Supporting Information

Visualized RNA detection of SARS-CoV-2 in a closed-tube by coupling RT-PCR with nested invasive reaction

Jingwen Shan,‡[a] Tao Wu,‡ [b] Wei Wei,[a] Jinling Huang,[a] Yijun Li,[a] Bingjie Zou,[a,c] Yi Ma,[d] Lunbiao Cui,*[b] Haiping Wu*[a,c] and Guohua Zhou*[a,c,d,e]

[a] *Department of Clinical Pharmacy, Jinling Hospital, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou, 510515, China*

[b] *NHC Key laboratory of Enteric Pathogenic Microbiology, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, 210009, China*

[c] *School of Life Science and Technology, China Pharmaceutical University, Nanjing, 210009, China*

[d] *State Key Laboratory of Analytical Chemistry for Life Science & Jiangsu Key Laboratory of Molecular Medicine, Medical School of Nanjing University, Nanjing, 210002, China*

[e] *School of Pharmacy, Nanjing Medical University, Nanjing, 211166, China*

Corresponding Authors

* Guohua Zhou, E-mail: ghzhou@nju.edu.cn

* Haiping Wu, E-mail: wuhaiping@smu.edu.cn

* Lunbiao Cui, E-mail: lbcui@jscdc.cn

Author Contributions

‡ Jingwen Shan and Tao Wu contributed equally to this work.

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Table S1. Sequences of PCR primers

Primers	Sequence (5′-3′)
N gene-F	GCAGTCAAGCCTCTTCTCGTTCCTCATCACGTA
N gene-R	AGTTTGGCCTTGTTGTTGTTGGCCTTTACCAGA
S gene-F	AAATGGAACCATTACAGATGCTGTAGACTGTGC
S gene-R	GGCGTTAAAAACTTCACCAAAAGGGCACAAGTT
Orflab-F	TACGCCAAGCTTTGTTAAAAACAGTACAATTCTG
Orflab-R	GGCATTAACAATGAATAATAAGAATCTACAACAGG
A570D-F	TGAGTCTAACAAAAAGTTTCTGCCTTTCCAACA
A570D-R	TGTAATGTCAAGAATCTCAAGTGTCTGTGGATCA
D80A-F	CCAATGTTACTTGGTTCCTGCTATACATGTCTC
D80A-R	ACTTCTCAGTGGAAGCAAAATAAACACCATCA
T547K-F	CAGCAACTGTTTGTGGACCTAAAAAGTCTACT
T547K-R	GCCAAATTGTTGGAAAGGCAGAAACTTTTTG
β-actin-F	CCCTGAACCCCAAGGCCAACCGCGAGAAGATGACC
β-actin-R	CATACCCCTCGTAGATGGGCACAGTGTGGGTG
T7-A570D-F	ACTCGTTAATACGACTCACTATAGGGAG
	TGAGTCTAACAAAAAGTTTCTGCCTTTCCAACA
T7-D80A-F	ACTCGTTAATACGACTCACTATAGGGAG
	CCAATGTTACTTGGTTCCTGCTATACATGTCTC
T7-T547K-F	ACTCGTTAATACGACTCACTATAGGGAG
	CAGCAACTGTTTGTGGACCTAAAAAGTCTACT

Note: F refers to forward primer; R refers to reverse primer; Underlined sequences are the T7 promoter.

Table S2. Sequences of probes

Probes	Sequence (5′-3′)
N gene-UP	TCTTGCTTTGCTGCTGCTTGT
N gene-DP	CGCGCCGAGG ACAGATTGAACCAGCTT
S gene-UP	TGATAGATTCCTTTTTCTACAGTGAAGGATC
S gene-DP	CGCGCCGAGG TTCAACGTACACTTTGTTTC
Orflab-UP	GAAATCATACCAGTTACCATTGAGATCTTGT
Orflab-DP	CGCGCCGAGG ATTATCTAATGTCAGTACACCA
A570D-UP	CGGACAGCATCAGTAGTGTCAC
A570D-DP	CGCGCCGAGG TCAATGTCTCTGCCAAAT
D80A-UP	GGGACCAATGGTACTAAGAGGTTTGT
D80A-DP	CGCGCCGAGG CTAACCCTGTCCTACCA
T547K-UP	CTCAGTAAGAACACCTGTGCCTC
T547K-DP	CGCGCCGAGG TTTAAACCATTGAAGTTGAAATTG
β-actin-UP	GCCAGAGGCGTACAGGGAC
β-actin-DP	CGCGCCGAGG TAGCACAGCCTGGATA

Note: UP refers to upstream probe; DP refers to downstream probe.

