SUPPLEMENTARY INFORMATION

Paper-based DNA Sensor enabling Colorimetric Assay Integrated with Smartphone for Human Papillomavirus Detection

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1. Synthesis of acpcPNA probe for HPV type 16

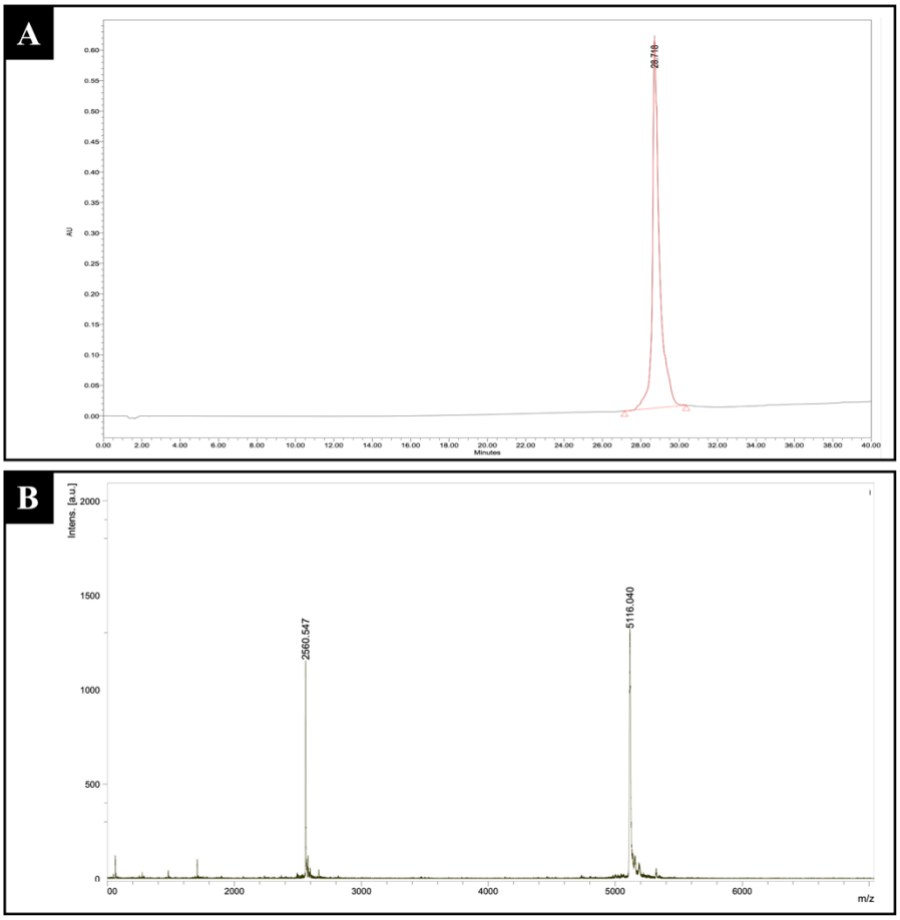

Fig. S1 (A) HPLC chromatogram and (B) MALDI-TOF mass spectrum of Bz-CATACACCTCCAGC-Lys_3-NH_2

