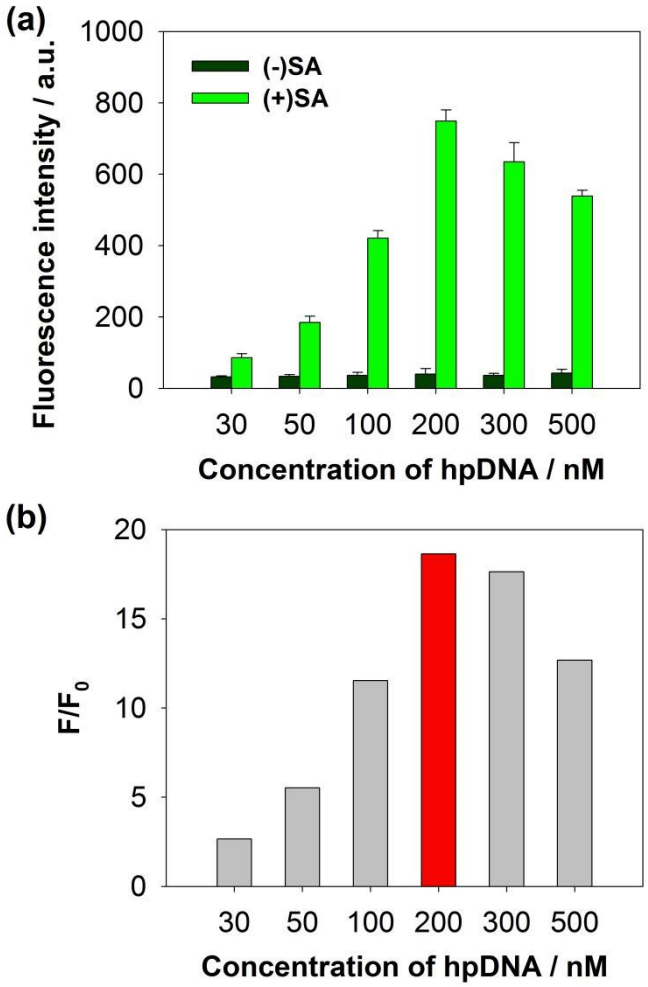

Fig. S2 (a) Fluorescence intensity of the detection system under different hpDNA concentration. (b) The effect of hpDNA concentration on the detection performance by the manner of signal-to-background (F/F_0), where F is the fluorescence intensity of the detection system in the presence of SA and F_0 is that in the absence of SA.

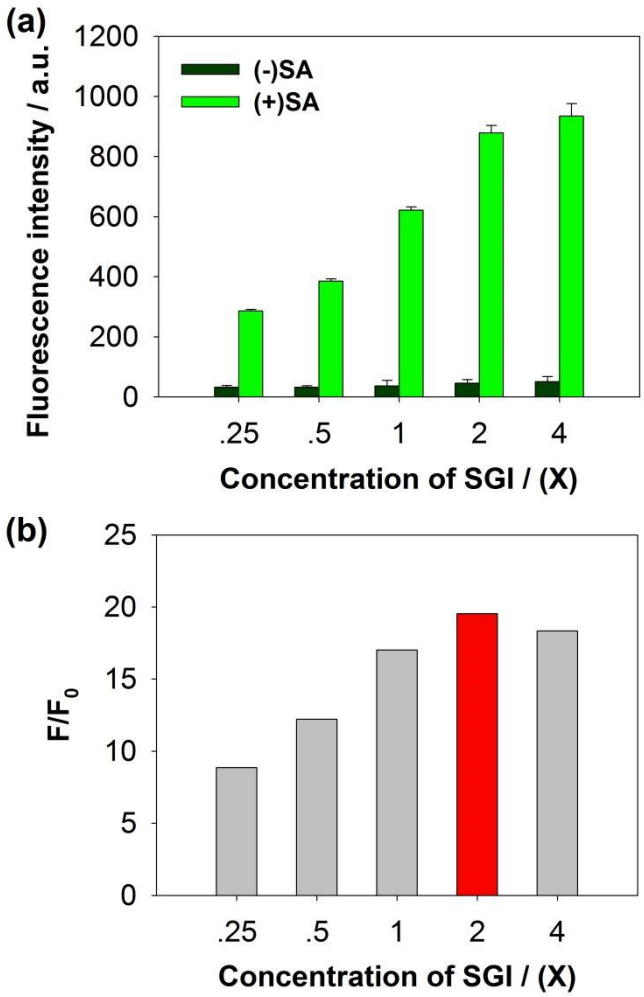

Fig. S3 (a) Fluorescence intensity of the detection system under different SGI concentration in the absence of SA (F_0) or in the presence of SA (F). (b) The effect of SGI concentration on the detection performance by the manner of signal-to-background (F/F_0).

