SUPPORTING INFORMATION

CRISPR-Cas12a based nucleic acid amplification-free DNA biosensor via Au nanoparticle-assisted metal-enhanced fluorescence and colorimetric analysis

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MATERIALS AND METHODS

Preparation of DNA-functionalized 20- and 60-AuNP

To prepare each of the DNA-functionalized Au nanoparticles, 1 ml of an aqueous suspension of colloidal Au nanoparticles (20 and 60 nm, 1 OD) was centrifuged for 10 min at 13,000 rpm, after which the supernatant was removed. The particles were then resuspended in 0.15 M PBS solution at pH 7.4, and thiolated ssDNA for 20-AuNP (FITC-5‘-CTGATAAGCTTTTTTTTTT-SH) and 60-AuNP (5‘-AAAAAAAAAA-AGCTTATCAGAAAAAAAAA-3‘-SH) were added to the Au solution to allow Au-thiol interactions. The solution was incubated on a rocking shaker for 3 h to allow the thiolated DNAs to bind to the AuNP surface. After washing (5 min at 13,000 rpm, resuspension in PBS, 0.05% Tween 20, 0.1% BSA), the nanoparticles were mixed and incubated on a shaker at a 5:1 ratio, after which the complementary binding of functionalized DNAs was performed for one h at room temperature (RT). For the passivation of the Au surface, 3% BSA in PBS was left in the AuNP solution overnight at 4 °C.

TEM analysis for the confirmation of functionalization and binding formation of 20- and 60-AuNP

The 20- and 60-AuNP complexes were functionalized with ssDNA. To confirm the functionalization of ssDNA on the surface of each AuNPs, field-emission TEM (FE-TEM) images were obtained with a JEM-2100 electron microscope with negative staining for 5 min using platinum blue ($[Pt_4(NH_3)_8(C_6H_{13}O_5)_4]$) from Nisshin EM Co., Ltd. (Tokyo, Japan). AuNP nanosensors (20- and 60-AuNP complexes) before and after CRISPR-Cas12a activation were also characterized by FE-TEM analysis.

Colorimetric analysis of target-mediated CRISPR-Cas12a reaction

To confirm the colorimetric detection of BRCA-1 (5'-GAACAAAAGGAAGAAAATCA-3') by the AuNP nanosensors, the absorbance spectra were measured with a UV/vis spectrophotometer (V530, Jasco) from 200 to 800 nm. The shift in the absorbance peaks of the 20-AuNP, 60-AuNP, 20- and 60-AuNP complementary binding complexes upon activation of the CRISPR-Cas12a complex with 1 μM and 1 nM BRCA-1 were measured and summarized in a bar chart.

BRCA-1 detection using MEF-based fluorescence measurements

BRCA-1 at concentrations ranging from 1 fM to 100 pM were added to the AuNP nanosensor solution with CRISPR-Cas12a (crRNA: UAAUUUCUACUCUUGUAGAUGAUUUUCUUCCUUUUGUUCA, Recombinant Acidaminococcus sp. BV3L6 Cas12a from IDT) to allow the trans-cleavage reaction of ssDNA between 20- and 60-AuNP for 30 min at room temperature. After the enzymatic reaction was completed, the AuNP nanosensor solutions were analyzed at 520 nm using a fluorescence spectrophotometer (Hitachi Model F-7000 high-performance fluorescence spectrophotometer) at a 490 nm excitation. To test its specificity, SM (1 nM), DM (1 nM), miR-21 (1 nM), and miR-141 (1 nM) were applied to the AuNP nanosensor solution with the same reaction times and temperatures.

SUPPLEMENTARY FIGURES

Supplementary Figure 1 Transmission electron microscope (TEM) images of AuNPs before and after DNA functionalization. a) 20-AuNP (left) and 60-AuNP (right) before DNA functionalization. Scale bars are 100 nm. b) 20-AuNP (left) and 60-AuNP (right) after DNA functionalization. Scale bars are 20 nm.

Supplementary Figure 2 Peak wavelengths of Au nanosensor with activated CRISPR-Cas12a with different target cfDNA, ranging from 1 nM to 1 mM.

Supplementary Figure 3 Transmission electron microscope (TEM) images of DNA-functionalized AuNPs with the CRISPR-Cas12a complex. a) DNA-functionalized AuNPs, which consisted of a 60-AuNP core surrounded by 20-AuNPs before the CRISPR-Cas12a activation reaction. b) DNA-functionalized 20- and 60-AuNPs after the CRISPR-Cas12a activation reaction. Scale bars are 10 nm.

