Electronic Supplementary Material (ESI) for Analytical Methods.

Biosensor Model Based on Single Hairpin Structure for Highly Sensitive Detection of Multiple Targets

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Specificity of detecting HCV DNA

To determine whether the biosensor system is selective for the HCV DNA, we used four interfering substances with the same base sequence length: complete mismatch (HCV-CMT), two bases mismatch (HCV-MT1), four bases mismatch (HCV-MT2), and six base mismatch (HCV-MT3) for testing. The required nucleic acid sequences are listed in the Table S2. We also ran a control trial without any interfering substances. According to Figure S1, the tested relative fluorescence intensities of the HCV-MT1, HCV-MT2, HCV-MT3, and HCV-CMT gene sequences were 86%, 81%, 57%, and 31%, respectively, under the identical experimental conditions. It is important to note that the relative fluorescence intensity is lowest when the target gene HCV (HCV-NC) is absent. However, HCV-MT1 and HCV-MT2 have a relative higher value compared to HCV-MT3 and HCV-CMT, this is due to the oligonucleotide sequences of HCV-MT1 and HCV-MT2 are only two and four different bases with the target gene. Based on this, HCV-MT1 and HCV-MT2 may additionally combine with HCV-HP to create a blunt end that λ exonuclease can cut. Nevertheless, their relative fluorescence intensity is lower than that of the target gene because their binding capacity is lower than the target gene's. However, HCV-CMT and HCV-MT3 differ by more than 5 bases from the target gene, resulting in relatively low fluorescence intensity. This can prove that our designed biosensor can effectively detect HCV DNA.

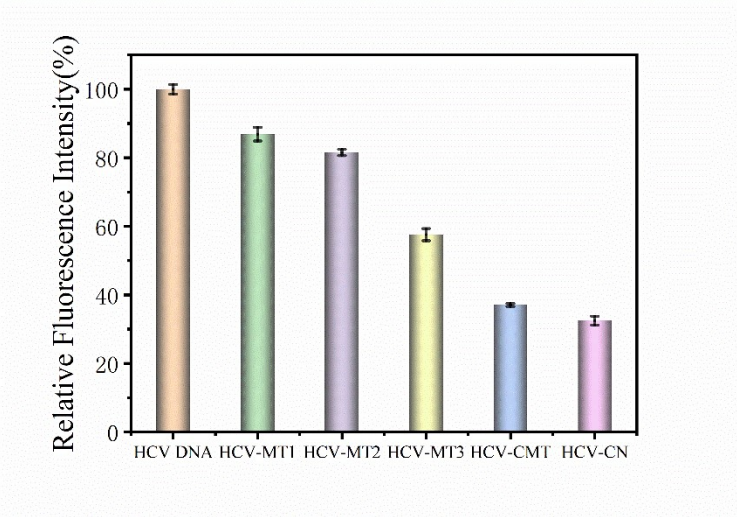

Figure S1. Specificity of detecting HCV DNA. Error bars: SD, n = 3. The desired concentrations, HCV-MT1, HCV-MT2, HCV-MT3 and HCV-CMT all are 0.25 μM. [HCV-HP]=0.5 μM, [λ exonuclease] =75 U/mL, [H1]=0.5 μM, [F-Q]=1 μM.

Table S1 Nucleic acid sequences required for specificity of detecting HCV DNA

oligonucleotide	Sequence (5'-3')
HCV DNA	TAAACCCGCTCAATGCCTGGAGTGT
HCV-MT1	TAAACCCGCTCAATGCCTGGAGTAA
HCV-MT2	TAAACCCGCTCAATGCCTGCCGTAA
HCV-MT3	TAAACCCGCTCAATTTCTGCCGTAA
HCV-CMT	TAGCTTATCAGACTGATGTTGA

The Influence of the Number of Bases on the Sensing Platform

In order to detect the target with different base numbers of the sensor platform, in addition to the HCV DNA and T-155 used in the experiment, we selected two different base number of target DNAs for testing (Figure S2). HCV DNA has 25 bases. Escherichia coli (E. coli) DNA has 17 bases and Dengue Fever virus (DF) DNA has 30 bases. The required nucleic acid

sequences are listed in the Table S2 We tested the sensor performance using three types of DNA as target DNA, and ultimately found that the fluorescence intensities of the three were not significantly different. Therefore, it can be concluded that the number of bases has no significant impact on the performance of the sensing platform.