Table S3. Probes for self-assembling of AuNP-probes

Name	Types	Sequences (5'-3')	Modification
Hp	Hairpin probe	GTCTTGTGGTACTGCACT CGTCTCGGTTTTCCGAGA CGAGTCCTCGGCGCGATC GTGATGAACCAT	3'-Spacer C3
3'-AuNP-1	Nanoparticle probe	GCAGTACCACAAGACAAA AAAAAAA	3'-SH C6
5'-AuNP-2	Nanoparticle probe	AAAAAAAAAAATGGTTCA TCACGAT	5'-SH C6

Note: AuNPs, gold nanoparticles; Hp, hairpin probe.

Table S4. Probes for RT-PCR coupled with invasive reaction

Probes	Sequence (5′-3′)
FAM-N gene-DP	ACGGACGCGGAG ACAGATTGAACCAGCTT
FRET-hairpin probe	[T(FAM)]CT[T(BHQ1)]AGCCGGTTTTCCGGCTAAGACTC CGCGTCCGT

Note: DP refers to downstream probe.

Table S5. Components of buffer used for each reverse transcriptase

Reverse transcriptase name (buffer)	Components
Tth DNA polymerase (kit-recommended buffer)	250 mM bicine/KOH (pH 8.2), 575 mM K-acetate and 40% glycerol (v/v)
M-MLV reverse transcriptase (kit-recommended buffer)	50mM Tris-HCl (pH 8.3), 75 mM KCl, 3mM $MgCl_2$, 10mM DTT, 0.5 mM [^{3}H]-dTTP and 0.4mM poly A·oligo$(dT)_{12-18}$
AMV reverse transcriptase (kit-recommended buffer)	250 mM Tris-HCl (pH 8.3), 250 mM KCl, 50 mM $MgCl_2$, 2.5 mM spermidine and 50 mM DTT
HiScript II reverse transcriptase (kit-recommended buffer)	Unavailable from the instruction
HiScript II reverse transcriptase (visualized closed-tube PCR buffer)	10 mM Tris–HCl (pH 8.5), 7.5 mM $MgCl_2{\cdot}6H_2O$, 30 mM NaCl, 0.05% NP-40 and 0.05% Tween-20

