Electronic Supplementary Material (ESI) for Analyst.

A new colorimetric assay method for the detection of anti-hepatitis C virus antibody with high sensitivity

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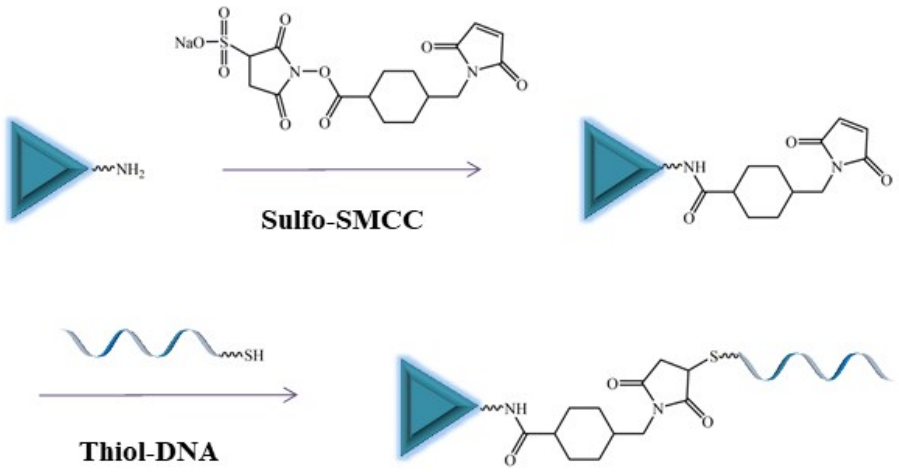
Table S1. Sequences and modifications of the DNA strands used in the study.

Name	Sequences and Modifications
Split DNA	5’-TTGGGTTAGGATCGTGTGGTTGGG-3’
Pre-cirDNA	5’-PO_4^{3-}-GATCCTAACCCAACCCGCCCTACCCAAAACCCAACCC GCCCTACCAAAACCCAACCCGCCCTACCCAACCACAC-3’
A strand	5’-SH-TAGCGAGTGTATTGAATGTGCAGGATCGTG-3’
B strand	5’-GATCCTGCACATTCAATACACTCG-3’
C strand	5’-SH-GCACATTCAATACACTCGCTA-3’
A strand -FAM	5’-SH-TAGCGAGTGTATTGAATGTGCAGGATC-FAM-GTG-3’
B strand - BHQ1	5’-BHQ1-GATCCTGCACATTCAATACACTCG-3’

The 3’-end of the A strand has a primer sequence of RCA (purple sequence). A FAM fluorophore (red) is modified near the 3’-end of the A strand-FAM. Accordingly, its quencher BHQ1 (green) are modified at the end of the 5’-end of the B strand- BHQ1.

Preparation of Antigen-AB Conjugates. The purified solution of sulfo-SMCC-activated antigen was mixed with A strand in the ratio of 1-to-3 at 25°C for 48 h. Instead of removing un-reacted A, an equal concentration of B strands were added to the above solution and incubated for another 4 h at 25°C. Finally, antigen-AB conjugates were purified by 30 K MWCO filter (Millipore) 8 times using buffer A to remove duplex AB which failed to conjugate with antigen.



Scheme S1. The conjugation of DNA and antigen by the heterobifunctional linker (sulfo-SMCC).

