Electronic Supplementary Information (ESI)

A sequence-specific plasmonic loop-mediated isothermal amplification (LAMP) assay with orthogonal color readouts enabled by CRISPR Cas12a

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S1. Experimental details

Materials. 100 × Tris-EDTA buffer solution (TE), 10 × phosphate buffer saline (PBS), TWEEN 20, magnesium chloride ($MgCl_2$), and sodium chloride (NaCl) were purchased from Sigma (Oakville, ON, Canada). EnGen Lba Cas12a (Cpf1), WarmStart LAMP Kit, OneTaq Master Mix, and 10 × NEBuffer™ 2.1 Buffer were purchased from New England Biolabs Ltd. (Whitby, ON, Canada). NANOpure H_2O (> 18.0 MΩ), purified using an Ultrapure Mili-Q water system, was used for all experiments. Two kinds of 20-nm gold nanoparticles (AuNPs) solutions were purchased from Sigma and BBI Solutions. All DNA samples and CRISPR RNA were purchased from Integrated DNA Technologies (Coralville, IA) and purified using high-performance liquid chromatography. The DNA sequences and modifications are outlined in Table S1.

Table S1.DNA sequence and modifications.

DNA name	Sequence (5'-to-3')
Color Readout System	
AuNP-A	HS-AAA AAA AAA ACC TCA CCA CCA ACA C
AuNP-B	HS-AAA AAA AAA ACA CAC ACA CTC ACA C
S	GTG TGA GTG TGT GTG GTG TTG GTG GTG AGG
Model Synthetic Target and crRNA	
NTS-35	GCT TGT GGC CG **TTTA** CGT CGC CGT CCA GCT CGA CC
TS-35	GGT CGA GCT GGA CGG CGA CG **TAAA** CGG CCA CAA GC
crRNA	UAA UUU CUA CUA AGU GUA GAU CGT CGC CGT CCA GCT CGA CC
HBV S-gene, LAMP primers, and crRNA	
TS_{HBV}	TCC TCA CAA TAC CGC AGA GTC TAG ACT CGT GGT GGA CTT CTC TCA ATT TTC TAG GGG GAA CTA CCG TGT GTC TTG GC **CAAA** ATT CGC AGT CCC CAA CCT CCA ATC ACT CAC CAA CCT CTT GTC CTC CAA TTT G TC CTG GTT ATC GCT GGA TGT GTC TGC GGC GTT TTA TCA TCT TCC TCT TCA TCC TGC TGC
NTS_{HBV}	GCA GCA GGA TGA AGA GGA AGA TGA TAA AAC GCC GCA GAC ACA TCC AGC GAT AAC CAG GAC AAATTG GAG GAC AAG AGG TTG GTG AGT GAT TGG AGG TTG GGG ACT GCG AAT **TTTG** GC CAA GAC ACA CGG TAG TTC CCC CTA GAA AAT TGA GAG AAG TCC ACC ACG AGT CTA GAC TCT GCG GTA TTG TGA GGA
$crRNA_{HBV}$	UAA UUU CUA CUA AGU GUA GAU GC CAA GAC ACA CGG TAG TTC
$F3_{HBV}$	TCC TCA CAA TAC CGC AGA GT
$B3_{HBV}$	GCA GCA GGA TGA AGA GGA AT
$F1P_{HBV}$	GTT GGG GAC TGC GAA TTT TGG CTT TTT AGA CTC GTG GTG GAC TTC T
$B1P_{HBV}$	TCA CTC ACC AAC CTC TTG TCC TTT TTA AAA CGC CGC AGA CAC AT
LF_{HBV}	GGT AGT TCC CCC TAG AAA ATT GAG
LB_{HBV}	AAT TTG TCC TGG TTA TCG CTG G