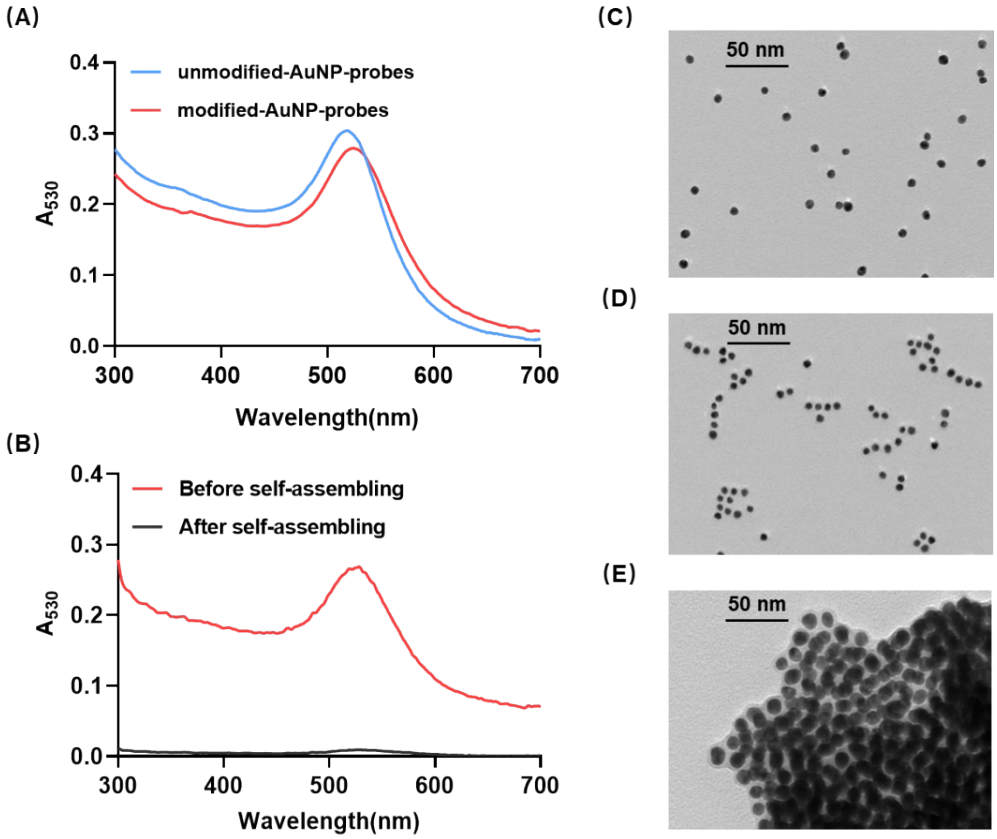

Fig. S1 Characterization of AuNP-probes. (A) UV-Vis absorbance (300-700 nm) of unmodified AuNPs and oligonucleotide probes-modified AuNPs. (B) UV-Vis absorbance (300-700 nm) for the AuNP-probes before and after self-assembling. The TEM images of gold nanoparticles (C), the free (D) and aggregated (E) AuNP-probes. Scale bar: 50 nm.

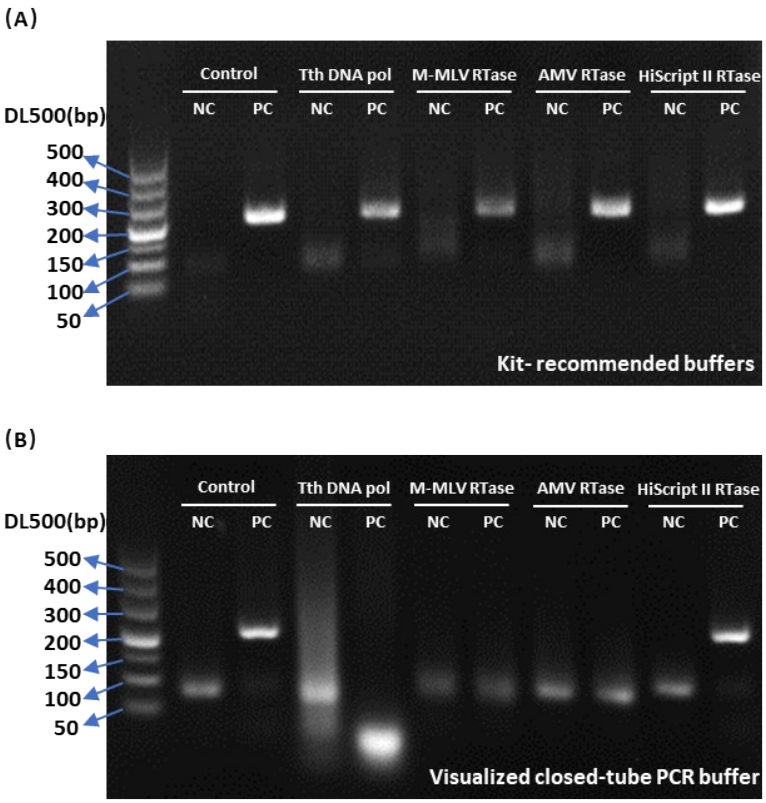

Fig. S2 Gel electrophoresis images of PCR products using cDNAs reverse-transcribed by each of Tth DNA polymerase, M-MLV reverse transcriptase, AMV reverse transcriptase and HiScript II reverse transcriptase at the corresponding kit-recommended buffers (A) and at the proposed visualized closed-tube PCR buffer (B). PC and NC mean PCR with and without the cDNA reverse-transcribed by individual reverse transcriptase indicated in each band, respectively; while the PC in control means PCR with the cDNA reverse-transcribed by HiScript II reverse transcriptase in kit-recommended buffer. A total of 2×10^5 copies of the N gene RNA of SARS-CoV-2 were reverse-transcribed into 20 μL, and a 2-μL aliquot was employed for each PCR.

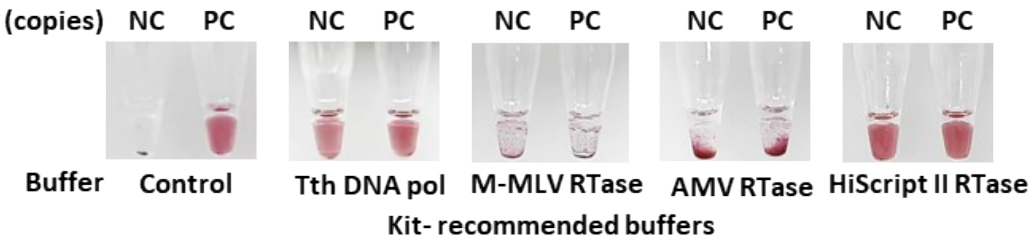

Fig. S3 Images of the visualized closed-tube RT-PCR at kit-recommended buffers for Tth DNA polymerase, M-MLV reverse transcriptase, AMV reverse transcriptase and HiScript II reverse transcriptase, respectively; while the control group was at the proposed visualized closed-tube PCR buffer. PC and NC mean with and without the target of 2×10^4 copies of the N gene RNA of SARS-CoV-2.