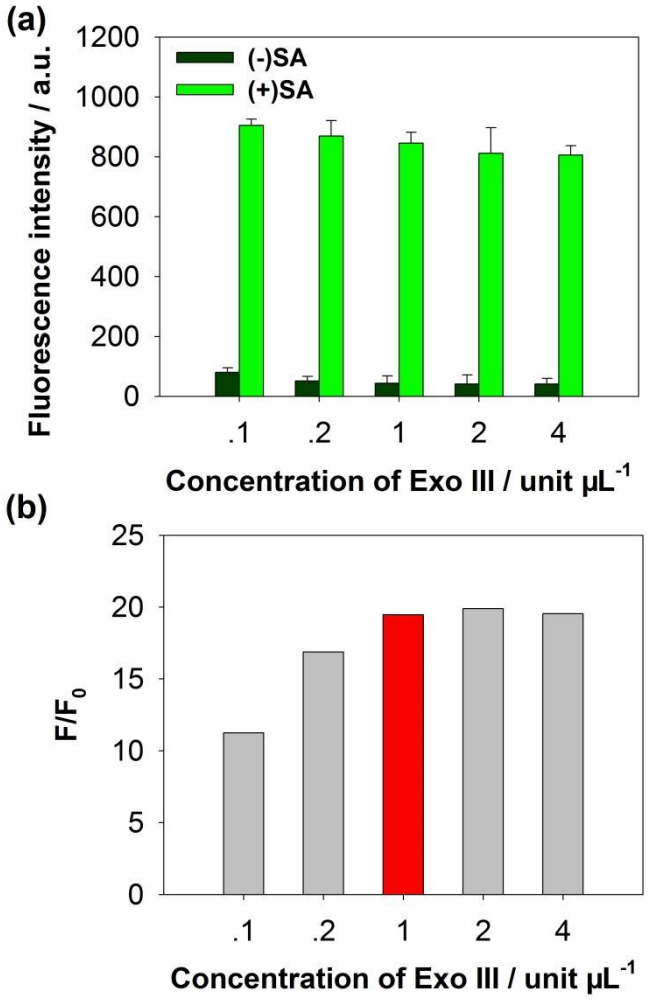

Fig. S4 (a) Fluorescence intensity of the detection system under different Exo III amount. (b) The effect of Exo III concentration on the detection performance by the manner of signal-to-background (F/F_0), where F is the fluorescence intensity of the detection system in the presence of SA and F_0 is that in the absence of SA.

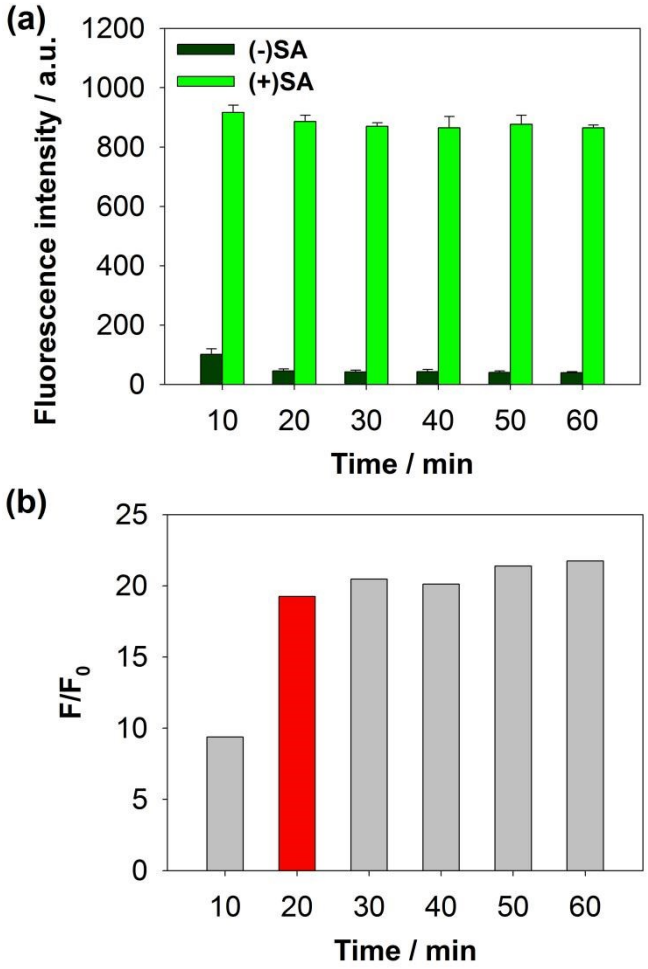

Fig. S5 (a) Fluorescence spectra of the detection system under different enzymatic reaction time. (b) The effect of reaction time on the detection performance by the manner of signal-to-background (F/F_0), where F is the fluorescence intensity of the detection system in the presence of SA and F_0 is that in the absence of SA.

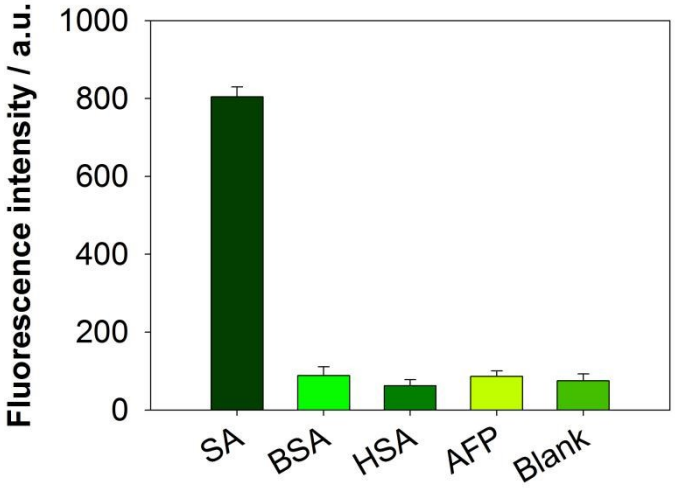

Fig. S6 Selectivity of the approach for SA detection in serum samples. The final concentration of SA, SA, HSA, and AFP was 10 μg mL^{-1}.