# Electronic Support Materials：

# AuPt nanoalloy with Dual Functionalities for sensitive detection of HPV16 DNA

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**Table S1.** The sequence of oligonucleotide DNA[a]

| DNA | Sequences (5'-3') |
|---|---|
| Detection DNA | SH-5-AAC AAA TAG TTG-3 |
| Capture DNA | 5-CCA ACA AAT GCC-3-Biotin |
| Control DNA | Biotin-5- CAA CTA TTT GTT-3 |
| Target DNA (HPV 16) | 5-GGC ATT TGT TGG GGT AAC CAA CTA TTT GTT-3 |
| HPV 11 | 5-AGG CAC ACG CTG CAA AGG GAA-3 |
| HPV18 | 5-TA T AA T GTT GTT TCT CTG CGTC-3 |
| HPV 31 | 5-TGT TGT TGG CTC TTG GT A CGT TTA-3 |

a：Oligonucleotide DNA was obtained by the solid-phase phosphoramidite approach.[1]

**Preparation of AuPt nanoalloy and Pt nanoparticles**

Typically, glassware and magnetic stirrers were cleaned with freshly prepared aqua regia and rinsed with deionized water. 700 uL of $HAuCl_4$ solution ($2.8\times10^{-2}$ M) and 300 uL of $K_2PtCl_4$ solution ($1.9\times10^{-2}$ M) were added to 100 mL of deionized water and stirred thoroughly. 1.5 mL of tri-sodium citrate ($3.9\times10^{-2}$ M) was added to the above mixture. The solution was stirred vigorously followed by addition of 1 mL of freshly prepared $NaBH_4$ solution ($4.0\times10^{-2}$ M). The reaction solution was stirred for 24 h at room temperature. The suspension was centrifuged, and the supernatant was discarded. Then the precipitate was re-dispersed in CTAB solution (0.01 M). Pt nanoparticles were prepared using the same method at the same molar concentration.

