Supplementary Information (SI)

Fe_3O_4-Au heterodimer nanoparticles-based lateral flow assay for rapid and simultaneous detection of multiple influenza virus nucleic acids

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1. Reagents and Instruments

Acetone was brought from Sinopharm Chemical Reagent Co., Ltd. Chloroauric acid ($HAuCl_4$) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) were obtained from Energy Chemical. Oleic acid, 1-octadecene, tetramethylammonium hydroxide (TMAH) and biotin were brought from Sigma-Aldrich. Trisodium citrate (TSC) was provided by Shanghai Macklin Biochemical Co., Ltd. Streptavidin (SA) was purchased from Amresco. HS-PEG-COOH was provided by Shanghai ToYong Bio-Tech. Co., Ltd. Albumin bovine serum (BSA) was obtained from Beijing Solarbio Science & Technology Co., Ltd. Sample pad, conjugate pad, nitrocellulose membrane (NC membrane), PVC substrate and absorbent pad were bought from Joey-biotech Co., Ltd. (Shanghai, China). The CN140 NC membrane was provided by Sartorius in Göttingen, Germany. Target cDNA of H1N1, H3N2, H9N2, probe DNA (P-DNA), T line DNA (T-DNA), and C line DNA (C-DNA) were purchased from Invitrogen Corp. The sequences were shown as follows:

Target cDNA sequences of H1N1, H3N2, H9N2:

H1N1: 5'-ATT CAA TCC AGA GGT CTA TTT GGA GCC ATT GCC GGT TTT ATT GAA-3'

H3N2: 5'-TAT GCC ACC CTT AGG TCA CTA GTT GCC TCA TCT GGC AAC CTG GAA-3'

H9N2: 5'-TAG AAG GGG TCA AGC TGG AAT CTG AAG GAA CTT ACA AAA TCC TCA-3'

P-DNA sequence:

P-H1: Biotin-5'-TTC AAT AAA ACC GGC AAT GGC TCC-3'

P-H3: Biotin-5'-TTC CAG GTT GCC AGA TGA GGC AAC-3'

P-H9: Biotin-5'-TGA GGA TTT TGT AAG TTC CTT CAG-3'

T-DNA sequence:

T-H1: 5'-AAA TAG ACC TCT GGA TTG AAT-3'-Biotin

T-H3: 5'-TAG TGA CCT AAG GGT GGC ATA -3'-Biotin

T-H9: 5'-ATT CCA GCT TGA CCC CTT CTA -3'-Biotin

C-DNA sequence:

C-H1: Biotin-5'-GGA GCC ATT GCC GGT TTT ATT GAA-3'

C-H3: Biotin-5'-GTT GCC TCA TCT GGC AAC CTG GAA-3'

C-H9: Biotin-5'-CTG AAG GAA CTT ACA AAA TCC TCA-3'

Absorption spectra were measured using a Shimazu UV-2450 spectrophotometer (Shimadzu). Transmission electron microscopy (TEM) images were performed by a microscope (JEOL, JEM 1400). High-resolution (HR) TEM and energy dispersive X-ray (EDX) elemental mapping were obtained by a microscope (FEI, Tecnai F30). Hydrodynamic diameters and zeta potentials were measured on a Zetasizer Nano ZS instrument (Malvern). The test strips were sprayed by HGS510 (AUTOKUN) and divided by HGS210 induction cutting machine (AUTOKUN).

S1. Particle size distributions of Fe_3O_4 nanoparticles and Au nanoparticles

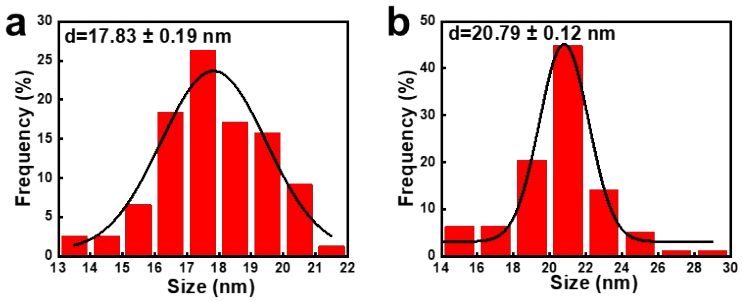

Figure S1. (a) Size distribution of Fe_3O_4 nanoparticles. (b) Size distribution of Au nanoparticles.

S2. Optimization of the BSA concentration in blocking

As shown in Figure S1, with the increase of BSA concentration, the grey intensity showed a trend of first increasing and then decreasing, and 5 wt % BSA produced the highest signal intensity, which was considered as the best blocking concentration.