HBV plasmid extraction. The HBV 1.3-mer WT replicon encoding 1.3 units of the Hepatitis B virus genome (subtype ayw) was a gift from Wang-Shick Ryu (Addgene plasmid # 65459).[1] The replicon was transformed in E.coli DH5α and received as a bacterial stab in Luria- Bertani (LB) agar tube. Some cells were picked from the stab culture and streaked on LB agar plate with 100 µg/mL of Ampicillin and incubated at 37°C for 18 hours. A single colony of E. coli was cultured in LB medium with 100 µg/mL of Ampicillin and incubated at 37°C for 18 hours. The HBV plasmid was isolated from 2 mL of the bacterial culture with QIAprep Spin Miniprep Kit (Qiagen, Toronto, ON, Canada) and confirmed with PCR amplification using specific primers in the S gene of HBV. The plasmid concentration was quantified by Implen Nanophotometer. The measurements were performed in triplicates without prior dilution with a sample volume of 1.0 µl. Different dilutions of the plasmid, 10 and 100 times was measured to confirm accuracy. A260/A230 and A260/A280 ratios were measured to ensure purity.

Preparation of DNA functionalized AuNPs. DNA functionalized AuNPs were prepared by conjugating thiolated DNA oligonucleotides onto the 20 nm AuNPs according to our previously established protocol.[2] Briefly, 40 µL of 5 µM Arm-A DNA strands or Arm-B DNA strands were mixed with 500 µL of 20 nm AuNPs (1.16 nM), respectively. The mixture was incubated at room temperature for 12 hrs and then slowly mixed with 16.5 µL of 3 M NaCl solution, followed by 10s of sonication. The salt aging process was repeated five times with 1h interval for 2 days. The mixture was then centrifuged at 13,500 rpm for 30 min to separate the DNA-AuNPs from excess thiolated DNA oligonucleotides. The supernatant was discarded and DNA-AuNPs were washed with 1 mL 1×PBS buffer (ph 7.4) containing 0.01% TWEEN 20. The washing steps were repeated for four times. The DNA-AuNPs was finally dispersed in PBS buffer at 5 nM final concentration and stored at 4℃.

LAMP protocol. For a typical LAMP reaction, 1 µL HBV plasmid was added to a reaction mixture containing WarmStart LAMP 2× Master Mix, 1.6 µM FIP and BIP, 0.2 µM F3 and B3, 0.4 µM LF and LB to a final volume of 25 µL. The mixture was incubated at 65°C for 30 minutes, then inactivated by heating at 80°C for 10 min.

CRISPR-Cas12a assay with dual color readouts. For a typical CRISPR-Cas12a assay, a reaction mixture containing 30 nM Cas12a, 30 nM of gRNA, 500 nM S and varying concentrations of target dsDNA or LAMP amplicon was incubated at 37°C for 2 hrs, followed by an enzyme denaturation step at 80 °C for 10 min. For the C1 readout system, 2 µL reaction solution was mixed with 18 µL of C1 reaction solution. The mixture containing 50 nM S (or P), 1 nM AuNP-A, and 1 nM AuNP-B was then incubated at 50°C for 20 minutes and then cooled to the room temperature for color development. For the C2 readout system, 10 µL Cas12a reaction solution was mixed with 10 µL of C2 reaction mixture. The reaction mixture containing 250 nM S (or P) and 1 nM unmodified AuNPs was then mixed with 10 mM Mg^{2+} to induce aggregation. The final colors of the two readout systems were recorded using a digital camera. The absorbance of each solution was then scanned from 450 nm to 700 nm at a resolution of 1 nm using a Multimode Microplate reader (SpectraMax i3, Molecular Devices).

Polyacrylamide gel electrophoresis (PAGE). A 5-µL solution containing PCR or LAMP amplicons was mixed with loading buffer and then loaded onto 6% PAGE gel. A voltage of 110 V was applied for driving the electrophoresis. After electrophoresis, the gel was stained with Ethidium Bromide and imaged using Gel Doc XR+ Imager System (BioRad).

