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## **Supporting information**

## A label-free electrochemical DNA biosensor used a printed circuit board gold electrode (PCBGE) to detect SARS-CoV-2 without amplification

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## Printed circuit board gold electrode (PCBGE)



**Figure S1**. Cyclic voltammograms (third cycle shown) of PCBEs in (A) 50 mM  $K_4Fe(CN)_6$  solution and (B) 1 mM RuHex solution at a scan rate of 0.1 V s<sup>-1</sup> were obtained using two different reference electrodes (REs) coated with silver (Ag) and gold (Au), respectively.

Table S1.	The calcul	ated $i_{\rm pa}/i_{\rm pc}$ v	value for two	different	PCBEs v	with silver (	(Ag) and	gold (	(Au)
coated ref	ference elec	trodes, resp	pectively in a	a 50 mM I	K <sub>4</sub> Fe(CN	$)_6$ solution	(Figure S	31A).	

Reference electrode (RE)	$i_{\rm pa}/i_{\rm pc}$	$E_{\rm peak}/{\rm mV}$	$E_{\rm mid}$ / mV
Gold (Au)	$1.06 \pm 0.02$	$186 \pm 22$	$-4 \pm 0.4$
Silver (Ag)	$1.26\pm0.06$	$212 \pm 12$	$153 \pm 9$

Table S2. The calculated  $i_{pa}/i_{pc}$  value for two different PCBEs with silver (Ag) and gold (Au) coated reference electrodes, respectively, in a 1 mM RuHex solution (Figure S1B).

Reference electrode (RE)	$i_{\rm pa}/i_{\rm pc}$	$E_{\rm peak}/{ m mV}$	$E_{\rm mid}$ /mV
Gold (Au)	$1.20 \pm 0.02$	$-71.6 \pm 3.7$	$-105 \pm 8$
Silver (Ag)	$1.30\pm0.02$	$-72.4 \pm 1.4$	$-299 \pm 11$



**Figure S2**. Suggested an equivalent circuit model utilized in convergently fitting the Nyquist plots from EIS measurements. Rs is the solution resistance, W is the Warburg constant, CPE is the constant phase element, and  $R_{ct}$  is the charge transfer resistance.

**Table S3.**  $R_{ct}$  values, fitting errors, convergence fit values, and convergence for the fitted Nyquist plots are shown in Figure 3B, based on an equivalent model circuit proposed in Figure S2.

Electrode	$R_{\rm ct}/{ m k}\Omega$	Fitting error /	Convergence fit (x <sup>2</sup> )	Convergence
		%		
Bare Au	0.15	2.24	0.075	Yes
Au-Thiolated ssDNA	0.58	1.02	0.053	Yes
MCH/Au-Thiolated	1.03	0.91	0.043	Yes
ssDNA				

**Table S4.**  $R_{ct}$  values, fitting errors, convergence fit values, and convergence for the fitted Nyquist plots are shown in Figure 4B, based on an equivalent model circuit proposed in Figure S2.

Electrode	$R_{\rm ct}/{\rm k}\Omega$	Fitting error /	Convergence fit (x <sup>2</sup> )	Convergence
		%		
DNA sensor	1.03	0.91	0.0426	Yes
DNA sensor + Target	3.06	3.84	0.2419	Yes
DNA (1µM)				



**Figure S3.** (A) Nyquist plots (fitting errors are shown in Table S5) and (B) charge-transfer resistance ( $R_{ct}$ ) values for DNA sensor-based PCBGE at different incubation times of target ssDNA (from 2 to 30 min). Statistical analyses were performed using the Tukey test. Data points are the mean values obtained in 3 independent experiments ± SD, and \*\*\* denotes p ( 0.001 and (ns) not significant.

Incubation time (min)	$R_{\rm ct}/{ m k\Omega}$	Fitting error / %	Convergence fit (x <sup>2</sup> )	Convergence
2	2.22	2.83	0.133	Yes
5	3.76	4.52	0.304	Yes
10	3.06	3.84	0.242	Yes
30	3.31	2.44	0.105	Yes

**Table S5.**  $R_{ct}$  values, fitting errors, convergence fit values, and convergence for the fitted Nyquist plots are shown in Figure S3, based on an equivalent model circuit proposed in Figure S2.

Table S6. $R_{ct}$ values, fitting errors, convergence fit values, and convergence for the fitted
Nyquist plots are shown in Figure 6A, based on an equivalent model circuit proposed in Figure
S2.

Electrode	$R_{ m ct}/ m k\Omega$	Fitting error / %	Convergence fit (x <sup>2</sup> )	Convergence
DNA sensor	1.03	0.91	0.0426	Yes
DNA sensor + Target DNA (1µM)	3.76	4.52	0.3041	Yes
DNA sensor + 1-Base Mismatch	1.11	0.91	0.0430	Yes

**Table S7.**  $R_{ct}$  values, fitting errors, convergence fit values, and convergence for the fitted Nyquist plots are shown in Figure 7A, based on an equivalent model circuit proposed in Figure S2.

Electrode	$R_{\rm ct}/{\rm k}\Omega$	Fitting error / %	Convergence fit (x <sup>2</sup> )	Convergence
DNA sensor	1.03	0.91	0.0426	Yes
1 µM	3.76	4.52	0.3041	Yes
3 µM	4.49	1.35	0.0230	Yes
5 μΜ	6.61	1.74	0.0044	Yes
8 μΜ	10.85	2.13	0.0582	Yes
15 µM	27.53	1.29	0.0322	Yes

**Table S8.**  $R_{ct}$  values, fitting errors, convergence fit values, and convergence for the fitted Nyquist plots are shown in Figure 7C, based on an equivalent model circuit proposed in Figure S2.

Electrode	$R_{ m ct}$ / k $\Omega$	Fitting error / %	Convergence fit $(x^2)$	Convergence
DNA sensor	1.03	0.91	0.0426	Yes
1 copy/µL	40.06	2.17	0.1262	Yes
10 copies/µL	70.19	3.58	0.2078	Yes
1000 copies/µL	82.56	2.91	0.1173	Yes
100000 copies/µL	166.25	2.83	0.1115	Yes



**Figure S4.** (A) Nyquist plots (fitting errors are shown in Table S9) for detection of synthetic RNA.

**Table S9.**  $R_{ct}$  values, fitting errors, convergence fit values, and convergence for the fitted Nyquist plots are shown in Figure S4, based on an equivalent model circuit proposed in Figure S2.

Electrode	$R_{ m ct}/ m k\Omega$	Fitting error / %	Convergence fit (x <sup>2</sup> )	Convergence
DNA sensor	0.879	1.356	0.064312	Yes
DNA sensor + Synthetic RNA (108 copies/µL)	13.59	1.60	0.0366	Yes

**Table S10.**  $R_{ct}$  values, fitting errors, convergence fit values, and convergence for the fitted Nyquist plots are shown in Figure 8A, based on an equivalent model circuit proposed in Figure S2.

Electrode	$R_{\rm ct}/{\rm k}\Omega$	Fitting error / %	Convergence fit (x <sup>2</sup> )	Convergence
DNA sensor	0.879	1.356	0.064312	Yes
DNA sensor + Negative sample A	1.8989	0.59	0.023294	Yes
DNA sensor + Negative sample B	1.8546	0.419	0.010508	Yes
DNA sensor + Negative sample C	1.702	0.614	0.02576	Yes
DNA sensor + Positive sample A	4.1693	0.744	0.037141	Yes
DNA sensor + Positive sample B	4.0779	0.549	0.02026	Yes
DNA sensor + Positive sample C	4.9554	0.699	0.032741	Yes