

Electronic supplementary information

Target triggered proximity combination-based fluorescent sensing strategy for adenosine detection

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Table S1 Sequences of oligonucleotides used in this work.^a

Name	Sequence (from 5' to 3')
Difunctional probe-0	GGG AGT TGA GTG CTG AGG ATG CGG AGG AAG GT TTT TT AC CTG GGG GAG TA
Difunctional probe-1	GGG AGT TGA GTG CTG AGG ATG CGG AGG AAG GT TTT TT AC CTG GGG GAG TAT
Difunctional probe-2	GGG AGT TGA GTG CTG AGG ATG CGG AGG AAG GT TTT TT AC CTG GGG GAG TAT C
Difunctional probe-3	GGG AGT TGA GTG CTG AGG ATG CGG AGG AAG GT TTT TT AC CTG GGG GAG TAT CC
Split probe-A	ACC TGG GGG AGT AT
Split probe-B	GGG AGT TGA GTG CTG AGG A TGC GG AGG AAG GT phosphate-AGT GCT GAG GAA ACC CAA CCC GCC CTA
Padlock probe	CCC GCT GAG GAA ACC CAA CCC GCC CTA CCC GCT GAG GGA GTT G

^a In the difunctional probe and split probe, the rose domains are adenosine aptamer sequences, the green domains are assistant sequences, and the black and blue domains are the nicking recognition site sequences and the polymerase/nicking template sequences. In the padlock probe, the blue domain is the polymerase/nicking template and partial nicking recognition site sequences, the black domain is the nicking recognition site sequences, and the purple domain is the complementary sequences of G-quadruplex.

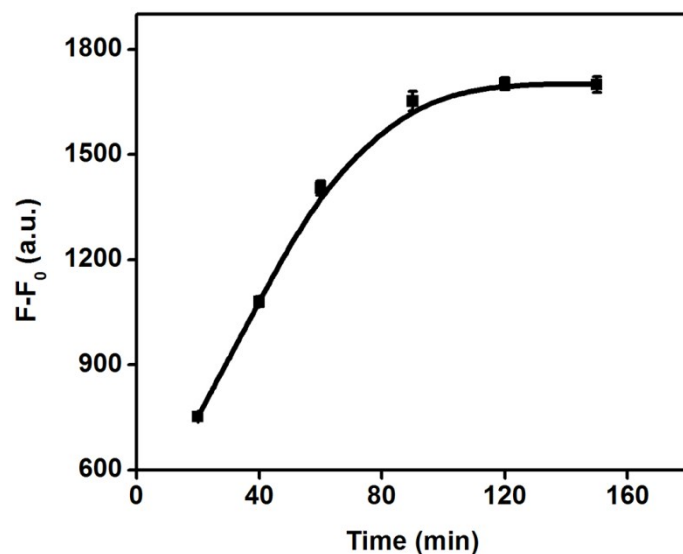


Fig. S1 The effect of the combination time on the fluorescence increase ($F-F_0$). Conditions: c (difunctional probe) = 5.0×10^{-8} M, c (adenosine) = 5.0×10^{-5} M, KF = 2.0 U, Nt.BbvCI = 6.0 U, c (padlock probe) = 4.0×10^{-7} M, T4 = 120.0 U, Phi29 = 3.0 U, c (ThT) = 1.5×10^{-5} M.

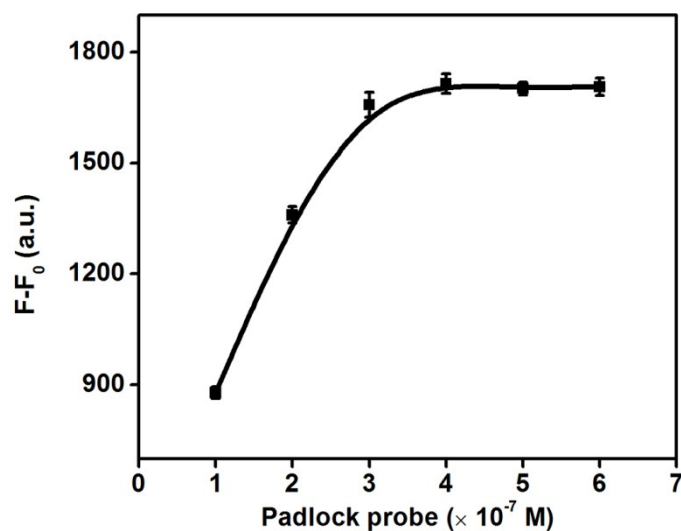


Fig. S2 The effect of the padlock probe concentration on the fluorescence increase ($F-F_0$). Conditions: c (difunctional probe) = 5.0×10^{-8} M, c (adenosine) = 5.0×10^{-5} M, KF = 2.0 U, Nt.BbvCI = 6.0 U, T4 = 120.0 U, Phi29 = 3.0 U, c (ThT) = 1.5×10^{-5} M.

