Electronic Supplementary Material (ESI) for Analytical Methods.

Naked-eye visualization of nucleic acid amplicons using hierarchical nanoassembly

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Supplementary Information

Supplementary Figure 1

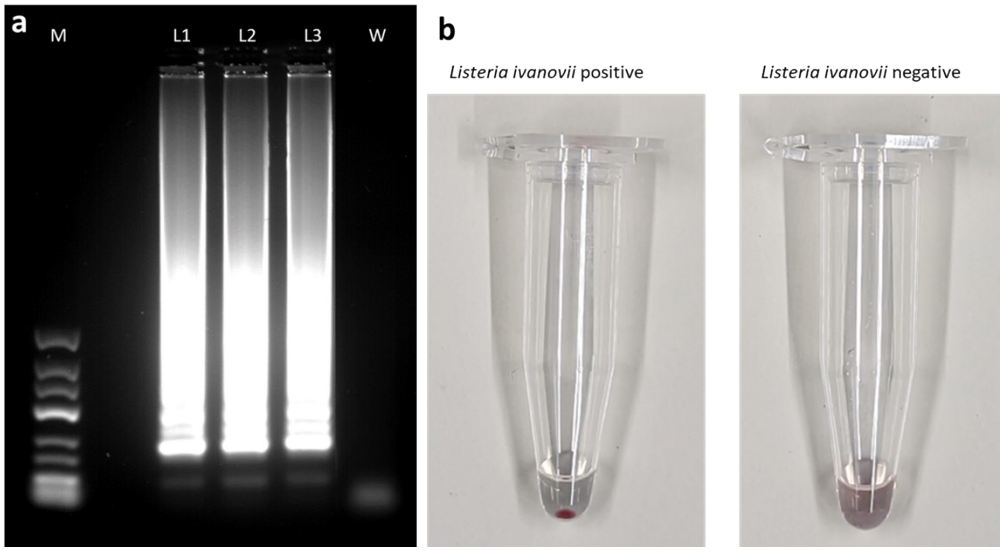
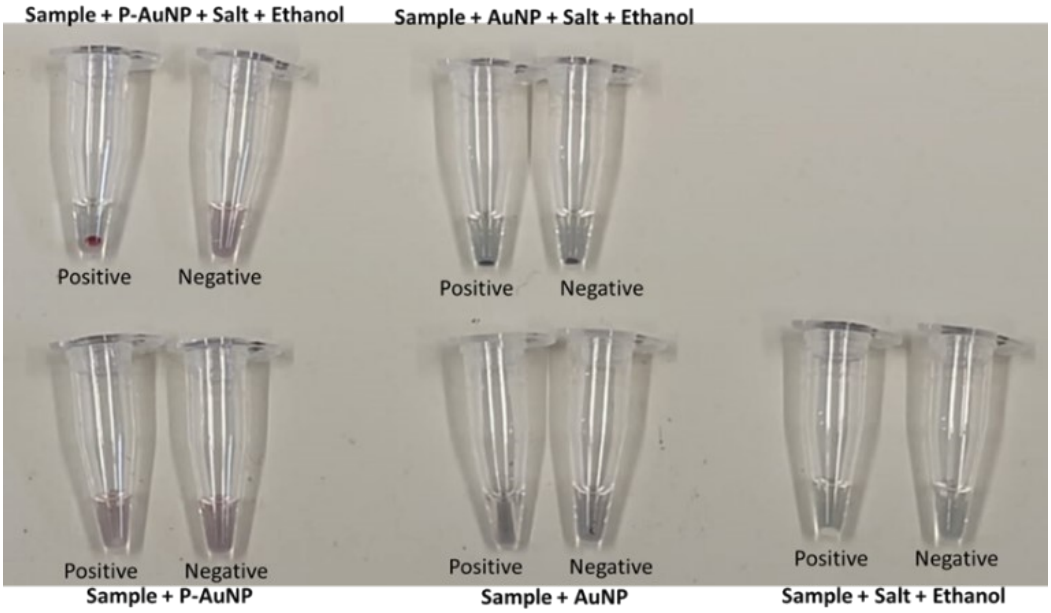


Fig. S1 (a) Agarose gel image of LAMP products obtained from amplifying *Listeria ivanovii* gene. Well M serves as a marker. Wells L1 to L3 contains *L. ivanovii* maintained as triplicate. Well W indicates the negative control, which is nuclease free water. (b) Visualization of *Listeria ivanovii* DNA amplicons (2.44 mg/ml) by nanoassembly with nuclease free water as negative control.

