Probing surface hydrophobicity of individual protein at single-molecule resolution using solid-state nanopores

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Solid-state nanopore is found to be a promising tool to detect proteins and their complexes. Nanopore-protein interaction is a fundamental and ubiquitous process in biology and medical biotechnology. By translocating phi29 connector protein through silicon nitride nanopores, we demonstrate preliminarily probing the surface hydrophobicity of individual protein at single-molecule resolution. The unique “double-level event” observed in the translocation and the ratio of two current drop levels suggest that the position where the interaction occurs is the hydrophobic surface of the protein. We provide a potential method to locate the hydrophobic region of a specific protein surface. This study is of fundamental significance in revealing the important role that hydrophobic interaction plays in nanopore-protein interaction and holds great potential for detecting local surface chemical property of individual protein using solid-state nanopores.

INTRODUCTION

Nanopores, both biological nanopore [1–5] and solid-state nanopore [6–8], have been developed to be very promising candidates for biological detection at single molecule level, such as DNA [7,9–11], RNA [12,13] and proteins [14–23]. The detection of single molecules is realized through monitoring the temporary ionic current blockage during single molecule translocation through nanopore which is driven by external electric field. By monitoring and statistically analyzing these blockade currents, their durations, and the shapes of the blockades, the properties of biomolecules, such as size, structure, conformation, and dynamic motion can be decoded. The usage of nanopore-based techniques for the detection of biomolecules does not require labeling, surface immobilization, or biomolecular modification. Therefore, nanopore-based techniques are powerful meth-

ods for sensing single molecules. Nanopore technique has been shown to be a versatile tool for studying size, charge, or folding state of proteins [14,21,24–26] according to the modulation of ionic current caused by their translocation. Solid-state nanopores have prominent advantages of characterizing single molecules including the versatility of pore dimensions, robustness, longer lifetime, and high chemical resistance to denaturing agents [10,27–34].

Spontaneous adsorption of proteins onto solid-state surfaces [35] is of importance in a broad spectrum of areas, such as the design of nanofluidic devices and functional biomaterials, biosensors, and biomedical molecular diagnosis. Although valuable insight has been put on study of protein adsorption at the liquid-solid interface [23,36–39], this phenomenon is still not comprehensively understood yet. Study of protein adsorption is rather complex due to a multitude of electrostatic and hydrophobic forces among the side chains of the proteins and the reactive groups at the solid-liquid interface [36]. In a sense, solid-state nanopores offer great platforms to study this type of interaction since they can detect protein translocation process at single-molecule level in a nanoscale confined system. Adsorption of single molecule of BSA protein onto SiN nanopore surfaces has been observed, which was attributed to nonspecific, random, and spontaneous adsorption [37]. Repeated adsorption/desorption events have been observed in protein translocation through the nanotube detention zone [23]. Our understanding of nanopore-protein interaction is still at an early stage and needs further investigation.

The bacterial virus phi29 DNA-packaging nanomotor, with its intricate channel, has inspired applications in nanotechnology [9,11,40,41]. It has been demonstrated to have

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the capability to detect single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) at single-molecule level by inserting into planar lipid bilayers with an appropriate size ion channel for single DNA translocation [11]. The protein hub of this motor is a truncated cone structure, termed phi29 connector protein (Figs 1a and b), which allows double-stranded DNA (dsDNA) to enter during maturation and exit during infection. The protein has different chemical characteristic along its surface: the two terminal parts are hydrophilic while the middle part is hydrophobic (Fig. 1a). This peculiar surface chemical property inspires us to study the protein-nanopore interaction since hydrophilic and hydrophobic region may result in different interaction with the solid surfaces.