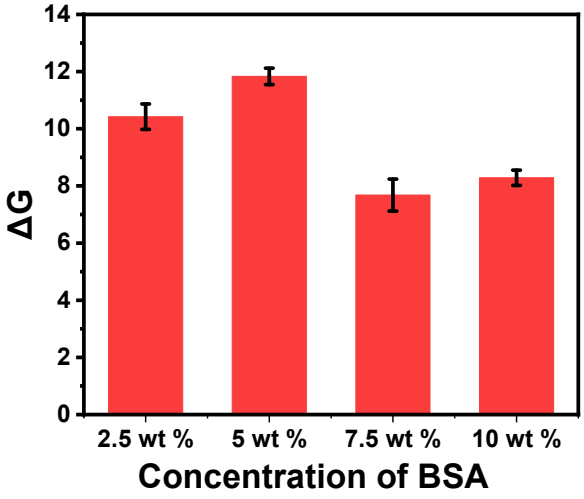

Figure S2. Plot of ΔG versus BSA concentration. Error bar = ±SD, n = 3.

S3. Optimization of the NC membrane type

Three kinds of NC membranes with different chromatographic speeds were selected for exploration. The chromatographic speed affects the residence time of the probes on the NC membrane thus affecting the sensitivity. As can be seen from Figure S2, CN95 NC membrane

produced the highest signal value, which was selected as the best reaction substrate.

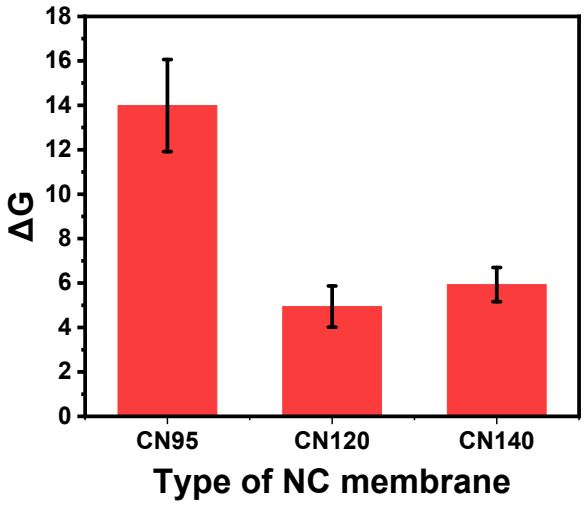

Figure S3. Plot of ΔG versus NC membrane type. Error bar = ±SD, n = 3.

S4. Optimization of the SA and biotin-DNA reaction ratio

Since there are 4 biotin binding sites on one SA, so we choose the reaction ratio from 1:1 to 1:4 for optimization. As shown in Figure S3, the strongest signal intensity was produced when the reaction ratio was 1:2, so 1:2 molar ratio of SA to biotin-DNA was selected.

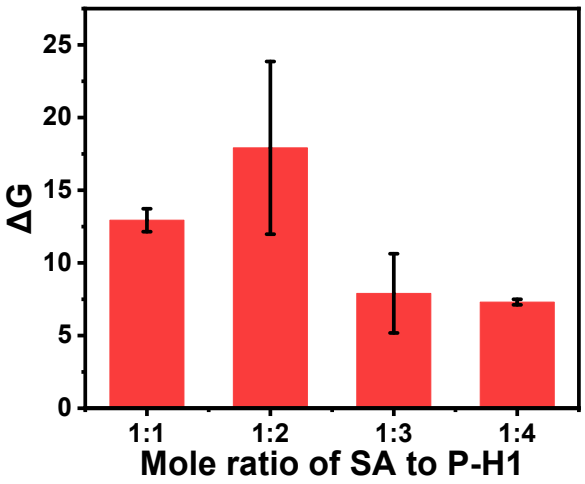

Figure S4. Plot of ΔG versus SA and biotin-DNA reaction ratio. Error bar = ±SD, n = 3.

S5. Optimization of the SA-DNA concentration for spraying T line

As shown in Figure S4, with the increase of SA-DNA concentration sprayed on T_1, the grey

intensity first increased and then decreased. The grey intensity was maximum when the concentration was 1 mg·mL^{-1}, and hence 1 mg·mL^{-1} was selected as the optimum spraying concentration.

