Electronic Supporting Information

Amplification-free CRISRP/Cas Based Dual-enzyme Colorimetric Nucleic Acid Biosensing Device

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Experimental methods

Synthesis and characterization of PMBA

PMBA was synthesized as previously reported.[1] A solution of 2-aminoethyl methacrylate hydrochloride (AEMA-HCl) (12.1 mmol) and trimethylamine (24.2 mmol) was prepared in chloroform (40 mL). Di-tert-butyl dicarbonate (13.3 mmol) was added to this mixture, and the reaction was carried out at 40 °C overnight. Upon completion, ethyl acetate (60 mL) was added, and resulting precipitate was removed by filtration. The filtrate was evaporated under vacuum to dryness, and the residue was washed three times with water, followed by freeze-drying overnight, yielding 78% of the Boc-protected AEMA.
MMA (54.8 mmol), Boc-AEMA (6.09 mmol) and AIBN (0.122 mmol) were dissolved in ethyl acetate (30.6 mL). 1, 3, 5-Trioxane (10 mmol) was added as a reference and the conversion rate was 91.9%. Oxygen was removed by nitrogen bubbling before polymerization, which was carried out overnight at 70 °C. The resulting copolymer was precipitated by dropping it into 1000 mL of n-hexane, followed by drying under vacuum overnight. NMR analysis was performed on a JNM ECA-500 (JEOL, Japan). The peaks and integral value was consistent with the previous work.

The weight-average molecular weight (Mw) and number average molecular weight (Mn) of the polymers were determined using a GPC system (Prominence, CMB-20A system controller, DGU-20A3R degasser, LC-20AR pump, SIL-20ACHT autosampler, RID-20A refractive index detector, and CTO-20AC column oven; Shimadzu, Kyoto, Japan). The columns used were a TSKgel guard column α and two TSKgelα-M colums (TOSOH, Tosoh, Japan) connected in series. The mobile phase was DMF containing 10 mM LiBr, used as eluent at a flow rate of 0.7 mL min^{-1} at 40 °C. The weight-average molecular weight (Mw) and polydispersity (Mw/Mn) of the synthesized polymer are shown in Table S2.

Investigating the influence of PVA residues on the enzymatic Cas and ALP reactions

A PVA membrane was added to 300 μL of NE buffer and incubated for 1h. A solution was prepared with the following final concentrations: Cas enzyme = 50 nM, crRNA = 100 nM, target DNA = 1 nM, and FQ reporter = 1000 nM (6-FAM-TTATT-IABkFQ, purchased from IDT). Here, the FQ reporter was used as a substitute for the AuNPs-ssDNA-ALP probe. After the activation of the Cas enzyme, the single-stranded DNA connecting the quencher and the fluorophore was cleaved, turning on the fluorescence emission of 6-FAM at 520 nm when excited by a 495 nm light source. Similarly, 100 μl of 1 μg/mL ALP solutions with and without PVA were prepared, followed by adding 20 μL of BCIP/NBT substrate. After a 10-minute reaction, the absorbance at 580 nm for the two situations was compared.

Table S1 Sequences of nucleic acids used in this work

Oligo	Sequence(5'-3')
Target DNA	AAAAAAAAAATCATGTAACGTTCTATGATTTACACTCCATGGT
	TTTTTTTTTTAGTACATTGCAAGATACTAAATGTGAGGTACCA
1 mismatched DNA	AAAAAAAAAATCATGTCACGTTCTATGATTTACACTCCATGGT
	TTTTTTTTTTAGTACAGTGCAAGATACTAAATGTGAGGTACCA
2 mismatched DNA	AAAAAAAAAATCATGTCACGTGCTATGATTTACACTCCATGGT
	TTTTTTTTTTAGTACAGTGCACGATACTAAATGTGAGGTACCA
crRNA	UAAUUUCUACUAAGUGUAGAUGUACAUUGCAAGAUACUAAA
ssDNA 30 base	BiosG-$(TTA)_{10}$-ThioMC6-D
ssDNA 100 base	BiosG-$(TTA)_{33}$T-ThioMC3-D

