Supporting information

Binding-Triggered Hybridization Chain Reaction Cascade Muti-site Activated CRISPR/Cas12a Signal Amplification Strategy for Sensitive Detection of α-Synuclein

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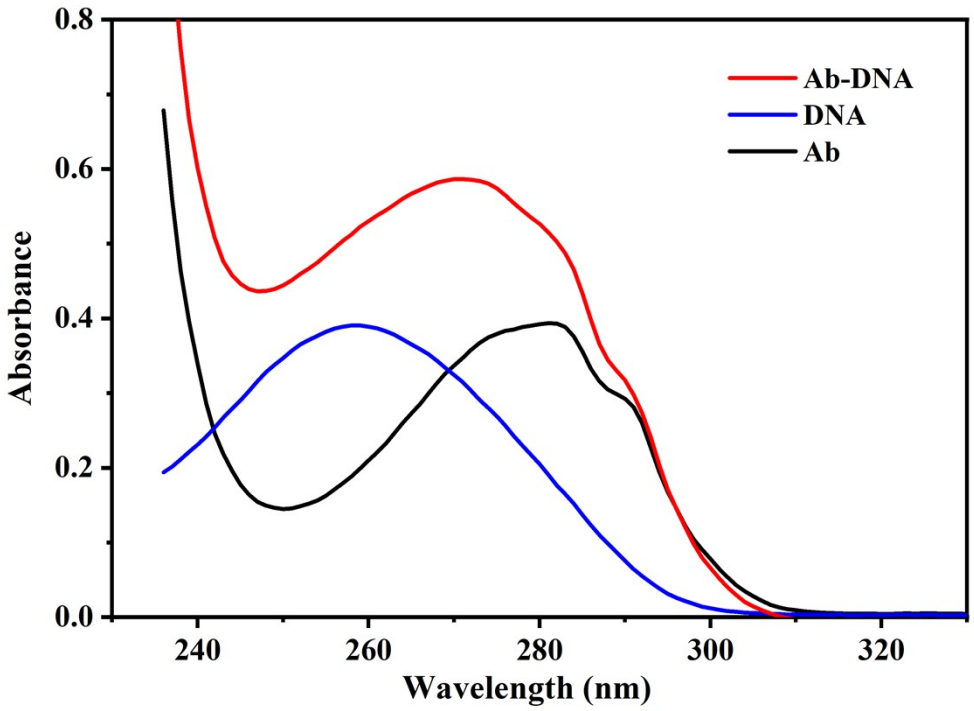

Fig. S1 UV-vis spectrum characterization of ab-DNA conjugates.