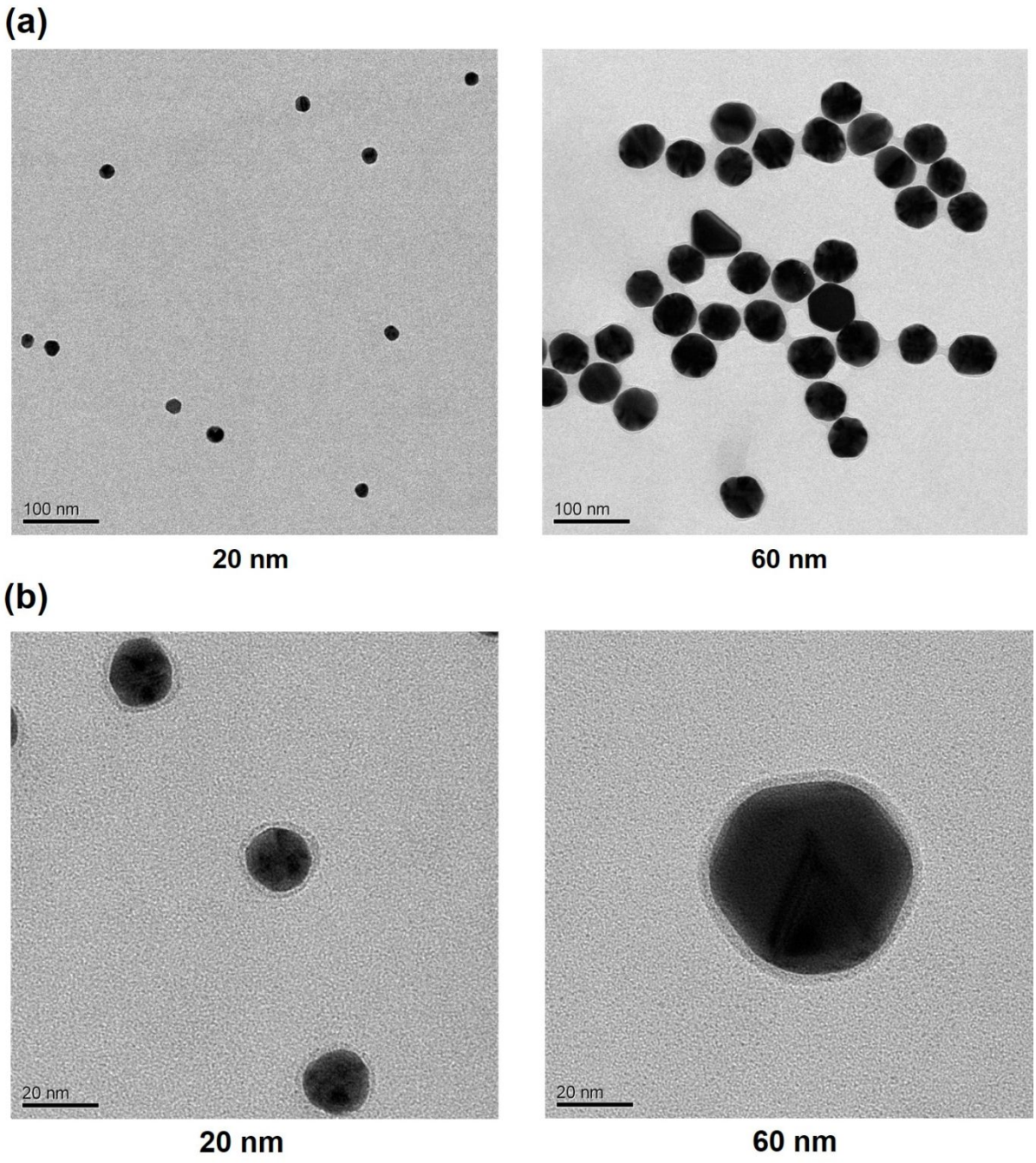
Supplementary Figure 4 Fluorescence intensities at 520 nm of the Au nanosensor without MEF effect. Target cfDNAs were applied as 1 fM, 1pM, and 1 nM with CRISPR-Cas12a complex.

