Supplementary material for:

Double-stem Hairpin Probe and Ultrasensitive Colorimetric Detection of Cancer-related Nucleic Acids

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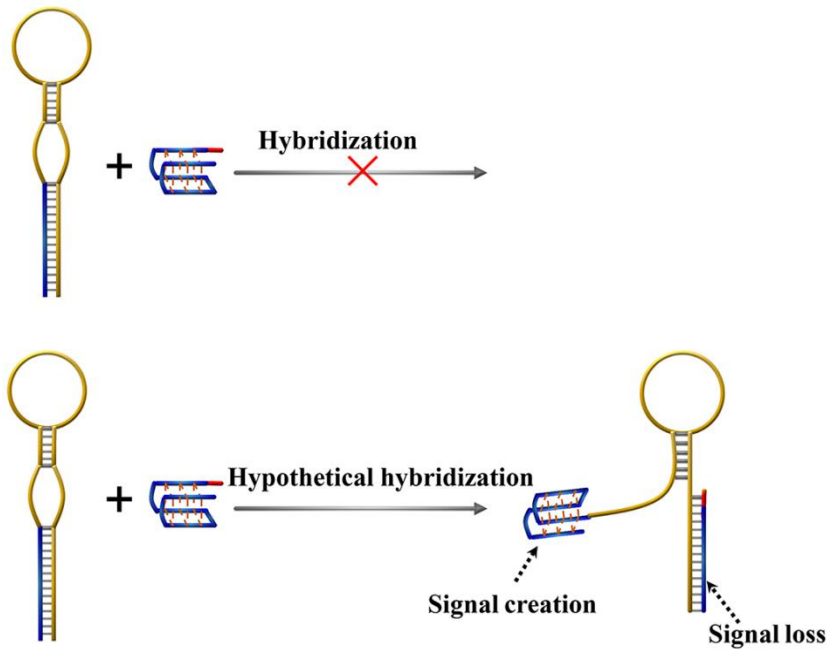
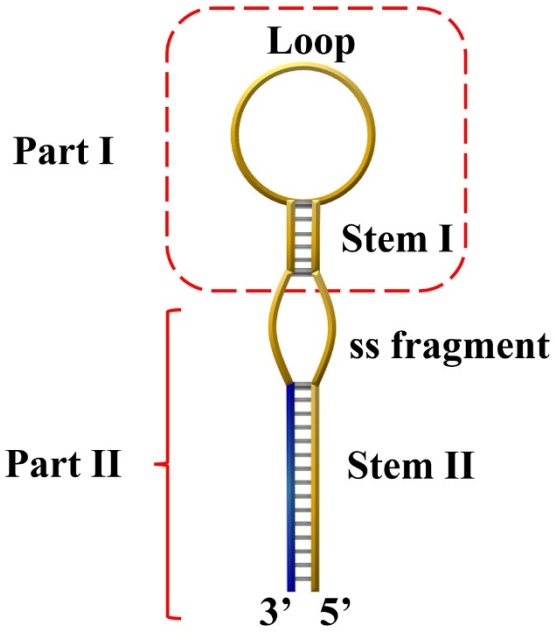


Scheme S1. Schematic showing of the mechanism of no signal loss. The upper panel indicates that the hybridization of DHP with Ns does not occur, while the lower panel shows that, even if Ns hybridize with DHPs, no any signal loss takes place.



Scheme S2. Functional fragments of DHP.

