Supporting Information:

**A Supported Lipid Bilayers-based Lab-on-a-chip Biosensor for Rapid Electrical Screening of Coronavirus Drugs**

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1. Simplified physical model of the FET detection mechanism

The circuit model of the simplified lipid membrane is shown in Figure S1, which mainly contains two capacitors, \( C_{\text{FET}} \) and \( C_{\text{DL}} \). \( C_{\text{DL}} \) represents the double capacitor between the sensitive interface and the electrolyte solution. \( C_{\text{FET}} \) includes the “gate” dielectric capacitance between the FET and the electrolyte, \( C_{\text{OX}} \), in series with the semiconductor capacitance of the underlying gold sensor interface, \( C_{\text{SI}} \). Before the biomolecular binding, \( \pm \sigma_{\text{DL}} \) and \( \pm \sigma_{\text{FET}} \) represent the charge on \( C_{\text{DL}} \) and \( C_{\text{FET}} \), respectively. References (Shoorideh, Kaveh, and Chi On Chui. PNAS 111.14 (2014) : 5111-5116) give the rationale for this equivalent circuit model.

As can be seen from Figure S1, \( \Delta \sigma \) represents the charge at the sensing interface, and \( \Delta \sigma \) represents the charge disturbance introduced by specific binding. Due to the DC isolation of \( C_{\text{DL}} \) and \( C_{\text{FET}} \), the charge between \( C_{\text{DL}} \) negative electrode and \( C_{\text{FET}} \) positive electrode should be conserved. Thus, \( \Delta \sigma = -\Delta \sigma_{\text{DL}} + \Delta \sigma_{\text{FET}} \) (Eq. S1).

\[
\Delta V_{\text{FET}} = \frac{C_{\text{DL}} \Delta V_{\text{DL}} - \Delta \sigma}{C_{\text{FET}}} \quad \text{(Eq.S2)}
\]

According to Figure S1, \( V_{\text{Bias}} = V_{\text{DL}} + V_{\text{FET}} \) (Eq.S3). Taking the derivative of both sides with respect to \( \sigma \) yields \( \frac{dV_{\text{Bias}}}{d\sigma} = \frac{dV_{\text{DL}}}{d\sigma} + \frac{dV_{\text{FET}}}{d\sigma} \). And since the applied reference voltage \( V_{\text{Bias}} \) is constant and independent of \( \sigma \), \( \frac{dV_{\text{DL}}}{d\sigma} + \frac{dV_{\text{FET}}}{d\sigma} = 0 \) is always true regardless of how big \( \Delta \sigma \) is. Thus, \( \Delta V_{\text{DL}} = -\Delta V_{\text{FET}} \) (Eq.S4).

According to Eq.S2 and Eq.S4, it can be derived that

\[
\varphi = \Delta V_{\text{FET}} = \frac{\Delta \sigma}{C_{\text{DL}} + C_{\text{FET}}}
\]

which also demonstrates the sensor can detect the voltage shift of the gate electrode induced by the charge fluctuation of the sensitive interface.
2. Characterizations of the SLB-based sensing interface and modification process

First, the process of spontaneous vesicles adsorbs and rupture to form SLB was monitored by QCM-D results as shown in Figure S2. Continuous frequency decrease and dissipation increase revealed a vesicle constantly deposited on the chip surface. At 15 min, the frequency began increase and the dissipation began decrease to a stable plateau, showing a typical frequency shift of 22 Hz and dissipation changes of less than $0.1 \times 10^{-6}$ induced by ruptures of lipid vesicles. After introducing the ACE2 receptor solution and extensive PBS rinsing, the current continuously decreased due to the its negative-charged property (-20.9 mV) as shown in Figure S3a, indicating that ACE2 receptors were successfully embedded into the lipid bilayers by the electric perspective of FET.

The integrity of the phospholipid film after modification was characterized by cyclic voltammetry as shown in Figure S3b. Cyclic voltammetry tests (CHI 660E, Huachen, Shanghai) were conducted using $K_3Fe(CN)_6$ as a redox probe. The significant decrease of current illustrates the good quality and integrity of the SLB.

FRAP experiments were performed to verify the formation of SLB. Texas-Red labeled SLB were fabricated before bleaching. We defined a 50 μm circle as bleaching point and exposure the SLB to high-intensity excitation with a wavelength of 595 nm for 5 mins. Then, fluorescence intensity in
the bleached area was continuously collected for 5 mins. As shown in Figure S3c, the red fluorescence in the bleached area gradually recovered, indicating good lateral fluidity and bioactivity of the SLB.

