Electronic Supplementary Information

DNA Detection on Lateral Flow Test Strips: Enhanced Signal Sensitivity Using LNA-conjugated Gold Nanoparticles

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EXPERIMENTAL PROTOCOLS

1. Reagents

Streptavidin from *Streptomyces avidinii*, dithiothreitol (DTT), triethylamine (TEA), ethyl acetate, $Na_3PO_4 \cdot 12H_2O$, sucrose, Tween 20, Triton X-100, sodium chloride-sodium citrate (SSC) buffer (pH 7.0), phosphate buffer saline (PBS, pH 7.4, 0.01 M), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. All chemicals were of analytical grade. All buffer solutions were prepared using double distilled water. Glass fibers (GFCP000800), cellulose fiber sample pads (CFSP001700), laminated cards (HF000MC100) and nitrocellulose membranes (HFB24004) were obtained from Millipore (Bedford, MA, USA). Plastic cassettes (MICA-120) were purchased from Diagnostic Consulting Network (Carlsbad, CA, USA). Sodium citrate stabilized gold nanoparticles (AuNP; ~15 nm diameter) were purchased from Ted Pella Inc. (Redding, CA, USA). All oligonucleotides except LP were obtained from Integrated DNA technologies, Inc. (Coralville, IA, USA). LNA-T and LNA-A^{Bz} amidites were obtained from Exiqon A/S (Vedbæk, Denmark). Thiol modifier (5'-/5ThioMC6; *S*-trityl-6-mercaptohexyl-1-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]-phosphoramidite), internal spacer phosphoramidite (iSp18; 18-*O*-dimethoxytritylhexaethyleneglycol,1-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]-phosphoramidite), and succinyl-linked 3'-dC-CPG solid support (5'-dimethoxytrityl-*N*-benzoyl-3'-deoxycytosine, 2'-succinoyl-long chain alkylamino-CPG 500) were obtained from Glen Research Inc. (Sterling, VA, USA).

2. Oligonucleotides:

Sequences for E. coli O157:H7:

ET: 34-mer hyla DNA target: 3'-CGGCCTTGTCAAGATAGTCCGTACCGAGAACTAC-5'

DP: Thiolated 13-mer DNA-AuNP probe: 5'-/5ThioMC6-D//iSp18/GCC GGA ACA GTT C-3'

LP: Thiolated 13-mer LNA-AuNP probe: 5'-/5ThioMC6-D//iSp18/GCC GGa ACa GTt C-3'

TP: Biotinylated 21-mer test line probe: 5'-TAT CAG GCA TGG CTC TTG ATG/iSp18//3BioTEG/-3'

CP: Biotinylated 13-mer control line probe: 3'-CGG CCT TGT CAA G/iSp18//5Biosg/-5'

Sequence for Salmonella typhimurium:

NT: 30-mer noncomplementary DNA: 3'-GGTGCAAGCCCGTTAAGCAATAACCGCTAT-5'

3. Preparation of DNA-AuNP

The disulfide bond of thiolated DNA probe (**DP**) was reduced using DTT as previously reported:[S1] A solution of thiolated DNA in water (**DP**; 1.0 OD; 196 µL) was added to a solution of 4 µL of triethylamine and 10 µL of DTT (300 mg/mL) in PB buffer (1.0 mM $NaH_2PO_4 \cdot H_2O$, 18.6 mM $Na_2HPO_4 \cdot 7H_2O$) and allowed to react for 1h at rt. Excess DTT was removed by extraction with EtOAc (5 × 800 µL). The reduced **DP** was added to a colloidal 2X solution of AuNPs (1 mL; 2.8×10^{12} AuNPs) in PB buffer. The 2X AuNP solution was obtained by centrifuging 2 mL of the commercial AuNP solution for 40 min at 8000 *g*, followed by resuspension in 1 mL PB buffer. After 24h incubation at 4 °C, 10 µL of 10% SDS was added to the DP-AuNP solution and salt aging was performed by adding of 2 M NaCl in portions every 0.5h up to a final concentration of 0.7 M NaCl, at this point the solution was allowed to stand at 4 °C for 24h. The DP-AuNP conjugates were then rinsed via the following protocol: centrifugation (8,000 *g*, 40 min.), decantation and resuspension of the red pellet in 1 mL of eluent buffer (20 mM Na_3PO_4, 5% BSA, 0.25% Tween, and 10% sucrose). This solution was used immediately to prepare conjugation pad.

