Supporting Information

An FET-Based Flexible Biosensor System For Dynamic Behavior Observation Of Lipid Membrane With Nanoparticles In Vitro

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1. Fabrication of SLB membrane

The SLB membrane was fabricated by vesicle fusion method. First, 100μL DOPC solution (10 $mg \cdot mL^{-1}$ dissolved in chloroform) was dried with a nitrogen gas stream in a glass vial, then vacuumized overnight. The vacuum-dried lipid film was then hydrated with 1mL PBS buffer (0.1M) and mixed to form a suspension with vesicles of various sizes. Next, the vesicle suspension was repetitively pushed through a polycarbonate membrane with 100 nm pore size in a Liposofast manual extruder (AVANTI) to obtain a size uniform vesicle solution.

Before SLB deposition, the PDMS substrate was treated by plasma for 5 mins to forming the hydrophilic surface. 100ul vesicle suspension was added on the plasma-processed channel surface and stand for 1h at room temperature to form the supported lipid bilayer via vesicle fusion method. Excess vesicles were removed by rinsing with 10mM PBS buffer for three times respectively after deposition.

2. Stretch test on the biosensor system

Real-time FET currents of the biosensor system under stretch test are shown in Figure S1. In this experiment, the chambers are filled with 1M KCl solution and 0.1M PBS in the trans chamber and cis chamber, respectively, with bias voltage of 0.3V. External stretch begins at 20 seconds, the stretch and release at the same rate. For bare sensor (without SLB membrane integration), the output current remains stable, showing that the properties of electrode and the spike signals in the output current are not significantly affected by the overall stretching of the device (Figure S1a). For biosensor that integrated with the SLB membrane, the stretch cause significant changes in the current signals, especially for spike amplitude (Figure S1b). This phenomenon may be owing to the

structural changes of the lipid bilayers under stretching due to the deformation of the substrate. During the stretching process, the SLB membrane increases in transverse area, the phospholipids narrow in longitudinal spacing, becoming structurally denser, resulting in a decrease in current spike amplitude. During the releasing process, the SLB membrane decreases in transverse area, the phospholipids are squeezed and rearranged violently, becoming structurally unstable, resulting in an increase in current spike amplitude. This change in current indicates that the SLB membrane is stretched as the device is stretched.

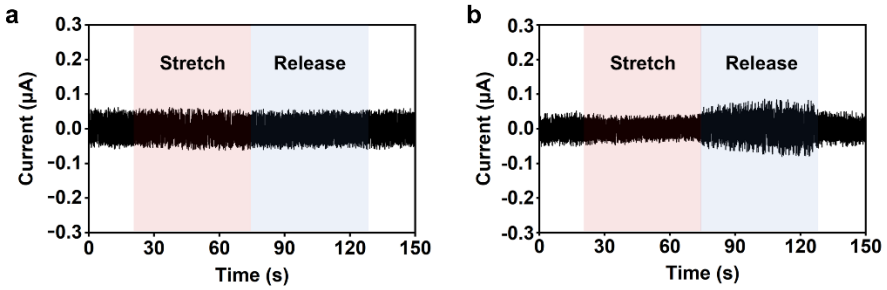

Figure S1 Real-time FET currents of the biosensor system under stretch tests. a) Stretch test on the bare sensor (without SLB membrane integration). b) Stretch test on the biosensor (with SLB membrane integration).

3. Characterization of Au Nanoparticles

The size of gold nanoparticles used in the experiment were characterized by TEM (Tecnai G2 F20, FEI), showed in Figure S2.