Table S2 Molecular weight of PMBA

Mn	Mw	Mw/Mn
43369	92532	2.13

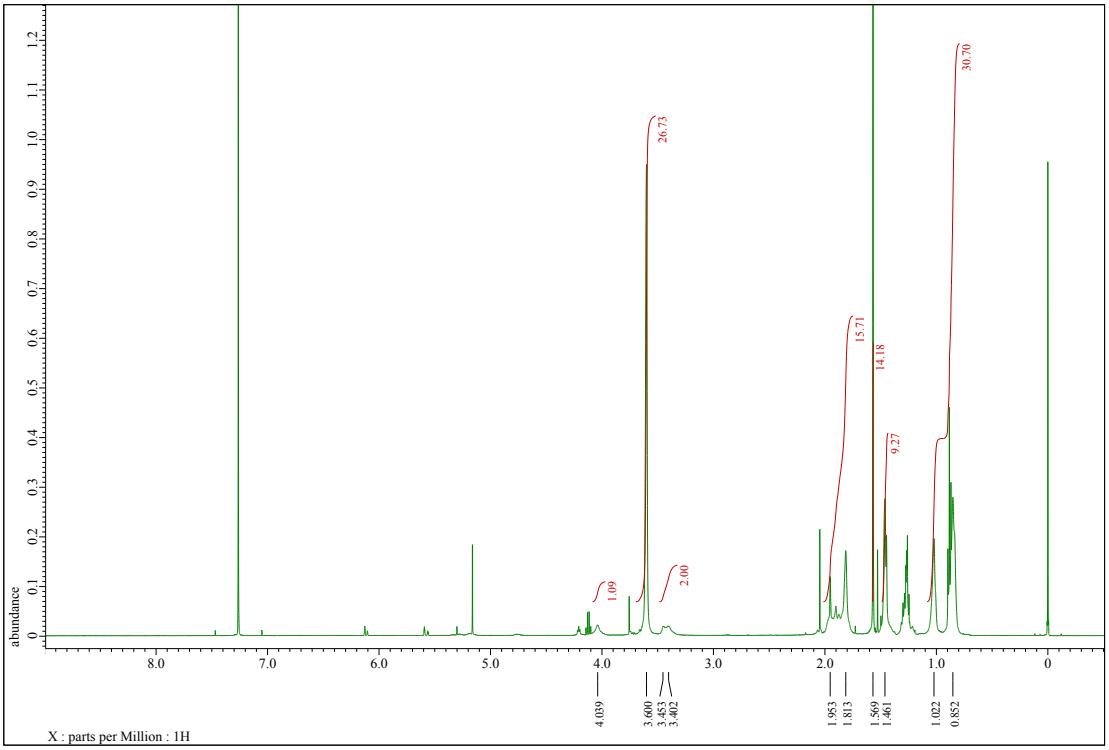

Fig. S1 NMR chart for PMBA

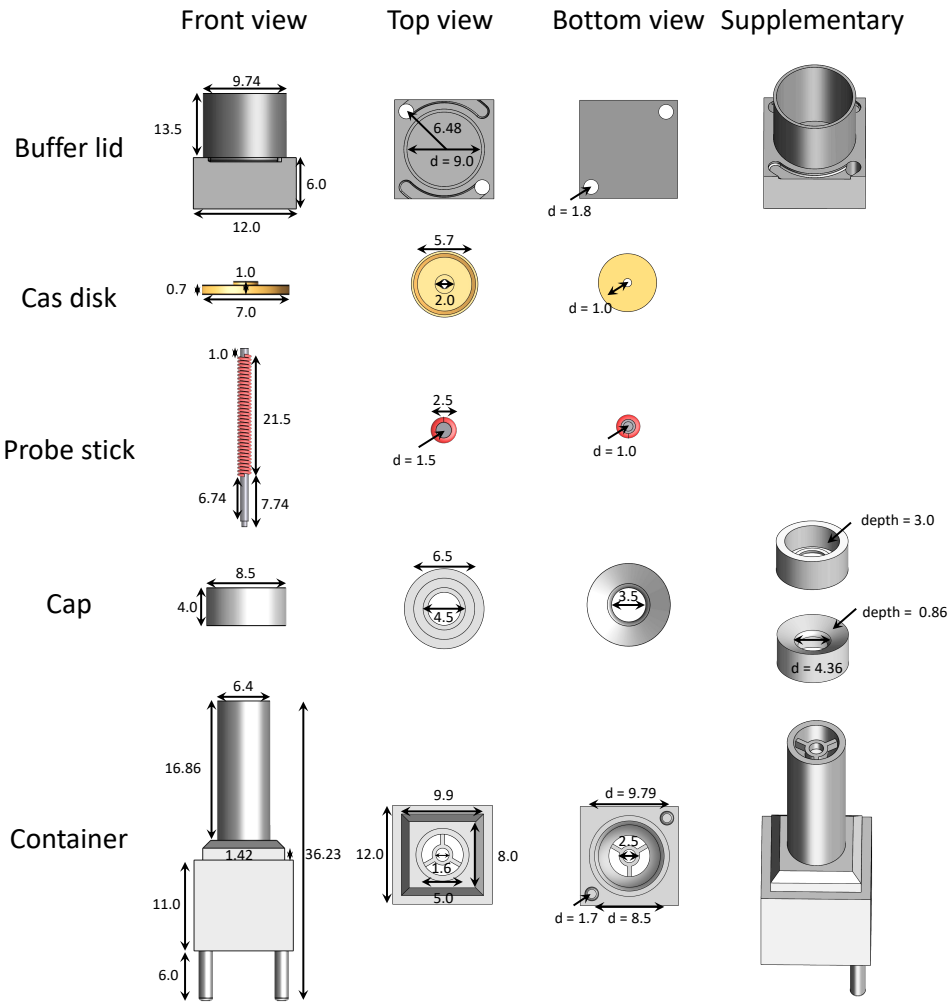

Fig. S2 Sketches and detailed scale for each component of the 3D-printed device (units: mm).