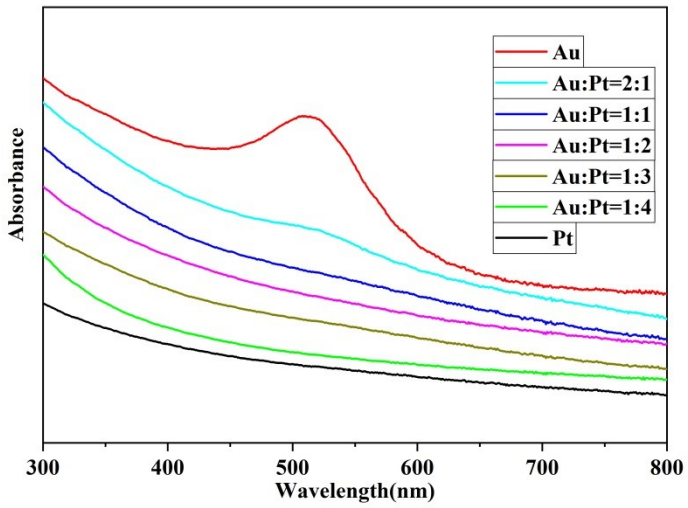


**Figure S1**. UV-vis spectra of AuPt Nanoalloys with different proportion of Au and Pt.

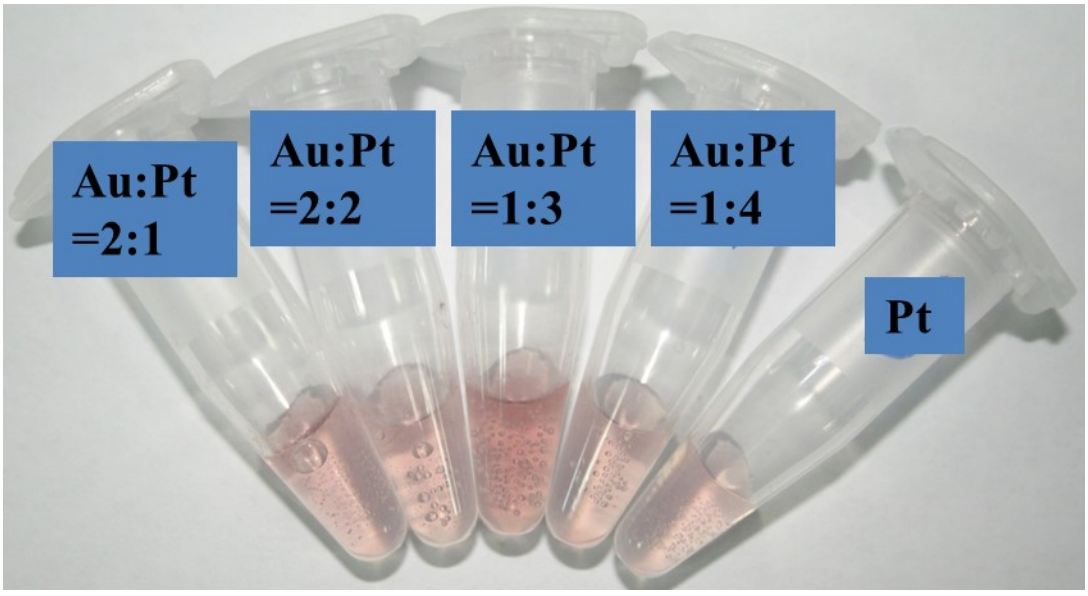


**Figure S2.** Comparison of catalytic performance of AuPt nanoalloy with different ratios (Au:Pt=2:1, 1:1, 1:2, 1:3, 1:4) and Pt.