4. Synthesis and purification of LNA probe (LP)

The synthesis of **LP** was performed on a 0.2 µmol scale using a DNA synthesizer (Expedite 8909; Biolytic Lab Performance Inc., Fremont, CA, USA) and succinyl-linked 3'-dC-CPG solid support. Standard protocols for incorporation of DNA phosphoramidites were used. A 50-fold molar excess of LNA-A^{Bz}, LNA-T, internal spacer (iSP18) and thiol modifier (5'-thiol-modifier C6) phosphoramidites in 200 µL anhydrous acetonitrile were used during hand-couplings, along with extended coupling (15 min, activator: 4,5-dicyanoimidazole), oxidation (45s) and double capping. Amidites were added in ten portions over 15 min. Cleavage from solid support and removal of protecting groups was accomplished with 32% aq ammonia (55 °C, 24h).

Purification of crude **LP** was performed on a HPLC system (Varian ProStar 410 HPLC Autosampler, UV-VIS and PDA detector 330, Dynamax solvent flow system, Rainin AI-200 Autocollector) equipped with an XTerra MS C18 column (5 µm, 4.6 × 150 mm; Waters, Milford, MA, USA) using the gradient protocol depicted in Table S1. The identity of **LP** was established through MALDI-TOF MS analysis using a quadrupole time-of-flight tandem mass spectrometer equipped with a MALDI source (positive ion mode; calc. [M+H] *m/z* = 4825; obs. [M+H] *m/z* = 4826; Fig. S1B). Purity (>90%) was verified by RP-HPLC running in analytical mode.

Table S1. RP-HPLC protocol for purification of **LP**.[a]

t [min]	Buffer A [v%]	Buffer B [v%]
0	100	0
3	100	0
48	35	65
48.1	0	100
51.5	0	100

[a] Buffer A: 0.05 M TEAA (triethyl ammonium acetate) pH 7.0, buffer B: 75% MeCN in H_2O v/v. A flow rate of 1.4 mL/min was used.

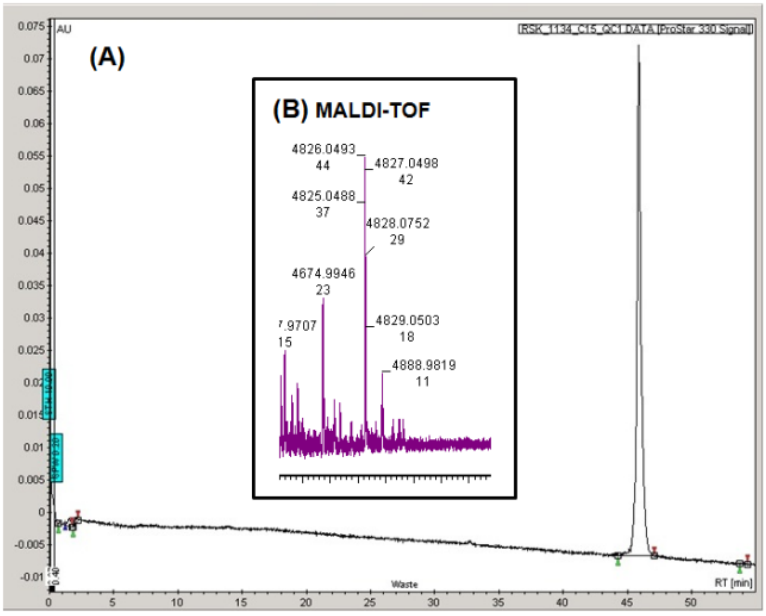

Figure S1: (**A**): RP-HPLC chromatogram and (B) MALDI-TOF MS of purified **LP**.