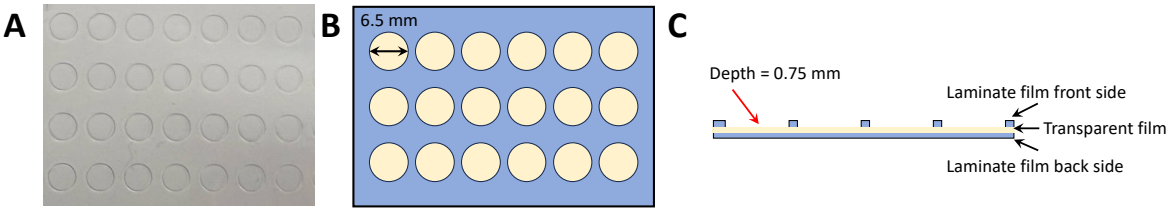

Fig. S3 (A) PVA membrane molding platform, (B) top view scale, and (C) cross-sectional view.

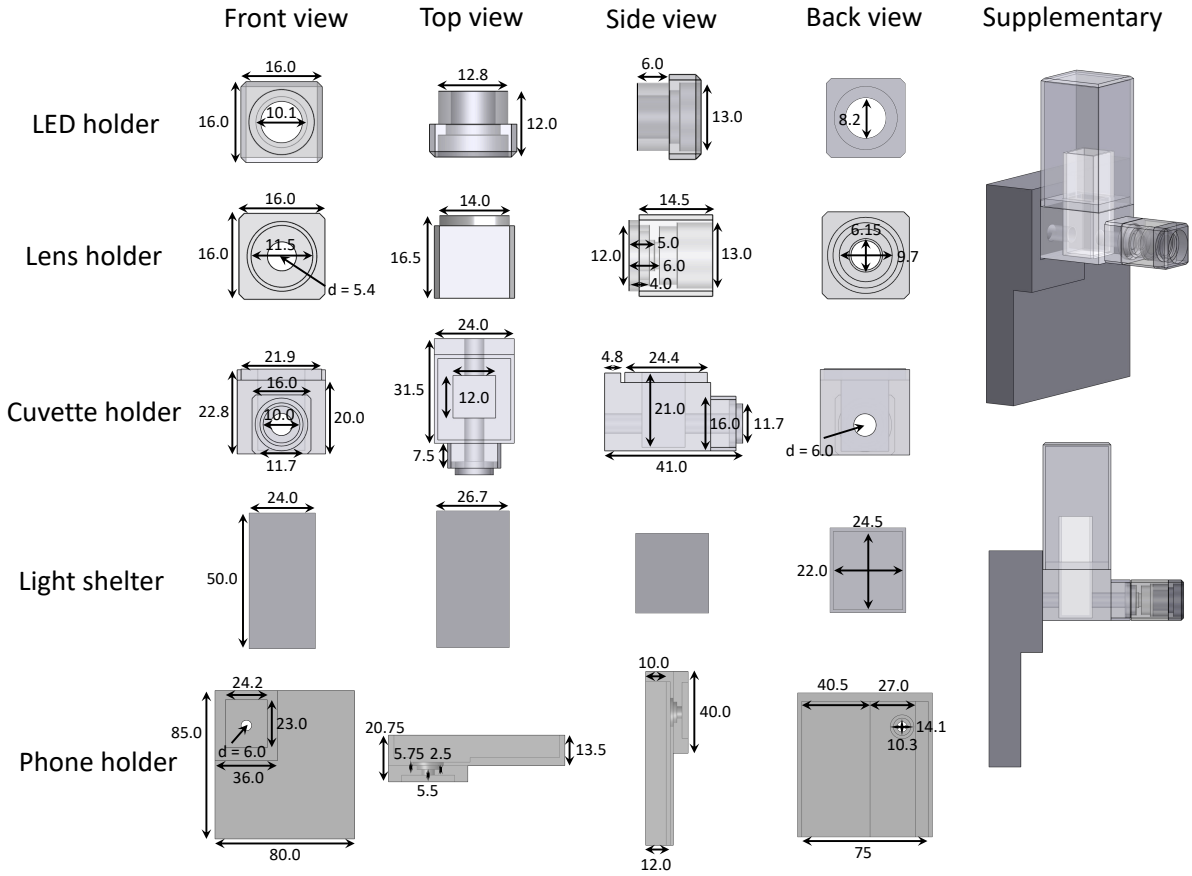

Fig. S4 Sketches and detailed scale for each component of the smartphone accessory (units: mm).