In this work, by studying the translocation behavior of the phi29 connector protein through SiN nanopores, we successfully probed the surface hydrophobicity of the

detected molecule at single-molecule resolution. The observed unique “double-level” translocation events can be explained by the hydrophobic interaction between the hydrophobic part of the protein and the SiN nanopore surface. The ratio of the two current drop levels of such “double-level event” indicates that it is the hydrophobic part of the protein where the interaction happens. To some extent, we provide a potential method to locate the hydrophobic region of a specific protein surface. Additionally, “anomalous” translocation of phi29 connector protein has been observed at pH 9. By comparison of measurement at pH 4, we attribute such “anomalous” translocation to the electroosmosis dominant protein transport. Our study is of fundamental significance in revealing the important role that hydrophobic interaction plays in nanopore-protein interaction. Solid-state nanopore provides unique platforms to study the interaction between protein and solid surfaces

Figure 1 (a) Side view of the phi29 connector protein showing the hydrophobic part, hydrophilic part and the diameter of the channel. “Big head” of the protein is the C terminal and “small head” is the N terminal. (b) Top view of the protein showing the diameter of the narrow part and wide part of the channel. (c) Schematic diagram of phi29 connector protein translocation experiment through SiN nanopore.

at single-molecular level.

RESULTS AND DISCUSSION

Fig. 1c shows the schematic diagram of the experimental configuration of the objective protein molecules (phi29 connector protein) translocation through a SiN nanopore. Generally, we fabricated a single nanopore in a freestanding membrane of 80 nm thick low-stress SiN membrane using the focused electron beam of a transmission electron microscope (TEM). The nanopore chip sealed with polydimethylsiloxane (PDMS) gaskets is mounted between two

polyether ether ketone (PEEK) chambers. The *trans* and *cis* chambers full of ionic solution and electrically contacted with Ag/AgCl electrodes are isolated by the chip so that the single SiN nanopore is the only transmembrane channel connecting these two parts. Charged phi29 connector proteins can be driven through the nanopore when a bias voltage is applied across the membrane. Change of the ionic current with time is monitored, implying the translocation of individual phi29 connector protein. The protein contains 12 copies of gp10 subunits, each having 3 α -helices, to form a central channel. The diameter of the widest and nar-

Figure 2 (a) Current trace of phi29 connector protein translocation measured under 100 mV (0.5 M KCl, 5 mM Tris, pH 9), presenting stable and smooth trace. Inset: TEM image of the used SiN nanopore. Scale bar: 40 nm. (b) Scatter plot of current blockage vs. event duration of phi29 connector protein translocation under voltage of 125 mV (blue, $N = 1683$) and 175 mV (red, $N = 1401$). (c, d) Histogram of event duration of Fig. 2b showing the exponential tail fit. Characteristic event duration, τ , is defined by an exponential decay fit to the tail of the event duration histogram. (e) Distribution of current blockage of Fig. 2b showing the most probable current blockage is in direct proportion to the applied voltage. (f) Calculated open current of 13 nA (black square) and normalized current blockage of 29 pA (red circle) as a function of nanopore radius and nanopore length.

rowest channel is 6 nm and 3.6 nm, respectively (Fig. 1b).

Fig. 2a shows a typical current trace after phi29 connector protein is added to the *cis* reservoir (pH 9, 0.5 M KCl, 5 mM Tris), presenting a stable and smooth trace with high throughput. We call each temporary drop in ionic current, which is attributed to a single phi29 connector protein translocation through the pore, “a translocation event”. Each translocation event can be characterized by two parameters: current blockage and event duration. Fig. 2b shows the scatter plot of current blockage *vs.* event duration of phi29 connector protein translocation under two different voltages. Event durations mainly fall between 1 and 10 ms, implying that phi29 connector protein has interaction with the hydrophobic surface of SiN membrane during translocation. Event duration of protein translocation in previous work was usually within several hundreds of microseconds, resulting in a limited temporal resolution hindering the characterization of many biomolecules [16,42,43]. Fologea *et al.* [16] successfully probed BSA molecules using SiN nanopores, which has similar molecular weight as phi29 connector protein, with most probable translocation time of nearly 110 μ s (0.4 M KCl, 120 mV, 16 nm diameter nanopore). Events with such short translocation time could be reasonably recognized as free translocations with no strong interaction between the protein molecule and the nanopore, which was also observed in our experiments. However, we also observed that more than 70% events had a translocation time more than 1 ms (both at 125 mV and 175 mV in Fig. 2b), implying strong interaction between the protein and the nanopore. The interaction dominated long-lived protein translocations have high temporal resolution and bear great potential to sense more specific structure of protein molecules. Histogram of event duration (Figs 2c and d) shows the mono exponential tail fit, indicating the presence of the protein-pore interaction [44]. Characteristic event duration, τ , defined by an exponential decay fit to the tail of the event duration histogram, declines as the bias voltage rises. Fig. 2e shows the Gaussian distribution of the current blockage under 125 (red) and 175 mV (blue). Mean current blockages are 36 and 49 pA, respectively, in direct proportion to the applied voltage, revealing similar behavior of DNA translocation where current blockage increases linearly with the applied voltage [45].

