Electronic Supplementary Material (ESI) for Analytical Methods.

Trimetallic Au@Pd@Pt nanozyme enhanced lateral flow immunoassay for detection of SARS-CoV-2 nucleocapsid protein

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Effect of different substances on the stability of Au@Pd@Pt NZs

The stability of Au@Pd@Pt NZs was performed. The details as following: 1440 μL PBS (pH=5), 20 μL 0.05 mg/mL of Au@Pd@Pt NZs, 20 μL H_2O_2 (8 mM), TMB (0.2 mM) and 20 μL H_2O, $(CH_3COO)_2Zn$ solution (0.1 M), $FeCl_3$ solution (0.1 M), $Co(NO_3)_2$ solution (0.1 M), OVA (1.0 wt%), BSA (1.0 wt%), MPA (1%). After mixing 15 min, the absorbance at 652 nm was measured.

Preparation of Au nanoparticles

Au nanoparticles (Au NPs) were synthesized according to the typical method.[1] Briefly, 1.0 mL of 1.0 wt% $HAuCl_4$ was added into 99 mL of ultrapure water, and refluxed at 100 ℃ to boiling. Then 1.5 mL of 1.0 wt% sodium citrate was added into the solution and maintained stirring for 15 min. Finally, a wine red uniformly dispersed solution was obtained.

Preparation of Au NPs-Ab_2 conjugates

The Au NPs-Ab_2 conjugates were prepared based on previous study.[2] First, the pH of the Au NPs solution (1mg/mL) was adjusted to 8.5 by adding 0.2 M K_2CO_3. Then, 200 μL Ab_2 (1mg/mL) was added into 20 mL Au NPs solution and incubated for 1 h at 4 ℃ with gentle shaking. Subsequently, 20 mg BSA was added to block nonspecific binding sites. After the mixture was shaken for 1 h, Au NPs-Ab_2 conjugates were centrifuged at 10000 rpm for 6 min. Finally, the obtained probes were dispersed in 5 mL PBS containing 1% BSA, 0.1% Tween-20, 10% sucrose, 5% maltose and 5% trehalose. The prepared conjugates stored at 4 ℃ for further use.

Preparation of test strips

The preparation of gold test strips was the same as those for Au@Pd@Pt NZs-

LFIA, except that the Au-Ab_2 conjugates were used.

The process of detection based on Au NPs-LFIA

First, 100 μL N-protein with different concentrations varying from 0.05 ng/mL to 100 ng/mL was dispensed on the sample pad. The liquid flow to the absorption pad due to capillary action, and the presence of T line and C line because of the formation of sandwich structure. After 10 min, a red band observed with naked eyes.

Immunoassay procedure based on ELISA

First, 100μL SARS-CoV-2 N-protein antibody 2D3 (5ng/mL) was dropped onto a 96-microwell plate and incubated 12 h at 4 ℃. After pouring out the liquid, 200 μL BSA was added into each well to block nonspecific binding sites. The blocking reaction was kept 37 ℃ for 1 h. Then, the well was washed with PBS containing 0.1 % Tween-20 (PBST) for three times and 100 μL of different concentrations of N-protein were added to each well. After incubating for 1 h at 37 °C, the well was washed five times with PBST to remove unbound N-protein. Subsequently, 100 μL biotinylated SARS-CoV-2 N-protein antibody 3F2 was added and incubated for 1 h at 37 °C. After unbound biotinylated SARS-CoV-2 N-protein antibody 3F2 was removed by washing with PBST, 100 μL streptavidin-HRP was added. After incubating for 30 min at 37 °C, the well was washed five times with PBST. Finally, 100 μL TMB substrate solution was added into each well and incubated for 15 min at 37 °C, followed by adding 50 μL stop solution to stop the reaction. The absorbance value at 450 nm was recorded with a microplate reader.

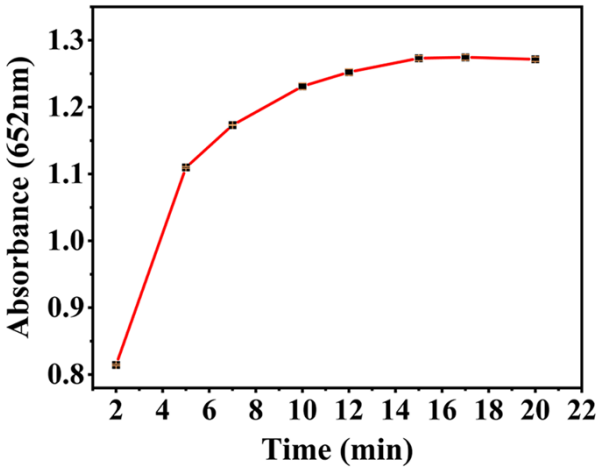

Fig. S1 The catalytical activity under different catalytical time.