2. Gadget camera structure and color intensity analyzing application

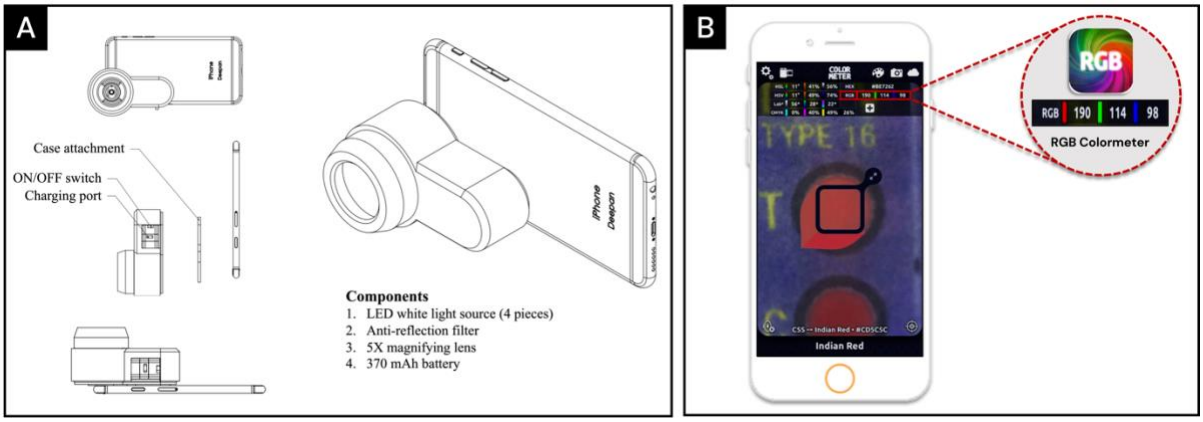

Fig. S2 (A) Structure of the home-made gadget camera coupled with smartphone readout device and its components designed by EOSCE group (B) Image of color intensity analyzing by using RGB Colorimeter application

3. Colorimetric detection of HPV DNA based on acpcPNA-induced d-AuNPs aggregation (proof-of-concept experiments)

The proof-of-concept experiment for this work was demonstrated as shown in Table S1. The color of d-AuNPs remained red when adding complementary DNA. In contrast, when the d-AuNPs solution was dropped onto the paper that was pre-deposited with the acpcPNA probe, the color turned to purple due to the aggregation of d-AuNPs. In the presence of the complementary DNA, the hybridization of DNA and acpcPNA probe occurred and the color remained red due to the depletion of free acpcPNA probe that can induce d-AuNPs aggregation.

Table S1 List of d-AuNPs conditions and their photographs

d-AuNPs in various conditions	Photographs
d-AuNPs	
d-AuNPs + complementary DNA	
d-AuNPs + acpcPNA probe	
d-AuNPs + acpcPNA probe + complementary DNA	

Next, the images of the d-AuNP solution on the paper were taken via a smartphone coupled with a home-made camera gadget for light control. Then, they were imported to the RGB Colorimeter application for measuring the color intensity. To interpret the results, the intensity in the red channel was used to quantitate the “redness” of the reaction. When the solution is redder, a larger numerical value is obtained in the red channel. When the solution turns purple, the red value in the red channel decreases.

Fig. S3 showed the color intensity of the d-AuNPs solution on the paper under each condition. All three channels (red, green, and blue) were compared. The d-AuNPs and the complementary DNA (d-AuNPs +DNA) provide the same color intensity as free d-AuNPs in the red channel indicating that the aggregation process is not affected by the negatively charged DNA. On the other hand, the aggregation of the d-AuNPs readily occurred

since the electrostatic repulsion was shielded when the positively charged acpcPNA probe was introduced into the d-AuNPs solution (d-AuNPs+acpcPNA). The color of the solution changed to purple, thus resulting in a decreased color intensity in the red channel. When the complementary DNA was also present in the system, the hybridization of the target DNA with the acpcPNA probe inhibited the aggregation of the d-AuNPs induced by the acpcPNA probe, thus the solution became redder. The red color intensity (d-AuNPs+acpcPNA+DNA) increased with the increasing of complementary DNA (d-AuNPs+acpcPNA). A small intensity change was also observed in the green channel, but not the blue channel. However, the intensity change in the red channel was the most obvious and was therefore chosen for the next experiments.