Several evidences demonstrate that the current blockage signal reveals a real translocation process of phi29 connector protein through nanopores. Firstly, linear increase of current blockage as a function of applied voltage (Fig. 2e) is a strong evidence for real translocation behavior. Secondly, Poisson-Boltzmann formulation was used to cal-

culate the openpore current and DNA translocation blockage in nanopores in our previous work [46]. By setting the DNA radius as 1.25 nm, due to the Stern layer effect on DNA solution interface, and associated with other original boundary condition setting, the pore-geometry-variation curves based on a specific openpore current or DNA blockage can be independently mapped. The crossing point of those two curves can determine the pore geometry (pore radius and pore length) at a certain openpore current and DNA blockage [31]. Here, by applying the Poisson-Boltzmann formulation and setting the original boundary conditions, we realize the simulation of pore-geometry-variation curves based on the specific openpore current and protein translocation blockage (Fig. 2f). In our experiment under 100 mV, the openpore current is 13 nA and the mean protein blockage is about 29 pA. We can get a pore radius of 34.5 nm by obtaining these two curves then finding the point of intersection, which is in agreement with the value 33 nm obtained from TEM figures (Fig. 2a inset). Thirdly, the current blockage can be used to estimate the volume of protein. Protein diameter can be estimated from the current blockage data based on [47]

$$d_{\text{pro}}^3 = s \frac{\Delta I}{I_0} (l_{\text{pore}} + 0.8d_{\text{pore}}) d_{\text{pore}}^2 \quad (1)$$

where d_{pro} is the equivalent protein diameter, d_{pore} is the pore diameter, l_{pore} is the pore length, ΔI is the statistical mean current blockage and I_0 is the mean openpore current. According to the Poisson-Boltzmann calculation based on the translocation experimental data, the calculated protein volume falls in a range between 483 and 642 nm³ by considering that the phi29 connector protein has a spherical geometry. The total volume of phi29 connector protein (560 nm³), according to simple calculation of Fig. 5a, fits well within the range calculated from conductance and current blockage, which is another strong evidence for real translocation of protein through nanopores.

During analyzing translocation event signal of phi29 connector protein, one kind of anomalous events have been observed (Fig. 3a). Such events have two different current blockage levels, I_1 and I_2 , and two time durations, t_1 and t_2 , correspondingly. We call such signals as “double-level events” compared to the normal “single-level events”. These double-level events cannot resemble the partially folded events in DNA translocation (Fig. 3b) since the protein will be stable in our solution surroundings without folding. Additionally, double-level events always show a shallower blockage I_1 first followed by a deeper blockage I_2 , which is different from the partially folded events of DNA translocation who has a deeper blockage first (Fig. 3b). We

Figure 3 (a) Current trace showing two types of typical events of phi29 connector protein translocation: single-level event (left) and double-level event (right). (b) Three types of typical translocation events of dsDNA through SiN nanopores. (c) Histogram of I_1/I_2 about all 160 detected double-level events in Fig. 2b (125mV: 94 and 175mV: 66) showing that most of the ratios fall into 0.5 to 0.8.