5. Preparation of LNA-AuNP

The *S*-trityl group of thiolated LNA probe (**LP**) was deprotected using silver nitrate according to recommendations from the vendor (Glen Research):[S2] A solution of 50 µL thiolated LNA probe in water (**LP**; 1.0 OD) was mixed with a solution of 20 µL triethylammonium acetate (TEAA; 0.1 M), 20 µL water and 30 µL $AgNO_3$ (0.1 mM), and allowed to react for 30 min. A solution of 45 µL of DTT (0.1 mM) was added to the above mixture and the solution was vortexed for 5 min at rt. and centrifuged (10,000 *g*, 5 min). After collecting the supernatant, the yellow precipitate was washed with 200 µL of TEAA (0.1 M), which was added to supernatant. Excess DTT was removed by extraction with EtOAc (5 × 400 µL). The reduced **LP** was added to a colloidal 2X

solution of AuNP in PB buffer and incubated for 24h at 4 °C. Salt aging was performed as described in Section 3. No aggregates were observed during handling, indicating good colloidal stability. LNA-AuNPs were directly used to prepare conjugation pads.

6. Preparation of the streptavidine-biotine-DNA conjugates

300 µL of a streptavidin solution - taken from a stock at 1.67 mg/mL in PBS (0.14 M NaCl, 0.14 M KCl, 5.5 mM $Na_2HPO_4{\cdot}7H_2O$, and 1.5 mM KH_2PO_4; pH 7.4) - and 60 µL of a 1 mM biotin-DNA probe (**TP** or **CP**) solution, were mixed into 140 µL of PBS, and incubated for 1h at rt. The reaction mixture was then filtered through a spin column with a 30K cutoff filter (Millipore; 3000*g*; 20 min) to remove excess of **TP** or **CP**. The resulting sterptavidine-biotin-DNA conjugates were washed with PBS (3 x 800 µL) dissolved in 500 µL of PBS and used for the preparation of the test pad.

7. Preparation of Pads:

Sample Application Pad

A 17 mm × 5 cm cellulose fiber pad was pretreated with saturation buffer (1 mL; 0.25% Triton X-100, 0.05 M Tris-HCl, 0.15 mM NaCl at pH 8.0), dried at rt and stored in desiccators at rt until LFTS assembly.

Conjugation pad

Suspensions of either DNA-AuNP or LNA-AuNP probes were placed over the entire area of the 8 mm × 15 cm glass fiber pads. The pads were dried at rt and stored in desiccators at 4 °C until LFTS assembly (up to two months, with no drop in performance).

Test pad

Test and control lines (see Fig. 1) were prepared by depositing streptavidin-biotin-DNA conjugates on nitrocellulose membrane (25 mm × 50 mm) using a manual microinjector (# 17961, Sutter Instrument Co., Novato, CA, USA) equipped with a microsyringe (Hamilton 81130) having a blunt-ended needle (Hamilton 7780-04). The conjugates were deposited at a speed of 0.6 µL per mm of nitrocellulose membrane. Test lines were deposited in four passes with a 20 minute wait period between each pass. Control lines were deposited in an identical

manner except that only three passes were performed. Test pads were dried at rt and stored in desiccators at 4 °C until LFTS assembly.

Absorbent pad

17 mm × 5 cm cellulose fiber pads were used without any pretreatment. Pads were stored in desiccators at rt until LFTS assembly.