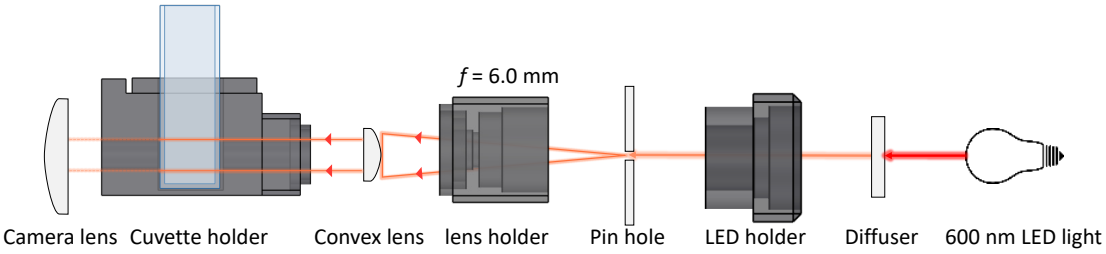

Fig. S5 Optical path scheme for smartphone-based signal detection.

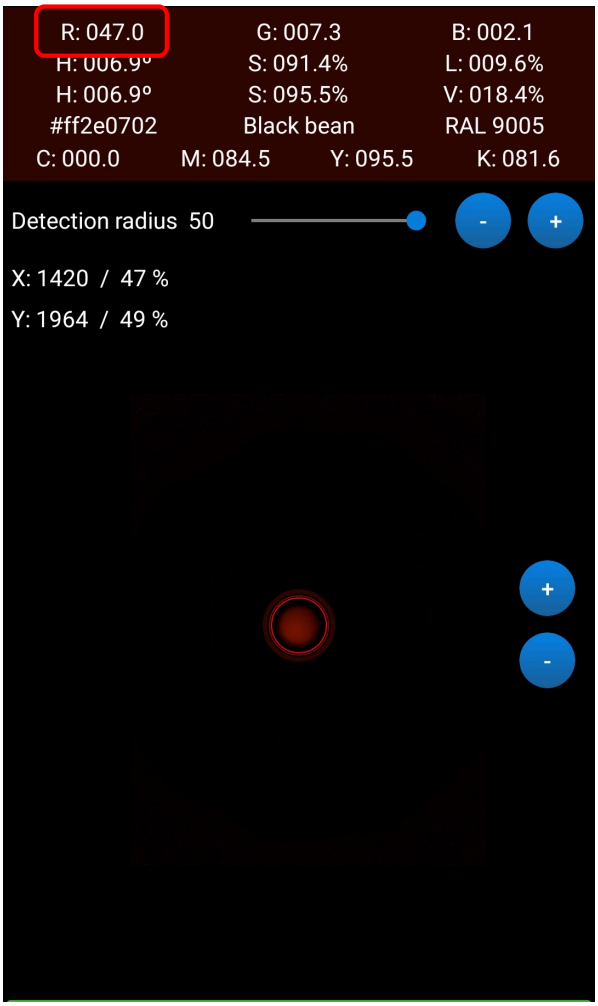

Fig. S6 Screenshot of the app used during smartphone-based signal detection; R represents the red intensity.