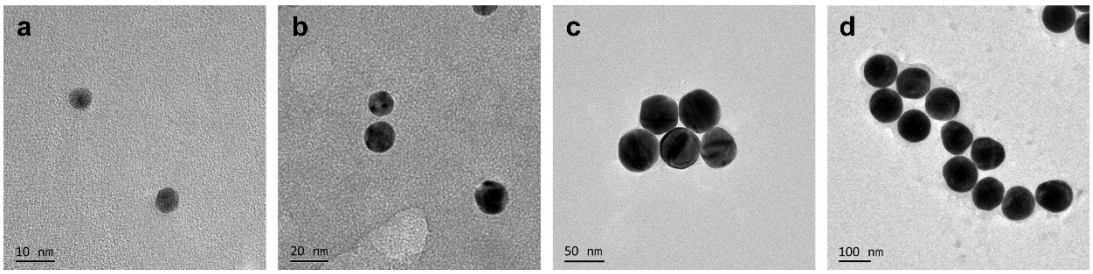

Figure S2. The TEM images of Au nanoparticles with diameter of 5nm (a), 15nm (b), 50nm (c) and 100nm (d).

4. Characterization of the SLB formation by EGFET-based biosensing system

The real-time FET current signals before and after SLB formation are shown in Figure S3. The chambers separated by the SLB are filled with buffer that have a large ion concentration difference (1M KCl solution in the trans chamber and 0.1M PBS in the cis chamber). The transmembrane currents are recorded in the constant bias of 0V, 0.1V, 0.2V, 0.3V respectively. The formation of the membrane results in a noticeable decrease in the current.

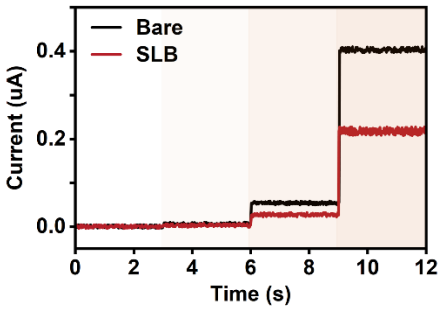

Figure S3 Characterizations of the SLB formation process by real-time FET currents.

5. Model of cell membrane interacted with a spherical nanoparticle

The model of the simplified interaction between the cell membrane and a spherical nanoparticle is shown in Figure S4. The grey sphere represents a nanoparticle which radius is *R*, and the curved lines represents the cell membrane, in which the black line represents the membrane segment contacting the NP and the green line represents the uncontacted membrane segment. The contact part (black line) produces the bending energy (C), the stretching energy (Γ) and the adhesion energy(W), and the uncontacted part (green line) produces the additional deformation energy (Λ). The up and down of the arrows of energies indicates that the energy prevents or promotes the particles to penetrate the membrane, respectively.

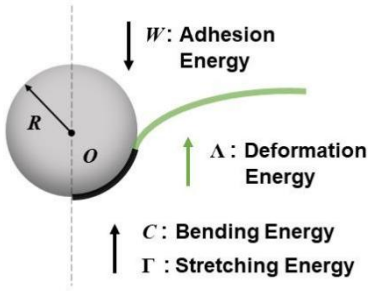

Figure S4. Model of NP-cell membrane Interactions.

6. Interaction between virus and lipid membrane

Figure S5 illustrate the statistics of mean spike amplitude of membrane currents in the interactions between virus and SLB membrane. As shown in Figure S5b, the SLB showed greater damage when interacted with larger viruses (seeing the 0% points), and the stretching of SLB promoted the internalization of virus through the membrane, similar to the statistic of spike numbers (Figure 7b).

Both damages that caused by two viruses to membrane cannot be recovered in a short time (Figure S5c). Compared to larger influenza viruses, the membrane damage caused by parvovirus appears to recover relatively well. With stretch of the membrane, the damage to the membrane caused by action of viruses is further deepened (Figure S5c and S5d). Notably, the damage induced by the influenza virus on membrane stretched for 20% becomes more severe and seems to be irreparable (Figure S5d).