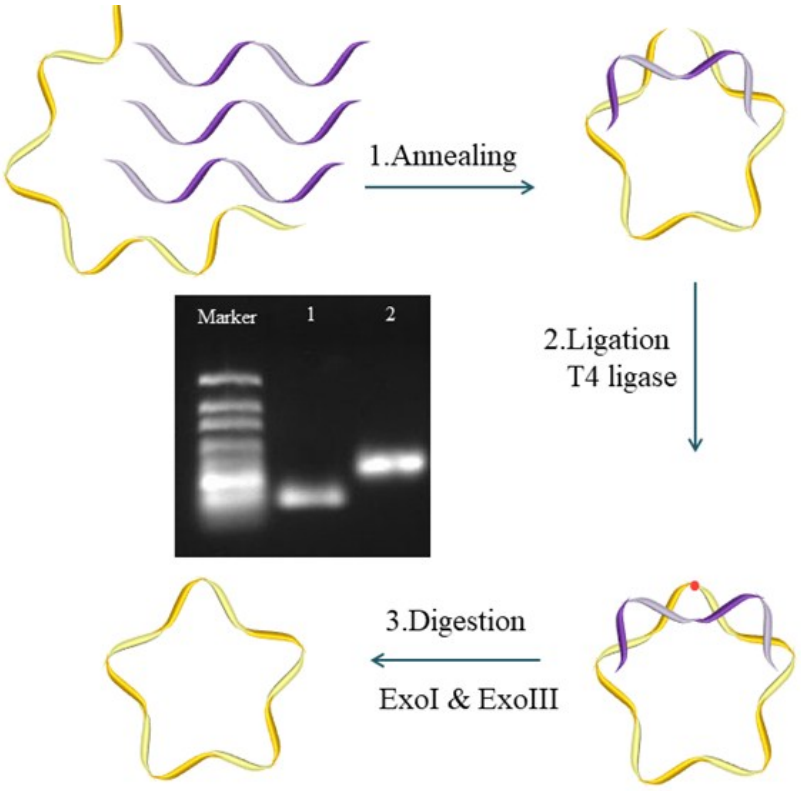


Figure S1. Preparation of cirDNA under the assistance of DNA tool enzymes. T4 ligase was used to link linear pre-cirDNA into cirDNA, ExoI and ExoIII were used to digest splint DNA and pre-cirDNA that was not formed cirDNA. The generated cirDNA was purified with a QIAquick Nucleotide Removal Kit, and the concentration of cirDNA was identified by a NanoDrop Lite UV-Vis Spectrophotometer (Thermo Fisher). The generated cirDNA was further verified by 1 % agarose gel analysis. Inset: the agarose gel electrophoresis result to verify the formation of the cirDNA. Lane 1, cirDNA; Lane 2, pre-cirDNA. When the linear DNA is looped, a relatively stable structure is formed. In the result of agarose gel electrophoresis, the band of cirDNA appears in front of pre-cirDNA due to smaller steric hindrance.