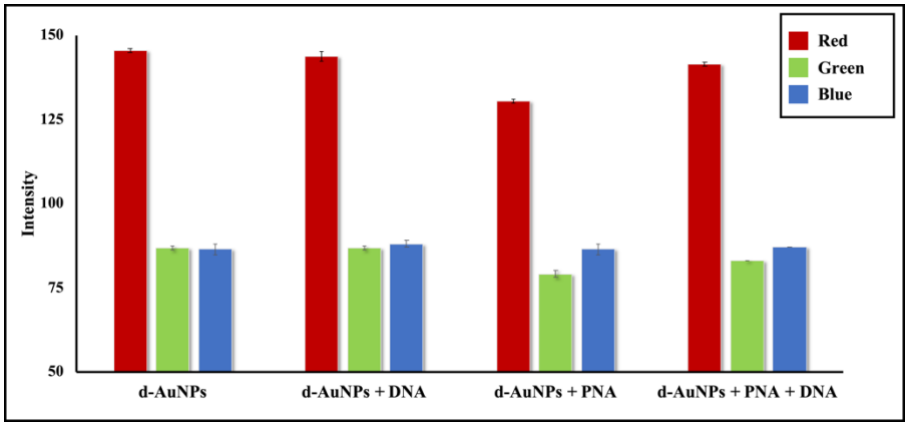

Fig. S3 Color intensity of d-AuNPs in various condition measuring by RGB Colorimeter application in red, green, and blue channel

To confirm the performance of the acpcPNA-induced d-AuNPs aggregation, d-AuNPs under various conditions were characterized. Firstly, the color change of the d-AuNPs which related to the d-AuNPs aggregation state was characterized by UV-vis absorption spectrophotometry in the solution phase. Fig. S4 shows the UV-vis absorption spectra of d-AuNPs under various conditions as shown in Table S2. The solution of d-AuNPs (red line) provides an absorption peak at 522 nm which was in good agreement with the previous work [1]. After adding complementary DNA (blue line), the peak at 522 nm which was similar to the peak of d-AuNPs was still present. This indicated that the complementary DNA did not affect the d-AuNPs aggregation state. In contrast, when the acpcPNA probe was added (purple line), the solution turned purple and the peak red-shifted to 552 nm due to the aggregation of d-AuNPs. When both the acpcPNA probe and its complementary DNA target were present in the d-AuNPs solution, a slight red-shifting of the absorption peak (at 526 nm) was also observed, and the solution remained red. This result indicated that some of the acpcPNA probes hybridized with the

complementary DNA, leading to lower amounts of the acpcPNA probe that can induce the d-AuNPs aggregation, resulting in a mixture of dispersed and aggregated forms of the d-AuNPs.

Table S2 List of reaction components in the UV-vis absorption spectrophotometry studies

Reagents	Concentration
d-AuNPs	10-fold diluted stock using Milli Q water
complementary DNA (C-DNA)	0, 50 µM
acpcPNA probe	0, 50 µM

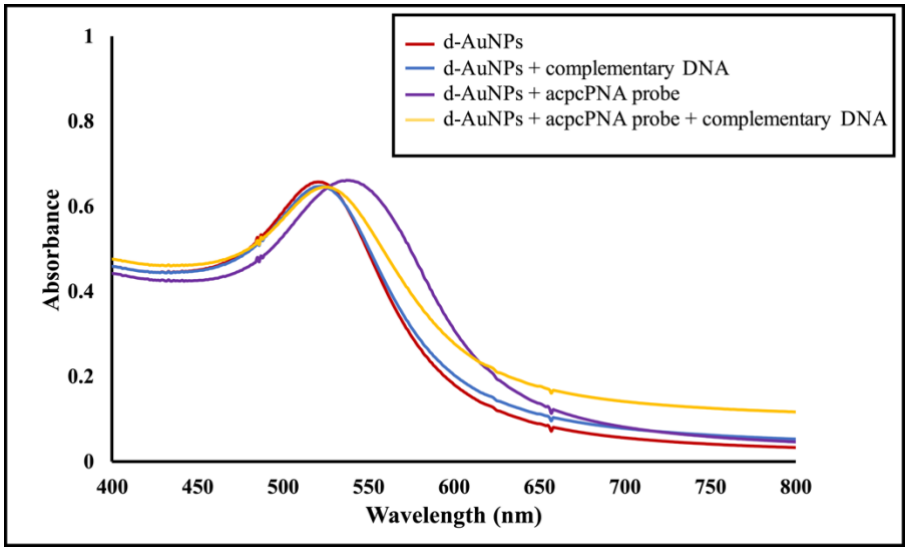

Fig. S4 UV–vis absorption spectra of d-AuNPs with different condition

Moreover, the dispersion and morphology of the d-AuNPs under each condition were investigated using transmission electron microscopy (TEM). Fig. S5 shows the TEM images of the d-AuNPs under different conditions. The morphology of the d-AuNPs (Fig. S5A) is spherical with the particle size of 17 nm. In the presence of the complementary DNA, the particle size remained the same as shown in Fig. S5B. After adding the acpcPNA probe, the particle size of the d-AuNPs increased due to aggregation of the d-AuNPs (Fig. S5C). The results indicated that the acpcPNA probe could induce the d-AuNPs aggregation while DNA could not.