Supplementary Figure 2



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Experimental Section

Materials

WarmStart® LAMP Kit (DNA & RNA) was purchased from New England Biolabs (Ipswich, MA). The oligonucleotide sequences for AuNP synthesis and primers for LAMP experiments were fabricated by Integrated DNA Technologies (Coralville, IA). All solutions were prepared using nuclease free water purchased from Stemcell Technologies (Vancouver, Canada). Gold (III) chloride trihydrate, trisodium citrate dehydrate, Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), and sodium chloride (NaCl) were obtained from Sigma-Aldrich (St. Louis, MO). GelRed® Nucleic Acid Stain 10000X Water and phosphate saline buffer (PBS) were purchased from Millipore Sigma (Burlington, MA). GeneRuler Low Range DNA Ladder, 10X UltraPure TAE Buffer and UltraPure DNase/RNase-Free Distilled Water were purchased from Thermo Fisher Scientific

(USA). Ethylenediaminetetraacetic acid (EDTA) was obtained from Boston Bioproducts (Milford, MA) and ethanol was procured from Fisher Scientific (USA).

Fungal culture and DNA extraction

Bretziella fagacearum (B. fagacearum) isolates were provided by the United States Forest Service Northern Research Station, NRS-16, Saint Paul, USA. *B. fagacearum* DNA was extracted using a commercial kit (QIAamp DNA Mini Kit) with modifications in the buffer volumes provided in the manufacturer's instructions. This was used as the sample for LAMP reactions.

Obtaining LAMP amplicons

The LAMP reaction mixture was prepared according to the manufacturer's instructions for the WarmStart® LAMP Kit (DNA & RNA). The total reaction volume was 25 µl and the amplification was carried out at 65°C for 30 minutes followed by enzyme denaturation at 85°C for 5 minutes. The final LAMP products were used for testing the developed assay.

LAMP visualization using agarose gel electrophoresis

Agarose gel electrophoresis of the obtained LAMP products was done to confirm the generation of DNA amplicons. Serial dilutions of the amplicons (2x, 4x, 6x, 8x, 10x, 20x and 40x) were also prepared and run alongside the original amplicons.

Synthesis of gold nanoparticle (AuNP)-oligos

All the glassware used for nanoparticle synthesis was cleaned using Nochromix solution followed by Aqua Regia based on standard laboratory protocol. The AuNPs were then synthesized using a modified version of the Turkevich's method.[1] Initially, 0.25mM $HAuCl_4$ was prepared in 90 mL of deionized water and boiled at 100°C with vigorous stirring. Then, 10 mL of preheated trisodium citrate (7.352 g/L) was added. The mixture was maintained at the same temperature and vigorously stirred until the solution turned deep red in color. The nanoparticles were later stored at 4°C and characterized using UV – visible spectrophotometer (Shimadzu 1800) and transmission electron microscope (TEM, FEI Tecnai T12).

Two different oligonucleotide sequences with disulfide modifications were designed for conjugation with the AuNPs. The oligonucleotides were determined based on the primer and probe sequences used for RT-PCR in a previous study to test for *B. fagacearum*.[2] These were:

Oligos 1: Thiol 5'-TGGCAGGGACTTCTTTCTTCA- 3'

Oligos 2: 5'-ATGTTTCTGCCAGTAGTATT-3' Thiol

It must be noted that although the chosen sequences were part of the target fungal DNA, they were non-complementary to the target amplicons obtained from the chosen primer set. This helped with understanding the nature of the oligonucleotide stabilized AuNPs' attachment to the LAMP amplicons to enable naked eye detection of infection. The conjugation of the oligonucleotides with AuNPs was done using a modified version of a previously developed protocol.[3] Briefly, stock solutions of the oligonucleotides were prepared at 100 µM concentration in PBS-EDTA buffer. This was followed by incubation with TCEP for 1 h at room temperature with gentle shaking at 400 rpm. This solution was then added to 5 mL of AuNPs solution and incubated at room temperature for 5 h. After this, 600 µL of 1M NaCl was added dropwise to stabilize the conjugates and incubated for another 16 h. Finally, the solution was centrifuged at 14,000 rpm for 30 minutes and resuspended in PBS-EDTA buffer. The AuNP-oligos conjugates (AuNP-O1 and AuNP-O2) were characterized by UV – visible spectrophotometer and TEM.