S2. Supporting Figures

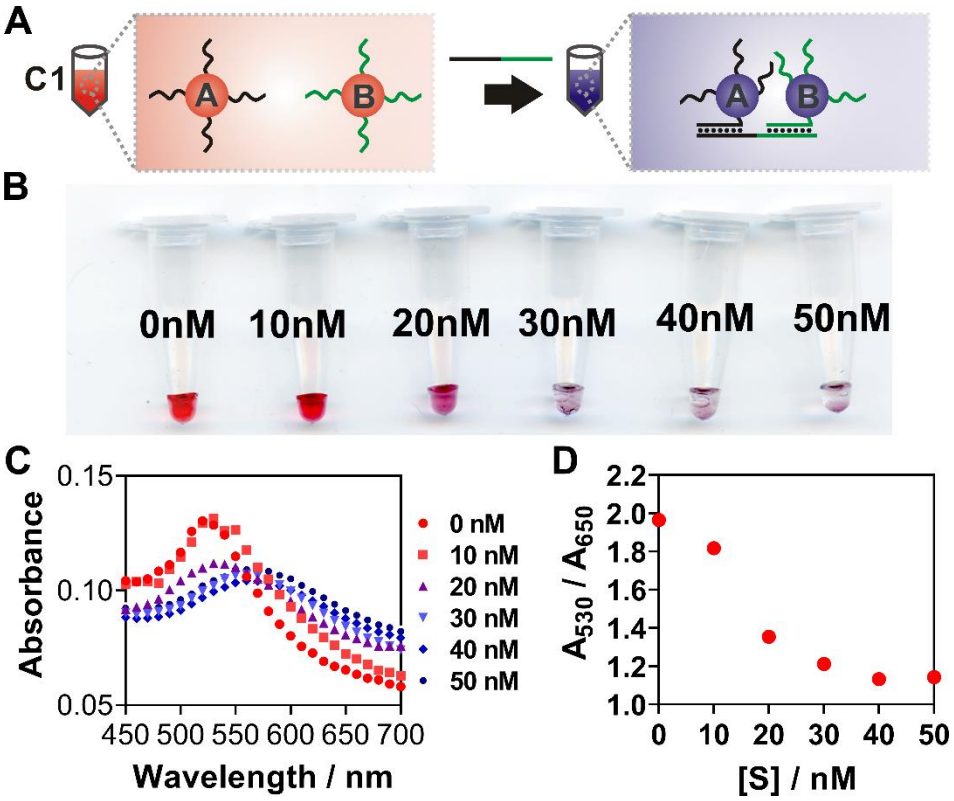

Figure S1. Characterization of C1 color readout system. (**A**) Schematic illustration of the mechanism of C1 system. (**B**) Color transitions in the presence of varying S from 0 nM to 50 nM. (**C**) Absorption spectra of reaction solutions in C1. (**D**) A_{530}/A_{650} plotted against varying S concentrations.