also found that the ratio of I_1 and I_2 fell into a certain range. 90% of the I_1/I_2 values of all detected double-level events fall within the range from 0.55 to 0.84, as shown in the distribution plot of Fig. 3c. Another three parallel experiments show similar findings of “double-level events” and the certain range of I_1/I_2 (Fig. 4). Over 90% of the I_1/I_2 values fall into the range of 0.55 to 0.84 in each experiment, as shown in Fig. 4. The certain value of I_1/I_2 suggests that it may be the certain region of protein molecules that interacts with nanopore wall. It should be noted that the “double-level events” only account for a small portion of the total detected events: for the three parallel experiments, the number of “double-level events” is 215, 443 and 651, respectively, with the total events number of ~3500, ~7000 and ~10,000, respectively.

Protein adsorption is a complex process involving van der Waals, hydrogen bonding, hydrophobic interaction and electrostatic interaction. Surface-protein interaction has been proved to be affected by underlying surface chemical property, structural information of protein surface and surface topography, among which surface chemical property has been shown to play a fundamental role in protein adsorption. From Fig. 1a, three segments of phi29 connec-

tor protein have different surface chemical properties, with two segments showing hydrophilic property and the middle segment showing hydrophobic property. The unique surface chemical property inspires us to probe the role it plays in the interaction between protein and the hydrophobic nanopore surface. Roach *et al.* [38,39] reported that proteins had a much higher binding affinity toward hydrophobic rather than hydrophilic surface. They implied that it was the hydrophobic region rather than the hydrophilic region of protein surface that contributed to surface-protein interaction, which can be explained as hydrophobic interaction. Then hydrophobic interaction possibly accounts for the adsorption (or interaction) between protein and inorganic surface (SiN), therefore, the hydrophobic region of the phi29 connector protein may prefer to interact with the hydrophobic SiN nanopore surface.

Next we try to explain why the “double-level event” shows a shallower blockage first followed by a deeper blockage and associates with hydrophobic interaction. Fig. 5a is the equivalent schematic showing the geometrical structure of phi29 connector protein. It can be regarded as three segments of cylinder with different inner and outer diameters as well as different surface chemical properties.

Figure 4 Another three experiments of phi29 connector protein translocation at pH 9 under voltage of 100 mV with nanopore radius of 21–23 nm. (a, b, c left) Scatter plot of current blockage vs. event duration of phi29 connector protein translocation events (with total number more than 3500, 7000 and 10,000 respectively). (a, b, c right) Histogram of I_1/I_2 about detected double-level events (with number of 215, 443 and 651, respectively) showing excellent accordance to Fig. 3c with more than 90% falls into 0.5 to 0.8.

The volume of the three segments of phi29 connector protein from top to bottom (Fig. 5a) are V_1 , V_2 , V_3 , respectively, and the middle part has hydrophobic surface while the top and bottom parts are hydrophilic. As is only 7.5 nm in length, the protein cannot fulfill the entire SiN nanopore along the longitudinal direction, compared to DNA translocation, since the effective nanopore thickness is usually more than 40 nm in our experiment. Considering that the blockage is in proportional to the nanopore volume occupied by the protein, therefore, a shallower current blockage is observed if the protein is partly inside the nanopore when the interaction occurs as shown in Figs 5b and c. The shallower current blockage I_1 (Fig. 3a) can be interpreted as

phi29 connector protein interacting with nanopore wall for a certain time (t_1 in Fig. 3a) with only part of its volume inside the nanopore. Then deeper I_2 is caused by desorption of protein from nanopore wall with its entire volume inside the nanopore (Fig. 5d).