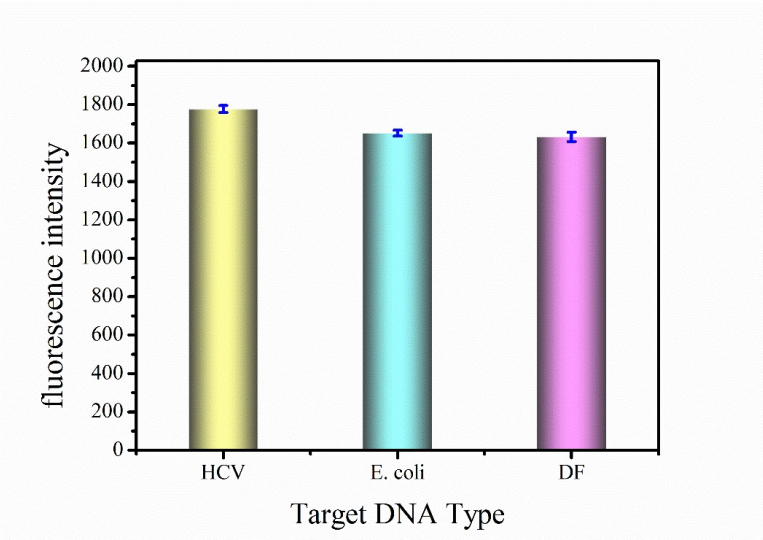

Figure S2. The effect of number of bases on the performance of detection platforms. The desired concentrations, target DNA all are 0.25 μM. Hairpin probe all are 0.5 μM, [λ exonuclease] =75 U/mL, [H1]=0.5 μM, [F-Q]=1 μM.

Table S2 Nucleic acid sequences required for the influence of the number of bases on the sensing platform

oligonucleotide	Sequence (5'-3')
Escherichia coli (E. coli)	CTAGTCGTATAGTAGGC
E. coli-HP	PO_4- GCCTACTATACGACTAGATAGTGACTGATAT TTCGACCGACATACCCGGTCGAAATATCAGT CACTAT
Dengue Fever virus DNA (DF)	AACCCAGCCTAAATGAAGAGCAGGACAAAA

DF-HP	PO_4- GCCTACTATACGACTAGATAGTGACTGATAT TTCGACCGACATACCCGGTCGAAATATCAGT CACTAT

Note: The underlined area represents the sequence in which the target DNA hybridizes with HP. The detection of different target genes can only be achieved by replacing the underlined sequence.

Target recovery rate in sample analysis

In order to obtain the recovery rate of target DNA in sample analysis, we added different concentrations of target DNA to 1% human serum, and the final fluorescence value was measured (F_S). The target fluorescence value measured under buffer condition is denoted as F_B, the recovery rate is expressed as F_S/F_B. We conducted experiments using two target DNA types, T-155 and HCV. The yellow in the figure represents the recovery rate of T-155, while the purple represents the recovery rate of HCV. We found that the value of F_S/F_B is approximately equal to 1, as shown in Figure S3. It shows that the biosensor we designed has a good recovery efficiency in a practical examination such as blood serum.

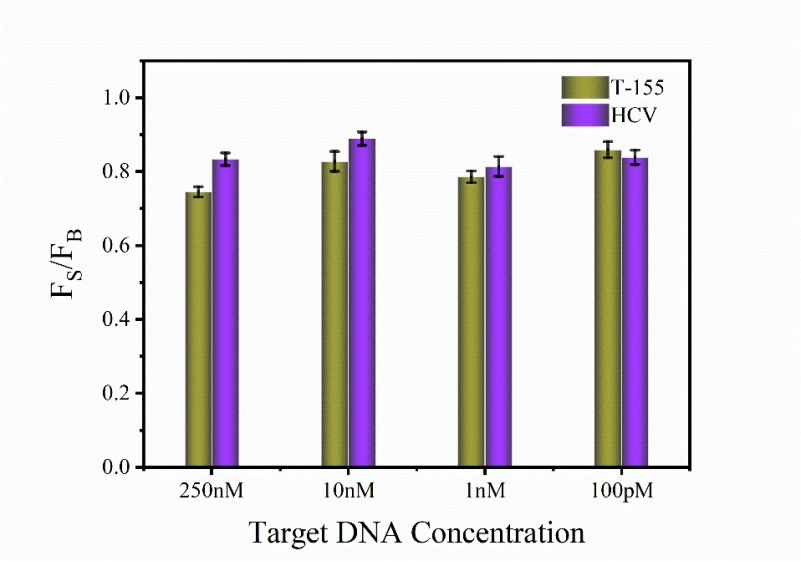

Figure S3. Target recovery rate in sample analysis. The desired concentrations, target DNA all are 0.25 μM. Hairpin probe all are 0.5 μM, [λ exonuclease] =75 U/mL,

[H1]=0.5 μM, [F-Q]=1 μM.