

Supplementary information

Table S1 Sequences of oligonucleotides

Name	Sequences (5'-3')
APT	GCTGTGTGACTCCTGCAAGGTCATTCAAGTCGTAGGTTTGCCTTCAGCCTCAACGCT TACGCAGCTGTATCTTGTCTCCTTTT-Biotin
APT-c1	TTTCGTTTGTACCATGATGTCATATTT <u>AATGGACCTTG CAGGAGTCACA</u>
APT-c1-17	TTTCGTTTGTACCATGATGTCATATTT <u>ACCTTG CAGGAGTCACA</u>
APT-c1-22	TTTCGTTTGTACCATGATGTCATATTT <u>AATGGACCTTG CAGGAGTCACA</u>
APT-c1-27	TTTCGTTTGTACCATGATGTCATATTT <u>GAATGGACCTTG CAGGAGTCACACAGC</u>
APT-c2	TTTCGTTTGTACCATGATGTCATATTT <u>CGTTGAGGCTGAAGGCAAAAC</u>
crRNA	UAAUUUCUACUAAGUGUAGAUAAAUAUGACAUCAUGGUAACAAA

* The underlined segment corresponds to the complementary region to the aptamer.

* The length-optimized APT-c1-22 is designated as APT-c1.

S2 Optimization results of complementary strand length

Experimental

Three complementary strands with complementary regions of 17 bp, 22 bp, and 27 bp were respectively designed, as detailed in Table S1. These three complementary strands (APT-c1-17, APT-c1-22, APT-c1-27) were separately annealed with the aptamer to form duplexes, which were then coated onto magnetic beads. 20 μ L of 5 μ M FF or 0 μ M FF was mixed with 20 μ L of magnetic bead suspension prepared with different-length complementary strand to perform replacement reaction, followed by fluorescence measurement.

Results

The length optimization results showed that: when the complementary region of APT-c1 was 17 bp, although the FF replacement efficiency was higher, the background signal of the negative control (NC) was excessively high; when the complementary region was 27 bp, although the NC background was very low, the FF replacement efficiency was significantly reduced; when the complementary region was 22 bp, both high FF replacement efficiency and low background signal were achieved, as shown in Figure S2A-B. These results indicate that if the complementary strand is too short, its binding to the aptamer is not stable enough, which is prone to lead to high background signal; if it is too long, the replacement efficiency will decrease, thereby affecting detection sensitivity.

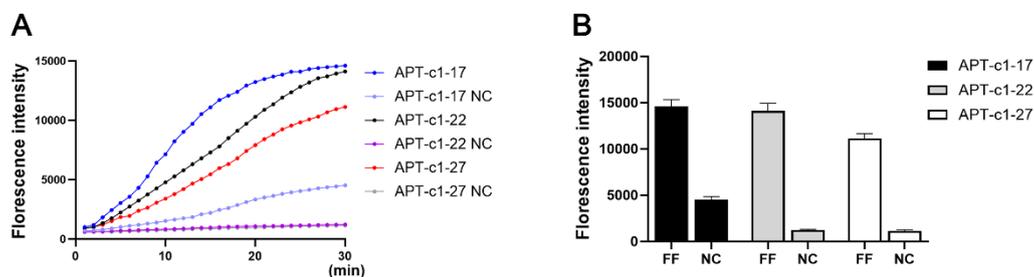


Fig S2. Optimization results of complementary strand length

(A) Real-time fluorescence curves at different length of complementary strand; (B) Final fluorescence intensities at 30 min of detection with different length of complementary strand.