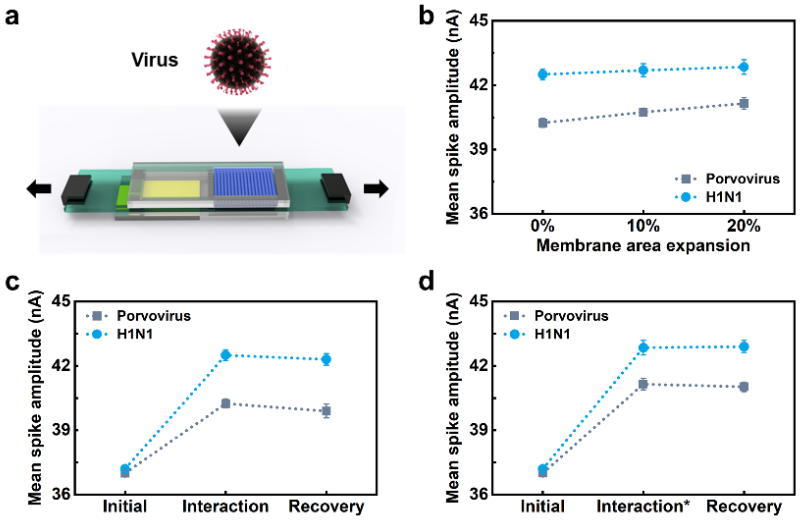

Figure S5. Interaction of viruses and SLB. a) Diagram of interaction between viruses and SLB. b) Effect of film expansion (area expansion 0%, 10% and 20%) on the interaction between two viruses and the SLB. c) Recovery from membrane damage from two viruses without stretching. d) Recovery from membrane damage from two viruses with membrane area expansion of 20%.

Table S1. Zeta potential of Au nanoparticles

Number	Sample	Potential (mV)	conclusion
(a)	AuNP 5nm	-7.2	Uncharged
(b)	AuNP 15nm	-3.4	Uncharged
(c)	AuNP 50nm	-2.4	Uncharged
(d)	AuNP 100nm	-5.4	Uncharged

7. Methods for Studying Nanoparticle-Membrane Interactions

Previous methods for studying nanoparticle–membrane interactions have mainly included cell experiments, model simulations, and in vitro membrane simulation systems. Model simulations allow for detailed dynamic modeling of the interaction process between nanoparticles and cell membranes [1], and the simulation studies of membrane–particle interactions have greatly contributed to the establishment of a theoretical framework. However, experimental data are still needed to validate the simulation results. Typical cell experiments can provide an overall view of the cellular morphology before and after nanoparticle treatment such as using fluorescence imaging method [2], without offering real-time membrane deformation or capturing the moment of particle internalization. Another type of cell experiment, such as ECIS [3], is a powerful method for measuring cell impedance and provides valuable insights into the overall changes in cells on a conductive substrate. However, researchers are also interested in understanding the transient changes in pure membranes induced by encounters with nanoparticles. In vitro membrane simulation systems typically use static membranes with no residual space beneath them [4]. In contrast, the sensor system proposed in this paper allows for quantitative stretching through an external machine, simulating the physiological activities of the lipid membrane, and also provides some space beneath the membrane.

Table S2. Comparison of research methods for nanoparticle-membrane interaction

Research Method	Research level of nanoparticle-membrane interactions	Whether dynamic simulation is possible	Whether it reflects the membrane transient changes	Reference
Model Simulation	Mechanism	Yes	Yes	1
Cell Experiment	Result	Partially	No	2, 3
Membrane simulation system in vitro	The process of nanoparticle embedding into the membrane	Yes	Yes	4
The method proposed in this paper	The process of nanoparticle penetration across the lipid membrane	Yes	Yes	-

Reference

[1] J. Lin, H. Zhang, Z. Chen and Y. Zheng, ACS Nano, 2010, 4, 5421-5429.

[2] B. D. Chithrani, A. A. Ghazani and W. C. Chan, Nano Lett, 2006, 6, 662-668.

[3] L. Schaller, K. Hofmann, F. Geiger and A. Dietrich, Applied Research, 2024, 3, e202400059.

[4] Y. Lu, H. Zhang, Z. Wang, S. Jeong, M.-C. Jo, M.-H. Park and X. Duan, Particle & Particle Systems Characterization, 2019, 36, 1800370.