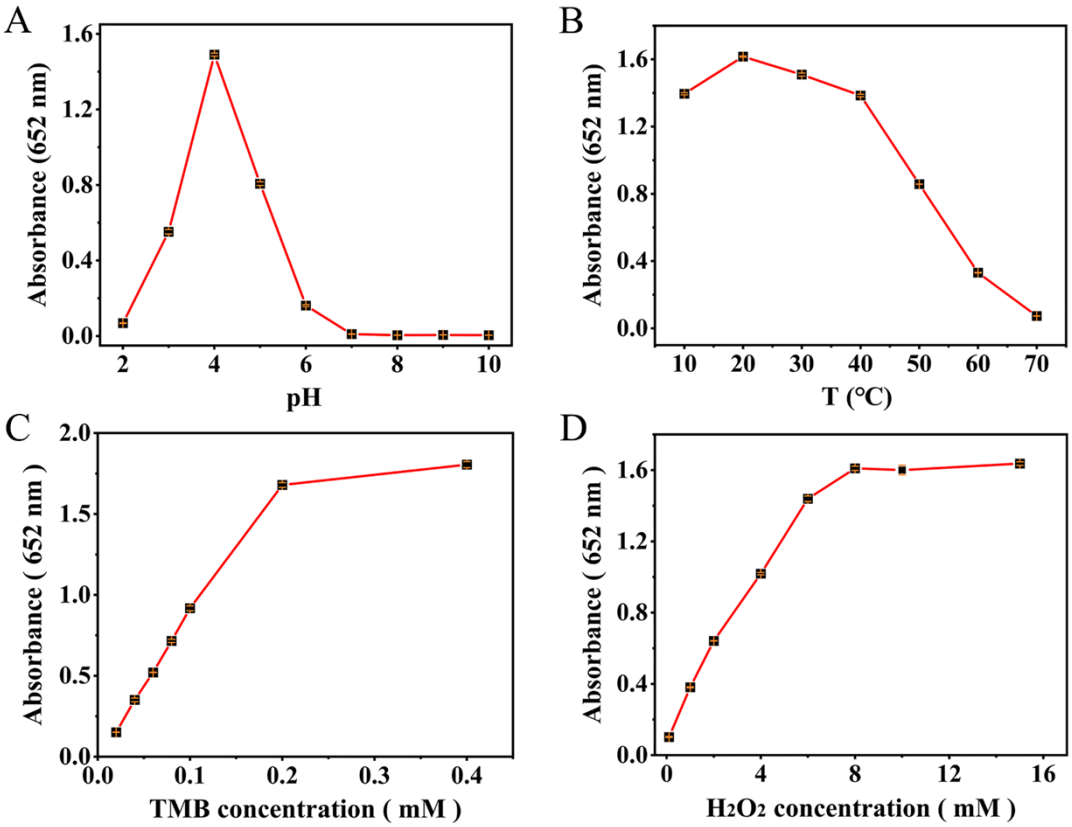

Fig. S2 Effects of different parameters on the peroxidase-like property of Au@Pd@Pt NZs: (A) pH; (B) temperature; (C) TMB concentration; (D) H_2O_2 concentration.

Table S1. Comparison of the K_m and V_{max} of Au@Pd@Pt NZs, HRP and other nanozymes.

Catalyst	K_m		V_{max} (10^{-8} Ms^{-1})		Ref.
	TMB	H_2O_2	TMB	H_2O_2	
HRP	0.434	3.70	10	8.71	3
Fe_3O_4	0.098	154	3.44	9.78	3
Au@Pt NPs	0.03	13.22	5.5	5.2	4
Au@Pd@Pt NZs	0.065	4.59	24.78	19.82	This work

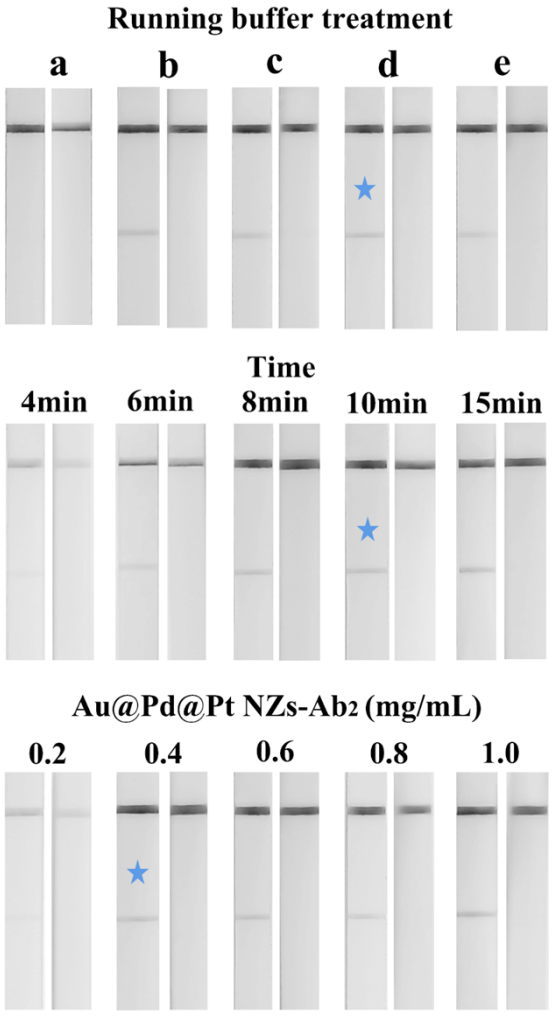

Fig. S3 The test strip images in the detection optimization.