In addition, the particle size of the d-AuNPs in the presence of both the acpcPNA probe and the complementary DNA target was investigated. Fig. S5D shows the mixture of small and large particles due to the presence of the d-AuNPs in both dispersion form and aggregation form. The results from the TEM technique were consistent with the UV-vis absorption spectrophotometry.

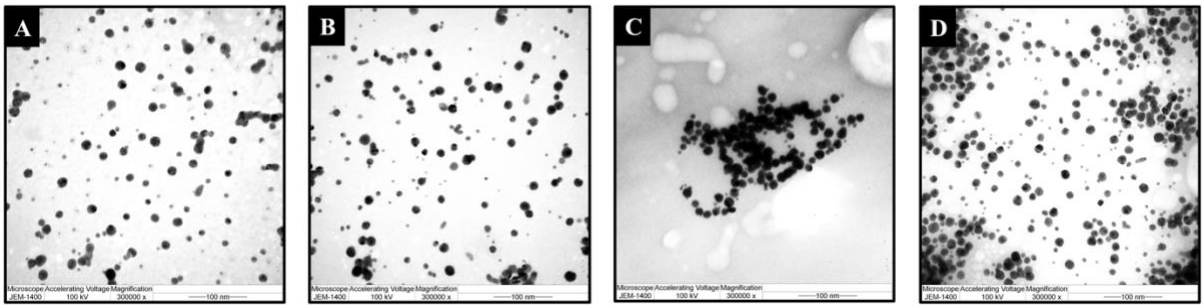

Fig. S5 TEM images of (A) d-AuNPs, (B) d-AuNPs +DNA, (C) d-AuNPs + acpcPNA and (D) d-AuNPs + acpcPNA + DNA

4. Optimization parameters

To find the optimal conditions for the assay, key experimental parameters including the d-AuNPs to buffer (0.01 M PBS, pH 7.4) ratio, the PBS mixing time, the acpcPNA probe concentration, and the incubation time were studied. The details of the optimization conditions are shown in Figure S6. In Fig. S6A, the color intensity in the red channel increased until the ratio of d-AuNPs:PBS reached 10:4 and tended to be stable after this point. This result indicated that beyond the ratio of d-AuNPs:PBS at 10:4, salts in the PBS solution did not affect the d-AuNPs aggregation. Hence, the ratio of 10:4 d-AuNPs:PBS was chosen for further experiments. Next, the effect of the mixing time of d-AuNPs and PBS buffer was evaluated. The color intensity of the d-AuNPs and PBS buffer mixture did not change when the mixing time was increased as shown in Fig. S6B. Therefore, the d-AuNPs and PBS buffer mixing time did not affect the d-AuNPs aggregation. Next, the effect of the acpcPNA probe concentration was investigated. We compromised between the acpcPNA concentration and volume to obtain the highest intensity which means the highest degree of aggregation. The acpcPNA probe concentration and volume were varied within a range of 1 to 50 µM and 2 to 5 µL, respectively. The differential color intensity (Δ intensity) was obtained from the intensity of d-AuNPs before and after the addition of the acpcPNA probe (Δ intensity = intensity $_{\text{without acpcPNA probe}}$ – intensity $_{\text{with acpcPNA probe}}$). The highest Δ intensity was observed at the

concentration of 50 µM and the volume of 3 µL and tended to be stable beyond this volume as shown in Fig. S6C. Therefore, 50 µM acpcPNA probe solution (3 µL) was selected as the optimal condition. Furthermore, the effect of incubation time in the presence of target DNA was next investigated. For this parameter, the differential color intensity (Δ intensity) obtained from the color intensity of d-AuNPs before and after the addition of the target DNA (Δ intensity = intensity $_{\text{with target DNA}}$ – intensity $_{\text{without target DNA}}$) decreased with the increase of incubation time and was constant after 15 min as shown in Fig. S6D. This result indicated that the reactions completely occurred after this incubation time. Therefore, 20 min of incubation time was employed for further experiments.