8. Assembly of lateral flow test strip

The lateral flow test strips are composed of four pads, i.e., sample application, conjugate, test, and absorption pad. The four pads were assembled on a plastic adhesive backing as shown in Fig. 1. The nitrocellulose membrane was the first pad to be placed on the adhesive backing. The conjugate pad was subsequently placed in a manner to give a ~2 mm overlap with nitrocellulose pad. The sample application pad was then placed to give ~2 mm overlap with the conjugate pad. Finally, the absorbent pad was placed to give a ~2 mm overlap with the nitrocellulose pad. After successful assembly of the pads, ~4 mm wide strips where cut using a rotary cutting tool and placed in plastic cassettes. The LFTS were stored in desiccators at 4°C until use.

9. Assay and Signal Amplification

A 130 µL aliquot of the DNA target in ¼X SSC buffer (0.6 M NaCl, and 60 mM sodium citrate dihydrate) was applied onto the sample application pad and allowed to migrate for 10 min. Visual detection (documented via digital photographs) was followed by quantification of signal intensities of test/control lines (peak areas) using a portable test strip reader (DT2032, Shanghai Kinbio Tech Co., LTD Shanghai, China) and integrated software (Kingbio strip reader ver. 2.0).

Gold-mediated amplification[S3] of test/control signal intensities was performed in the following manner: 130 µL amplifier solution (1:1 v/v of 0.2 mM $HAuCl_4 \cdot 2H_2O$ in 1 M HCl and 37% HCHO) was dropped directly onto the nitrocellulose membrane, and allowed to react for 10 min. A color change from pink to bluish-red was observed. Next, 130 µL 4X SSC solution was dropped on the test pad to flush the test strip, and intensities of test/control lines were recorded after 10 min using the portable test reader.

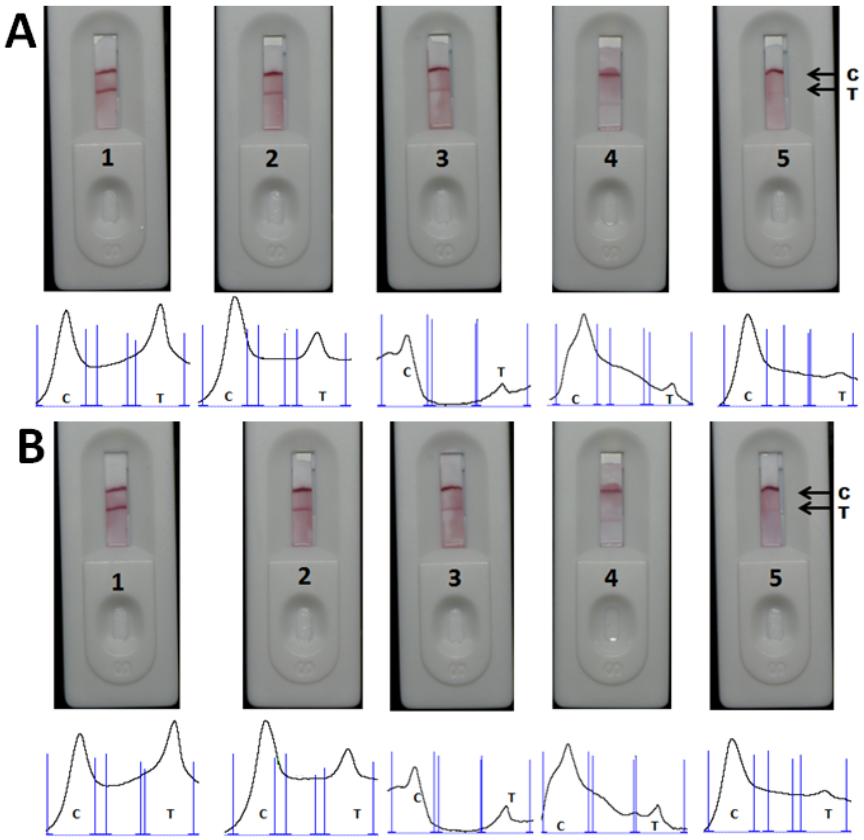

Figure S2: Images of test strips and peak areas using the DNA-AuNP signaling probes for *E. coli* O157:H7 target detection, before (A) and after (B) signal amplification. Target DNA **ET** was added at a concentration of 12.5, 6.25, 3.13, 1.56 or 0.78 nM (strips 1-5, respectively). "C" and "T" denotes control and test lines respectively.