The certain value of I_1/I_2 inspires us to investigate the certain region that the hydrophobic interaction occurs. From the dimension shown in Fig. 5a, we can get $V_1 = 305.5 \text{ nm}^3$, $V_2 = 165.7 \text{ nm}^3$, $V_3 = 88.6 \text{ nm}^3$, and total $V = V_1 + V_2 + V_3 = 559.8 \text{ nm}^3$, respectively. Assuming that C terminal (big head) of phi29 connector protein moves in the front and considering that the interaction site is among the hydrophobic region V_2 , I_1 should be caused by V_1 at least,

Figure 5 (a) Equivalent schematic diagram of phi29 connector protein showing three segments with corresponding channel sizes and different surface properties. (b–d) Schematic illustration indicates the location where double-level events might occur. (b, c) The adsorption of protein occurs as part of the phi29 connector protein is inside nanopore and part is outside of nanopore. The interaction occurs at the hydrophobic part of protein, as indicated by grey color. (d) After desorption, during translocation, protein has its entire volume inside the nanopore.

and $V_1 + V_2$ at most, which is in the range of $V_1 \sim V_1 + V_2$, as shown in Figs 5b and c. I_2 is caused by the entire V , which is $V_1 + V_2 + V_3$, as shown in Fig. 5d. Thus the value of I_1/I_2 should be in the range of V_1/V and $(V_1 + V_2)/V$. The calculation results of I_1/I_2 give a range from 0.55 to 0.84, revealing good accordance with the experimental value of I_1/I_2 (Figs 3c and 4), in which the value is mainly distributed between 0.5 and 0.8. I_1/I_2 should not present such value if the interaction occurs at location in V_1 or V_3 . Therefore, the observed anomalous “double-level events” can be reasonably attributed to the hydrophobic interaction, and the interaction occurs at, or at least, near the hydrophobic region (middle part) of phi29 connector protein. It is essential to make it clear that the double-level events are only part of the interaction-dominated events. If the interaction happens when the protein is totally inside the nanopore, events only present long translocation time without “double-level” shape, as supported by our data in Figs 2b and 4. As the protein size is much smaller than the pore, the small amount of double-level events (5.2% of all the events) is reasonable as there is big probability that the protein interacts with the pore totally inside. To some extent, we offer a promising method to probe the surface hydropho-

bicity of a specific protein on its surface region during its translocation through solid-state nanopores, which holds great potential on more accurate detection of local surface physical or biochemical modification of proteins and other biomolecules with solid-state nanopores. Further study of the orientation of protein translocation and more specific location will be conducted.

We also observed “anomalous” translocation of phi29 connector protein through SiN nanopores at pH 9: negatively charged proteins crossed the pore toward the negatively charged electrode. The net charge of phi29 connector protein as a function of pH is shown in Fig. 6a by calculation according to the pKa of every amino acid of the protein. At pH 9, the entire phi29 connector protein is negatively charged. We observed that the negatively charged phi29 connector proteins passed across the nanopore from the positively charged electrode toward the negatively charged one at pH 9. As the electric force exerted on the protein is toward the *cis* side where we added the sample, there should be some other force or effect that pushes phi29 connector protein passing through the nanopore from *cis* to *trans* side. In fact, such “anomalous” translocation across SiN nanopore was reported in previous work and the elec-

trosmotic (EO) effects were proved to play a vital role in these translocations [48–50]. EO effect may enhance or counteract electrophoresis and it is the combined action of electrophoretic (EP) and EO forces that governs the translocation direction of protein across the nanopores [48].

The protein and wall of SiN nanopore is negatively charged on the whole at pH 9 so the electrophoretic transport is toward the *cis* side while the EO transport is toward the *trans* side. The observed “anomalous” translocation indicates that EO effect overwhelms electrophoretic effect at pH 9 (Fig. 6b). Phi29 connector protein contains only −11.46 unit charges at pH 9, which are nearly negligible compared to that of DNA with the same molecular weight, so the electric force on the protein is several orders smaller. Meanwhile, phi29 connector protein is more inclined to be pushed forward by the axial force of EO flow while linear DNA is mainly affected by the tangential force of EO flow. That may qualitatively account for why such “anomalous” translocation is mostly observed in protein translocation. The disappearance of any translocation signal at the reverse voltage (*cis* side: electrically grounded, *trans* side: 100 mV) also proves this speculation. As the EO flow exists near the

nanopore surface, it will exert bigger force on the protein during its translocation through smaller pores. Therefore the translocation time will be generally shorter. That could help to explain the smaller portion of the interaction-dominated events in Fig. 4 compared to Fig. 2b.