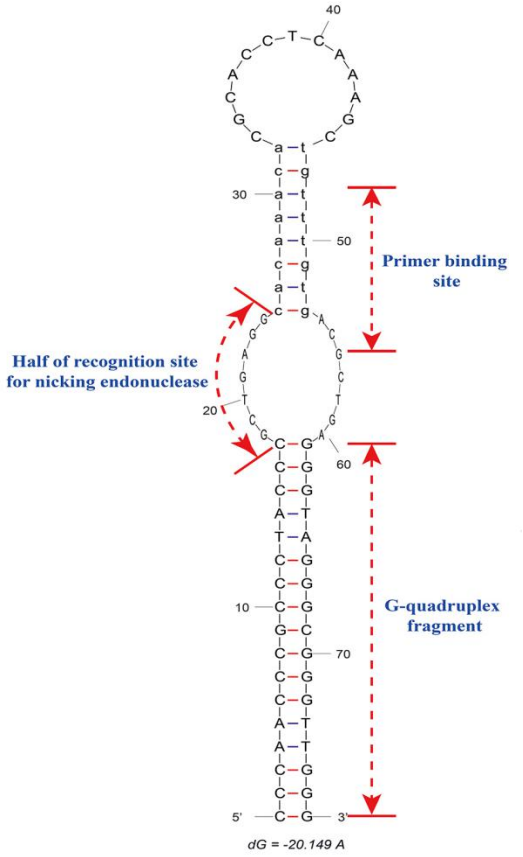
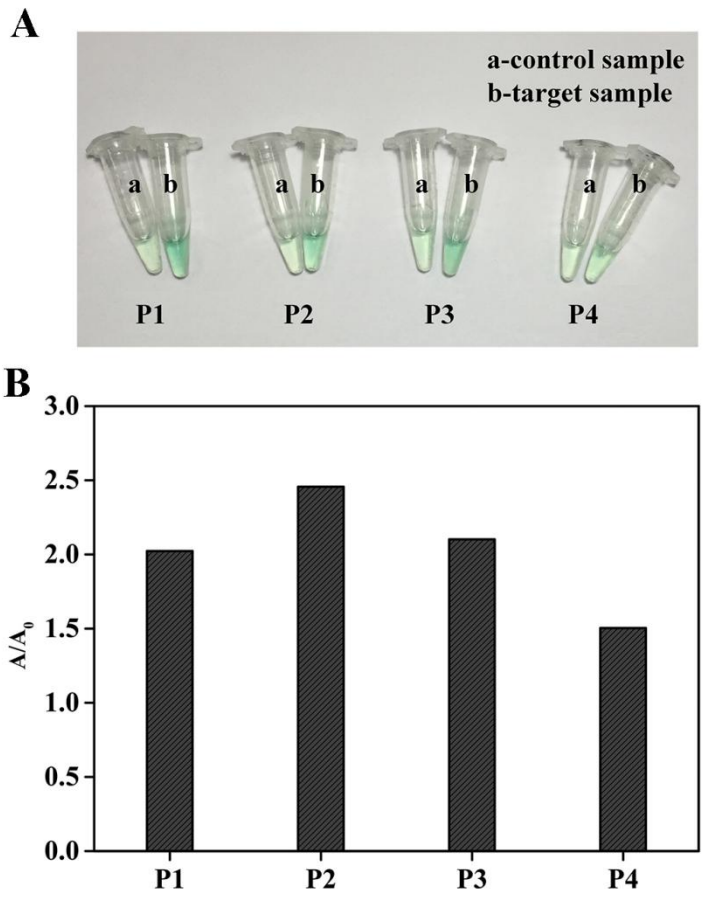


Figure S1. Secondary structure of DHP predicted by using "mfold" program.



C: Thermodynamic data of DHP/primer duplexes

	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS (kcal/mol)	T_m (℃)
P1	-11.2	-85.9	-240.7	49.4
P2	-8.8	-70.2	-197.9	40.9
P3	-6.7	-60.3	-173.0	30.5
P4	-5.0	-44.9	-128.6	17.9

Figure S2. (A) Comparative photographs of colorimetric DNA assays using different primers in the absence (a) and presence (b) of 150 μM target DNA. P1 to P4 indicated the sensing systems where the corresponding primers are employed; (B) The signal-to-background ratio calculated from UV-vis absorption spectra of the same sensing systems. The concentration of species involved is shown below: [DHP]= [P1]= [P2] = [P3]= [P4]= 500 nM; [hemin]= 3 uM; [H_2O_2]= 83.33 uM; [ABTS]= 0.75

mM. (C) Predicted thermodynamic data on DHP/primer duplex stability under selected experimental conditions.