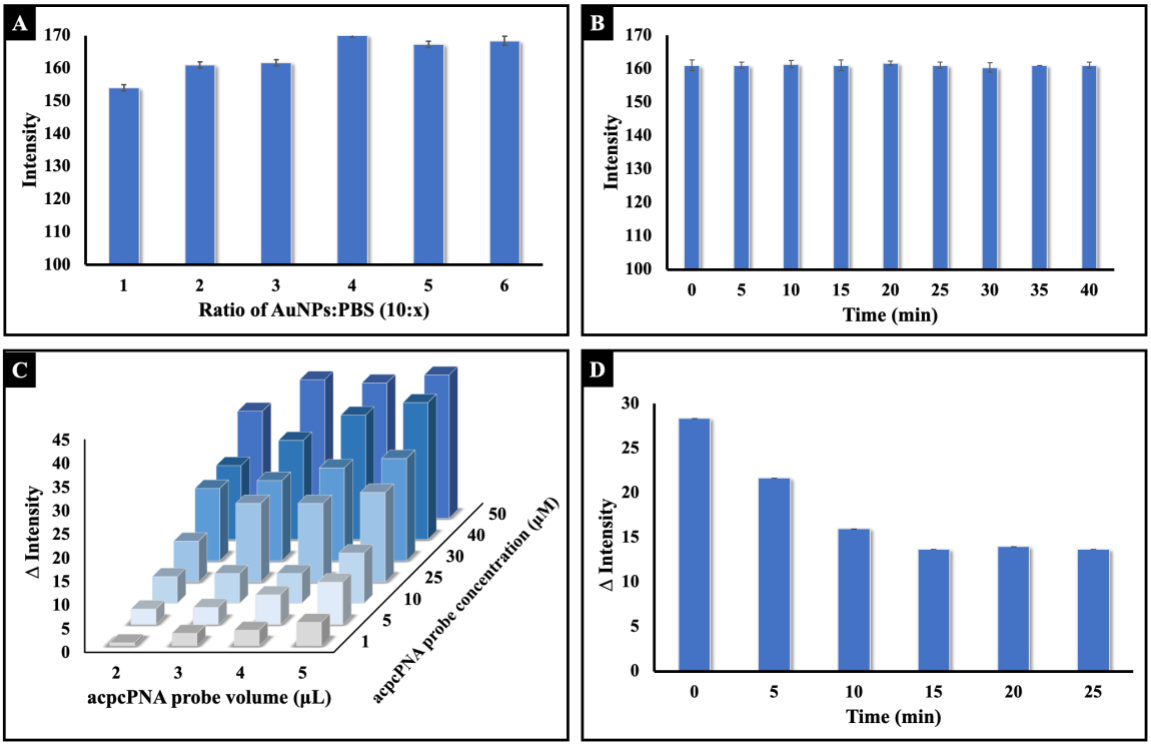

Fig. S6 Optimization of (A) d-AuNPs:PBS buffer ratio, (B) d-AuNPs and PBS mixing time, (C) acpcPNA probe concentration and (D) incubation time. The error bars showed one standard deviation (SD) obtained from three independent measurements (n=3).

5. Analytical performance in the absence of $MgCl_2$

The analytical performance of the proposed colorimetric DNA sensor was next evaluated using the paper-based sensors pre-deposited with the acpcPNA probe (50 µM, 3 µL). Under the optimal conditions, the photographic results of the proposed colorimetric DNA sensor (Fig. S7A), and a linear correlation between the

Δ Intensity and logarithmic DNA concentration in the range of 1 to 100 µM was observed (Fig. S7B, inset) with a correlation coefficient (R^2) of 0.9959. The experimental limit of detection (LOD) was found to be 1 µM.