**Detailed discussion of the optimization of experimental parameters**

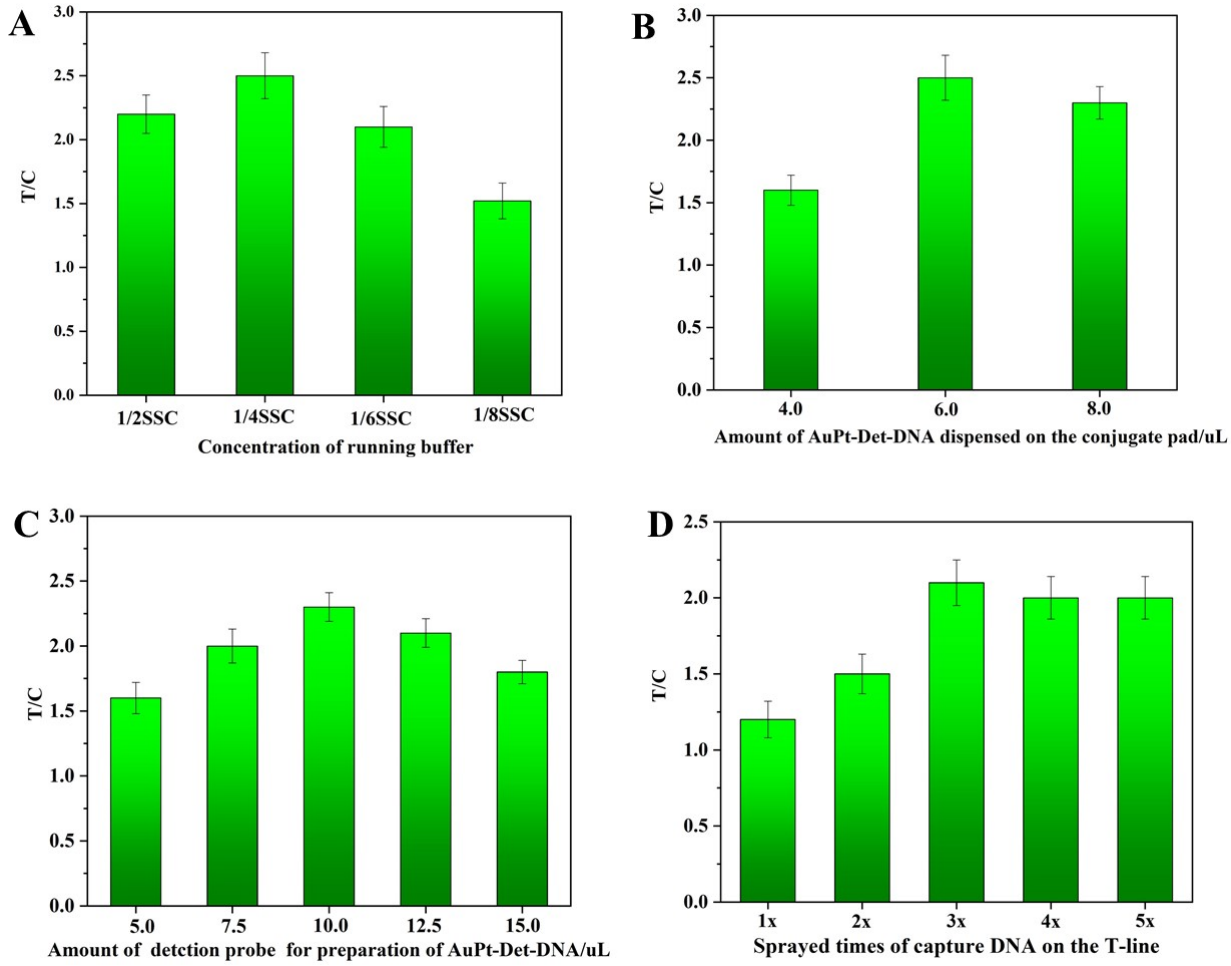


**Figure S3.** Optimization of experimental parameters.

Running buffer is considered as one of the most important parameters in the optimization of LFNAB. Due to SSC is the best buffer for DNA hybridization reactions, SSC was selected as the running buffer and the concentration was optimized. 2 times, 4 times, 6 times, and 8 times dilutions of stocked SSC (i.e., 1/2 SSC, 1/4 SSC, 1/6 SSC, and 1/8 SSC) were compared in our work, respectively. As shown in Figure S2A, it can be seen the highest S/N ratio was obtained via 1/4 SSC, which was used as the running buffer for all the assays.

In the current study, the AuPt-Det-DNA conjugates were dispensed on the conjugate pad. The amount of conjugates affected the intensities of T-line and C-line. Correspondingly, the amount

of AuPt-Det-DNA conjugates prober on the conjugate pad was controlled by the sprayed volume of the conjugates solution. To obtain high signal intensity, the sprayed volumes of AuPt-Det-DNA conjugates dispensing on conjugate pad were investigated. As shown in Figure S2B, the S/N ratio of the assay increased up to 6 μL on the conjugate pad, which was used as the optimal volume of the AuPt-Det-DNA conjugates for the following the experiments. The further increasing of the sprayed volume of AuPt-Det-DNA conjugates would lead to a decrease in the S/N ratio due to the increasingly nonspecific adsorption.

Sulfhydryl-modified detection DNA (Det-DNA) was used to prepare the signal probe of AuPt-Det-DNA conjugates via Au-S and Pt-S bonds. The amount of Det-DNA on AuPt nanoalloy surface would affect the response of LFNAB. Five kinds of consumed amount of Det-DNA were tested to reach a maximum performance. As shown in Figure S2C, the S/N ratio increased with increasing consumed amount from 5.0 uL to 15.0 uL of Det-DNA at the concentration of 0.5 OD $mL^{-1}$, then tended to balance till 10.0 uL. But with the continued increase of Det-DNA, a relatively signal decline emerged. An excess quantity of Det-DNA immobilized onto the AuPt surface cause reduction of chance of complimentary reaction with target DNA which directly decrease signal on the test line. So, 10 uL of 0.5 OD/mL detection DNA was chosen for the preparation of AuPt-Det-DNA conjugates probe.

