ESI for

Sensitive detection of genetically modified maize based on CRISPR/Cas12a system

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1. Reagents

Synthetic double-stranded DNA (dsDNA) oligonucleotides, including pCaMV35S and M810 Cry1Ab dsDNA, and ssDNA were purchased from Comate Bioscience Co., Ltd (Changchun, China), crRNA was purchased from GenScript (Nanjing, China) (Table 1). LbCas12a was also purchased from GenScript (Nanjing, China). Silver nitrate ($AgNO_3$) was purchased from Shanghai Chemical Reagent Co., LTD (Shanghai, China). Gold (III) chloride trihydrate ($HAuCl_4$) was purchased from Aladdin Industrial Corporation (Shanghai, China). Magnetic beads (MBs) were purchased from Wuxi Qirui Aisi Biotechnology Co., Ltd. Citric acid monohydrate ($C_6H_8O_7•H_2O$) and sodium hydroxide (NaOH) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Sodium chloride (NaCl), sodium dihydrogen phosphate ($NaH_2PO_4•H_2O$), and sodium citrate ($C_6H_5Na_3O_7$) were purchased from Beijing Chemical Works (Beijing, China). Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethane sulfonic acid (HEPES), and 4-mercaptobenzoic acid (4-MBA) were obtained from Aladdin (Shanghai, China). RNase-free ddH_2O was purchased from Nanjing Vazyme Biotech Co., Ltd (Nanjing, China). The plant gene extraction kit was purchased from Tiangen Biochemical Technology Co., Ltd (Beijing, China).

2. Synthesis of gold nanoparticles

First, 2.0 mL, 50 mM of $HAuCl_4$ and 98 mL of water were added to a three-necked flask and heated and stirred. When the solution started to boiling, we quickly added 10 mL of 38.8 mM sodium citrate solution, and let the mixture refluxed for 20 min. The colour of the solution gradually changed from light yellow to burgundy. We stopped heating and cool the solution to room temperature. The obtained gold colloid was stored at 4 ℃ away from light for subsequent use.

3. Synthesis of silver nanoparticles

Silver nanoparticles (AgNPs) were prepared according to the method developed by Lee et al.[1] First, 0.018 g of $AgNO_3$ and 100 mL of ultrapure water were added to a boiling flask and heated to slightly boiling. Then, 2.0 mL of 1 % sodium

citrate was quickly added. After the solution gradually turned into greyish-green and no more changes, the solution was kept at 90 °C for 40 min. After the ripening of AgNPs was completed, it was cooled to room temperature and stored at 4 °C for further use.

4. Apparatus

The sizes and morphologies of AuNPs and AgNPs were characterized by a transmission electron microscope (TEM, JEOL, Tokyo, Japan, JEM-2100F). Ultraviolet-visible (UV–vis, Ocean Optics, USB4000) and dynamic light scattering (DLS, Malvern Zetasizer Nano ZS) spectroscopy were used to measure the plasmonic properties of these nanoparticles. SERS measurements were carried out by a confocal Raman Spectrometer (LabRAM Aramis, HORIBA Jobin Yvon) with a laser wavelength of 633 nm. The outstretched scan spectra with a spectral range from 1000 to 1700 cm^{-1} were collected with an integration time of 10 s and one accumulation. The 520.7 cm^{-1} vibrational band of a silicon slide was applied for the wavenumber calibration.

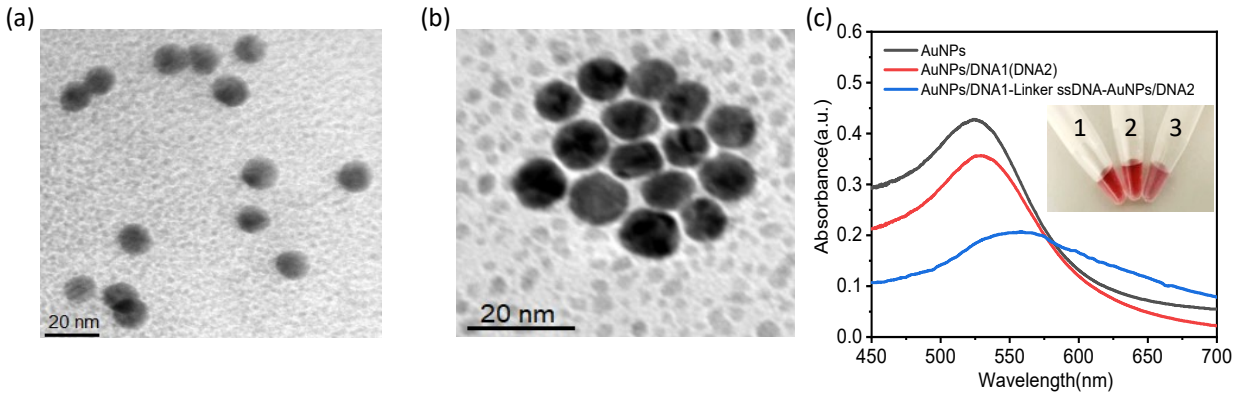

Fig. S1 (a) TEM image of the AuNPs. **(b)** TEM image of the AuNPs/DNA1-Linker ssDNA- AuNPs/DNA2. **(c)** UV-vis spectra of AuNPs, AuNPs/DNA1(DNA2), and AuNPs/DNA1-Linker ssDNA- AuNPs/DNA2.