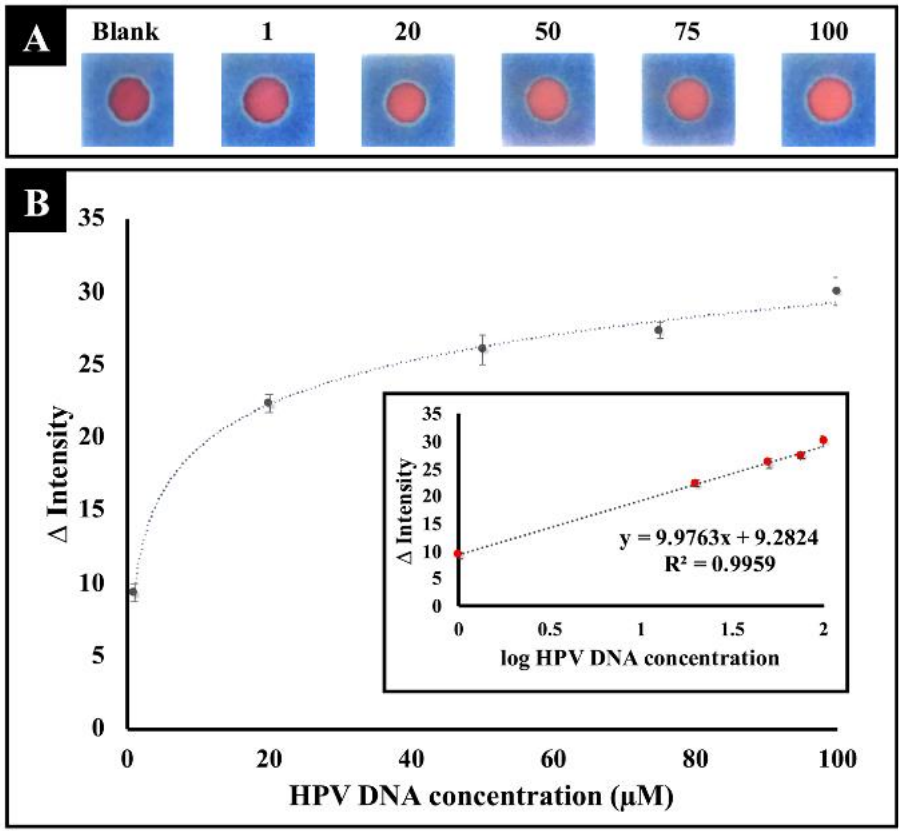

Fig. S7 (A) Photographic results of the proposed sensor with HPV DNA concentration in the range of 1-100 µM. (B) Calibration plot between Δ Intensity vs DNA concentration and calibration plot between Δ intensity and log DNA concentration (inset) for HPV DNA detection. The error bars showed one standard deviation (SD) provided from three independent measurements (n=3).