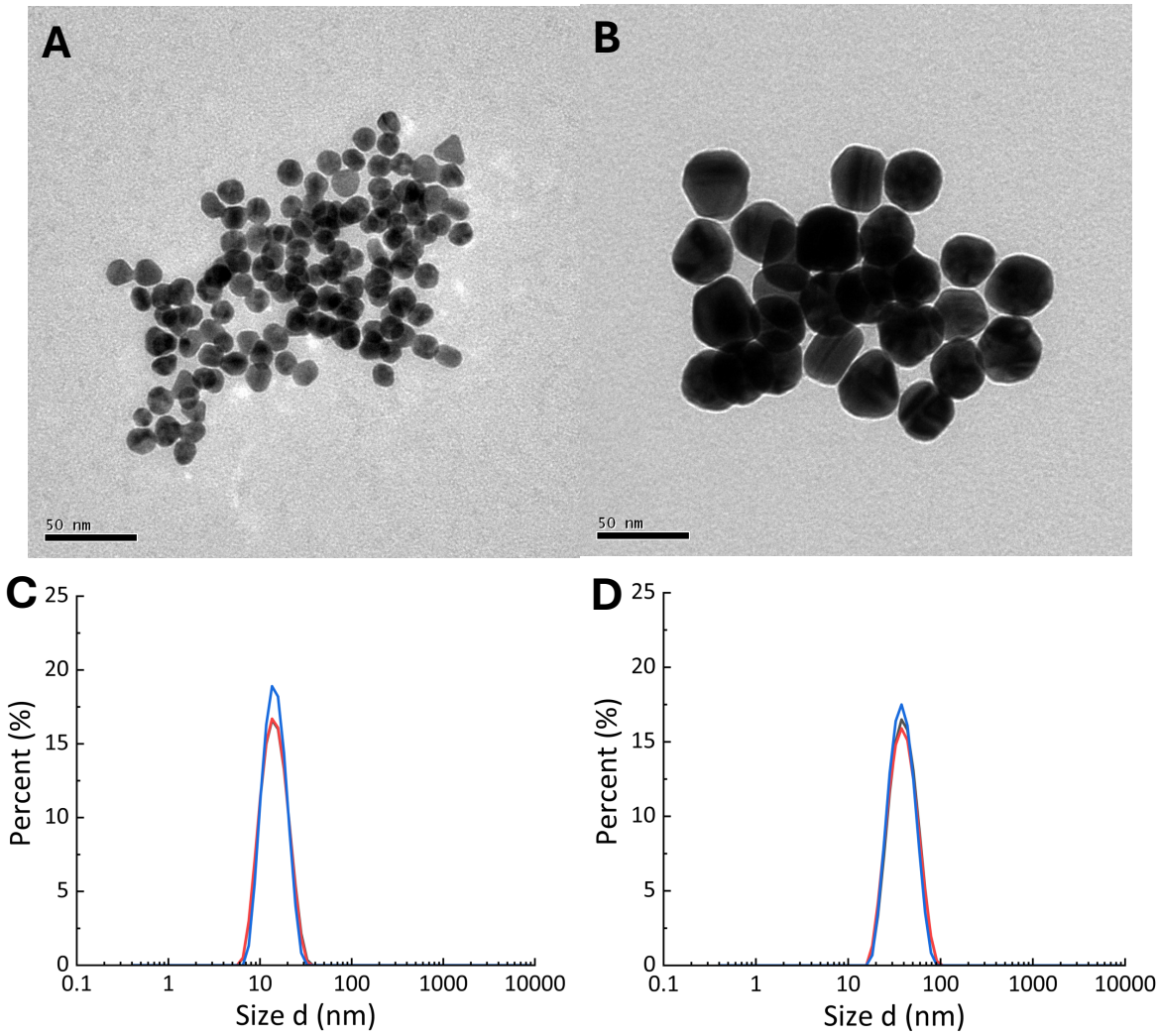

Fig. S7 TEM images of synthesized AuNPs: (A) 13 nm and (B) 30 nm diameter particles. Hydrodynamic particle size distribution by intensity of synthesized AuNPs: (C) 13 nm (Z-average: 13.44 nm, PDI: 0.118), and (D) 30 nm (Z-average: 34.31 nm, PDI: 0.144) diameter particles; the different color traces represent the results of 3 repeated measurements.

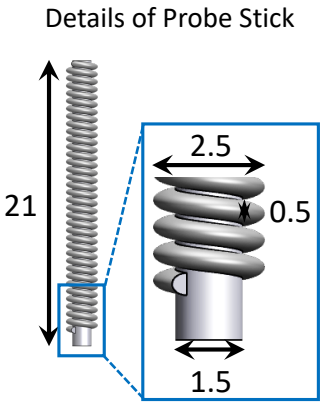

Fig. S8 Sketch and detailed scale for the probe stick, the blue frame shows a zoomed-in section (units: mm).

