Supporting Information

Binary split fluorescent aptasensor based on Lettuce DNA aptamer for label-free and enzyme-free analysis of Hepatitis B viral DNA

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Content

1. Experimental Section

1.1 Determination of the selectivity and competition in bsFDA

sLet-1 and sLet-2 were both added with different types DNA oligomer were incubated in reaction buffer (1×, 20 mM Hepes, 5 mM KCl and 8 mM $MgCl_2$, pH 6.5) for 1.5 h at 37 ℃, and then 2 μM DFHO were added into a final volume of 200 μL. To test the G4 ligand competition, bsFDA was mixed with different concentrations of TMPyP4 in reaction buffer solution. The fluorescence intensity values at 520-700 nm were collected using an RF-5301PC fluorescence spectrophotometer, and the slit width of excitation was kept at 10 nm and the slit width of emission was kept at 5 nm using a 2 mm×10 mm quartz cuvette. The excitation was performed at 510 nm and the fluorescence signal at 558 nm was recorded.

1.2 Spacer optimization assay

sLet-1 and sLet-2 with different spacer ranging from A_0 to A_2 were severally incubated in reaction buffer (1×, 20 mM Hepes, 5 mM KCl and 8 mM $MgCl_2$, pH 6.5) for 1.5 h at 37 ℃, and then 2 μM DFHO were added into a final volume of 200 μL. The fluorescence intensity values at 520-700 nm were collected using an RF-5301PC.

1.3 Experimental condition optimization

Different incubation time (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 h) were adopted for bsFDA incubation, and then the fluorescence of each group was detected using an F-2710 fluorescence spectrophotometer with an excitation wavelength of 510 nm. After obtaining the optimal incubation time, the same procedure was followed to optimize the concentrations of Mg^{2+} (1, 3, 5, 7, and 10 mM) and the reaction buffer pH values (6.0, 6.5, 7.0, 7.5, 8.0).

2.Supplemental Tables and Figures

Table S1. Sequences and secondary structures of DNA oligomers applied in this study*.

Oligonucleotide	Sequence (5' - 3')	Structure type
Lettuce	CTTAGTAGGGATGATGCGGCAGTGGGC TTCATCTATA TAAGATGAGGGGACTAAG	Single strand
HDs	GTC TGT GCC TTC TCA TCT GC	Single strand
sLet-1	CTT AGT AGG GAT GAT GCG GCA GTG GGC TTC ATC TAG CAC AGA C	Single strand
sLet-2	GCA GAT GAG AAG AGA TGA GGG GAC TAA G	Single strand
msLet-1	CTT AGT ATT GAT GAT GCG GCA GTT ATC TTC ATC TAG CAC AGA C	Single strand
msLet-2	GCA GAT GAG AAG AGA TGA TAT GAC TAA G	Single strand
sLet-1/A0	CTT AGT AGG GAT GAT GCG GCA GTG GGC TTC ATC TG CAC AGA C	Single strand
sLet-1/A1	CTT AGT AGG GAT GAT GCG GCA GTG GGC TTC ATC TAG CAC AGA C	Single strand
sLet-1/A2	CTT AGT AGG GAT GAT GCG GCA GTG GGC TTC ATC TAAG CAC AGA C	Single strand
sLet-2/A0	GCA GAT GAG AAG AGA TGA GGG GAC TAA G	Single strand
sLet-2/A1	GCA GAT GAG AAG A AGA TGA GGG GAC TAA G	Single strand
sLet-2/A2	GCA GAT GAG AAG AA AGA TGA GGG GAC TAA G	Single strand