6. Destabilizing of d-AuNPs by salt-induced aggregation

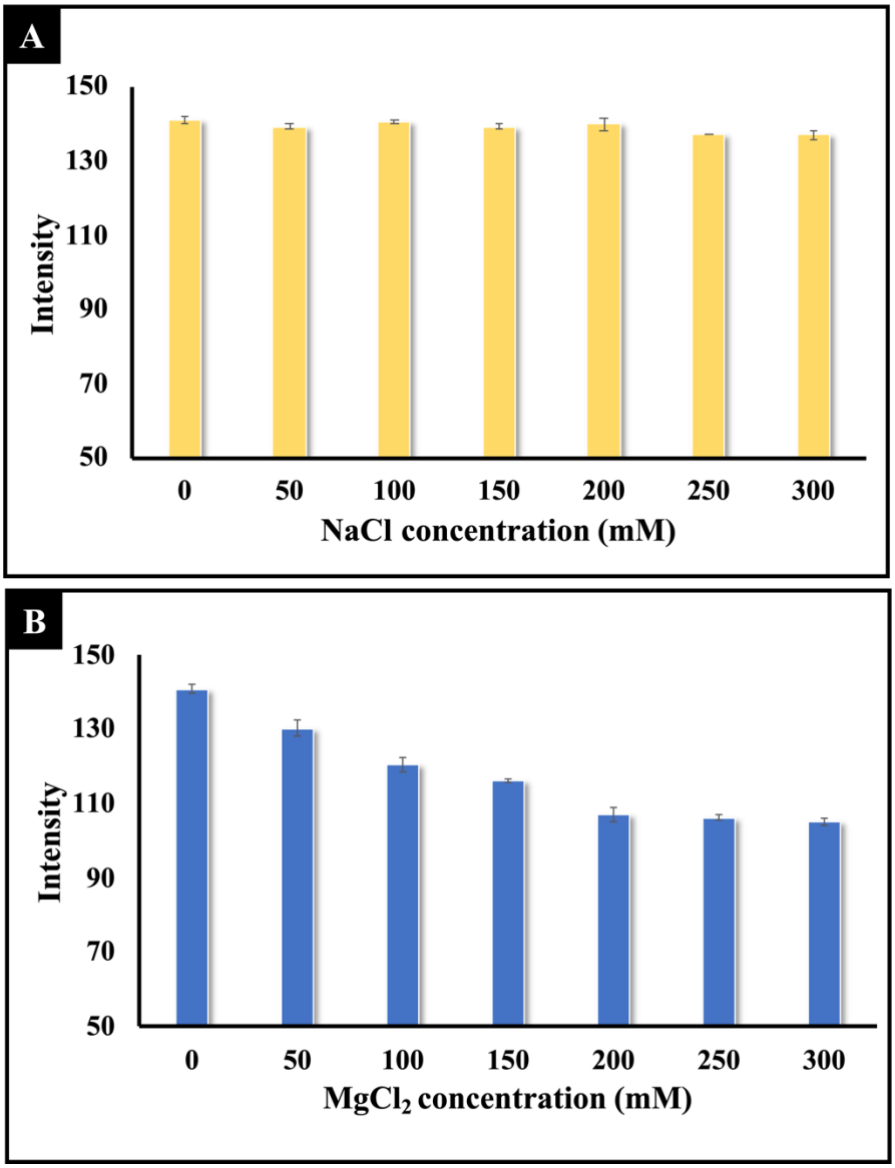

Fig. S8 The effect of salt-induced aggregation of d-AuNPs (A) NaCl and (B) $MgCl_2$ without acpcPNA probe. The error bars show one standard deviation (SD) provided from three independent measurements (n=3).

7. Stability and reproducibility studies

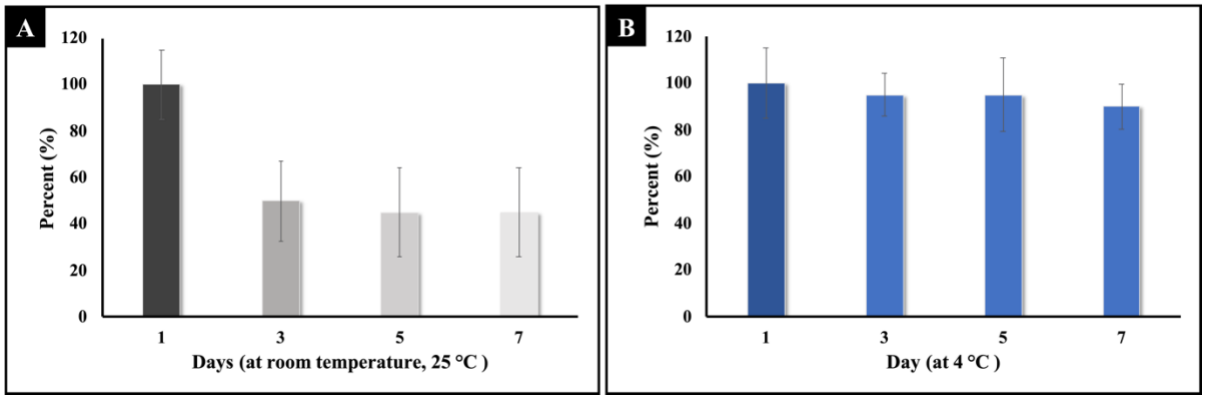

Fig. S9 Storage lifetime of the proposed colorimetric HPV DNA sensor at (A) room temperature (25°C) and (B) 4 °C. The error bars show one standard deviation (SD) provided from three independent measurements (n=3).