The amount of capture DNA (Cap-DNA) probes immobilized at the test line also affects the LFNAB response. To obtain the best response, the amount of Cap-DNA was optimized by spraying different amount of streptavidin-modified Cap-DNA complexes. This was achieved by increasing the sprayed times on the T-line. As shown in Figure S2D, three sprayed times of the capture DNA probe exhibited the highest S/N ratio.

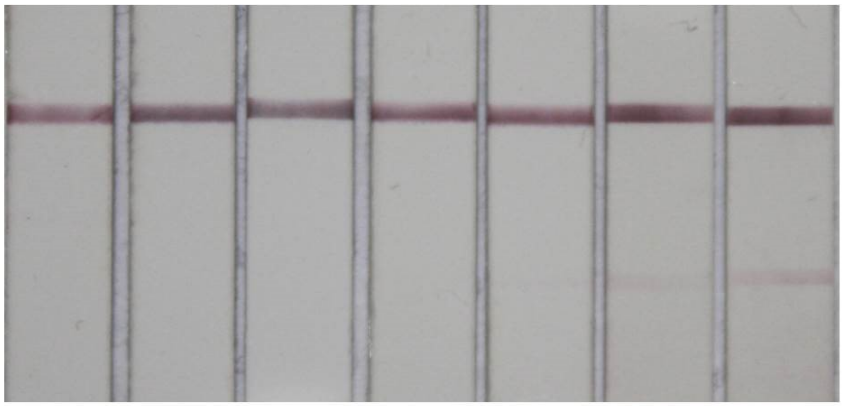

**Figure S4.** Au Nanoparticles (4 nm) as label to detect HPV16 DNA in LFNAB. The target concentration from left to right is 0, 2, 5, 10, 20, 50, 100 pM.

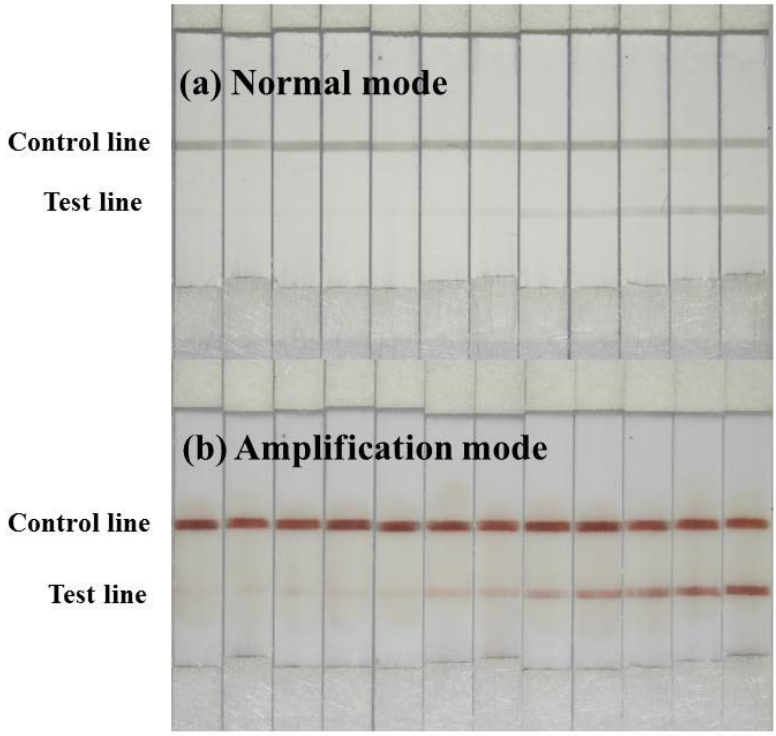


**Figure S5.** AuPt Nano Flowers (AuPt NFs) as label to detect HPV16 DNA in LFNAB. The target concentration from left to right is 0, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, 5000 pM.

## References

1. S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, **48**, 2223-2311.