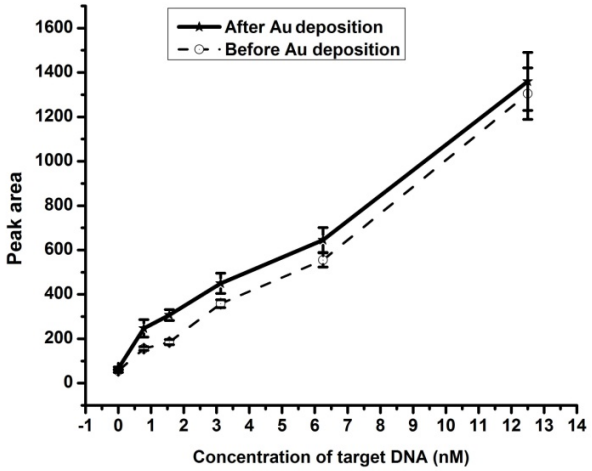

Figure S3: Signal intensity (peak area) before or after gold deposition (solid line) during detection of *E. coli* O157:H7 target DNA (**ET**) using DNA-AuNP probes.

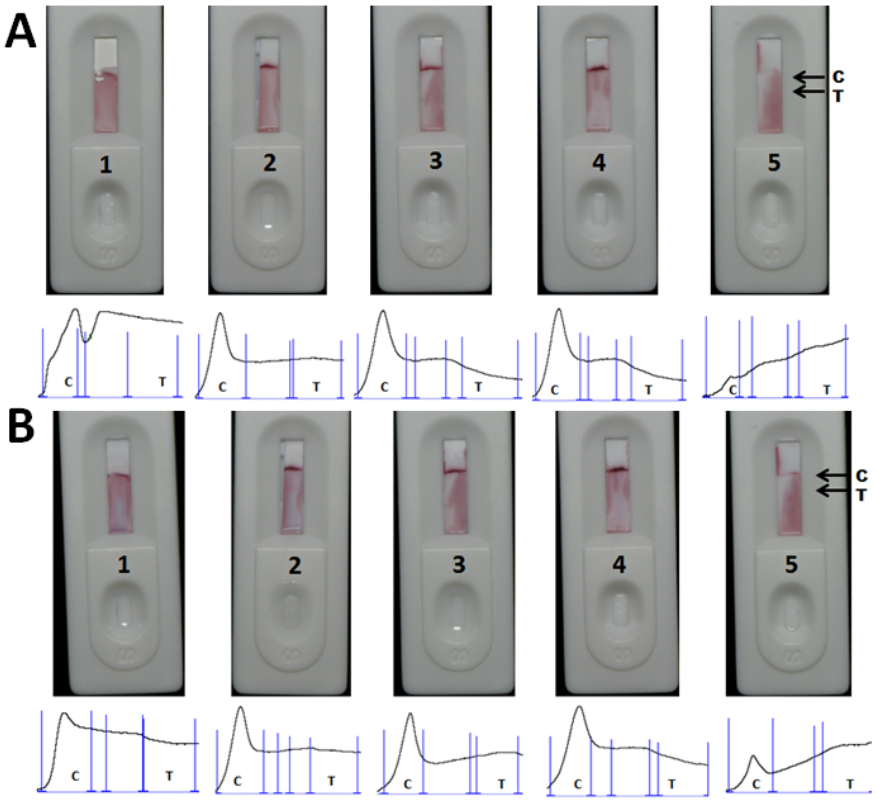

Figure S4: Images of test strips and peak areas using DNA-AuNP signaling probes specific for *E. coli* O157:H7 in the presence of non-complementary DNA target **NT** (*S. typimurium*) before (A) or after (B) signal amplification. **NT** was added at a concentration of 1.56, 3.13, 6.25, 12.5 or 25.0 nM (strips 1-5, respectively).