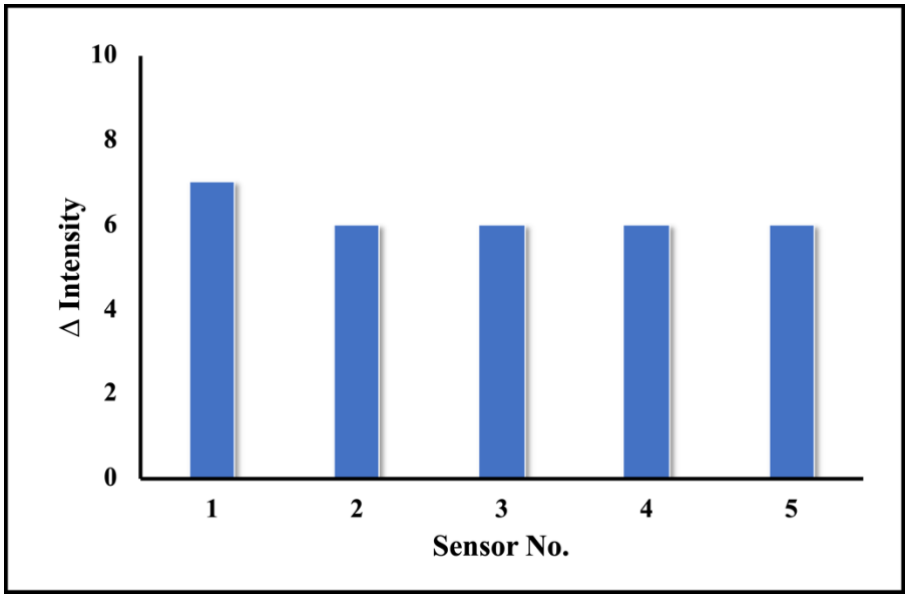

Fig. S10 Reproducibility of the five independently prepared sensors.

8. Comparison with other DNA-based HPV detection methods

Table S3 Comparison of the proposed DNA sensor with other HPV DNA detection methods

References	Analytical method	Format	Type of probe	Instrument	Linear range	LOD
[2]	Fluorometry	Solution	DNA	Microplate reader	0.05-200 µM	100 pM
[3]	Colorimetry	Paper	acpcPNA	Scanner + imageJ	20–2500 nM	1 nM
[4]	Electrochemistry	Paper	acpcPNA	Potentiostat	0.5–100 nM	150 pM
This work	**Colorimetry**	**Paper**	**acpcPNA**	**Smartphone + RGB colormeter**	**1-1000 nM**	**1 nM**

References:

[1] A.M. Baetsen-Young, M. Vasher, L.L. Matta, P. Colgan, E.C. Alocilja, B. Day, Direct colorimetric detection of unamplified pathogen DNA by dextrin-capped gold nanoparticles, Biosensors and Bioelectronics, 101(2018) 29-36.

[2] A.M. Jimenez Jimenez, M.A.M. Rodrigo, V. Milosavljevic, S. Krizkova, P. Kopel, Z. Heger, et al., Gold nanoparticles-modified nanomaghemite and quantum dots-based hybridization assay for detection of HPV, Sensors and Actuators B: Chemical, 240(2017) 503-10.

[3] P. Teengam, W. Siangproh, A. Tuantranont, T. Vilaivan, O. Chailapakul, C.S. Henry, Multiplex Paper-Based Colorimetric DNA Sensor Using Pyrrolidinyl Peptide Nucleic Acid-Induced AgNPs Aggregation for Detecting MERS-CoV, MTB, and HPV Oligonucleotides, Analytical Chemistry, 89(2017) 5428-35.

[4] S. Jampasa, W. Siangproh, R. Laocharoensuk, P. Yanatatsaneejit, T. Vilaivan, O. Chailapakul, A new DNA sensor design for the simultaneous detection of HPV type 16 and 18 DNA, Sensors and Actuators B: Chemical, 265(2018) 514-21.