Figure S3. Characterizations of the SLB formation process. a) The real-time FET current trace of the SLB formation and the ACE2 insertion. b) Characterization of the integrity of SLB by cyclic voltammetry. c) FRAP results of lipid bilayer. The images contain four periods: pre-bleaching, bleaching (2 min), 2 frames of post-bleaching (5 min).

The complete picture of the assembly results of ACE2 receptor with FITC fluorescence label is shown in Figure S4. The green fluorescence spots illustrate the successful assembly of ACE2 receptors and the uniform lateral distribution.

Figure S4. The full fluorescent images. a) SLB. b) The complex of ACE2 receptors with FITC label.

3. ACE2 receptors assembly optimization and density calculation
After the formation of SLB, we tested the assembly effect of ACE2 receptors with different concentrations through QCM-D to optimize the assembly of ACE2. As shown in Figure S5,
frequency changes of ACE2 were successively introduced into the QCM-D system at a series concentration of 0.01-50 μg/mL, and it was found by growth curve fitting that the maximum response change rate was obtained when the concentration was 1 μg/mL, indicating that the optimal assembly effect was obtained at this concentration. The assembly time was 30 mins, sufficient for the system to achieve a stable frequency response.

![Figure S5. Concentration optimization of ACE2 receptor assembly](image)

As shown in Figure S5, the fitting results show that ΔF of five times frequency at 1 μg/mL is 0.65 Hz and shows the maximum response change rate. Hence, 1 μg/mL was employed for next experiment.

4. characterization of sensor linearity and sensitivity

After SLB formation and ACE2 modification on the FET sensor, spike protein solution with concentration range of 0.001-50 μg/mL was introduced and the electrical response of FET was normalized as ΔI/I₀. As shown in Figure 3b, fitting of the linearity between the FET response and concentration indicates that our sensor has good linearity (R² = 0.99).

In addition, the sensitivity of the biosensor to spike protein was calculated to be 19.6 μA/log c[spike protein]. Here, LOD is defined in equation: 

\[
LOD = \frac{3 \times RMS_{noise}}{S}
\]

where RMS_noise is the noise standard deviation of FET sensor and S is the sensitivity as mentioned. Based on the sensitivity through linearity fit, the LOD was calculated to be 2.5 pM, significantly smaller than the report methods², indicating the great capability for sensitive detection.

5. characterization of sensor specificity

We also characterize the specificity of the sensor.

After SLB formation and ACE2 receptors assembly, we injected 1 μg/mL target molecules (α-hemolysin, spike protein, H1N1 virus, coronavirus) for 30 mins successively, as shown in Figure S6, the introduction of α-hemolysin and H1N1 virus basically generates rather smaller response compared with the introduction of spike protein and coronavirus respectively, indicating excellent specificity of sensor interface.
6. Calculation of the binding affinity

Figure S7a and S7b illustrate the real-time response of a gradient of spike protein solution (3, 6, 12, 25, 50, 100 µg/mL), respectively. The gradual decrease in QCM-D frequency illustrated the successful binding of spike protein and ACE2 receptors (Figure S7a). The higher the concentration, the larger the shift, and it reached saturation at approximately 12 µg/mL. Figure S7b shows that the wavelength of SPR shifted in sequence with an increase in spike protein concentration. The SPR and QCM-D results coincide, confirming that SLB membrane-doped ACE2 receptors were successfully constructed on the device surface and the SLB-based biosensor could stably detect the interaction between the ACE2 receptors and spike protein. The R-square values of the Hill fit by QCM-D and SPR methods were 0.98209 and 0.96403, respectively, indicating the goodness of fit.

Figure S7. The real-time trace of the capture of spike protein and the hill fit of the response. (a) The QCM-D frequency shift response of gradient spike protein. (b) The SPR wavelength shift response of gradient spike protein. (c) The hill fit of the relationship between the frequency shift with the gradient spike protein. (d) The hill fit of the relationship between the SPR wavelength shift with the
gradient spike protein. In Figure S7a and b, different background colors represent different solutions; Light gray: PBS buffer, light brown: ACE2 receptors solution, light blue: spike protein solution.