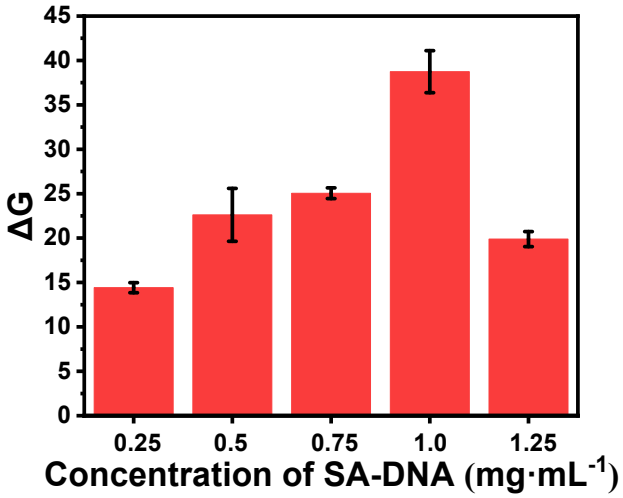

Figure S5. Plot of ΔG versus SA-DNA concentration for spraying T line. Error bar = ±SD, n = 3.

S6. Optimization of the DNA dosage in probe preparation

As shown in Figure S5, with the dosage of DNA increase, the signal intensity increased and then decreased. When the dosage of DNA was 0.25 OD·mL^{-1}, the signal intensity reached the maximum, which was the optimal dosage.

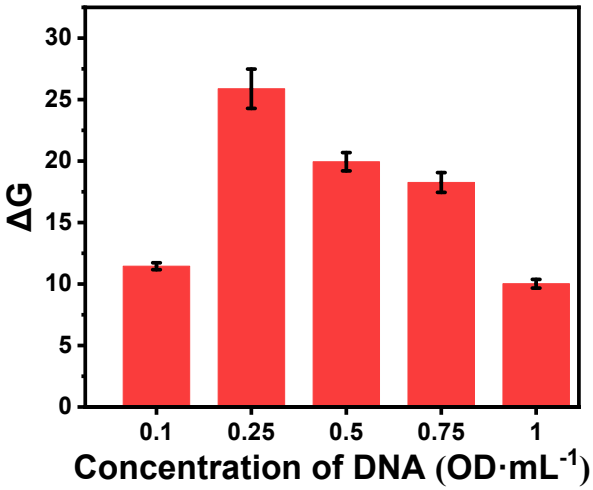

Figure S6. Plot of ΔG versus DNA dosage in probe preparation. Error bar = ±SD, n = 3.

S7. Optimization of the incubation time

Finally, we optimized the pre-incubation time. With the extension of the pre-incubation time, the signal intensities were almost the same, indicating that the pre-incubation time hardly influenced the detection signal. Considering saving time, we directly loaded the samples without incubation.

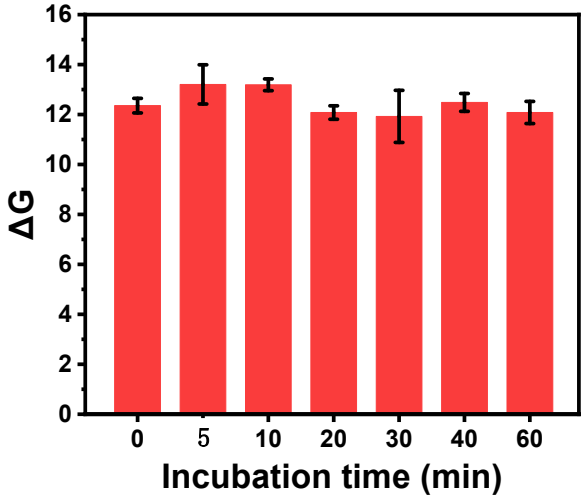

Figure S7. Plot of ΔG versus incubation time. Error bar = ±SD, n = 3.

Table S1. A comparison of the LOD of other reported LFA methods for detection of influenza virus

Materials used	**Methods combined with LFA**	**Target**	**Signal**	**Detection limit**	**Linearity range**	**Ref.**
ZnCdSe/ZnS QDs	Reverse transcription-free exponential amplification reaction	H1N1	Fluorescence	4.8 aM	10^4-10^9 aM	1
Au Nanoparticles	Reverse transcription recombinase polymerase amplification	H9N2	Color	0.15 pg	Qualitative	2
Au Nanoparticles	Strand displacement amplification	H1N1 and influenza B virus	Color	2×10^1 copies/μL and 6.3×10^1 copies/μL	Qualitative	3
Au Nanoparticles	Duplex recombinase polymerase amplification	influenza A and B virus	Color	500 and 50 copies/reaction	Qualitative	4
Carbon	Recombinase polymerase amplification	H7N9	Color	32 fg	Qualitative	5
Fe_3O_4-Au	/	H1N1, H3N2 and H9N2	Color	2.5, 2.5 and 0.5 nM	Qualitative	This work

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