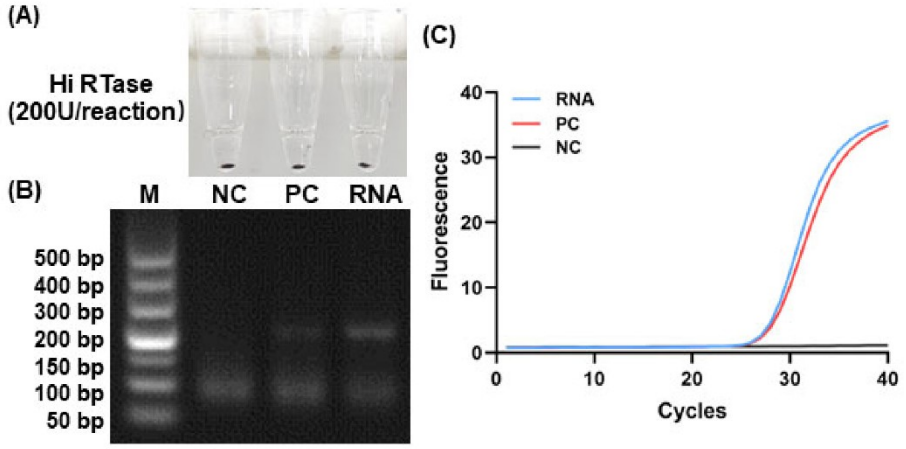

Fig. S4 Results of the visualized closed-tube RT-PCR using the kit-recommended amount (200 U) of HiScript II reverse transcriptase per reaction. (A) Image of the tubes after the visualized closed-tube RT-PCR. (B) Image of the agarose gel electrophoresis analysis of the products from the visualized closed-tube RT-PCR. (C) Real-time fluorescence analysis of RT-PCR (using HiScript II reverse transcriptase for RT) coupled with invasive reaction based on FRET hairpin probes to report signals. RNA means the N gene RNA (10^4 copies) of SARS-CoV-2. PC means the use of the DNA template (10^4 copies) amplified from the cDNA of the N gene of SARS-CoV-2 for assay. NC is the control without any target.

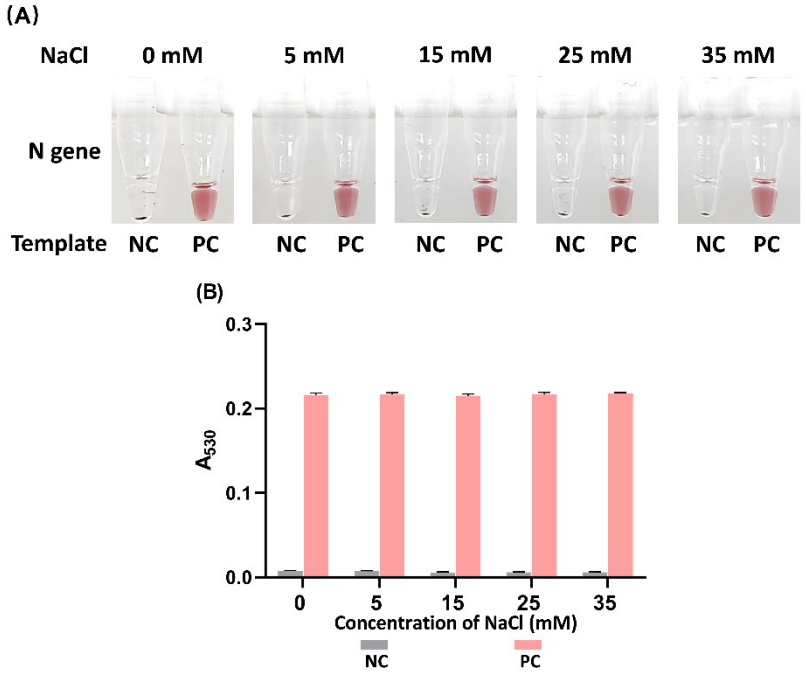

Fig. S5 Effect of the concentrations of NaCl on self-assembling of AuNP-probes in the visualized closed-tube RT-PCR. Images (A) and A_{530} of the PC and NC by the visualized closed-tube RT-PCR at 0 mM, 5 mM, 15 mM, 25 mM and 35 mM NaCl. PC means the use of the DNA template (10^4 copies) amplified from the cDNA of the N gene of SARS-CoV-2 for assay. NC is the control without any target.