Supplementary Information

Programmable CRISPR/Cas12a Activity by Adjusting Guide RNA ConformationforNon-nucleic Acid Markers Analysis andLogic Gate Applications

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Contents

Table S1. DNA oligonucleotides sequences used in this work.

Oligonucleotide name	Sequence (5' to 3') description
crRNA	UAAUUUCUACUAAGUGUAGAUGGUUGAUAACUAAACCUGG G
Apt-T1	ACCTGGGGGAGTACCAAGTACGAA
Apt-T2	TAATTTCTACTGGTGCGGAGGAAGGT
blocker1	*TTCGT*ACTTAGTAGAAATTA
blocker2	TACACTT/iHS-SH/AGTAGAA
blocker2a	TACACTTAGTAGAA
blocker3	TACACTTA8oxo-GTAGAAA
blocker3a	TACACTTAGTAGAAA
S	CCCAGGTTTAGTTATCAACC
FQ	*FAM*-TTATT-*BHQ*
blocker1a	*TTCGT*CTACACTTAGTAGAAATTA
blocker1b	*TTCGT*CTACTTAGTAGAAATTA
blocker1c	*TTCGT*TTAGTAGAAATTA
Apt-T1a	ACCTGGGGGAGTACCAAGTGTAGACGAA
Apt-T1b	ACCTGGGGGAGTACCAAGTAGACGAA
Apt-T1c	ACCTGGGGGAGTACCAAACGAA
ATP G1	CTAAACCTGGGGGAGTATTGCGGAGGAAGGT
ATP G2	ACCTGGGGGAGTATTGCGGAGGAAGGTTGATAA

The underlined part of Apt-T1 and Apt-T2 is the split aptamer of ATP, the underlined part of ATP G1 and ATP G2 is the complete aptamer of ATP. The middle of the blocker 2 was decorated with the disulfide bond (iHS-SH). The middle of the blocker 3 was decorated with the 8-oxoadenine (8oxo-G). The 3' and 5' ends of FQ were modified with FAM and BHQ, respectively.

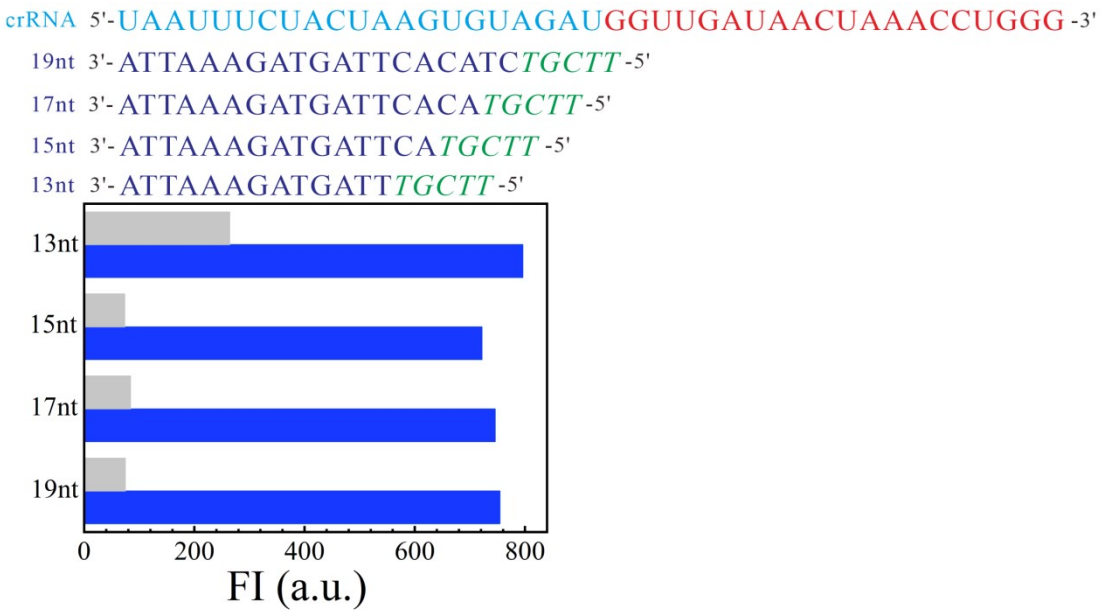

Fig. S1. Effect of length of blocker 1 on the fluorescence signal. 13, 15, 17, 19 nt blocker 1 was used.Gray: fluorescence background signal intensity without adding the target; Blue: The intensity of the fluorescent signal when the target is added.