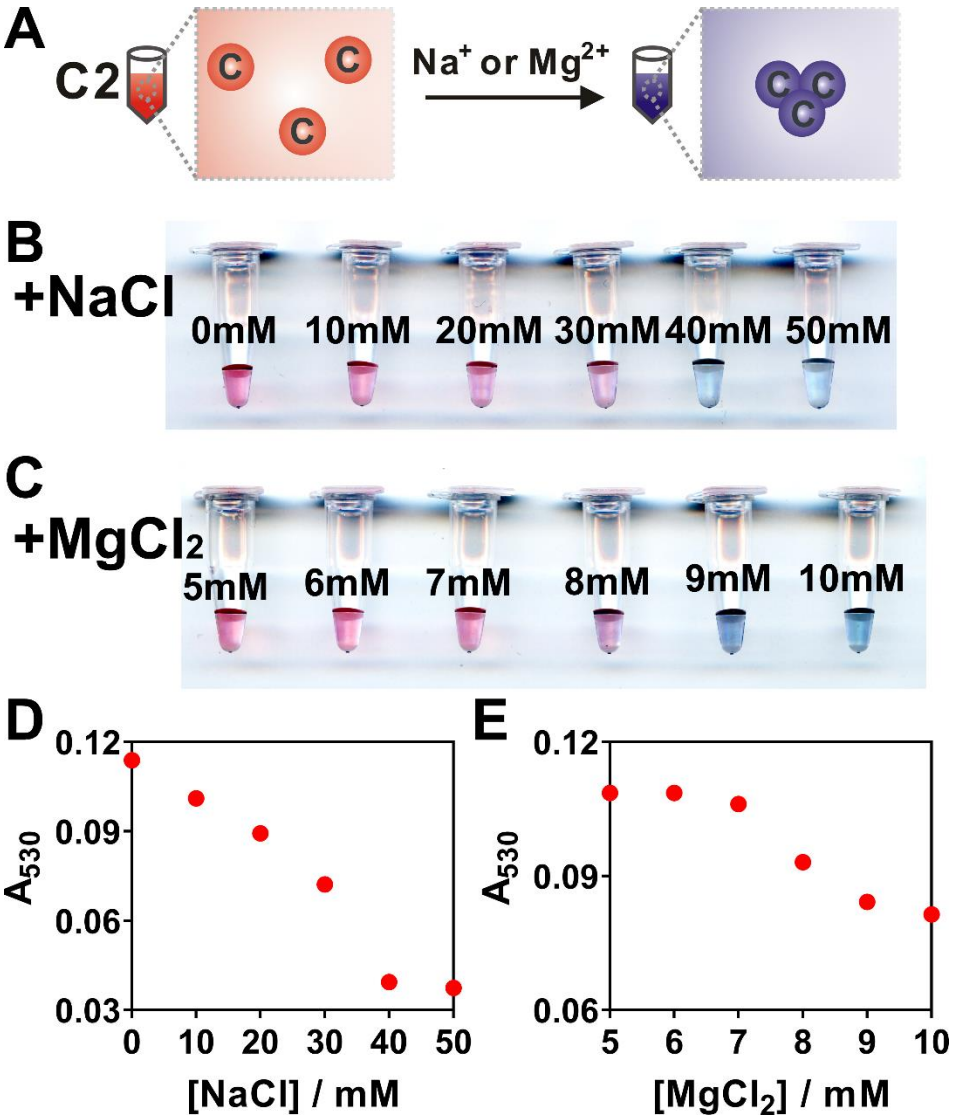

Figure S2. Characterization of salt-induced aggregation of unmodified AuNPs. (**A**) Schematic illustration of salt-induced aggregation of AuNPs. (**B**) Color transitions of AuNPs and aggregations induced by varying concentrations of NaCl. (**C**) Color transitions of AuNPs and aggregations induced by varying concentrations of Mg_2Cl. (**D**) A_{530} plotted against varying NaCl concentrations. (**E**) A_{530} plotted against varying Mg_2Cl concentrations.