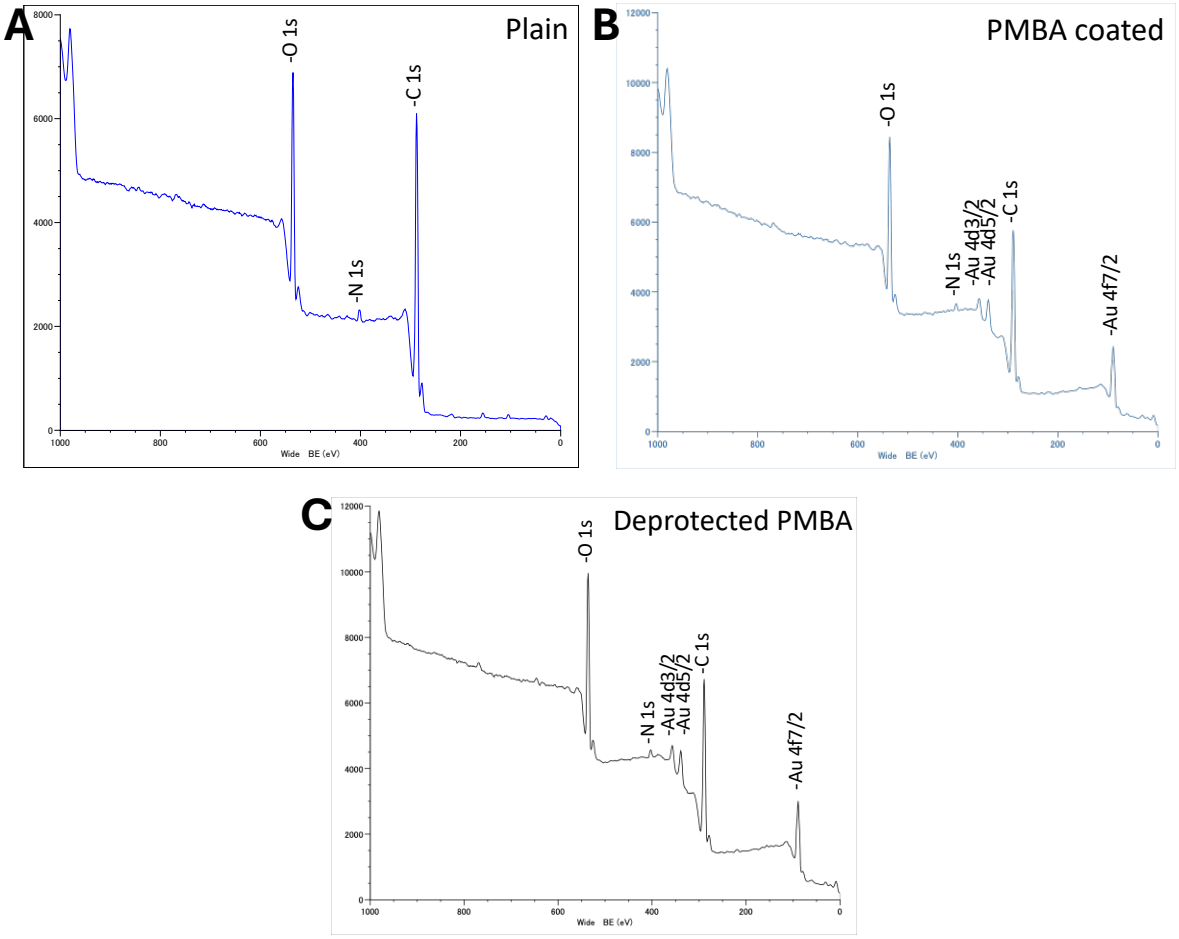

Fig. S9 XPS charts for probe sticks after AuNP (13 nm) immobilization onto: (A) plain probe stick, (B) PMBA coated probe stick, and (C) deprotected PMBA coated probe stick.

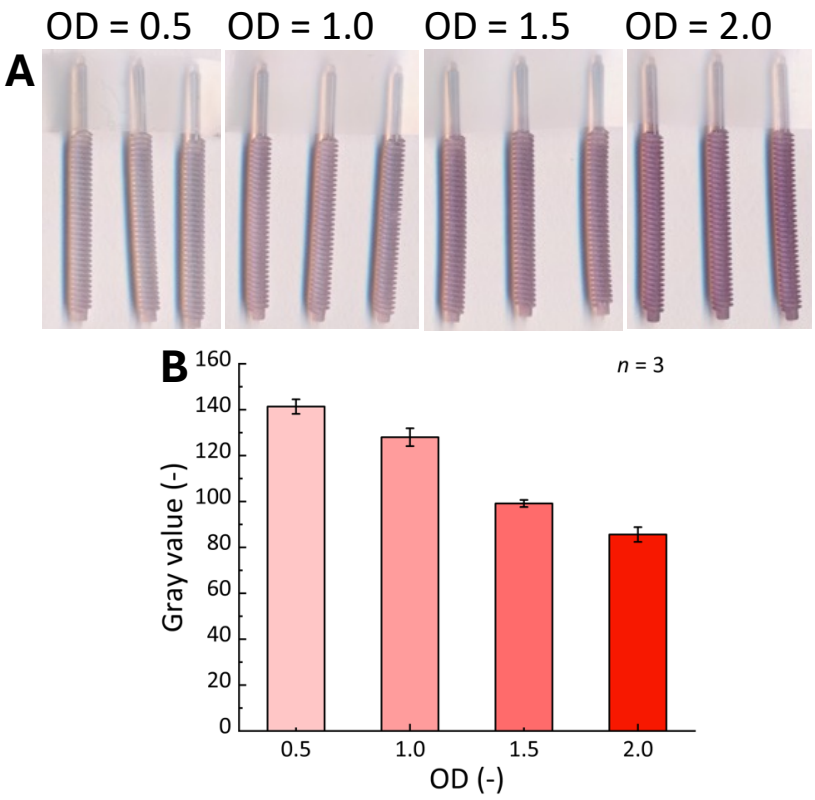

Fig. S10 Scanned images (A) and gray values (B) for probe sticks coated with different concentrations of 13 nm AuNPs solution.