7. HD5 IC\textsubscript{50} validated by QCM-D
Inhibition of HD5 peptides was also validated with a commercial QCM-D. As shown in Figure S8, the response of HD5 inhibition show a typical s-shape growth, reaching saturation at about 50 \( \mu g/mL \). The calculated IC\textsubscript{50} value is 2.98 \( \mu g/mL \).

![Figure S8](image)

**Figure S8.** The IC\textsubscript{50} value of HD5 peptide was monitored and calculated by QCM-D

8. Preparation of vesicles
Large vesicles were prepared by extrusion method in order to form SLB on gold substrates using vesicle deposition method. Briefly, 100 \( \mu L \) solution of DOPC (10 \( mg/mL \)) in chloroform was desiccative by gentle nitrogen stream and removed residual solvent under high vacuum overnight to obtain a lipid thin film. The film was then hydrated with 1mL PBS buffer and mixed to form a suspension vesicle with polydisperse size and lamellarity. Next, the mixed solution was repetitively pushed through a polycarbonate membrane with 100 nm pore size in a Liposofast manual extruder (AVESTIN) to obtain a size uniform vesicle solution.

9. Quartz crystal microbalance (QCM-D) measurements
QCM-D measurements were performed with the Q-sense xx instrument (Biolin Scientific, Sweden) to monitor the frequency shift \( \Delta f \) and dissipation change \( \Delta D \). Quartz crystals with Au and SiO\textsubscript{2} coatings (diameter 14 mm, thickness 0.3 mm and resonant frequency 4.95 MHz) were used. The quartz sensors were cleaned before each measurement by sonication in 99.7% ethanol for 5 mins, rinsed by Milli-Q water, dried under nitrogen stream, and treated by air plasma for 1 min. All measurements were carried by a peristaltic pump with a flow rate of 50 \( \mu L/min \). Firstly, the buffer was pumped in to obtain a stable baseline and afterwards the vesicle solution were circularly added over the sensor chip for 30 mins. After the SLB formation, the buffer was pumped again for 10 min to rinse remaining vesicle. Secondly, the ACE2 receptor solution was circularly injected over 30 min to stabilize the insertion stage and rinsed by buffer again. Finally, the spike protein solution with different concentrations (0.001, 0.01, 0.1, 1, 10, 50 \( \mu g/mL \)) were added, respectively.

10. Surface plasmon resonance (SPR) analysis
SPR experiments were performed using the P4SPR portable instrument (Affinité Instruments,
Canada). It uses a SPR chip and a fluidic cell comprising three sensing channels and a reference channel. Thus, all samples were measured in triplicate. The vesicle solution was injected on the plasma pre-processed surface of dove prisms to form the SLB. The following steps was carried as same as the QCM-D measurements.

11. **Atomic force microscopy (AFM) analysis**

AFM (Nanowizard4, Bruker, German) was used to measure the morphology of SLB with ACE2 receptors and the complex of ACE2 receptors and SIV. In AFM imaging, we introduced dilution concentration of 100 times of ACE2 receptors assembled in lipid bilayers to obtain clear image scanning. Each step of the measurement was rinsed with plenty of PBS buffer after assembly to ensure the measurement process is free from interference by dissociative particles. All measurements were performed under QI mode using an SNL-10 probe with 2 $nm$ tip radius and a spring constant of 0.35 $N/m$ on mica substrate immersed in PBS buffer. All measurements were conducted in an isolation box at room temperature.

| Number | Sample          | Potential (mV) | conclusion       |
|--------|-----------------|----------------|------------------|
| (a)    | Vesicle         | -10            | Uncharged        |
| (b)    | ACE2            | -20.9          | Negative charged |
| (c)    | Spike protein   | -23.8          | Negative charged |

**Table S1.** Zeta potential of our samples

1. Shoorideh, K. & Chui, C. O. On the origin of enhanced sensitivity in nanoscale FET-based biosensors. *Proc. Natl. Acad. Sci. U. S. A.* 2014. **111**, 5111–5116.

2. Gao, B., Rojas Chavez, A. A., Malkawi, W. I., Keefe, D. W., Smith, R., Haim, H., Salem, A. K. & Toor, F. Sensitive detection of SARS-CoV-2 spike protein using vertically-oriented silicon nanowire array-based biosensor. *Sens. Bio-Sensing Res.* 2022. **36**, 100487.