sLet-1/T0	CTT AGT AGG GAT GAT GCG GCA GTG GGC TTC ATC TG CAC AGA C	Single strand
sLet-1/T1	CTT AGT AGG GAT GAT GCG GCA GTG GGC TTC ATC TTG CAC AGA C	Single strand
sLet-1/T2	CTT AGT AGG GAT GAT GCG GCA GTG GGC TTC ATC TTTG CAC AGA C	Single strand
sLet-2/T0	GCA GAT GAG AAG AGA TGA GGG GAC TAA G	Single strand
sLet-2/T1	GCA GAT GAG AAG T AGA TGA GGG GAC TAA G	Single strand
sLet-2/T2	GCA GAT GAG AAG TT AGA TGA GGG GAC TAA G	Single strand
ssDNA1	CCAGTTCGTAGTAACCC	Single strand
ssDNA2	GGGTTACTACGAACTGG	Single strand
ds26	CAATCGGATCGAATTCGATCCGATTG	Duplex
HP1	GGGTGGGTGGACTCAGCATTGCCCAAG AAGATGGGCAATGGTGGGT	Hairpin
HP2	GGGTGGGTGGTGGGCAATGCTGAGTCA TTGCCCATCTTCTGTGGGT	Hairpin
Hras	TCGGGTTGCGGGCGCAGGGCACGGGCG	G-quadruplex
Kras	AGGGCGGTGTGGGAAGAGGGAAGAGG GGGAGG	G-quadruplex
H-Telo	GGGTTAGGGTTAGGGTTAGGG	G-quadruplex
Chlas	GGGTTTTAGGGTTTTAGGGTTTAGGG	G-quadruplex
c-Kit2	CCCGGGCGGGCGCGAGGGAGGGGAGG	G-quadruplex
Hum21	GGGTTAGGGTTAGGGTTAGGG	G-quadruplex
G3T3	GGGTTTGGGTTTGGGTTTGGG	G-quadruplex
Oxy	GGGGTTTTGGGG	G-quadruplex

PS2M	GTGGGTAGGGCGGGTTGG	G-quadruplex
T3TT	GGGTTTGGGTGGGTGGG	G-quadruplex
SG4	GGGTGGGTAGGGTGGG	G-quadruplex
VEGF	GGGAGGGTTGGGGTGGG	G-quadruplex
T95	GGGTGGGTGGGTGGGT	G-quadruplex
HT	TTGGGTTAGGGTTAGGGTTAGGGA	G-quadruplex
HDs-M1	GTTTGTGCCTTCTCATCTGC	Single strand
HDs-M2	ATCTGTGCCTTCTCATCTGC	Single strand
HDs-M3	GTCTGCGCCTTCTCATCTGC	Single strand
HDs-M4	GTCTGTGGCTTCTCATCTGC	Single strand
HDs-M5	GTCTGTGCGTTCTCATCTGC	Single strand
HDs-M6	GTCTGTGCCTTATCATCTGC	Single strand
HDs-M7	GTCTGTGCCTTCTCACCTGC	Single strand
HDs-M8	GTCTGTGCCTTCTCATCTGT	Single strand
HDs-M9	GGCTGTGCCTTCTCATCTGA	Single strand
HDs-M10	GTCTGTGTGTTCTCATCTGC	Single strand
HDs-M11	GTCTGTGACTTCTCATCTGA	Single strand
HDs-M12	GTCTGGGCCTTCTCATCCGC	Single strand
HDs-M13	GTCTGTGCCTTCGAATCTGC	Single strand
HDs-M14	GAGTGTGCCTTCTCATCTGC	Single strand
HDs-M15	GTCTGTGCCTTCTCATGAGC	Single strand

* dsDNA was obtained from the hybridization between ssDNA1 and ssDNA2.

Table S2. Detection performance of previously reported methods.

Detection method	Linear Range	LOD	Methods	Reference
G-quadruplex-hemin DNAzyme method	0.5-100 nM	0.2 nM	Colorimetry	[1]
DNA walker and CHA signal amplification fluorescent probe	0.5-50 nM	0.2 nM	Fluorescence	[2]
Hyper-branched RCA	0.1-40 nM	0.05 nM	Fluorescence	[3]
A label-free fluorescent molecular beacon based on DNA-Ag NCs	5-200 nM	6.8 nM	Fluorescence	[4]
MIP multifunctional fluorescent sensor	0.5-90 nM	5.3 pM	Fluorescence	[5]
Au nanoparticles on covalent Organic framework	0.5-10 nM	150 pM	Fluorescence	[6]
bsFDA	**1-1000 nM**	**0.9 nM**	**Fluorescence**	**This work**

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1501: GTCTGCCGTTCCGACCGACCACGGGGCGCACCTCTCTTTACGCGGACTCCCCGTCTGTGC
1561: CTTCTCATCTGCCGGACCGTGTGCACTTCGCTTCACCTCTGCACGTCGCATGGAGACCAC
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Scheme S1. Sequence alignment from +1501 to + 1620 of HBV DNA genome for obtaining the HDs sequence which marked in red[7].

