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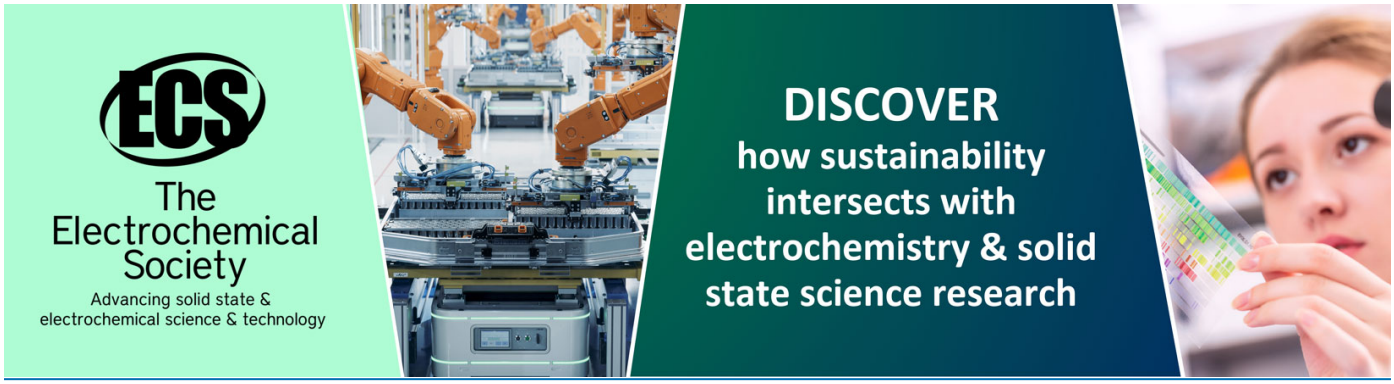

Formation of MspA channel on Nanopore-Spanning Lipid Bilayer

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Abstract. Biological nanopores are novel and versatile single-molecule sensor for individual label-free biopolymer detection and characterization. However, their studies and applications require a stable lipid bilayer to maintain protein function. Herein, we provided a method for producing lipid bilayers across nanopore array on a silicon nitride substrate by vesicle technique and MspA was embedded into lipid bilayer membrane formed ion channel. The formation process and stability of lipid bilayer membrane and translocation of samples were monitored real-time by patch clamp. The hybrid nanopore system exhibit excellent electrical properties and enhanced stability, making this system ideal for high-throughput DNA sequencing.

1. Introduction

Nanopore has attracted wide attentions in the past two decades as a method of single molecule detection and characterization. Traditional nanopore platforms include biological nanopore and solid-state nanopore. Biological nanopore mainly uses channel proteins which contain a narrow constriction for sequencing such as alpha hemolysin(α-HL)[1], mycobacterium smegmatis porin A(MspA)[2, 3], Aerolysin(AeL)[4, 5], etc. Solid-state nanopore is normally fabricated in thin membranes by using ion beam sculpting[6], the track-etch technique[7], the anodic oxidation[8] method, etc.

Due to the high sensitivity and versatility of nanopore sensing, nanopore platforms have obtained great development. But there remains a number of limitations with these systems mentioned above. For biological nanopore, it is difficult to change the protein pore size and the lipid bilayer lacks stability under different conditions. However, solid-state nanopore does not have such low signal noise compared with biological nanopore.

In order to combine the merits and avoid the shortcomings of solid-state and biological nanopore platform, hybrid nanopore platform is developed by researchers. For instance, insert a α-HL pore within a SiNx pore[9], embed carbon nanotubes within a lipid bilayer[10], the insertion of 3D DNA origami structures into SiNx nanopores[11] and so on. These methods bring various application but remain complicated. Furthermore, the BLMs are spanned over anodized porous alumina[12, 13], porous polycarbonate membranes[14] and glass nanopores[15, 16] in other researches. It has been proved that the lifetime of BLMs formed on solid-state nanopore array is longer than that of BLMs created over the micrometer-sized apertures in Teflon. In this work, we develop a novel hybrid nanopore using the method of producing lipid bilayer across nanopore array on a silicon nitride substrate by painting[17] and inserting mut-MspA M3. For the study of formation of the lipid bilayer membrane and ion channel

functions, patch clamp is chosen by us as the technique is able to monitor the actions of the lipid bilayer membrane and ion channels in real time, with high sensitivity and temporal resolution in the order of microseconds and sub-Pico amperes. Silicon nitride is chosen to form the lipid bilayer on the surface of it because of its smooth surface and stability under physical and acoustic vibration. MspA is a porin that its geometry reduces the sensing region to a short constriction (1.2 nm diameter and 0.6 nm length), which significantly improves separation of the current levels for nucleobases compared to the α-hemolysin which has a long β-barrel to produce a composite current signal. In our group previous study, we designed and expressed a novel mutant MspA in E. coli. The mut-MspA had good channel-formation ability and slowed down the DNA translocation. The hybrid nanopore is tested by translocating DNA sample. Such hybrid nanopore will open up a variety of applications including high-throughput analysis of ion-channel proteins.