Supplementary Figure 5 Electrophoresis images of a) DNA ladder, b) ssDNA, c) dsDNA, d) inactivated CRISPR-Cas12a complex, e) activated CRISPR-Cas12a complex, f) activated CRISPR-Cas12a complex with ssDNA, and g) activated CRISPR-Cas12a complex with dsDNA**.**

Supplementary Figure 6 Optical property of the 20-60 AuNPs pairs after the centrifugation. (a) Optical images of the 20-60 AuNPs pairs after centrifugation at 8,000 rpm for 10 min. Left microtube represents the before CRISPR-Cas12a reaction, whereas right one represents the after CRISPR-Cas12a reaction. (b) UV/vis spectrum of the supernatants from before- and after the CRISPR-Cas12a reaction.

Supplementary Figure 7 Fluorescence spectrum of Au nanosensor with activated CRISPR-Cas12a for the quantitative measurement of cfDNA ranging from 1 fM to 100 pM concentrations, corresponding to Figure 3b.

Supplementary Figure 8 (a) Fluorescence intensities of Au nanosensor with activated CRISPR-Cas12a at high concentrations (1 nM, 10 nM, and 100 nM) of cfDNAs. (b) Saturated curve of the fluorescence intensity versus BRCA-1 concentration.



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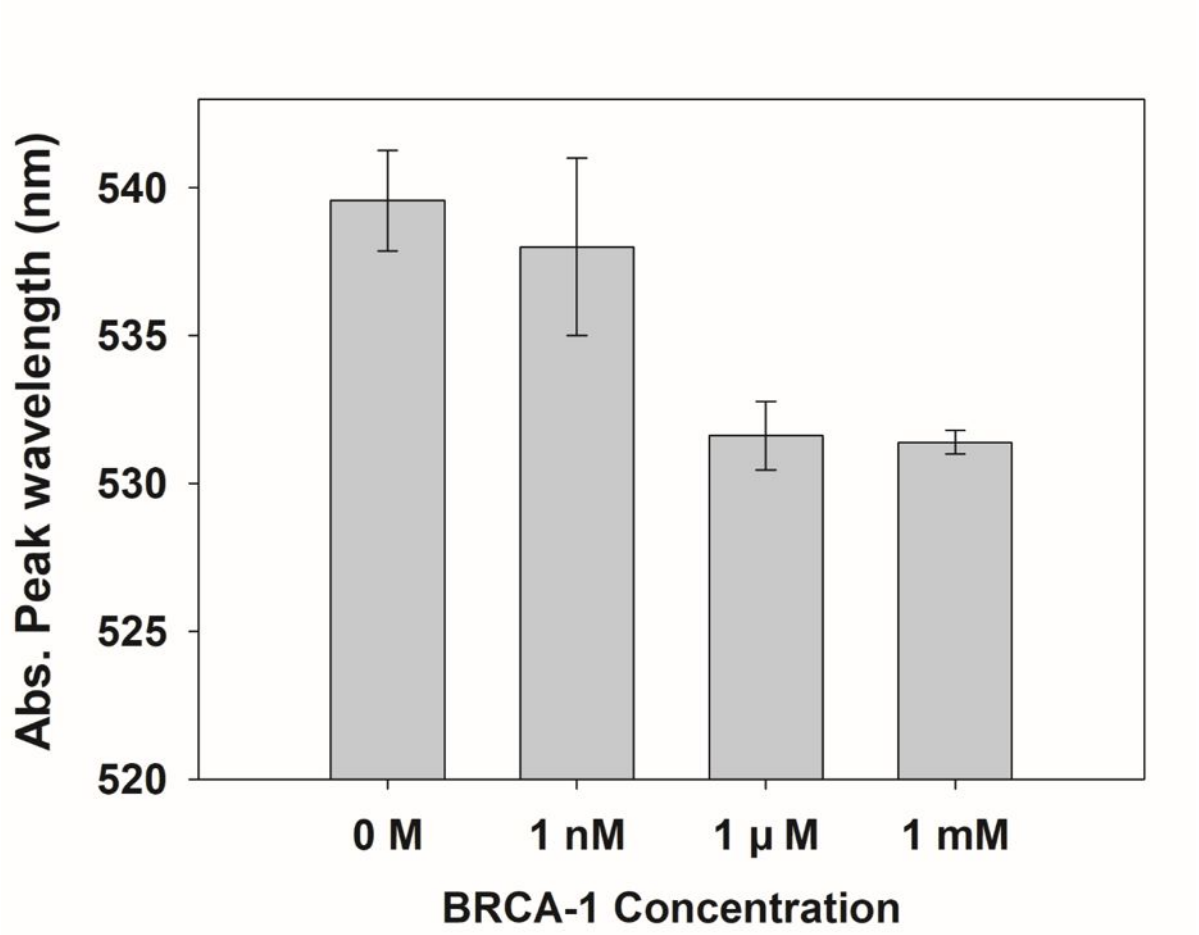
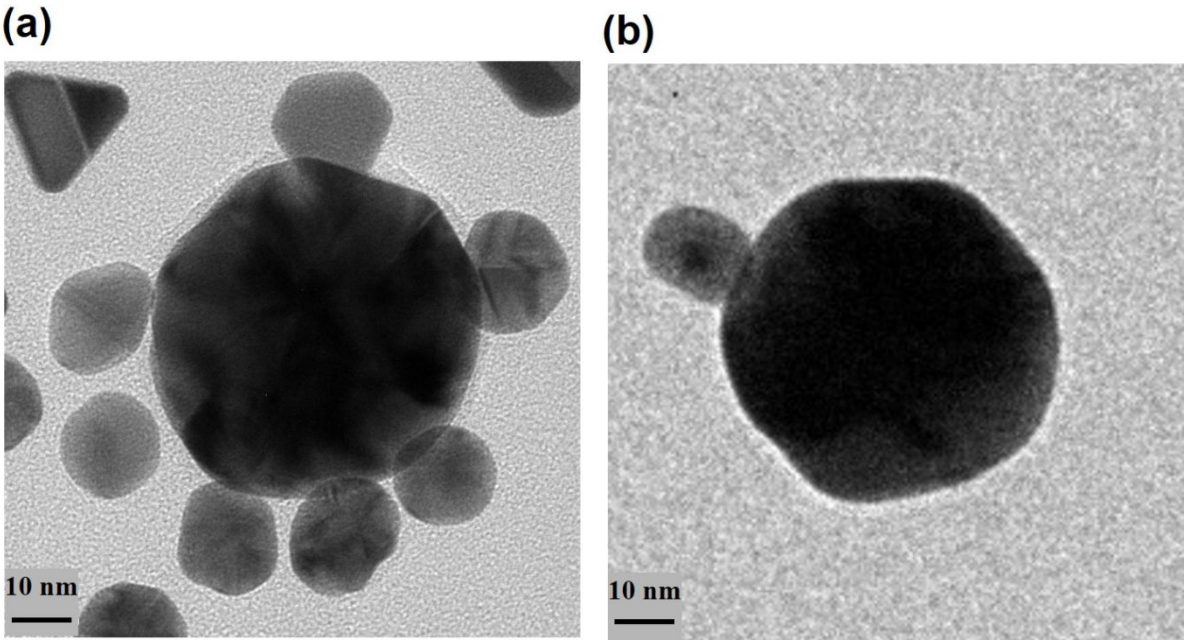