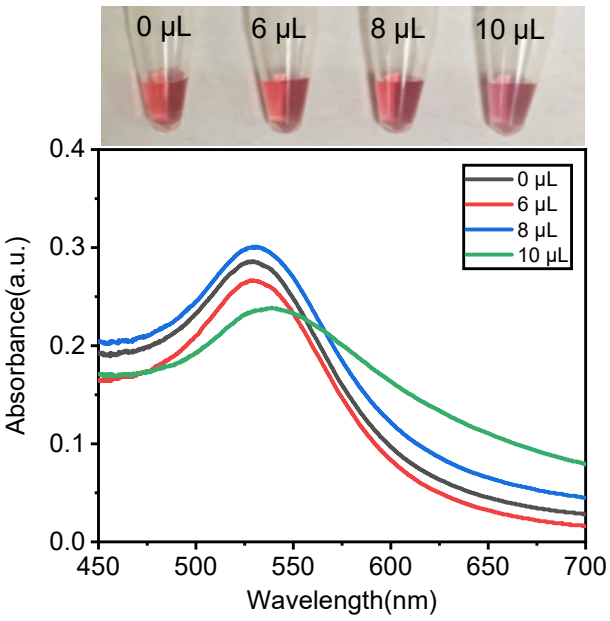

Fig. S2 Optimization of Linker ssDNA volume in this CRISPR/Cas12a system.

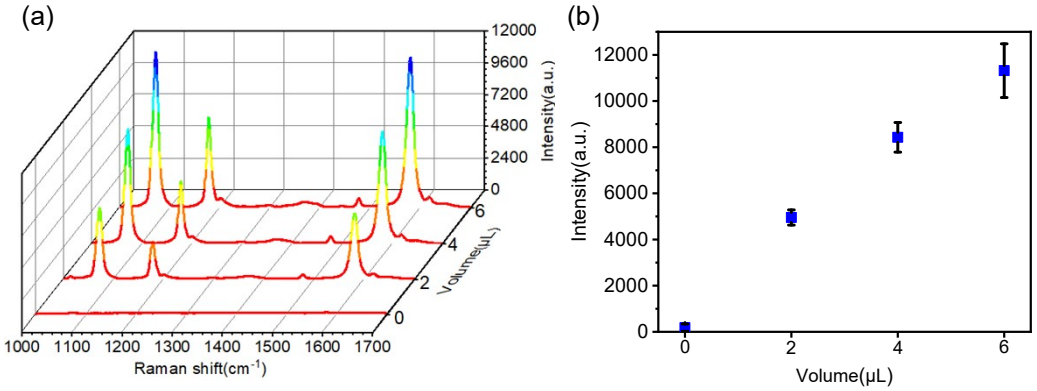

Fig. S3 (a) Optimized SERS spectra of Linker ssDNA (1 μM) volumetric amount. **(b)** SERS intensity of Raman feature peak 4-MBA at 1585 cm-1 versus concentration of added Linker ssDNA (1 μM) volumetric amount.

When no Linker ssDNA appeared, AgNPs/4-MBA/DNA1 could fail to attach the surface of MBs/DNA3. Thus, no SERS signal would be observed on the magnetic beads. Once Linker ssDNA existed, AgNPs/4-MBA/DNA1 was gradually captured by MBs/DNA3. Also, with the increase of Linker ssDNA volume, the SERS signal was gradually enhanced as well. When the added volume was raised to 6 μL, the SERS sensing probe captured by the magnetic beads reached saturation. The SERS signal intensity reached the maximum, and the subsequent experimental system was chosen to add 6 μL of Linker ssDNA with a concentration of 1 μM.