2. Experiment

2.1. Materials

1, 2-Diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) was purchased from Avanti Polar, Lipids, Inc. Tridecafluoro-1, 1, 2, 2-tetrahydrooctyl trichlorosilane (F_{13}-OTCS) was got from Gelest Inc. Potassium chloride, N-hexadecane and decane were bought from Sigma-Aldrich. 5 mM PBS at pH 7.4 was used to construct BLMs. Alcohol and acetone were purchased from Sigma-Aldrich be used to process silicon nitride nanopores hydrophilization. MspA and DNA were obtained from Sangon Biotech Co (Shanghai, China). All solution was prepared with ultrapure water from Milli-Q water purification system (resistivity of 18.2 MΩ.cm, 25±1ºC, Millipore. US) and filtered through 0.02 μm Anotop filter (Whatman). The Dual-Beam Focused Ion Beam(DB-FIB) system was Helios 600i provided by Laboratory of solid state microstructures of Nanjing University.

2.2. Nanopore fabrication

First, two Si_3N_4 membranes (100nm and 500 nm thickness) were deposited on both sides of a 300-μm-thick Si substrate by low-power chemical vapor deposition(LPCVD). Followed by photolithography, a 500×500 μm^2 open window was formed on the thick membrane. Then, form a 50×50 μm^2 square on the other side of Si substrate by reaction ion etching and KOH etching. Finally, nanopore was drilled using Ga+ ions to bombard the surface of the thin membrane using DB-FIB system at an acceleration potential of 30 kV. The nanopore was characterized by Scanning Electron Microscope(SEM).

2.3. Formation of lipid bilayer and ion channel

A schematic of the experimental setup was presented in Figure 1. The Si_3N_4 layer was precoated with a layer of n-hexadecane by dropping a 5 μL aliquot of 0.1% n-hexadecane in chloroform, and then set it in the middle of a Teflon chamber. The chip separated two 1.4 mL compartments in the chamber. The chambers on both sides of the aperture were filled with 1.4 mL of buffer composed of 0.1 M KCl and 10 mM PBS at pH 7.4. After a stable bilayer was formed by vesicle method, 5 μL MspA in 10 mM PBS buffer was added to the cis side of the bilayer and +100 mV potential was applied. Typically, a single MspA channel inserted in 30-50 min. Successful insertion was indicated by an abrupt current increase to ~300 ± 10 pA.

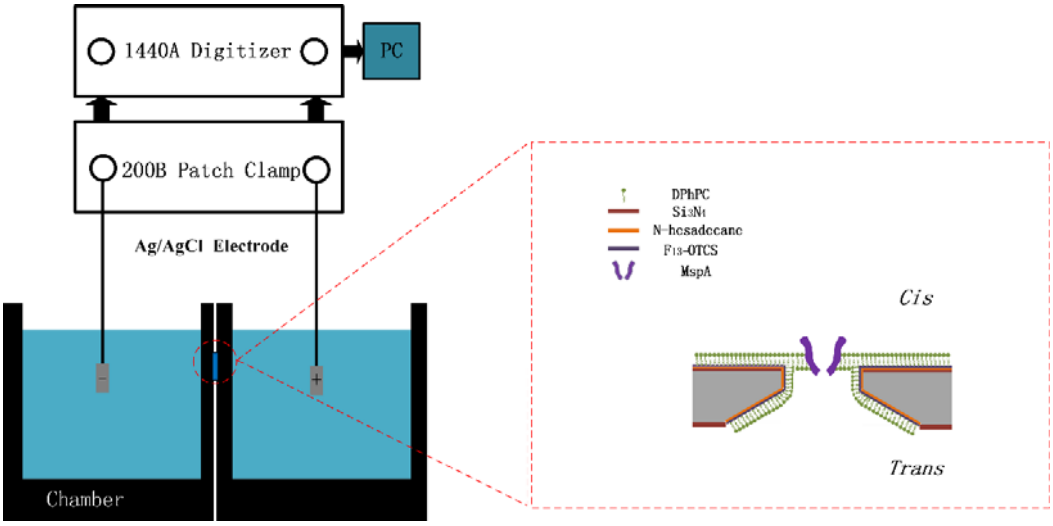

Figure 1. schematic of the experimental setup

3. Results and Discussion

3.1. Hydrophobic characterization

The contact angle measurement is illustrated in the case of drops (2 μL) of deionized water. Generally, if the water contact angle is smaller than 90°, the solid surface is considered hydrophilic. If the water contact angle is larger than 90°, the solid surface is considered hydrophobic. Contact Angle was measured by Theta Lite TL101(Biolin Scientific, Sweden). The hydrophobic treatment result of Si_3N_4 layer was presented in Figure 2.