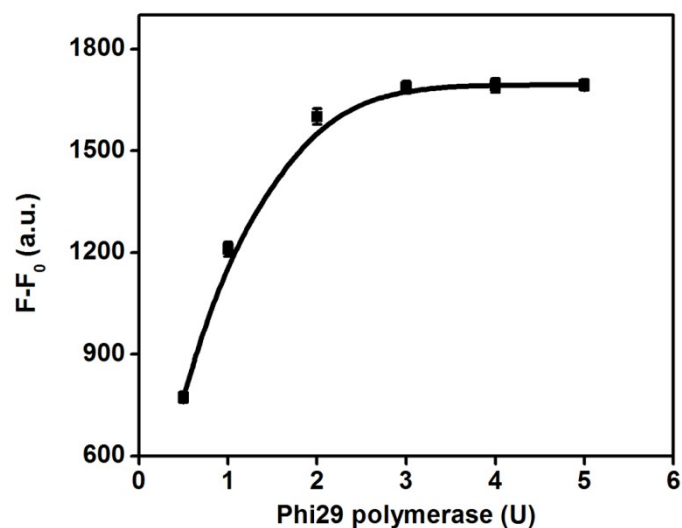


Fig. S3 The effect of the amount of Phi29 polymerase on the fluorescence increase ($F-F_0$). Conditions: c (difunctional probe) = 5.0×10^{-8} M, c (adenosine) = 5.0×10^{-5} M, $KF = 2.0$ U, $Nt.BbvCI = 6.0$ U, c (padlock probe) = 4.0×10^{-7} M, $T4 = 120.0$ U, c (ThT) = 1.5×10^{-5} M.

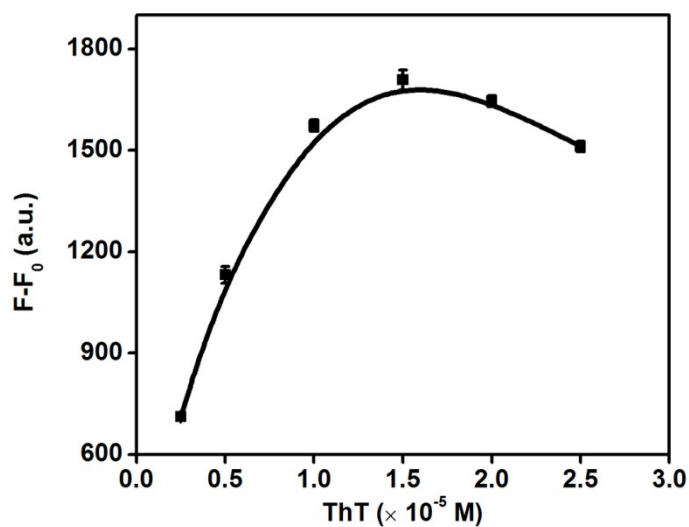


Fig. S4 The effect of the ThT concentration on the fluorescence increase ($F-F_0$). Conditions: c (difunctional probe) = 5.0×10^{-8} M, c (adenosine) = 5.0×10^{-5} M, $KF = 2.0$ U, $Nt.BbvCI = 6.0$ U, c (padlock probe) = 4.0×10^{-7} M, $T4 = 120.0$ U, $\text{Phi29} = 3.0$ U.

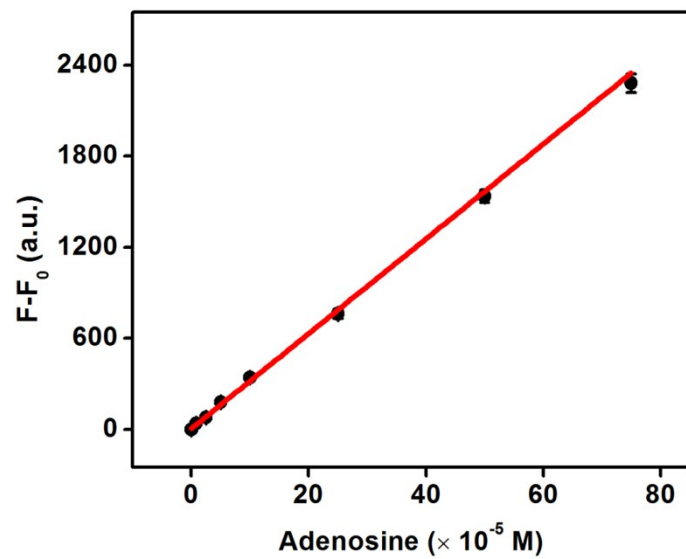


Fig. S5 The linear relationship between the fluorescence increase ($F-F_0$) and adenosine concentration. Conditions: c (Split probe-A) = 5.0×10^{-8} M, c (Split probe-B) = 5.0×10^{-8} M, c (padlock probe) = 4.0×10^{-7} M, KF = 2.0 U, Nt.BbvCI = 6.0 U, T4 = 120.0 U, Phi29 = 3.0 U, c (ThT) = 1.5×10^{-5} M.

Table S2 Comparison of detection limits between this work and reported fluorescent methods for adenosine detection.

References	Aptamer recognition mode	Detection limit
1	Aptamer/cDNA duplex	2×10^{-6} M
2	Aptamer/cDNA duplex	6×10^{-6} M
3	Aptamer/cDNA duplex	4.2×10^{-7} M
4	Aptamer/cDNA duplex	5×10^{-5} M
5	Aptamer/cDNA duplex	2×10^{-5} M
6	Aptamer/cDNA duplex	1.1×10^{-4} M
7	Aptamer/cDNA duplex	1.4×10^{-6} M
8	Aptamer/cDNA duplex	5×10^{-6} M
9	Split aptamer fragments	6×10^{-5} M
10	Split aptamer fragments	1×10^{-6} M
11	Split aptamer fragments	1×10^{-6} M
This work	Connected split aptamers	8.4×10^{-8} M

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