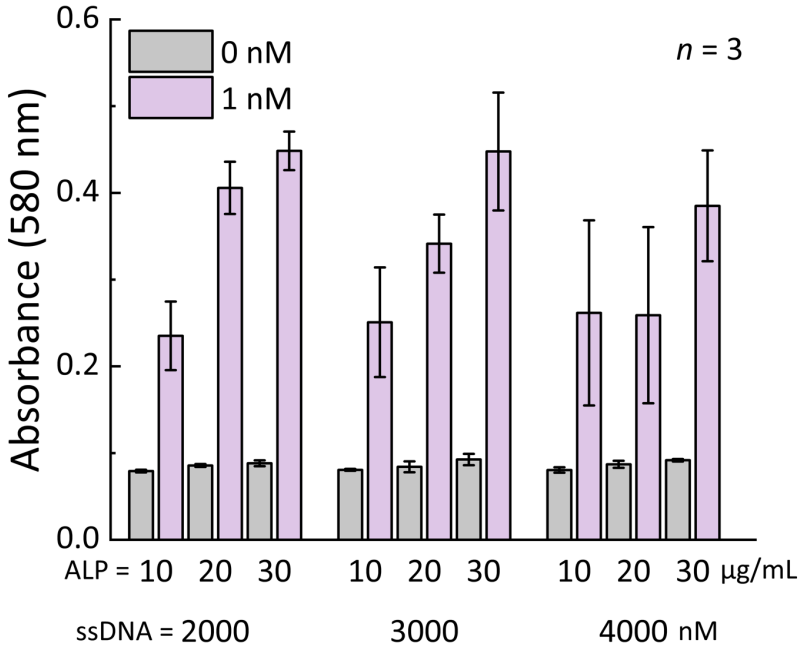

Fig. S11 Absorbance after dual enzymatic reaction (trans cleavage reaction: 30min, ALP reaction: 30min) of probe sticks with samples in the absence (0 nM) or presence (1 nM) of target DNA, when using different concentrations of ssDNA and ALP for probe stick modification.