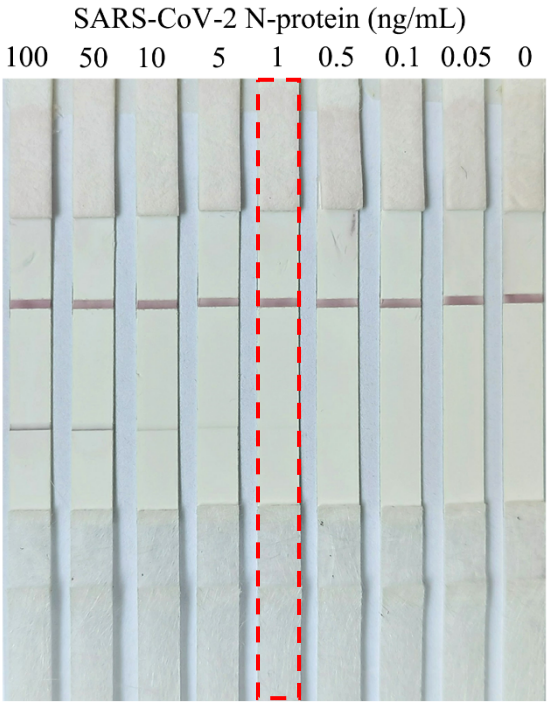

Fig. S4 The results based on Au NPs-LFIA with different N-protein concentrations ranging from 0 to 100 ng/mL.

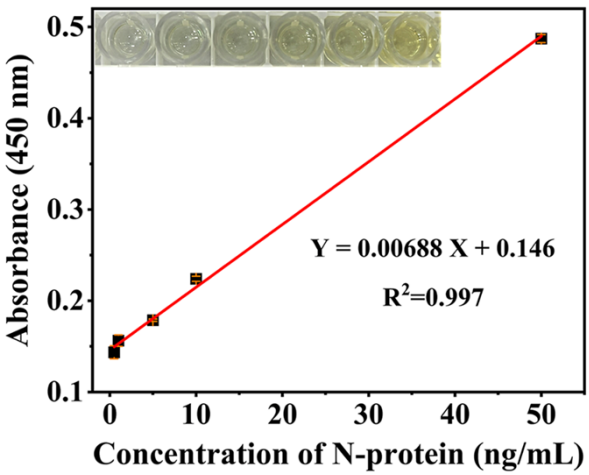

Fig. S5 The linear relationship between absorbance and the different concentrations of N-protein (0.5, 1, 5, 10, 50 ng/mL).

Table S2 Comparison of this work and reported LFIA for detection of N-protein.

Method	Material	Assay time	LOD (ng/mL)	Ref.
LFIA	Au NPs culster	20 min	0.038	5
LFIA	carboxylic red latex beads	20 min	0.65	6
LFIA	platinum-coated gold nanozymes	40 min	0.1	7
LFIA	Au@Pd@Pt NZs	25 min	0.037	This work

Table S3 Spiked-recovery test of N-protein spiked in human serum and artificial saliva (n = 3).

Sample	Added (ng/mL)	Detected (ng/mL)	Recovery (%)	RSD (%)
Human serum	1	1.08	107.9	1.88
	5	4.62	92.5	3.94
	10	9.97	99.7	1.06
Artificial saliva	1	1.04	104.4	1.05
	5	4.90	98.1	0.38
	10	9.78	97.8	3.73

References

1. G. Frens, *Nature*, 1973, **241**, 20–22
2. K. Zeng, D. Wei, Z. Zhang, H. Meng, Z. Huang and X. Zhang, *Sensors and Actuators B: Chemical*, 2019, **292**, 196-202.
3. L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett and X. Yan, *Nat Nanotechnol*, 2007, **2**, 577-583.
4. C. Lu, L. Tang, F. Gao, Y. Li, J. Liu and J. Zheng, *Biosens Bioelectron*, 2021, **187**, 113327.
5. H. K. Oh, K. Kim, J. Park, H. Im, S. Maher and M. G. Kim, *Biosens Bioelectron*, 2022, **205**, 114094.
6. B. D. Grant, C. E. Anderson, J. R. Williford, L. F. Alonzo, V. A. Glukhova, D. S. Boyle, B. H. Weigl and K. P. Nichols, *Anal Chem*, 2020, **92**, 11305-11309.
7. D. W. Bradbury, J. T. Trinh, M. J. Ryan, C. M. Cantu, J. Lu, F. D. Nicklen, Y. Du, R. Sun, B. M. Wu and D. T. Kamei, *Analyst*, 2021, **146**, 7386-7393.