Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

DNA tweezer-actuated regenerated DNAzyme nanoreactor: a fluorescence sensor for miRNA and adenosine detection

Xueying Chen, Xinrui Fu, Yuanyu Wu, Yifan Jin and Wei Li*

Key laboratory of Pharmaceutical Quality Control of Hebei Province, College of Pharmaceutical Science, Hebei University, Baoding 071002, P. R. China

*Corresponding author: Tel: +8618131235217, Email: liwei_91@126.com

Table S1 Oligonucleotide sequences used in this study

Name	5'-3'
T1	TTTT CGAC CGAG CGTG AATT AGTG ATCC GGAA CTCG CGCA ATGA ACC TTTT
T2	TTTT TCAG CTGG CCTA TCTA AGAC TGAA CTCG CACC GCCG GCAT AAGC TATG CGCT CTGC CGC TTTT
T3	TTTT TTAG GAGA TGGC ACGT TAAT GAAT AGTC TCCA CTTG CATC CGAG ATCC GAAC TGCT GCC TTTT
T4	TTTT CGAG AGAA GGCT TGCC AGGT TACG TTCG TACA TCGT CTGA GTT TTTT
T5	TTTT GGCA GCAG TTCA GGCC AGCT GA TTTT
T6	TTTT GGTT CATT GCGG AGTT CAGT CTTA GATG GATC TCGG ATGC AAGG CCTT CTCT CG TTTT
T7	ACTA TTCA TTAA CGTG TGTA CGAA CGTA ACCT GGCA ATGG AG TTT TAGC TTAT CAGA CTGA TTT GGTG CCGA GTTC CGGA TCAC TAAT TCCA TAGC TTAT GCCG GC
T8	CATC TTCT CCGA GCCG GTCG ACGAT ACAAA C TTTT TTTT GCGG CAGA GCGA CGCT CGGT CG TTTT
T9	AACT CAGA CGAC CATG TCCT AA TTTT AAAA GTTTG TATCG T AAAT AGTT GGT
Set strand	TCAA CATC AGTC TGAT AAGC TA
Fuel strand	TAGC TTAT CAGA CTGA TGTT GA
miR-21	UAGC UUAU CAGA CUGA UGUU GA
ST-miR-21	UAGC UUAU CAGA CUGA UGUA GA
DT-miR-21	UAGC UUAU CAGA CUGA AGUA GA
miR-155	UUAA UGCU AAUC GUGA UAGG GGU
Let-7a	UGAG GUAG UAGG UUGU AUAG UU
T8-BHQ-3	CATC TTCT CCGA GCCG GTCG ACGAT ACAAA C TTTT TTTT (BHQ-3) GCGG CAGA GCGA CGCT CGGT CG TTTT
T9-Cy5	AACT CAGA CGAC CATG TCCT AA (Cy5) TTTT AAAA GTTTG

	TATCG T AAAT AGTT GGT
MB	6-FAM-CCAC CACA TTCA AATT CACC AACT AT rA GG AAGA TGTT ACGA GGCG GTGG TGG-Dabcyl
T8 with mutant DNAzyme	CATC TTCT CCTA GCCT GTCG ACGAT ACAAA C TTTT TTTT GCGG CAGA GCGA CGCT CGGT CG TTTT
T7 for AD	ACTA TTCA TTAA CGTG TGTA CGAA CGTA ACCT GGCA ATGG AG TTT ACCT TCCT ACTC CCCC TTT GGTG CCGA GTTC CGGA TCAC TAAT TCCA TAGC TTAT GCCG GC
Set for AD	ACCTGGGGGAGTATTGCGGAGGAAGGT