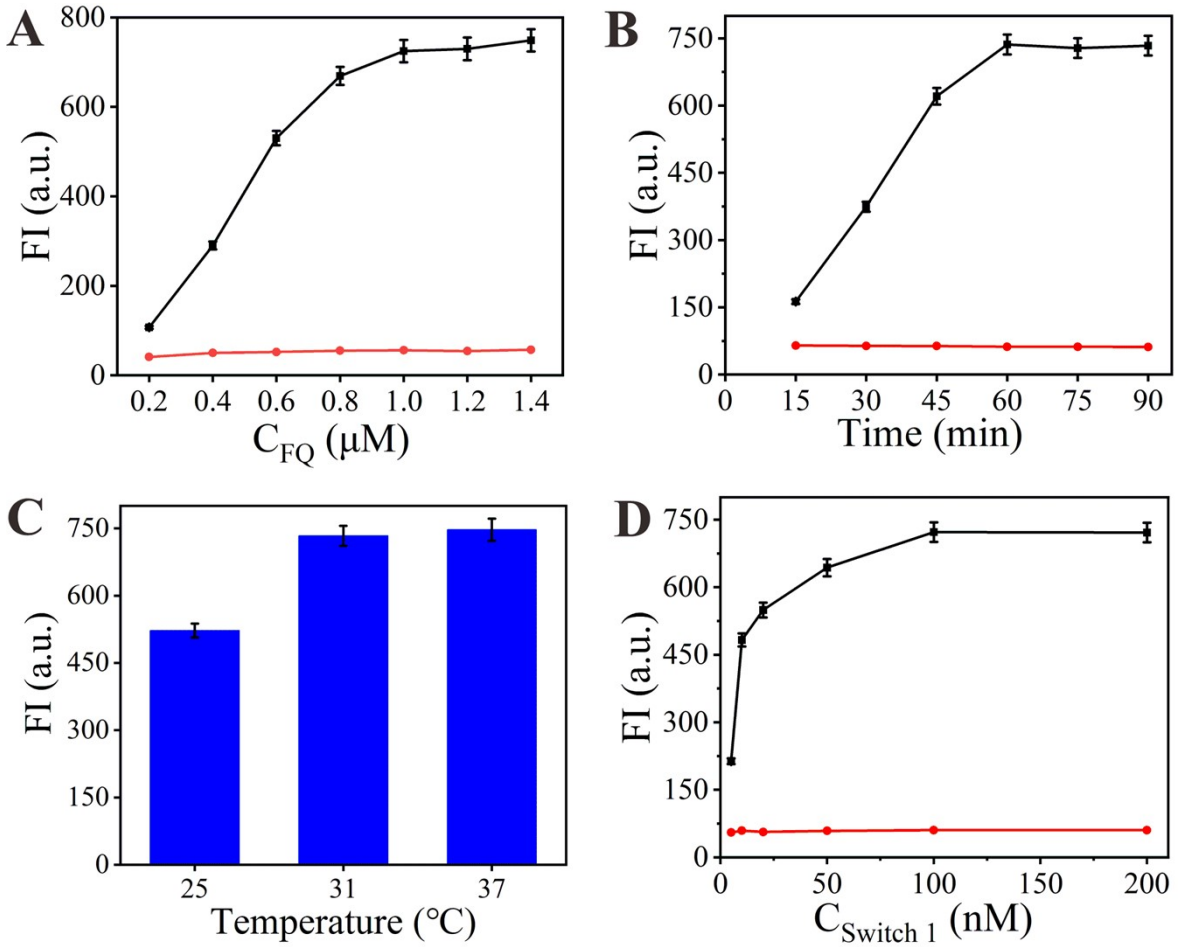

Fig. S2. Optimal experiment.(A) Effect of the concentration of FQ on the fluorescence signal of the biosensor in the presence (black) and absence (red) of 10 μM ATP.(B) Effect of the reaction time on the fluorescence signal of the biosensor in the presence (black) and absence (red) of 10 μM ATP. (C) Effect of the reaction temperature on the fluorescence signal of the biosensor. (D) Effect of the concentration of Switch 1 on thefluorescence signal of the biosensor in the presence (black) and absence (red) of 10 μM ATP.