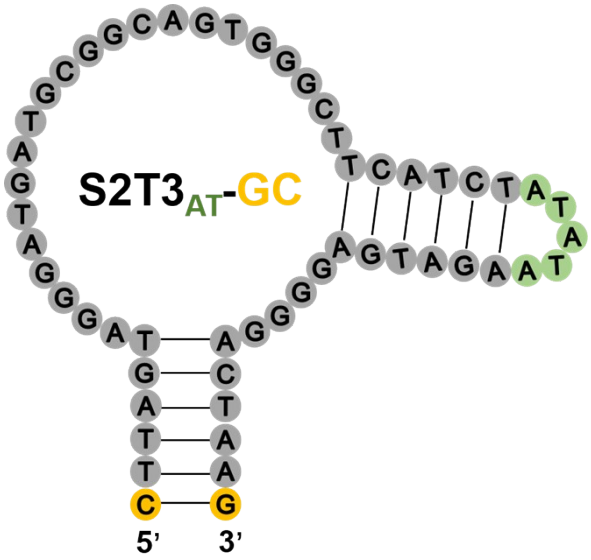

Figure S1. Sequence alignment and DNA structure of Lettuce aptamer.

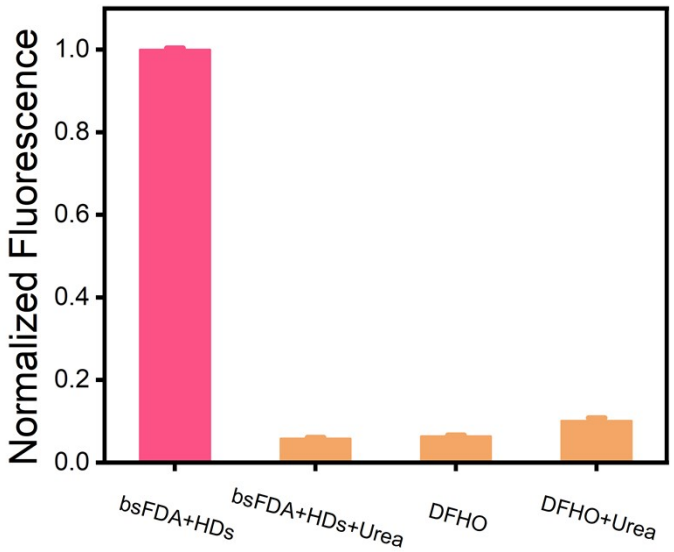

Figure S2. The normalized fluorescence of bsFDA/HDs and DFHO in the presence or absence 7 M urea.

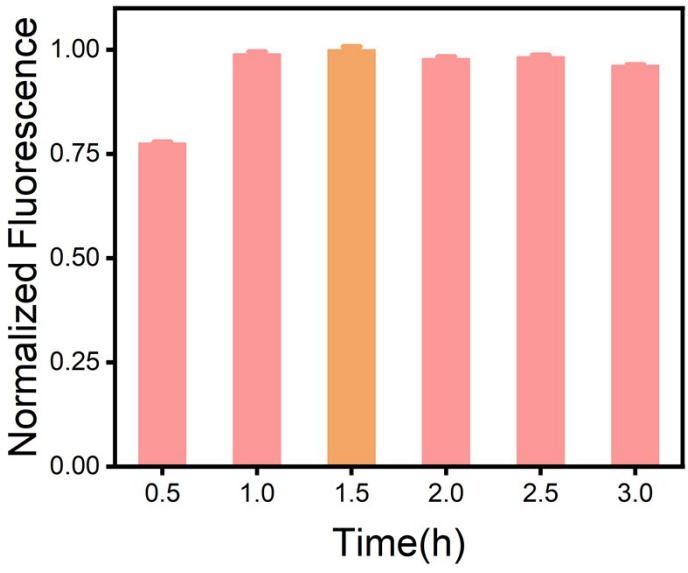

Figure S3. Fluorescent optimization of bsFDA for HDs recognition in different incubation time.