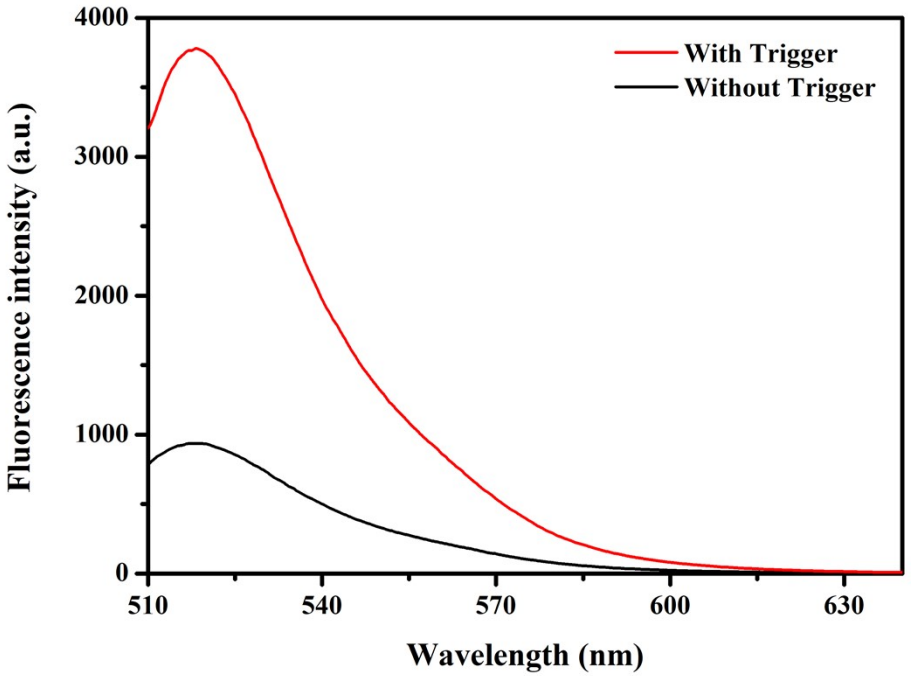

Fig. S2 Fluorescence emission spectra of the split trigger triggered HCR cascade muti-site activated CRISPR/Cas12a signal amplification strategy under different conditions.

