Electronic Supplementary Information

Rapid, amplification-free and high-throughput SARS-CoV-2 RNA detection via reduced-graphene-oxide based fluorescence assay

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Table S1. Sequences of oligonucleotides.

Name	Description	Sequence (5′-3′)
Probe R	DNA in RdRP gene	GTGAAATGGTCATGTGTGGCGGTTCACTATATGTTAAACCAGGTGGAAC-Cy3
Target	Complementary RNA	GUUCCACCUGGUUUAACAUAUAGUGAACCGCCACACAUGACCAUUUCAC
M 1-1	One base-pair mismatch	**A**UUCCACCUGGUUUAACAUAUAGUGAACCGCCACACAUGACCAUUUCAC
M 1-2	One base-pair mismatch	GUUCCACCUGGUUUAACAUAUAGUGAACCGCCACACAUGACCAUUUCA**A**
M 1-3	One base-pair mismatch	GUUCCACCUGGUUUAACAUAUAGU**A**AACCGCCACACAUGACCAUUUCAC
M 2-1	Two base-pair mismatches	**A**UUCCACCUGGUUUAACAUAUAGUGAACCGCCACACAUGACCAUUUCA**A**
M 2-2	Two base-pair mismatches	GUUCCACCUGGUUUAACAUAUAGU**AU**ACCGCCACACAUGACCAUUUCAC
M 2-3	Two base-pair mismatches	GUUCCACCUGGUUUAACAUAU**U**GUGAACC**A**CCACACAUGACCAUUUCAC
Mis-RNA	Non-complementary RNA	AAGAAUACCACGAAAGCAAGAAAAAGAAGUACGCUAUUAACUAUUAACGUACCUGU
Probe E1	DNA in E gene	ACAGGTACGTTAATAGTTAATAGCGTACTTCTTTTTCTTGCTTTCGTGGTATTCTT-Cy3
Probe E2	DNA in E gene	GCTAGTTACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATA-Cy3

Note: Probe R was used in detections of above-mentioned short RNAs. Probe E1 and Probe E2 were only used in detection of SARS-CoV-2-pseudovirus long RNA, which contains ORF1ab consequence, N gene and E gene.

ORF1 a/b consequence:

ATCGTGTTGTCTGTACTGCCGTTGCCACATAGATCATCCAAATCCTAAAGGATTTTGT

GACTTAAAAGGTAAGTATGTACAAATACCTACAACTTGTGCTAATGACCCTGTGGGTT

TTACACTTAAAAACACAGTCTGTACCGTCTGCGGTATGTGGAAAGGTTATGGCTGTAG

TTGTGATCAACTCCGCGAACCCATGCTTCAGTCAGCTGATGCACAATCGTTTTTAAAC

GGGTTTGCGGTGTAAGTGCAGCCCGTCTTACACCGTGCGGCACAGGCACTAGTACTG

ATGTCGTATACAGGGCTTTTGACATCTACAATGATAAAGTAGCTGGTTTTGCTAAATT

CCTAAAAACTAATTGTTGTCGCTTCCAAGAAAAGGACGAAGATGACAATTTAATTGA

TTCTTACTTTGTAGTTAAGAGACACACTTTCTCTAACTACCAACATGAAGAAACAATT

TATAATTTACTTAAGGATTGTCCAGCTGTTGCTAAACAT

N gene:

ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGA

CCCTCAGATTCAACTGGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCGATCAAAA

CAACGTCGGCCCCAAGGTTTACCCAATAATACTGCGTCTTGGTTCACCGCTCTCACTC

AACATGGCAAGGAAGACCTTAAATTCCCTCGAGGACAAGGCGTTCCAATTAACACCA

ATAGCAGTCCAGATGACCAAATTGGCTACTACCGAAGAGCTACCAGACGAATTCGTG

GTGGTGACGGTAAAATGAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTAGGAA

CTGGGCCAGAAGCTGGACTTCCCTATGGTGCTAACAAAGACGGCATCATATGGGTTG

CAACTGAGGGAGCCTTGAATACACCAAAAGATCACATTGGCACCCGCAATCCTGCTA

ACAATGCTGCAATCGTGCTACAACTTCCTCAAGGAACAACATTGCCAAAAGGCTTCT

ACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCACGTAGTC

GCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGAACTTCTCCTGCTAGAA

TGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTGCTGCTTGACAGATTGAACCA

GCTTGAGAGCAAAATGTCTGGTAAAGGCCAACAACAACAAGGCCAAACTGTCACTAA

GAAATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTACTGCCACTAAAGC

ATACAATGTAACACAAGCTTTCGGCAGACGTGGTCCAGAACAAACCCAAGGAAATTT

TGGGGACCAGGAACTAATCAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGC

ACAATTTGCCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCATTGGCATGGAAGTC

ACACCTTCGGGAACGTGGTTGACCTACACAGGTGCCATCAAATTGGATGACAAAGAT

CCAAATTTCAAAGATCAAGTCATTTTGCTGAATAAGCATATTGACGCATACAAAACAT

TCCCACCAACAGAGCCTAAAAAGGACAAAAAGAAGAAGGCTGATGAAACTCAAGCC

TTACCGCAGAGACAGAAGAAACAGCAAACTGTGACTCTTCTTCCTGCTGCAGATTTG

GATGATTTCTCCAAACAATTGCAACAATCCATGAGCAGTGCTGACTCAACTCAGGCCT

AA

E gene:

ATGTACTCATTCGTTTCGGAAGAGACAGGTACGTTAATAGTTAATAGCGTACTTCTTT

TTCTTGCTTTCGTGGTATTCTTGCTAGTTACACTAGCCATCCTTACTGCGCTTCGATTG

TGTGCGTACTGCTGCAATATTGTTAACGTGAGTCTTGTAAAACCTTCTTTTTACGTTTA

CTCTCGTGTTAAAAATCTGAATTCTTCTAGAGTTCCTGATCTTCTGGTCTAA

Table S2. Comparison of two detection strategies

Strategy	Reactions	Read-out Time	Signal intensity
Strategy 1	Quench and Recovery	20 min	1-fold
Strategy 2	Hybridization and Quench	11 min	3-fold

Table S3. Comparison of different SARS-CoV-2 RNA detection methods

Method	LOD	Stability	Cost	Time	Ref
RT-PCR	100 cp/mL	high	~$3/reaction	4 h	[1]
RT-qPCR	5 cp/reaction	high	~$300/96 reactions	~1 h	[2]
LAMP	4.8 cp/μL	high	$750/96 reactions	30 min	[3]
ReadiLAMP	20 cp/reaction	high	$870/100 reactions	~30 min	[4]
Sherlock CRISPR	6.75 cp/μL	high	~$10/reaction	~1 h	[5]
CRISPR DETECTR	10 cp/μL	high	~$10/reaction	~30 min	[6]
Capacitive biosensor	10 nM	low	N/A	~1 h	[7]
Triple-mode biosensor	160 fM	high	N/A	40 min	[8]
Dual-functional plasmonic biosensor	0.22 pM	low	N/A	~ 15 min	[9]
rGO-FET Biosensor	223 cp/μL	low	N/A	~30 min	[10]
rGO-based biosensor	0.684 pM	high	~$3/ 96 reactions	15 min	This work

Note: "Time" is the consumed time from sample loading to signal reading. As for the stability, Figure 6C demonstrated the PL intensity of reaction system. The reaction liquid needs to be stored in black since the fluorescence degrades quickly when exposed to light. LAMP and PCR based detection is also based on the fluorescence intensity reading, and has similar stability with the proposed work. While plasmonic, capacitor and FET based biosensors are easily affected by storage environment after reaction, and the stability is relatively low. In Table S3, it is hard to quantify the stability by detailed data, so only "high" and "low" are used.

Table S4. Comparison of GO-derived biosensors

Material	Method	Target	LOD	Time	Ref.
rGO	Electrochemical	30 nt	100 fM	4 h	[11]
rGO	Fluorescence	60 nt	0.8 pM	35 min	[12]
GO	Fluorescence	30 nt	5 pM	1 h	[13]
GO	Fluorescence	30 nt	1.18 nM	30 min	[14]
rGO-FET	Electrical	22 nt	100 fM	1 h	[15]
rGO-FET	Electrical	25 nt	0.37 fM	~30 min	[10]
rGO	Fluorescence	49 nt	0.684 pM	15 min	This work

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