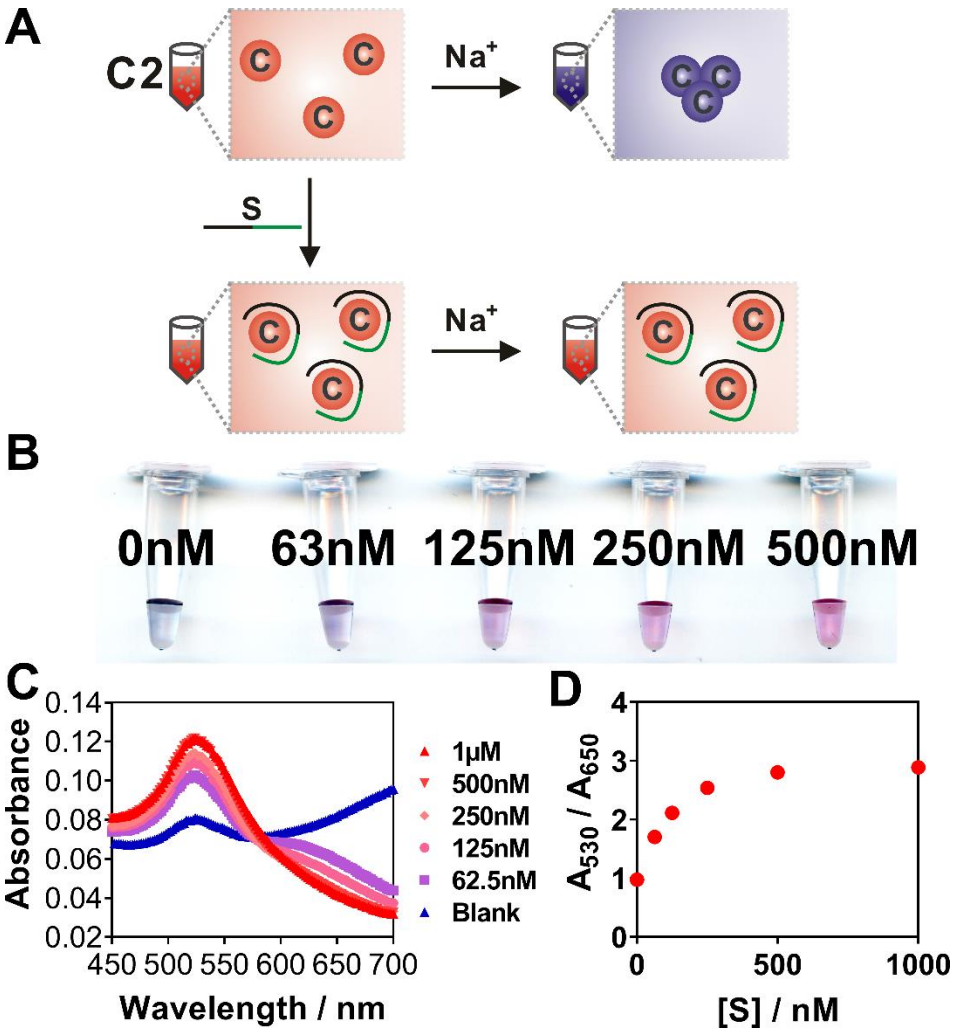

Figure S3. Characterization of preventing AuNPs from aggregation using S in C2 color readout system. (**A**) Schematic illustration of stabilizing unmodified AuNPs using S. (**B**) Color transitions in the presence of varying S from 0 nM to 500 nM. (**C**) Absorption spectra of reaction solutions in C2. (**D**) A_{530}/A_{650} plotted against varying S concentrations.