Figure S5: Images of test strips and peak areas using LNA-AuNP signaling probes specific for *E. coli* O157:H7 target detection, before (A) and after (B) signal amplification. Target DNA **ET** was added at a concentration of 0, 3.13, 1.56, 0.78 or 0.39 nM (strips 1-5, respectively). "C" and "T" denotes control and test lines respectively.

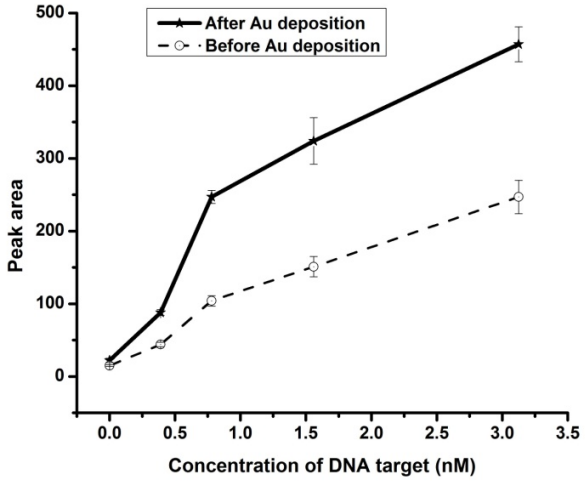

Figure S6: Signal intensity (peak area) before or after gold deposition during detection of *E. coli* O157:H7 target DNA (**ET**) detection using LNA-AuNP probes.

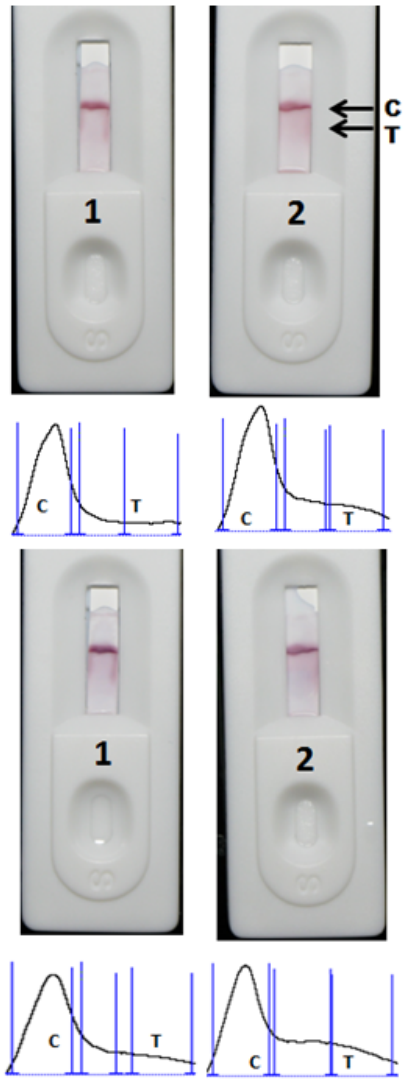

Figure S7: Images of test strips and peak areas using LNA-AuNP probes specific for *E. coli* O157:H7 in presence of a non-complementary DNA target (**NT**) (*S. typimurium*) before (A) and after (B) signal amplification. **NT** was added at a concentration of 25 or 50 nM (strips 1 and 2, respectively). "C" and "T" denotes control and test lines respectively.

References

S1. X. Mao, Y. Ma, A. Zhang, L. Zhang, L. Zeng, and G. Liu, *Anal. Chem.,* 2009, **81**, 1660.

S2. http://www.glenresearch.com/ProductFiles/10-1926.html

S3. K. T. Liao, and H. J. Huang, *Anal. Chem. Acta*, 2005, **538**, 159.