Polymerization primer optimization

In this section, four primers, P1 to P4, were respectively designed to have 10, 9, 8, and 7-base fragment complementary to the DHP to optimize the proper polymerization primer before investigating the colorimetric assay performance. Figure S2A shows the direct comparison of color change between different primer-based sensing systems. To evaluate accurately their signal intensities, the signal-to-background ratio (A/A_0) was calculated from the UV-vis absorption spectrum, and the comparative data are provided in Figure S2B. The results shows that P2 can generate the highest colorimetric signal. As indicated in Figure S2C, the stability of DHP/primer duplex increases with increasing the paired base number, causing the increasing background intensity. Predictably, the P1 produces the highest background, while the response signal is relatively low even at high target DNA concentration when the DHP/primer duplex is not stable enough to ensure the subsequent reactions. According to the melting temperature of DHP/P3 and DHP/P4 duplexes, they are unable to exist stably under experimental conditions (at 37 ℃ for 40 min), and the primer hybridization-induced polymerization, nicking and signal conversion inefficiently proceed, decreasing the colorimetric signal intensity. Considering that P1, P3, and P4 would compromise the assay ability, the P2 was adopted for following experiments.

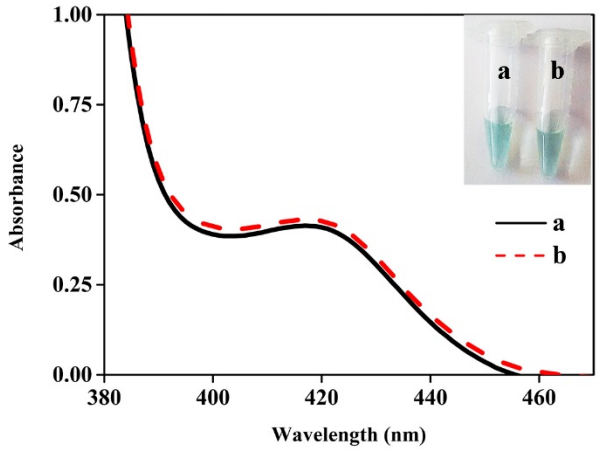


Figure S3. The UV-vis spectra, line a and line b, collected from Ns-contained solution and the mixture of Ns and DHP, respectively. Experiments were carried out according to the procedure described in "Experimental section", but, besides Ns and DHP, only hemin, H_2O_2 and ABTS were involved. Their concentrations are described below: [hemin]= 3 uM; [H_2O_2]= 83.33 uM; [ABTS]= 0.75 mM.

Comparative study of Ns and the mixture of Ns/DHP in terms of colorimetric signal

In order to examine whether there is signal loss for the proposed DHP- based sensing system, we conducted the comparative study of Ns and the mixture of Ns/DHP in terms of the capability to generate colorimetric signal. As can be seen in Figure S3, including the absorption spectra and photographs, the absorption peak of Ns/DHP mixture consisting of 250 nM Ns and 500 nM DHP is almost completely consistent with that of 250 nM Ns solution, revealing that no signal loss occurs and all the produced G-quadruplexes during DNA detection process can bind to hemin and act as catalytic DNAzyme to contribute to the colorimetric signal.

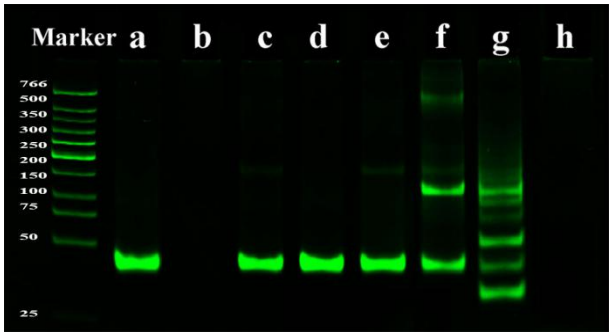


Figure S4. Native-PAGE graphs of the proposed sensing system and several controls. (a) DHP; (b) Target DNA; (c) DHP and target DNA; (d) DHP and P2; (e) DHP, target DNA and P2; (f) DHP, target DNA, P2 and polymerase; (g) DHP, target DNA, P2, polymerase and nickase; (h) Ns.