Supplementary Figure 2. Peak wavelengths of Au nanosensor with activated CRISPR-Cas12a with different target cfDNA, ranging from 1 nM to 1 mM.



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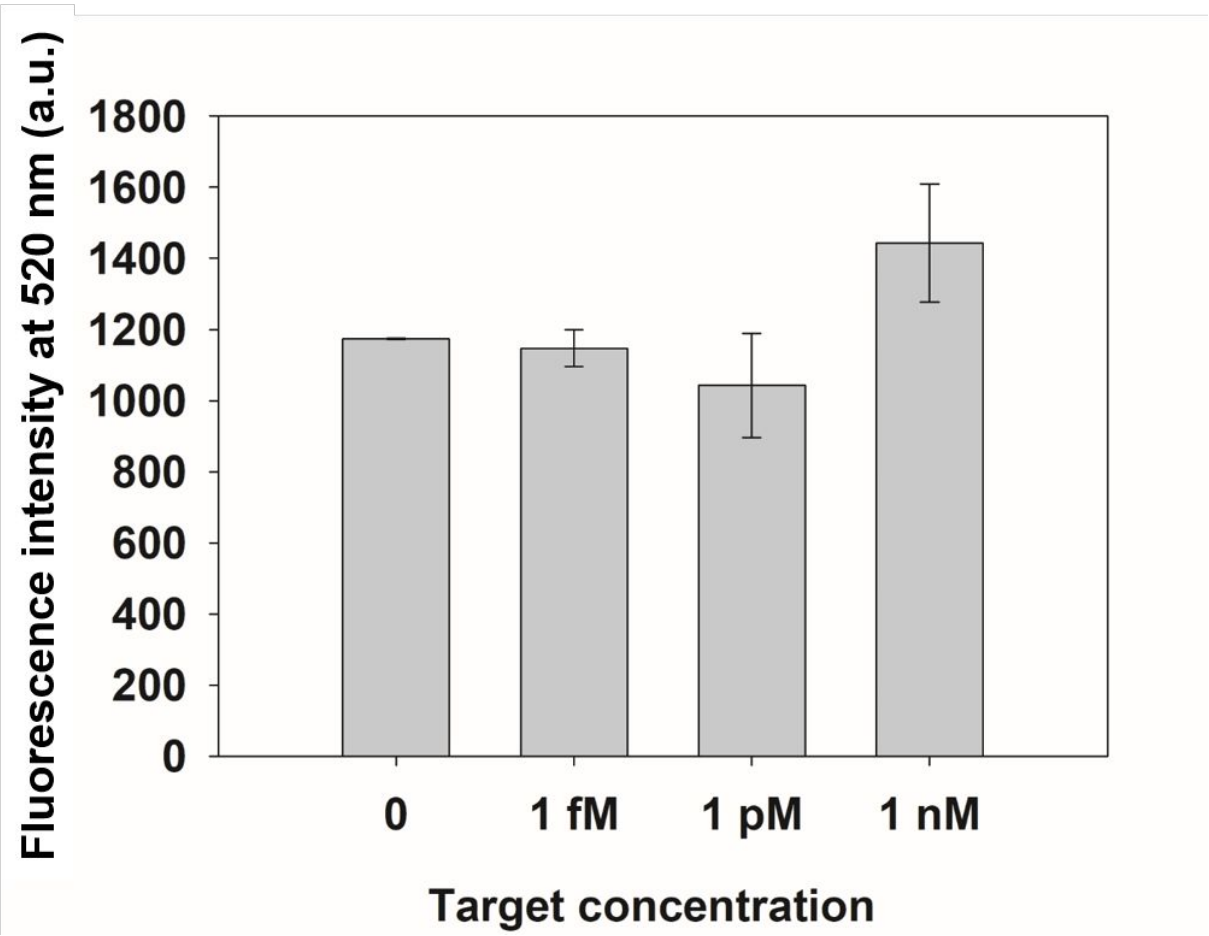

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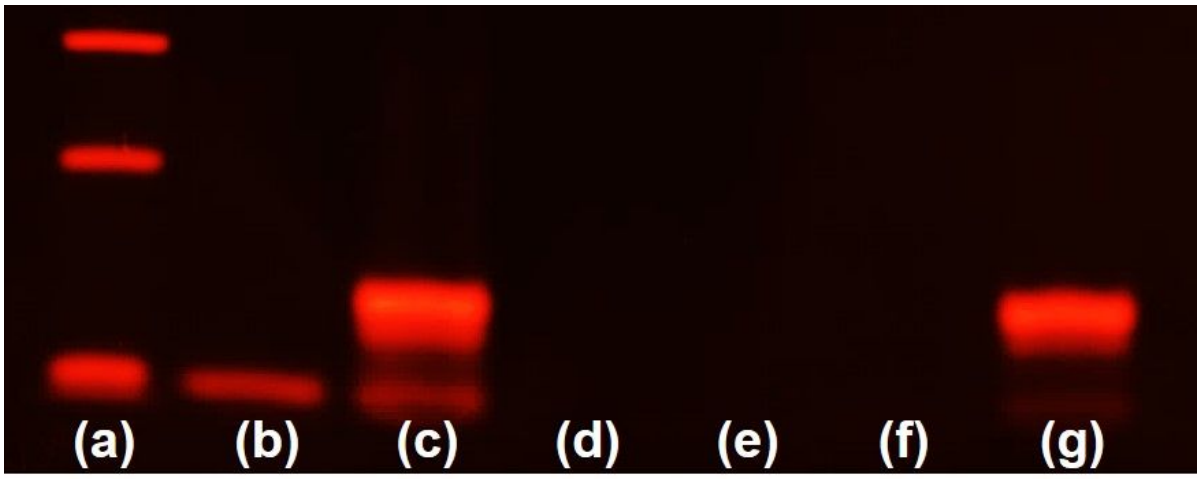