The same process was carried out with two oligonucleotide sequences that were complementary to the target amplicon sequence to see if the visualization technique worked differently. The following are the sequences used for this test:

Probe 1: 5'-ATGCCTAGCAGAATACTGC -3'Thiol

Probe 2: Thiol 5'- ACCTGATCCGAGGTCAAC-3'

Optical detection of LAMP products with AuNP-oligos

Initially, a small volume of each LAMP reaction product, including the diluted amplicons, was incubated separately for 5 minutes with AuNP-O1 and AuNP-O2 to enable attachment to

amplicons. Then, 2 M NaCl and 100% ethanol were added in equal amounts (same as AuNP-O1 and AuNP-O2 volumes) and incubated for 2 minutes to precipitate the amplified DNA-AuNP-oligos conjugates in target samples. The concentration of NaCl and ethanol were chosen to allow maximum precipitation of DNA molecules in the small volume of sample. This was based on conditions usually applied during a DNA extraction process with some modifications.[4] Finally, the samples were centrifuged for 1 minute at 6000 rpm (MyFuge™ Mini Centrifuge with 2 rotors from Benchmark Scientific) and distinguished as positive or negative based on their final appearance. Given the objective was to develop a rapid and simple method to visualize the amplicons, the tested incubation times were chosen to be lower to start with, and slowly increased until a difference between positive and negative samples could be seen.

Evaluation of the AuNP-oligos-amplicon assembly

To quantify the amount of AuNP-oligos that formed an assembly with the varying concentrations of the LAMP amplicons, the difference in the gray value of the supernatant and the red pellet was evaluated using the ImageJ software. Measuring the variation in the color intensity of the supernatant with varying amplicon concentration helped measure the amount of unassembled AuNP-oligos left in the supernatant. Fig. S3 illustrates this process.

Supplementary Figure 3

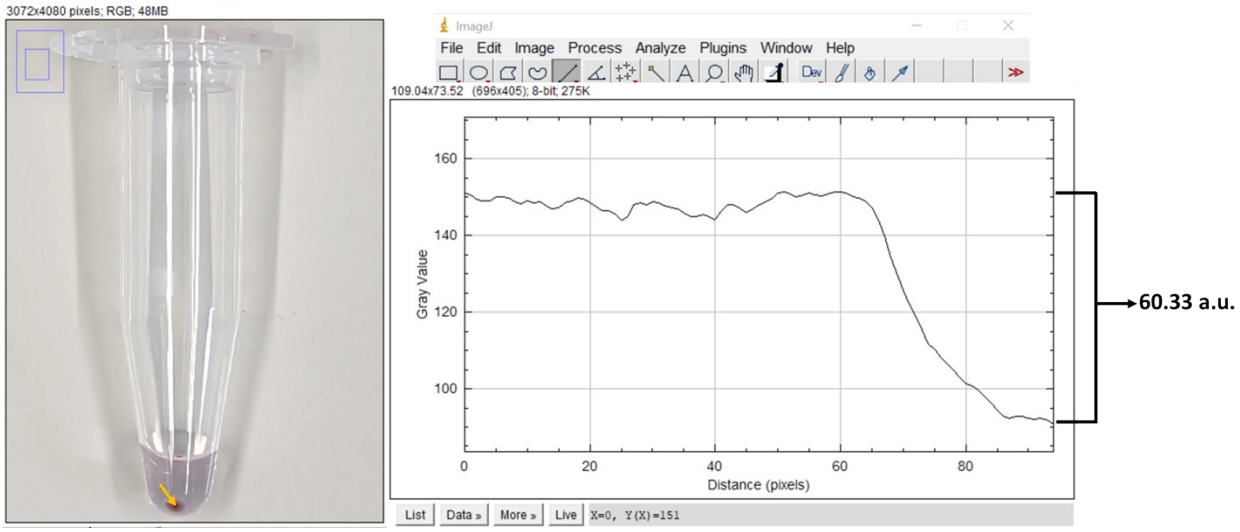

Fig. S3 The process of using ImageJ software to analyse the amount of AuNP-oligos remaining in the supernatant by measuring the gray value. The yellow arrow on the sample image shows the direction in which the measurement was done. The graph on the left indicates the gray value between the supernatant (highest value) and the pellet (lowest value). Higher difference between the two indicate that more AuNP-oligos assembled with the DNA amplicons to form the red pellet. The measurements were done in triplicates for every sample.

References

1 M. Wuithschick, A. Birnbaum, S. Witte, M. Sztucki, U. Vainio, N. Pinna, K. Rademann, F. Emmerling, R. Kraehnert and J. Polte, *ACS Nano*, 2015, **9**, 7052–7071.

2 A. Yang and J. Juzwik, *Plant Dis.*, 2017, **101**, 480–486.

3 R. Singh, A. Feltmeyer, O. Saiapina, J. Juzwik, B. Arenz and A. Abbas, *Sci. Rep.*, 2017, **7**, 1–9.

4 R. P. Hearn and K. E. Arblaster, *Biochem. Mol. Biol. Educ.*, 2010, **38**, 161–166.