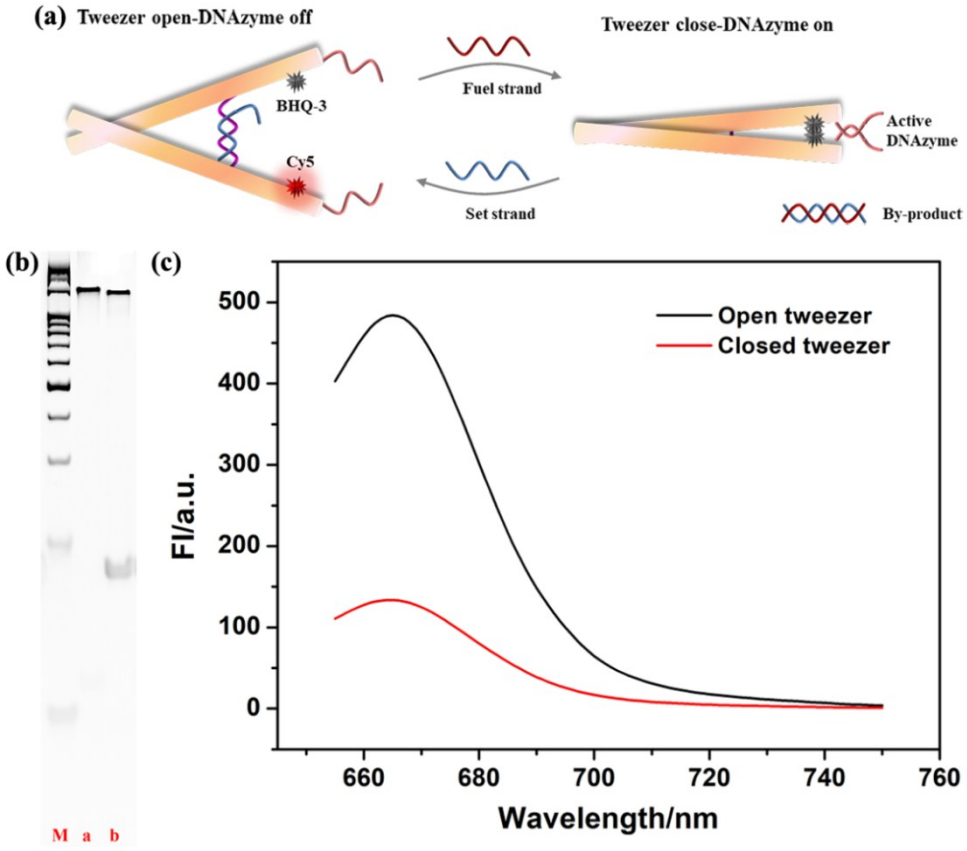

Figure S1 (a) Conformational switch of the DNA tweezer labeled with Cy5/BHQ-3 dye pairs. (b) Native PAGE for characterization of the assembled DNA tweezer. Lane a, assembled open tweezer; lane b, assembled open tweezer + fuel strand; lane M, DNA ladder marker. (c) Fluorescence spectra of Cy5 in assembled open tweezer and closed tweezer.

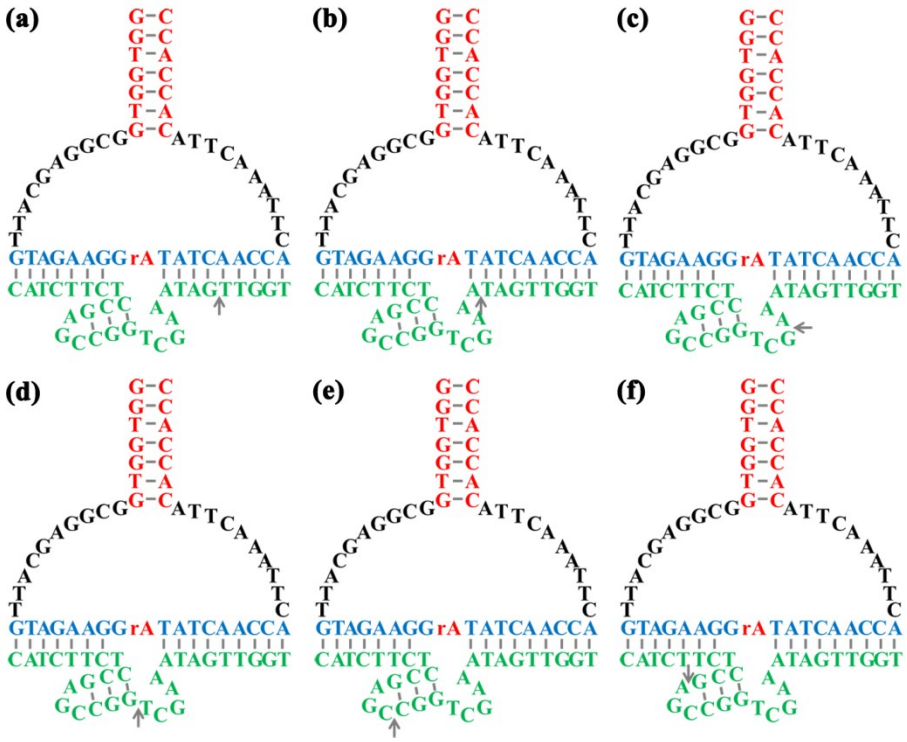

Figure S2 Schematic of the designed split DNAzymes. The gray arrows represent the split sites.