We also conducted the translocation experiments at pH 4 when phi29 is positively charged to further confirm whether the explanation of EO flow was reasonable in determining the translocation direction. At pH 4, only when negative potential was applied to the *trans* side did we observe translocation signals in the current traces which signified phi29 connector proteins passing through nanopore. At pH 4, the protein is positively charged (Fig. 6a) and EO flow can be negligible since the SiN nanopore is nearly electro neutral on the wall at this pH [51]. So translocation can only be observed when the negative potential is applied to *trans* side as the electrophoretic force drives the protein to pass through. The “normal” translocation is observed at pH 4 at the absence of EO flow, which confirms our explanation of the “anomalous” translocation at pH 9. Fig. 6c shows the scatter plot of current blockage vs. event duration at $V = 100$ (red) and 150 mV (blue) at pH 4. Due to

Figure 6 (a) Calculated net charge of phi29 connector protein as a function of pH. (b) Schematic illustration of phi29 connector protein translocation experiments at pH 9 and pH 4. Arrows represent electroosmotic and electrophoretic effects, and arrow length represents only relative value. (c) Scatter plot of phi29 connector protein translocation events at $V = 100$ mV (red) and 150 mV (blue), pH 4, 0.5 M KCl, 5 mM Tris. (d) Distribution of current blockage at $V = 100$ mV (red) and 150 mV (blue).

the absence of EO flow at pH 4, small electrophoretic force makes the translocation slower compared to that at pH 9, and several event durations nearly reach 10 seconds. The most probable current blockage shown by Gaussian fitting (Fig. 6d) is proportional to the applied voltage, which agrees with what has been analyzed at pH 9.

CONCLUSION

In conclusion, the observed unique “double-level event” in translocating single phi29 connector protein molecules through SiN nanopores reveals the presence of protein-nanopore interaction. The certain value of I_1/I_2 indicates that hydrophobic interaction between protein and nanopore occurs in the region of hydrophobic surface of the protein molecule. Meanwhile, we observed electroosmotic dominant protein transport at pH 9. In a word, we provide a potential method of precisely positioning the surface hydrophobic region of a specific protein inside nanopore at single-molecular resolution. Our work offers an insight into probing surface chemical property of proteins and provides a platform to study the protein-nanopore interaction.

EXPERIMENTAL SECTION

Nanopore fabrication

2 μm thick silicon oxide buffer layer and 80 nm silicon nitride were deposited on both sides of a 400 μm thick (100) Si wafer. A 20 μm square of freestanding membrane was formed by removing thin SiN and SiO₂ film windows on one side using reactive ion etching (RIE), followed by KOH (40%, 80°C) etching of the Si wafer. Then focused ion beam (FIB, DB235) was used to remove about 1.5 μm thick silicon oxide with size of 1 μm from the center of freestanding membrane. 2 μm square free-standing membrane of silicon nitride was finally formed after the remaining 500 nm thick silicon oxide was etched by 6:1 buffered oxide etching (BOE). The nanopores were drilled in the center of silicon nitride membrane using a focused 300 kV electron beam from TEM (Tecnai F30).