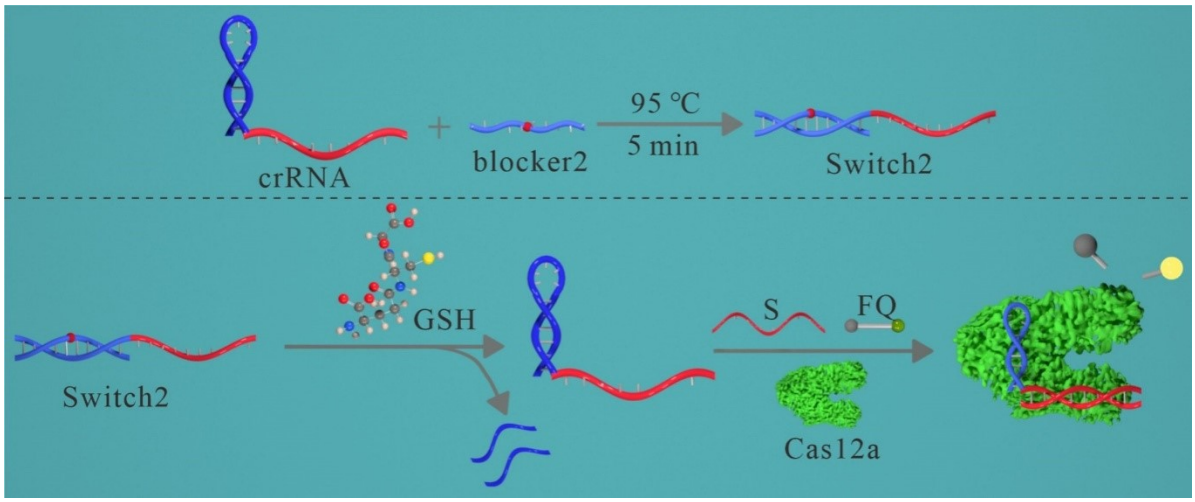

Fig. S3. Schematic illustration of the proposed universal biosensing strategy for GSH detection.

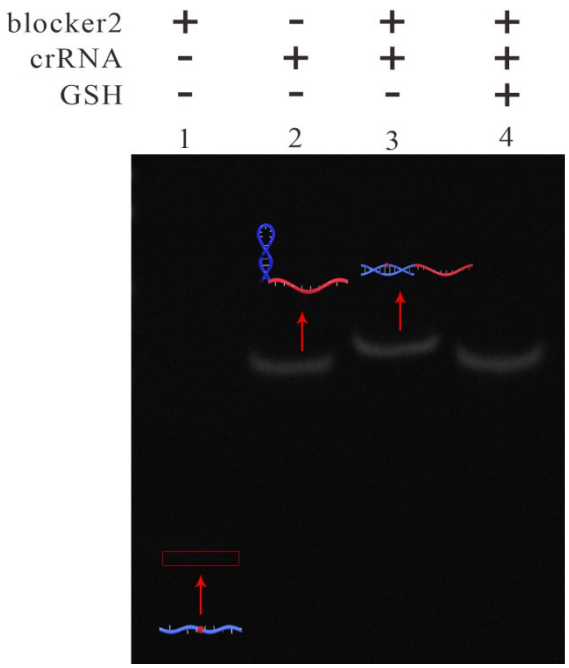

Fig. S4. PAGE characterizes the feasibility of the universal biosensor for GSH detection.