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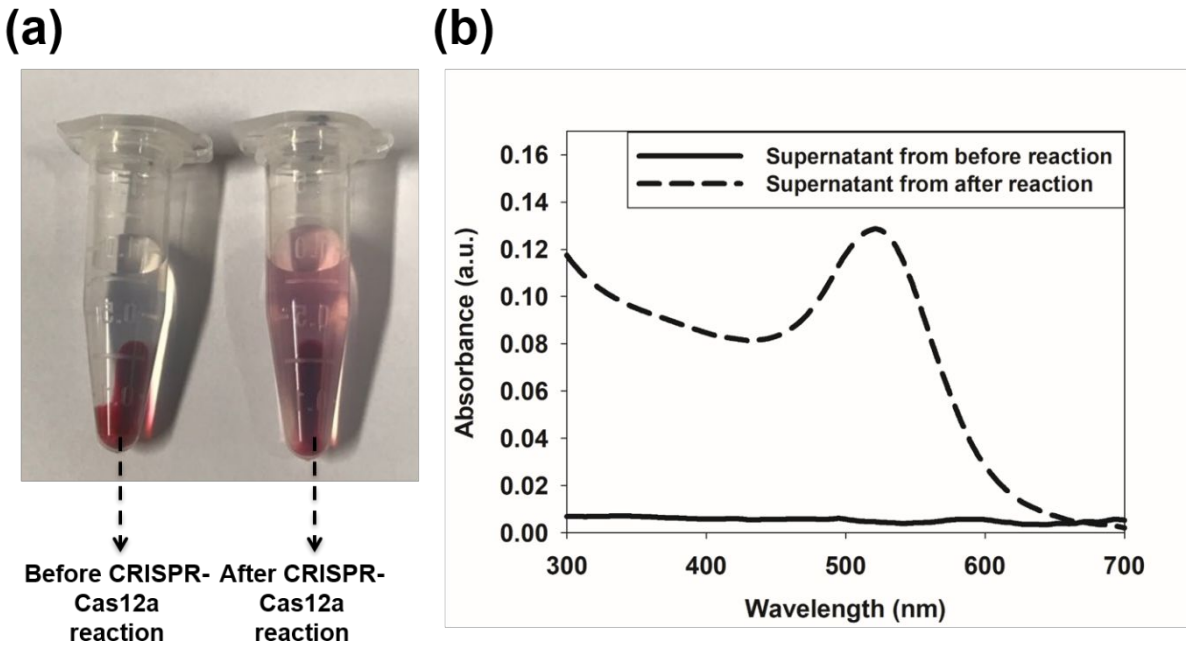