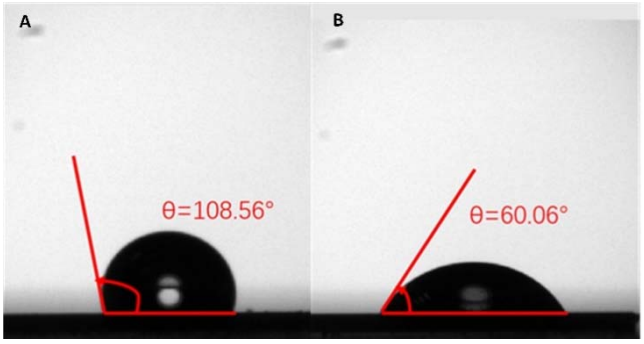

Figure 2. the contact angle measurement of Si3N4 after and before hydrophobic treatment

3.2. Bilayer stability and lifetime

Monitoring single ion channel activities in bilayers requires a membrane resistance Rm>1.0 GΩ. We took this value as a standard to assess the lifetime of lipid bilayers in nanopores. We investigated membrane lifetime of 90 nm (3×3 array), 120 nm (3×3 array) and 150 nm (3×3 array) diameter nanopores without incorporated channels containing 0.1 M KCl and 10 mM PBS buffer pH 7.4 at the applied voltage (±100 mV). The membrane resistances were monitored until it dropped significantly below the standard value of 1.0 GΩ. The results clearly demonstrate that the lifetime of lipid bilayers can be considerably improved with reducing pore size. Figure 3 shows the membrane lifetime of 90 nm (3×3 array), 120 nm (3×3 array) and 150 nm (3×3 array) diameter nanopores.

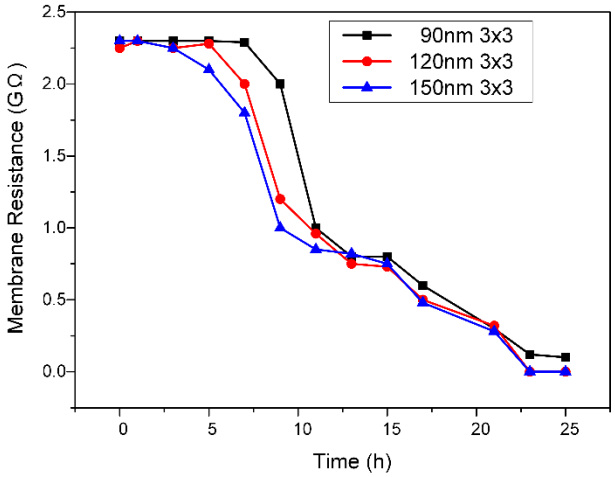

Figure 3. comparison on the time dependence of resistance of lipid bilayers with different sizes

3.3. Translocation of Poly dC100 through hybrid nanopore

After MspA channel was formed, bias potential (50mV and 100mV) was applied and 10 μL of poly (dC_{100}) samples (100 μM) was added into the cis chamber. Figure 4 shows the result of the type of all the translocation events.

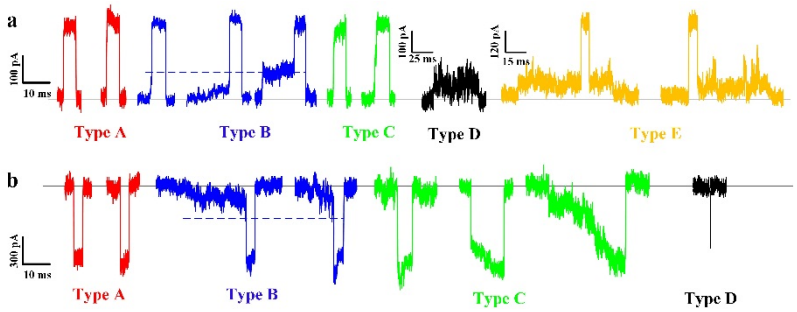

Figure 4. different types of ploy dC100 translocation events under 50mV and 100mV

4. Conclusion

In summary, we have succeeded in forming stable lipid bilayer membranes on nanopore array using vesicle method and insert MspA ion channel, which was carried out in the nanofluidic developed in our lab. The formation process and stability of lipid bilayer membrane and translocation of samples were monitored by patch clamp. The membrane capacitance (Cm) of the device with the bilayer was investigated to be ~20±2 pF and the membrane resistance (R m) was above 1.0 GΩ. The silicon nitride surface and aperture edge improve lipid bilayer membrane stability remarkably. The membrane lifetime was 5-24 h applied potential ±100 mV. A single MspA channel inserted in 30-60min applied potential +100 mV. The MspA channel current was recorded at ~ 300 ± 10 pA. This hybrid nanopore platform broadens the versatility of nanopore platform and paves the way for novel applications including high throughput DNA sequencing.

Acknowledgments

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