Native-PAGE characterization

12% native-PAGE experiments of different solutions were performed to demonstrate the mechanism of our detection strategy. As shown in Figure S4, only one bright band is clearly seen in lane a (containing DHP), whose position indicates the formation of single hairpin probe via intramolecular interaction rather than the intermolecular sticky-end pairing-based dimer of DHP. No detectable band could be observed in lane b (target DNA sample). When DHP was mixed with target DNA, a new weak band appears in lane c with a relatively lower mobility compared with lane a, indicating the hybridization between DHP and target DNA. After addition of primer with a low molecular weight, the position of this new band almost does not change while its brightness slightly increases (seen in lane e). The appearance of obvious new bands in lane f and lane g demonstrates that ploymerase and endonuclease does work during the operation of DHP to signal the target DNA. By the way, no any band is seen in

lane h (containing only Ns), implying that the nicked/displaced products can fold into G-quadruplex structure in the presence of hemin and plays a catalytic role but does not emit fluorescence under present experimental conditions.

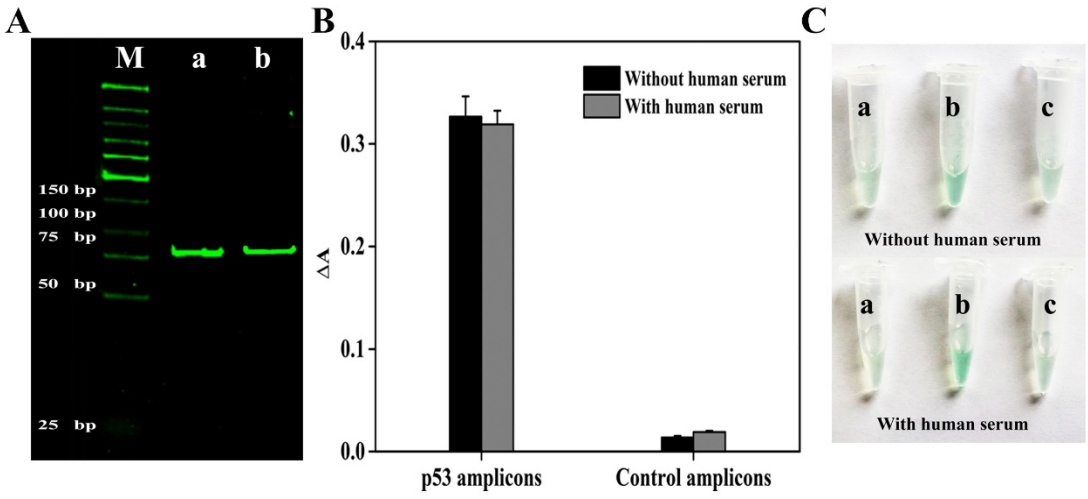


Figure S5. Analysis of real samples. (A) Native-PAGE demonstration of asymmetric PCR amplicons. Lane a: target amplicons, Lane b: control amplicons. (B) Background-subtracted bars corresponding to signal increase of real samples in the absence and presence of 1% human serum, respectively. The value of absorption increase (ΔA) is calculated from the following formula: $\Delta A = A - A_0$, where A and A_0 are the absorption peaks in the presence and absence of asymmetric amplicons, respectively. Error bars were deduced from N=3 experiments. (C) Comparative images of the colorimetric detection system in the absence (a), and presence of target amplicons (b) and control amplicons (c), respectively.

In order to demonstrate that the proposed DHP is applicable for real samples detection, the asymmetric PCR products obtained from human lung cancer cell line A549 genomic DNAs were detected under the aforementioned conditions. As shown in Figure S5A, a bright band was appeared both in lane a and lane b, confirming the successful amplification. Notably, the band of single-strand DNA (ssDNA) products could not be seen since the poor intercalation ability of the Sybr

Green I dye into the ssDNAs. Figure S5B displayed the signal change when interrogating different amplicons. One can observe that, a significant signal increase was detected for the samples of target amplicons, while a negligible signal output was obtained for the control amplicon. Moreover, the signal intensity almost does not change whether the human serum was involved. Figure S5C shows a distinct color change, and we can see that green color can be detected only in the presence of target amplicon. Clearly, these data suggest that the developed DHP holds the potential application for the detection of real genomic samples, and no substantial impact of human serum was verified.