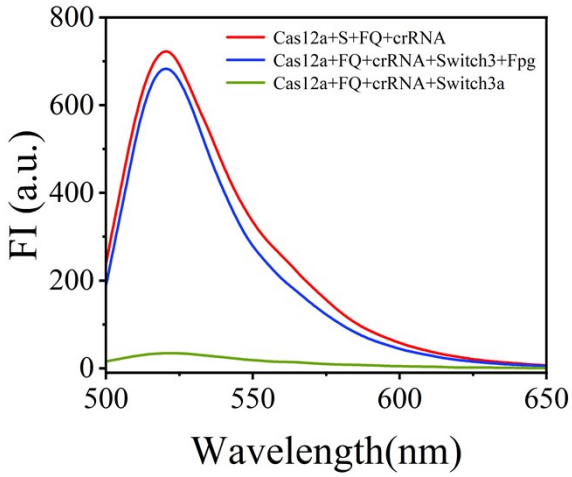

Fig. S5. Fluorescence spectra of the biosensor obtained upon the different control conditions.

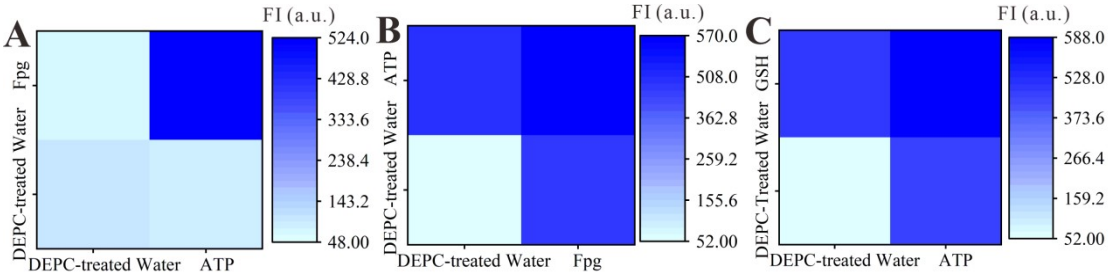

Fig. S6. Fluorescence intensity of "AND" logic gate and "OR" logic gate.(A) Fluorescence intensity of "AND" logic gate with ATP and Fpg as inputs. (B) Fluorescence intensity of "OR" logic gate with ATP and Fpg as inputs. (C) Fluorescence intensity of "OR" logic gate with ATP and GSH as inputs.

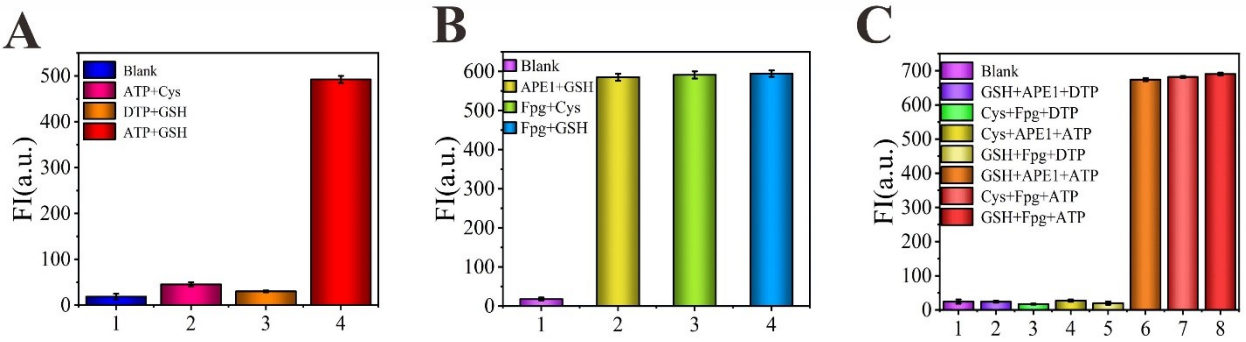

Fig. S7. The feasibility validation of this approach to implement an arbitrary logic circuit. (A) The fluorescent peak intensity of the "AND" logic gate by varying different input units. (B) The fluorescent peak intensity of the "OR" logic gate by varying different input units. (C) The fluorescent peak intensity of the three-input two-layered logic gate by varying different input units.

Table S2. Comparison of different methods for ATP determination.

Detection methods	LOD(μM)	Detection range(μM)	References
Fluorescence	19	125-2000	[1]
Fluorescence	2	5-2500	[2]
Fluorescence	1	5-600	[3]
Colorimetric	0.1225	0.2-3	[4]
Electrochemiluminescence	0.64	0.64-1000	[5]
Fluorescence	0.046	0-12.8	This work

References

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