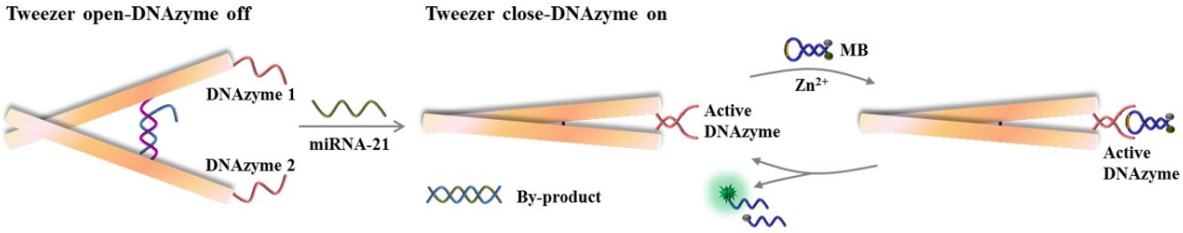

Figure S3 Schematic of the DNA tweezer actuated DNAzyme nanoreactor for miR-21 detection.

Table S2 Repeatability of this sensor for miR-21 detection

Concentration	Sample 1 (FI)	Sample 2 (FI)	Sample 3 (FI)	Average (FI)	RSD (%)
50 nM	2420	2395	2460	2425	1.35
0.5 nM	1438	1456	1419	1438	1.29
10 pM	874.1	844.8	854.3	857.7	1.74

Table S3 Reproducibility of this sensor for miR-21 detection

Concentration	Sample 1 (FI)	Sample 2 (FI)	Sample 3 (FI)	Average (FI)	RSD (%)
50 nM	2420	2466	2386	2424	1.66
0.5 nM	1438	1402	1411	1417	1.32
10 pM	874.1	825.4	811.3	836.9	3.94

Table S4 Recoveries of this sensor by spiking miR-21 in 100-fold diluted healthy serum

Spiked	Detected	Recovery	RSD
50 nM	50.51	101.0%	2.1%
0.5 nM	0.52	104.0%	2.3%
10 pM	9.87	98.7%	3.7%

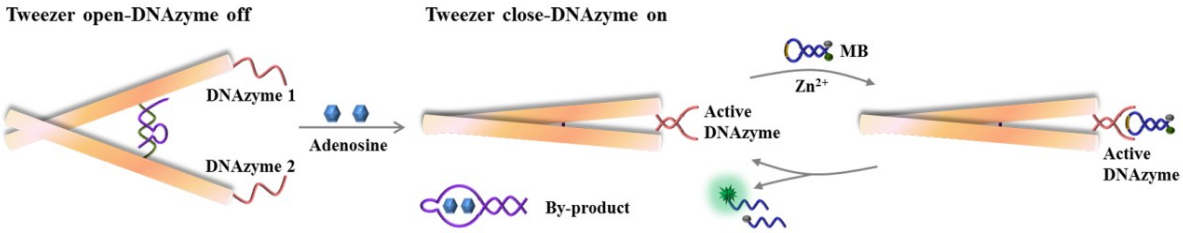

Figure S4 Schematic of the DNA tweezer actuated DNAzyme nanoreactor for adenosine detection.