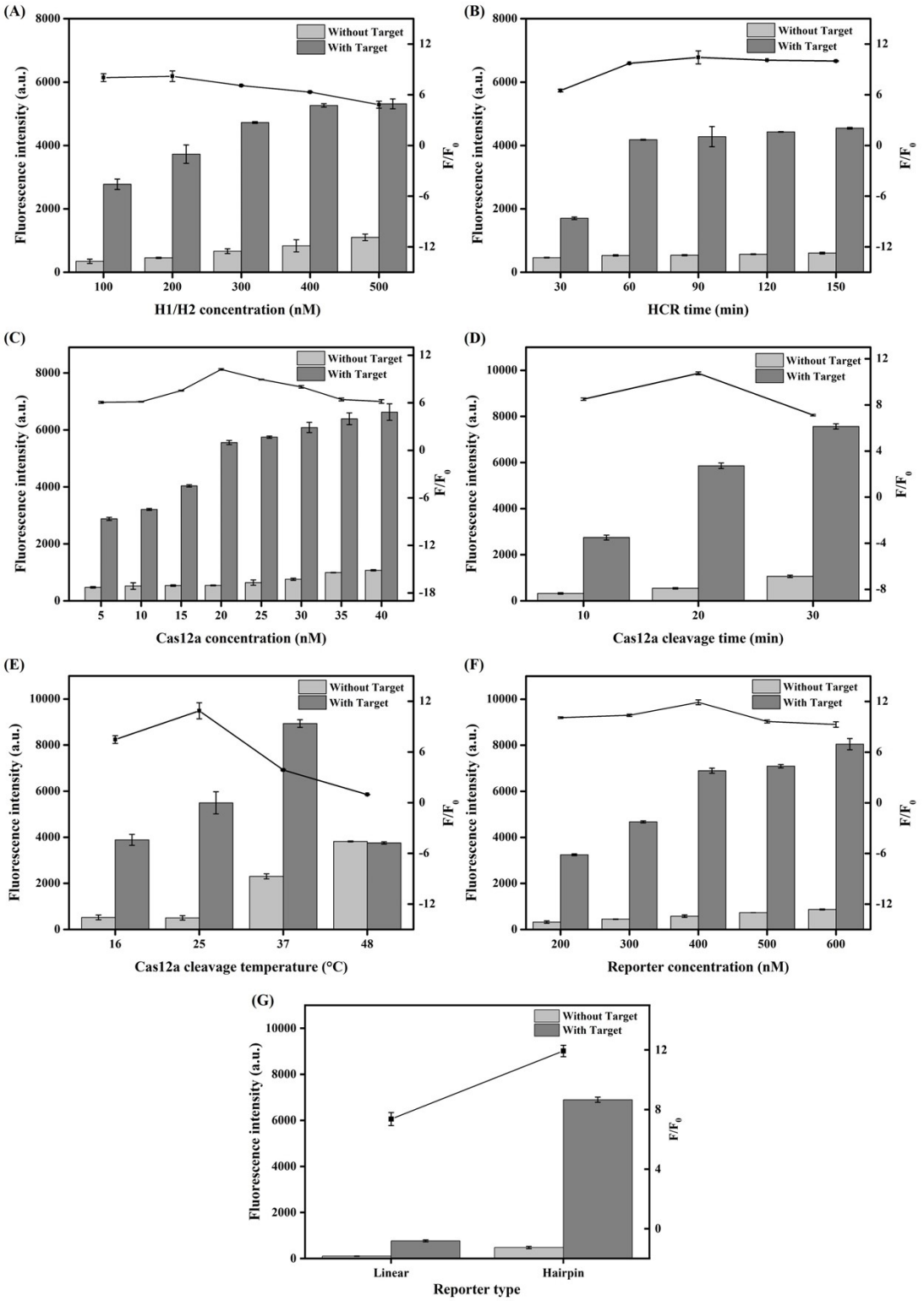

Fig. S3 Effect of the operation conditions of our strategy on the fluorescence signal intensity and F/F_0. The histogram shows the intensity of the fluorescence signal with and without α-syn, the curve shows the ratio of F/F_0. F and F_0 represent the fluorescence with and without α-syn, respectively.

(A) H1/H2 concentration, (B) HCR time, (C) Cas12a concentration, (D) Cas12a cleavage time, (E) Cas12a cleavage temperature, (F) reporter concentration, (G) reporter type.

Table S1. Sequences of oligonucleotides used in this study.

Name	Sequences (5′→3′)
DNA1’	CGTCAGGTAGTCCGT GGCGTGGGTTAA
DNA2’	AGTCTAGGATTC TTTACGGACTACCTGACG
Trigger	AGTCTAGGATTC GGCGTGGGTTAA
DNA1	Biotin-TACGTCCAGAACTTTACCCATCTTTTTTGTCCTGGGCGTGGGTTAA
DNA2	AGTCTAGGATTCTTTCAGGACTTTTTTTATCACATCAGGCTCTATGCTATTG-Biotin
H1	TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGGATTTCGGCGTG
H2	AGTCTAGGATTCGGCGTGGGTTAACACGCCGAAATCCTAGACTACTTTG
crRNA	UAAUUUCUACUAAGUGUAGAUGGCGUGUUAACCCACGCCGAAU
Hairpin reporter	(6-FAM)-CTCTCATTTTTTTTTTAGAGAG-(BHQ1)
Linear reporter	(6-FAM)-TTATT-(BHQ1)

The underlined letters are complementary sequences between DNA1’ and DNA2’, DNA1 and DNA2, and H2 and crRNA. PAM sequences are in red. The target sequences of crRNA are in blue.

Table S2. The results of α-syn assay precision test.

Concentration (ng mL^{-1})	**Sample 1 (ng mL^{-1})**	**Sample 2 (ng mL^{-1})**	**Sample 3 (ng mL^{-1})**	**RSD (n = 3)**
5.00	5.37	4.87	4.86	5.80%
10.0	11.0	11.1	11.7	3.47%
15.0	15.9	15.4	15.4	2.02%

Table S3. The results of α-syn assay reproducibility test.

Concentration (ng mL^{-1})	**Sample 1 (ng mL^{-1})**	**Sample 2 (ng mL^{-1})**	**Sample 3 (ng mL^{-1})**	**RSD (n = 3)**
5.00	5.37	4.91	5.14	4.50%
10.0	11.0	11.3	11.6	2.82%
15.0	15.9	15.7	16.5	2.48%

Table S4. Recovery tests of α-syn detection in human serum samples.

Sample	Target added (ng mL^{-1})	Target detected (ng mL^{-1})	Target recovery	RSD (n = 3)
1	5.00	4.64	92.8%	2.25%
2	10.0	10.0	100%	2.76%
3	15.0	15.6	106%	3.91%

Table S5. Comparison of the detection performance for α-syn with some reported works.

Method	Linear range	LOD	Reference
Surface plasmon resonance	70-700 nM	70 nM	[1]
Electrochemistry	10-000 ng mL^{-1}	1.13 ng mL^{-1}	[2]
Colorimetry	20-3000 nM	10 nM	[3]
Fluorescence	1-8 μM	4.36 μM	[4]
Fluorescence	1-2.5 μM	1 μM	[5]
Fluorescence	2-20 ng mL^{-1}	9.33 pM (0.13 ng mL^{-1})	This work

References

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