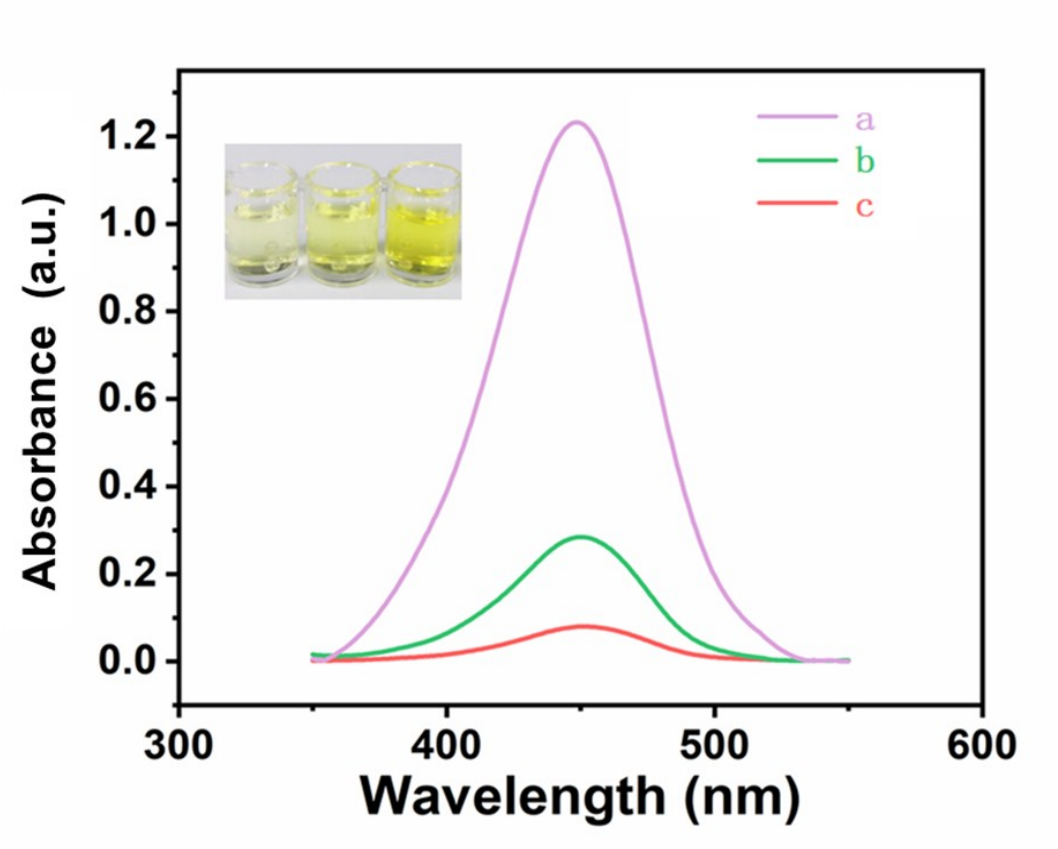


Figure S2. Feasibility of RCA reaction. UV-vis absorption spectra in different conditions: (a) the analysis system with 30 min RCA reaction; (b) the analysis system with 1 μM G-quadruplex sequences; (c) the analysis system without RCA reaction. Inset: digital image of the reaction mixtures under corresponding conditions.

Magnification of Colorimetric Signal by RCA Reaction. As shown in Figure S2, after 30 min RCA reaction, the analysis system shows maximum Abs. value at 450nm. This demonstrates that RCA reaction is successfully constructed and the multiple tandemly repeated G-quadruplex sequences could be generated by RCA reaction. After the addition of hemin, these G-quadruplex sequences can bind hemin to form peroxidase-like G-quadruplex DNAzymes, which can catalyze the oxidation of TMB by H_2O_2 to TMB^{2+}, resulting in a sharp increase of the absorption signal at 450 nm. An RCA primer could generate multiple G-quadruplex DNAzymes, and each DNAzyme would catalyze the formation of many copies of TMB^{2+}. In addition, the resulted tandemly repeated G-quadruplex units are linked head-to-tail, which can provide highly active hemin-binding sites. Thus these tandemly repeated G-quadruplex DNAzymes have higher catalytic activity. Taking all above into account, the magnification of colorimetric signal guarantees the high sensitivity of the proposed method for antibody detection.