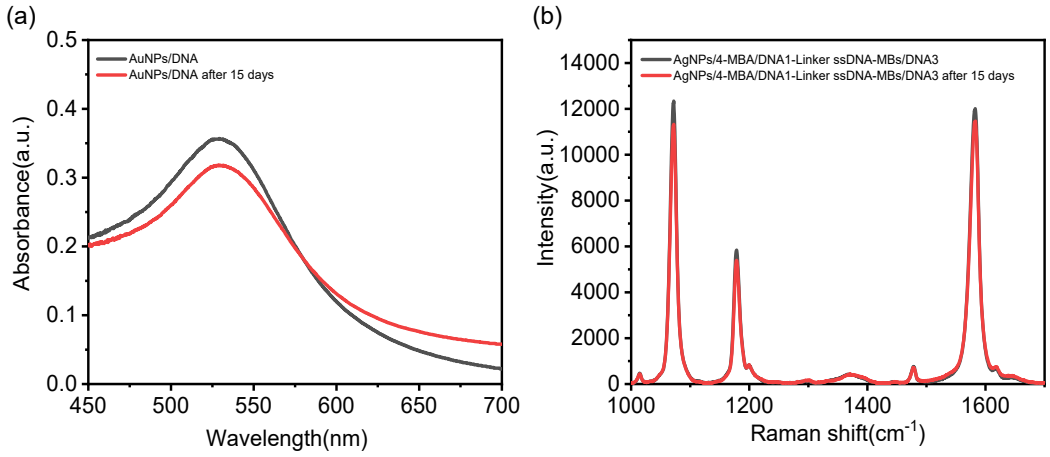

Fig. S4 (a) Uv-vis spectra of the freshly prepared probes and the ones after 15 days. **(b)** SERS spectra of the freshly prepared AgNPs/DNA1-Linker ssDNA-MB/DNA3 and the ones after 15 days.

The stability of analytical reagents is crucial in assay analysis. The probes we prepared can be stored for at least 15 days, and their plasmonic profiles and SERS signal intensities were recorded. As shown in **Fig. S4**, they showed almost no changes when they were stored in a refrigerator at 4°C.

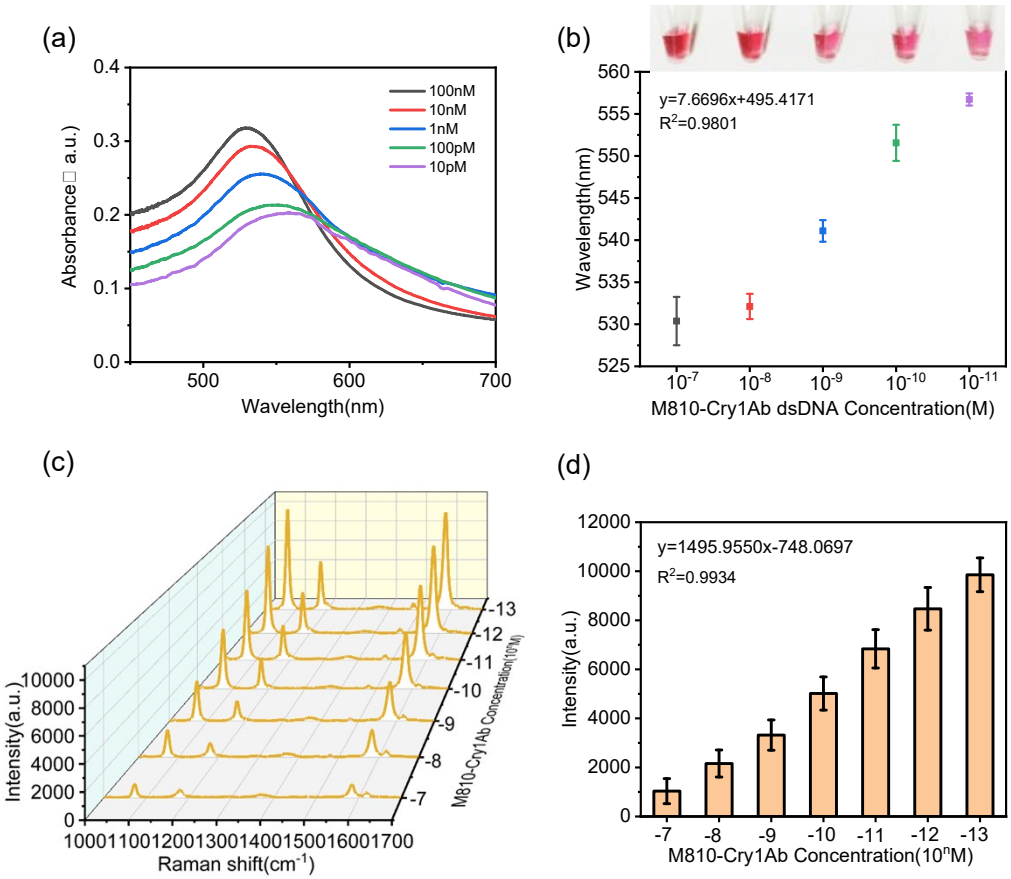

Fig. S5 (a) UV-Vis spectra of AuNPs/DNA with different concentrations of M810-Cry1Ab dsDNAs in the CRISPR/Cas12a system. **(b)** Plots of maximum absorption peak shift as a function of M810-Cry1Ab dsDNA concentrations and the colour changes. **(c)** SERS spectra of the CRISPR/Cas12a system for the detections of M810-Cry1Ab dsDNA.**(d)** SERS intensity of Raman signature peak 4-MBA at 1585 cm^{-1} in relation to the concentration of M810-Cry1Ab dsDNA.

References:

1. P. C. Lee and D. Meisel, *The Journal of Physical Chemistry*, 1982, **86**, 3391-3395.