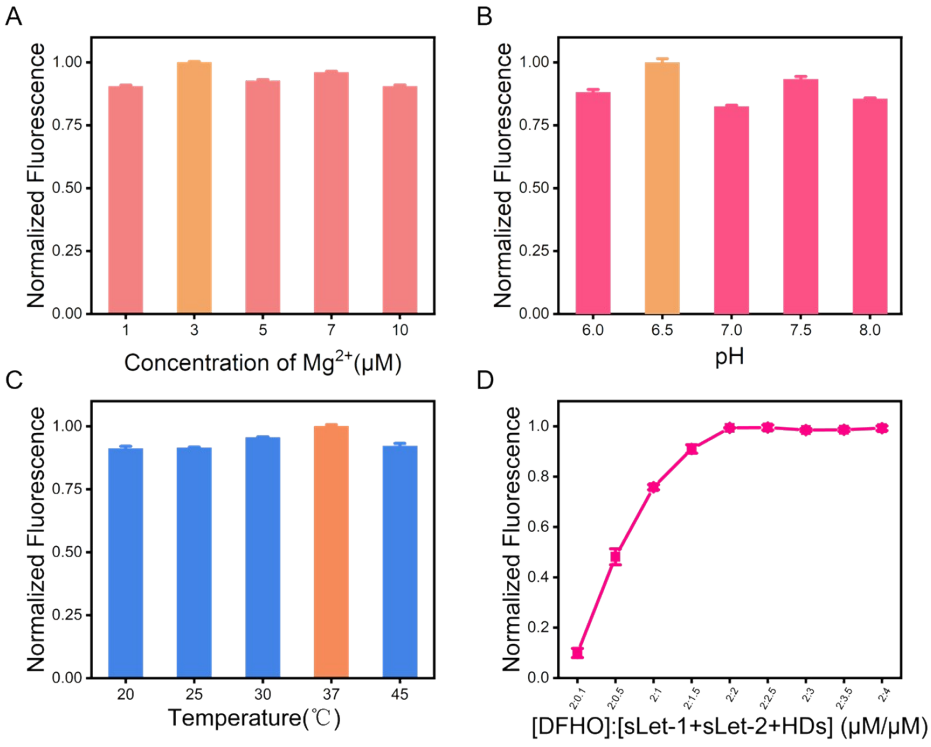

Figure S4. Fluorescent optimization of the concentrations of Mg^{2+} for DNA hybridization, the pH values in reaction buffer, the incubation temperature, and the concentration ratios between DFHO and the triplex DNA structure.

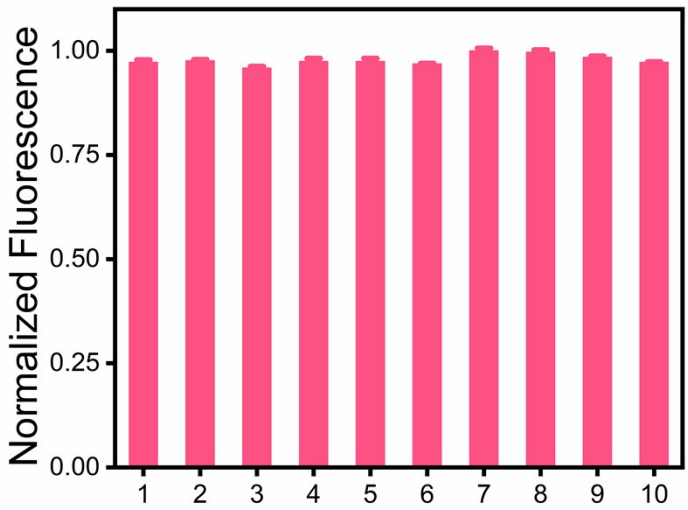

Figure S5. The fluorescent reproducibility of bsFDA for HDs detection in 10 times repetition.

4. References

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