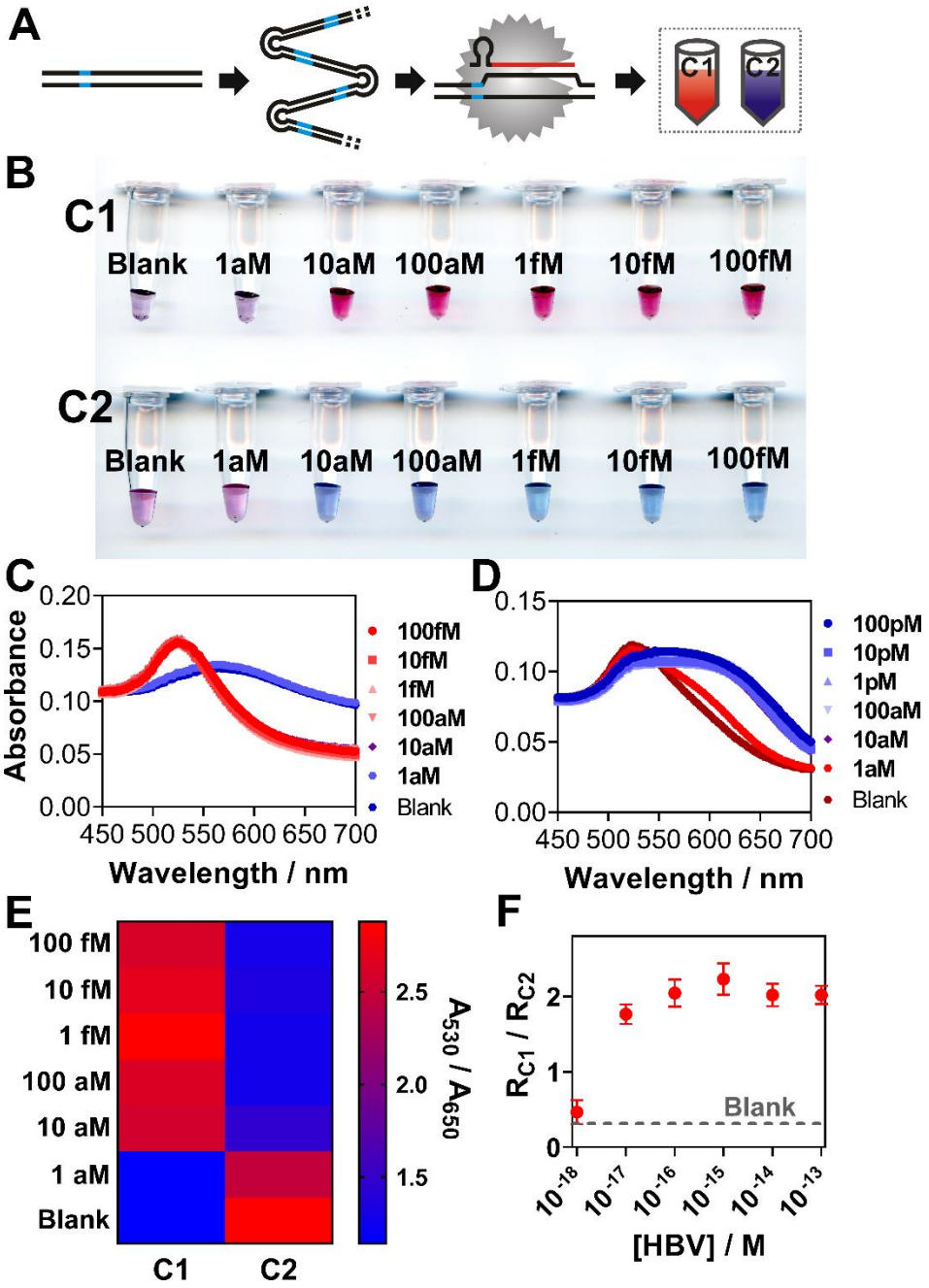

Figure S4. Analyzing HBV plasmid using plasmonic LAMP assay with complementary color readouts. (**A**) Schematic illustration of the assay workflow. (**B**) Color transitions in the presence of LAMP amplicons produced using varying concentrations of HBV plasmid in both C1 and C2 channels. (**C**) Absorption spectra of reaction solutions in C1 channel. (**D**) Absorption spectra of reaction solutions in C2 channel. (**E**) A heatmap comparing color transitions in C1 and C2 channels. (**F**) R_{C1}/R_{C2} plotted against varying concentrations of HBV plasmid.

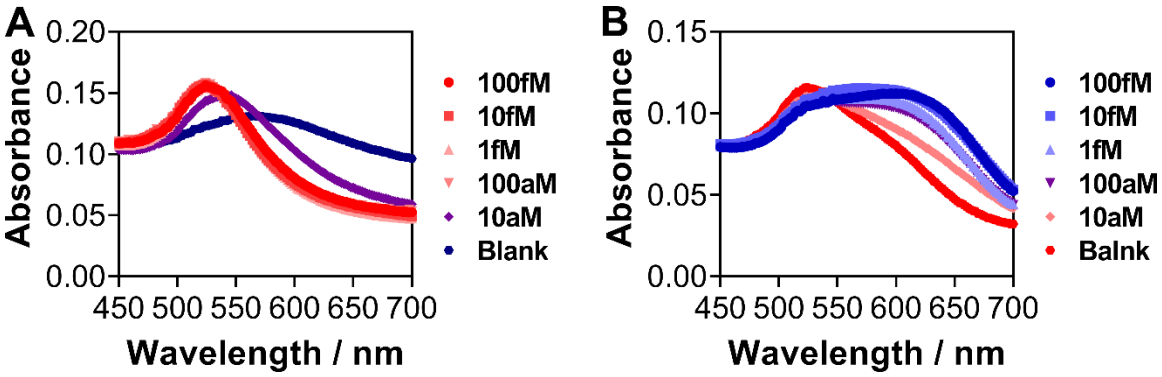

Figure S5. Detection of HBV plasmid in undiluted human serum samples using plasmonic LAMP assay. (**A**) Absorption spectra of reaction solutions in C1 channel. (**B**) Absorption spectra of reaction solutions in C2 channel.

References

1. H. Wang, S. Kim, W.S. Ryu, *Journal of Virol*ogy. **2009,** 83, 5815- 5824.

2. Y. Li, H. Mansour, T. Wang, S. Poojari, F. Li, *Analytical Chemistry*, **2019**, 91, 18, 11510-11513.