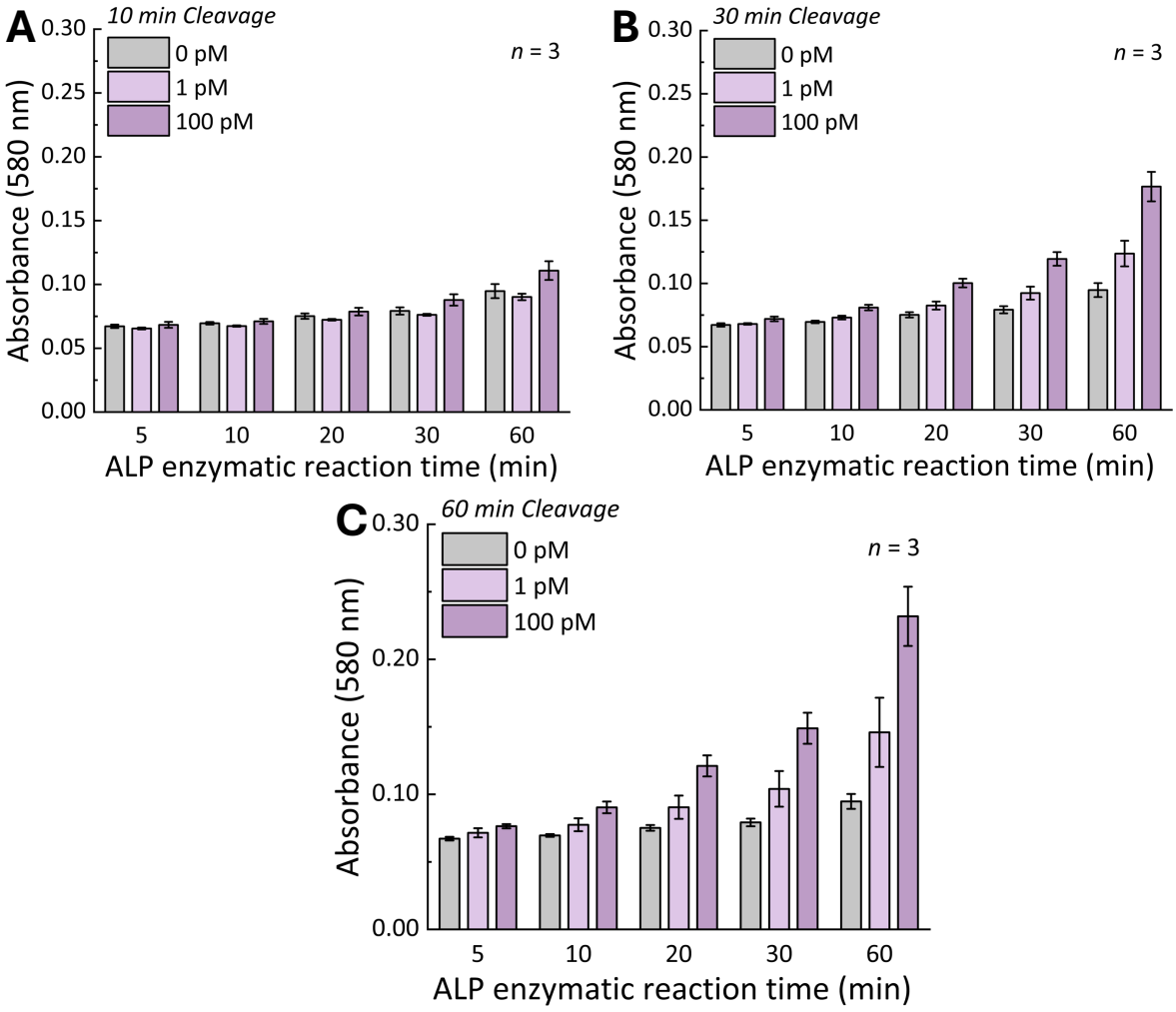

Fig. S12 Absorbance after dual enzymatic reaction for different reaction time combinations: (A) 10 min trans cleavage time, (B) 30min trans cleavage time, and (C) 60 min trans cleavage time.

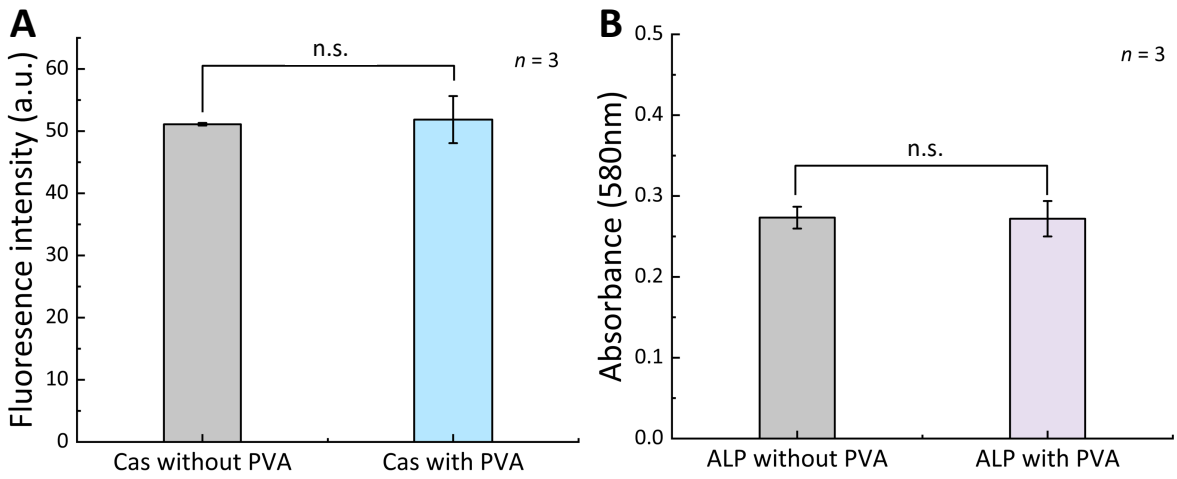

Fig. S13 Influence of PVA residues on (A) CRISPR/Cas reaction, (B) ALP reaction.

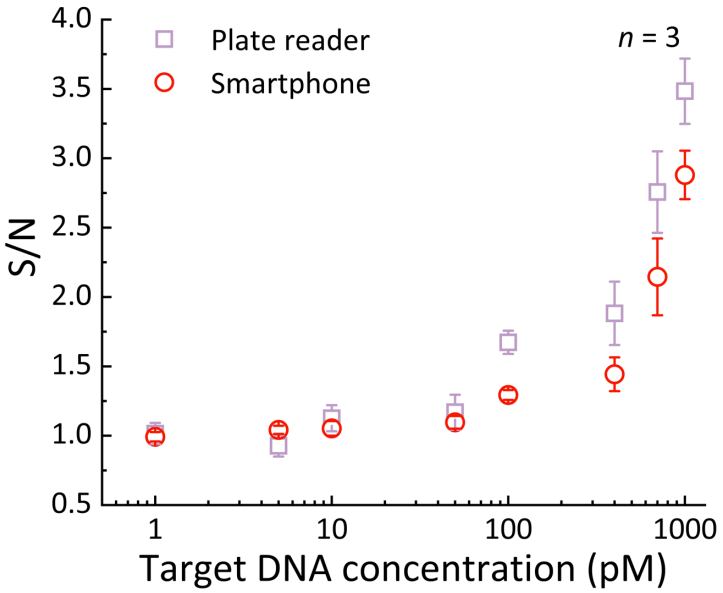

Fig. S14 Comparison of the S/N ratio between results recorded with a microplate reader and with the smartphone setup.

Reference:

1 M. Matsumoto, K. Kaneko, M. Hara, M. Matsui, K. Morita and T. Maruyama, *RSC Adv.*, 2021, **11**, 23409–23417.