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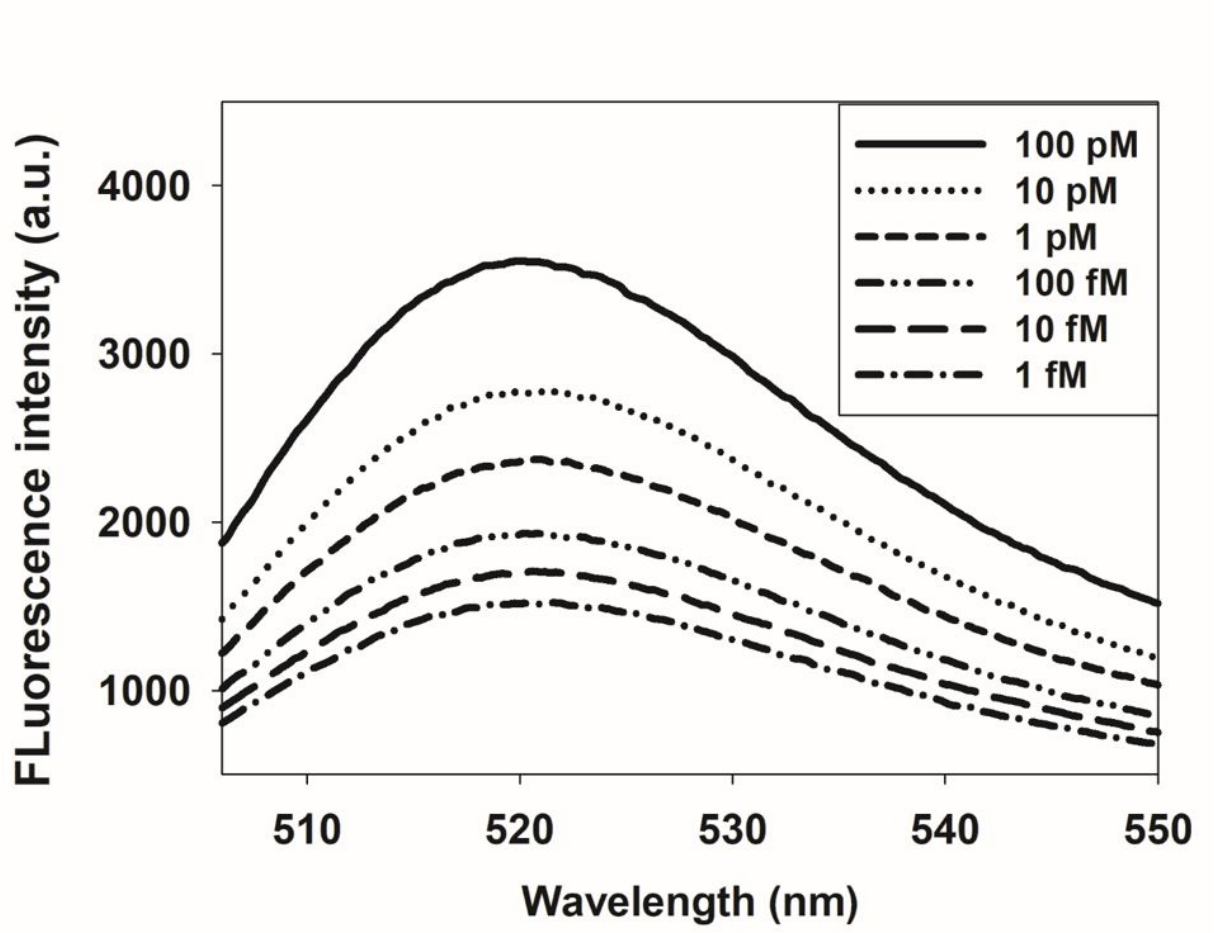
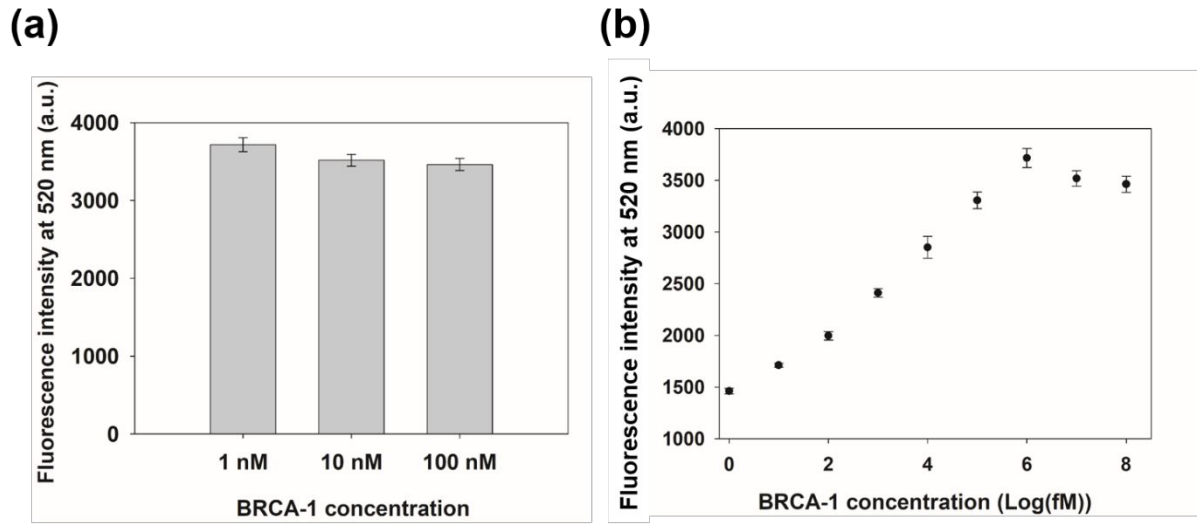


Supplementary Figure 7. Fluorescence spectrum of Au nanosensor with activated CRISPR-Cas12a for the quantitative measurement of cfDNA ranging from 1 fM to 100 pM concentrations, corresponding to Figure 3b.



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