Protein preparation

Gp10 C-his affinity tag plasmids were constructed from vector pET-21a (+) (Novagen) with a two-step polymerase chain reaction (PCR). Then the plasmids constructed previously were transformed into the *E. coli* strain HMS174 (DE3) for protein expression. Inoculate 10 mL LB broth and Ampicillin (50 $\mu\text{g mL}^{-1}$ 1:1000 dilution) with frozen stock. 1–2 μL bacterial were added to the liquid culture and then incubated overnight at 37°C, 180 rpm. On the sec-

ond day, Incubate 500 mL broth with 10 mL of an overnight culture until the culture achieves an optical density of 0.4–0.6. A final concentration of 0.4 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) was added into the cultured medium, which was incubated for another 3 h. The cells were harvested by centrifugation at 6000 rpm for 20 min in a Beckman JS-21 rotor, followed by French Press, and digested with DNase I and RNase A on ice for half an hour. Cell lysate was clarified by centrifugation and filtration. Then purification of C-His-tagged connector proteins was conducted with one-step immobilized metal affinity chromatography (IMAC). 2 mL Ni-NTA His-bind resin (Novagen, San Diego, CA) was added into a column and regenerated with 10 mL charge buffer (50 mM NiCl₂) and 10 mL His-wash buffer (15% glycerol, 500 mM NaCl, 50 mM ATP, 50 mM imidazole, and 100 mM Tris-HCl, pH 8.0). Cell pellets were resuspended in His-bind buffer (15% glycerol, 500 mM NaCl, 50 mM ATP, 10 mM imidazole, and 100 mM Tris-HCl, pH 8.0). Cell lysate was clarified by centrifugation at 12,500 rpm, 4°C (Beckman J2-21, rotor JA-20) for 20 min and filtration followed by loading onto His-bind resin by gravity flow. 10 mL His-wash buffer was used to remove impurities. 5 mL aliquots of His-elution buffer (15% glycerol, 500 mM NaCl, 50 mM ATP, 500 mM imidazole, and 100 mM Tris-HCl, pH 8.0) was applied to elute His-tagged gp10 protein. Then the sample was collected with every 0.5 mL elution coming out.

Phi29 connector protein translocation measurements

Each chip with a nanopore was rinsed by a standard process including ultrapure water, nitrogen gas and plasma cleaner, then sealed with polydimethylsiloxane (PDMS) gaskets which were later mounted between the polyether ether ketone (PEEK) chambers. The *trans* and *cis* chambers full of ionic solution (0.5 M KCl with 5 mM Tris) were electrically contacted with Ag/AgCl electrodes and bias voltage was applied by an Axon 200B patch clamp amplifier (Molecular Devices, Sunnyvale, CA) operating with an 8-pole, 40 kHz, low pass Bessel filter. Then the electrical measurement was conducted in a dark Faraday cage after the phi29 connector proteins were added in the *cis* chamber. Amplifier output was digitized at 250 kHz and continuously recorded to a disk using an Axon Digidata 1440A digitizer and pClamp 10 software.

Simulations

Calculations of open current through the nanopore, current blockage of protein in the nanopore at different pore radius, and pore length were performed using finite-element numerical calculations of a Poisson-Boltzmann for-

mulation, as previously described [46]. To determine the geometry parameters of the nanopore, we have done a series of ionic current calculations with and without the protein inside the nanopore respectively. Since the thickness of the nanopore membrane inside the nanopore is unchangeable, the only two parameters that determine the nanopore geometry are the pore radius and the effective length (the length of the cylindrical part of the nanopore channel). We swept a large variation in both these two parameters and compared the calculated current and blockage value with the experimental result until they matched up. In this way, the exact geometry parameters of the nanopore can be determined in experiment.

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Conflict of interest The authors declare that they have no conflict of interest.

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中文摘要 纳米孔是一种有效的探测蛋白质以及蛋白质复合物的工具, 蛋白质与纳米孔的相互作用是生物和生物医学领域普遍存在的基础过程. 本文通过分析phi29蛋白穿过氮化硅纳米孔产生的电信号, 初步实现了单分子层面对蛋白疏水表面的探测. phi29蛋白穿孔过程中独特的“双层事件”以及双层事件深度的比例表明相互作用发生在phi29蛋白的疏水表面. 本文提供了一种在蛋白表面定位疏水区域的潜在方法, 揭示了疏水相互作用在蛋白和纳米孔的相互作用中扮演着重要的角色. 另外, 该研究在固态纳米孔探测蛋白表面化学性质等领域也具有巨大的潜在意义.