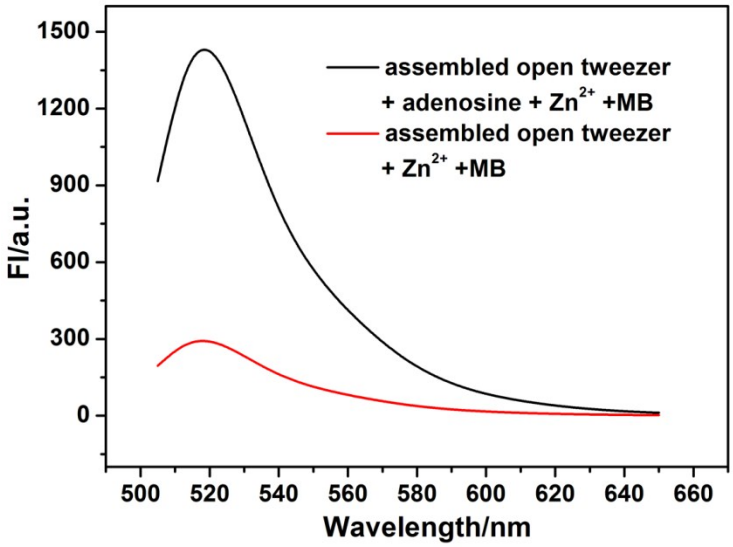

Figure S5 Fluorescence emission spectra with (in black) and without (in red) adenosine.

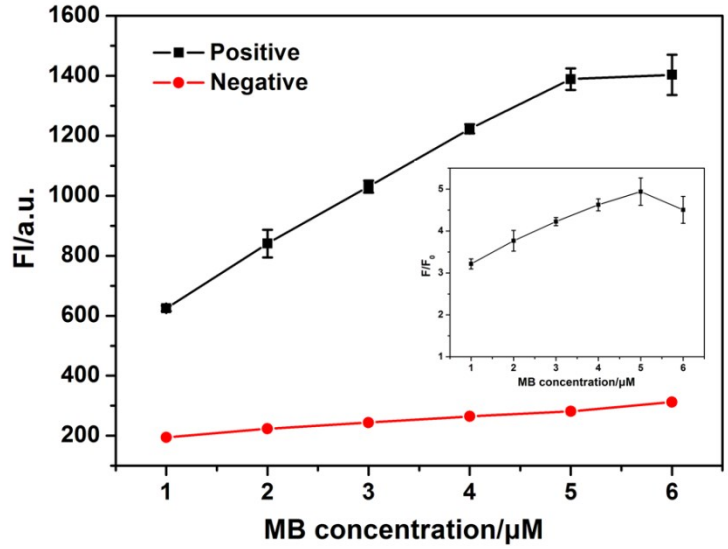

Figure S6 Effect of the MB concentration on fluorescence response. Inset shows the tendency of F/F0 under different MB concentration.

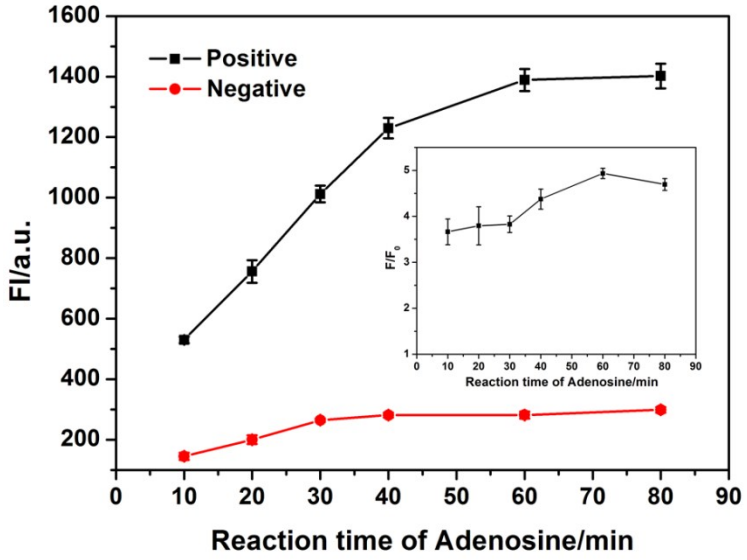

Figure S7 Effect of the adenosine reaction time on fluorescence response. Inset shows the tendency of F/F0 under different reaction time.

Table S5 Repeatability of this sensor for adenosine detection

Concentration	Sample 1 (FI)	Sample 2 (FI)	Sample 3 (FI)	Average (FI)	RSD (%)
10 μM	2127	2098	2115	2113.3	0.69
1.0 μM	1364	1372	1430	1388.7	2.59
0.5 μM	767.3	779.2	786.9	777.8	1.27

Table S6 Reproducibility of this sensor for adenosine detection

Concentration	Sample 1 (FI)	Sample 2 (FI)	Sample 3 (FI)	Average (FI)	RSD (%)
10 μM	2127	2131	2087	2115	1.15
1.0 μM	1364	1384	1413	1387	1.77
0.5 μM	767.3	782.5	815.9	788.6	3.15