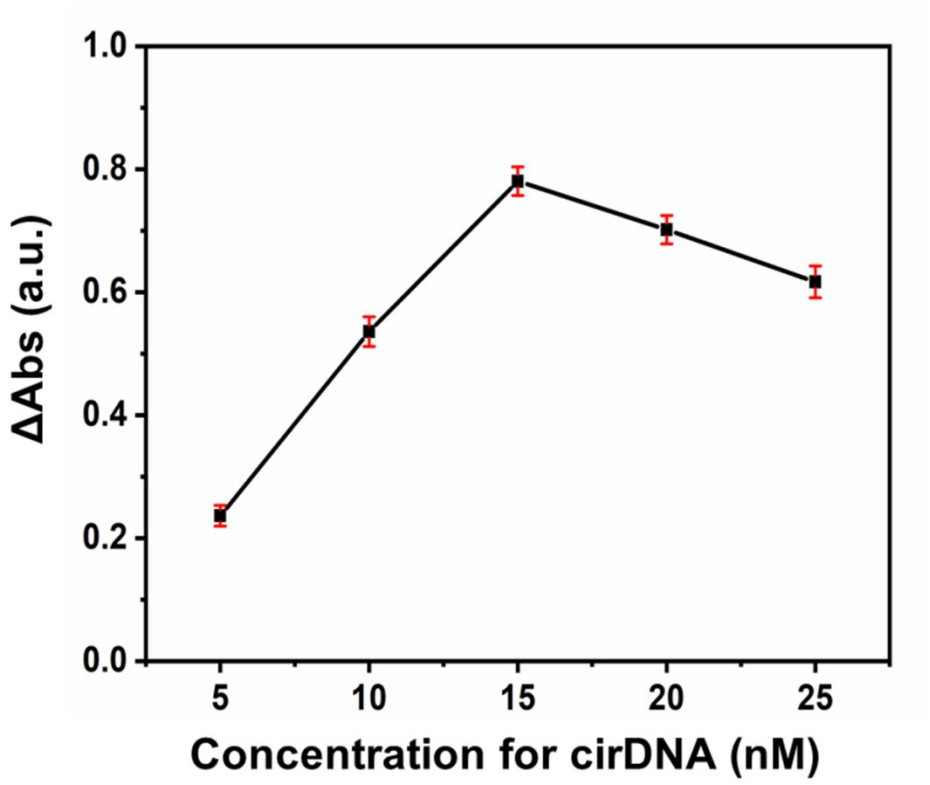


Figure S3. Effect of the concentration for cirDNA on absorption intensity at 450 nm of the proposed assay method. Error bars are the standard deviations of three parallel experiments.

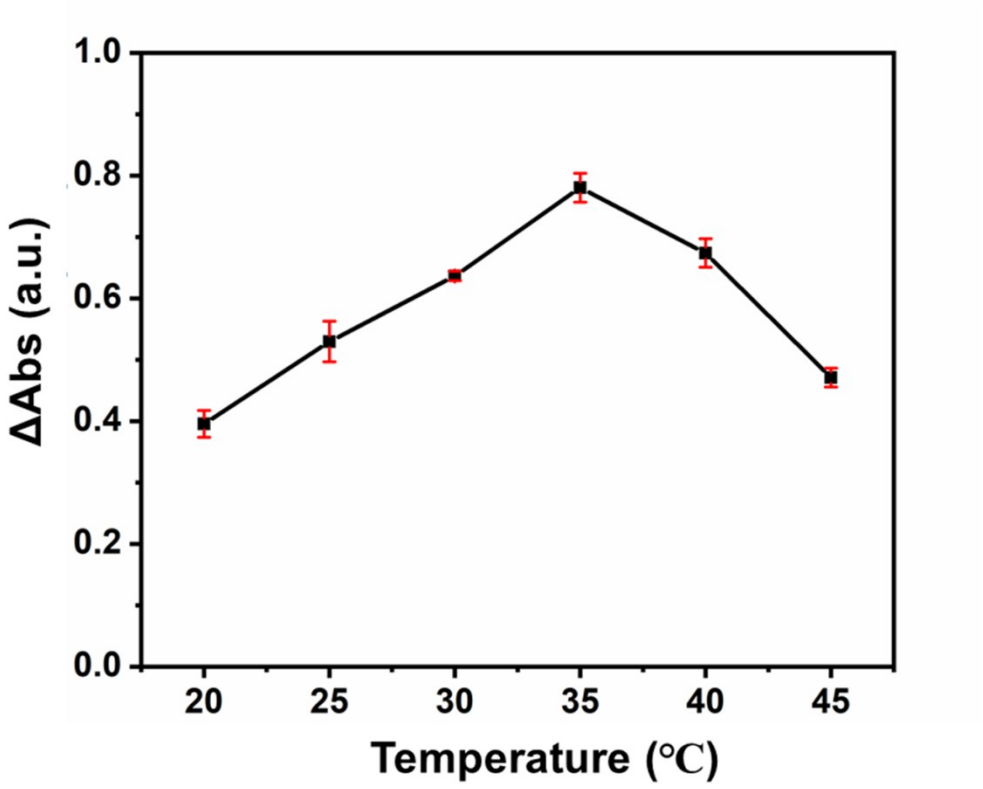


Figure S4. Effect of the temperature on absorption intensity at 450 nm of the proposed assay method. Error bars are the standard deviations of three parallel experiments.