Translocation of DNA and protein through a sequentially polymerized polyurea nanopore
Translocation of DNA and protein through a sequentially polymerized polyurea nanopore†

Hyung-Jun Kim,‡a Ui-Jin Choi,‡b Hyein Kim,†b Kidan Lee,a Kyeong-Beom Park,a Hyun-Mi Kim,a Dong-Kyu Kwak,c Seung-Wook Chi,c Jin Seok Lee,d‡b and Ki-Bum Kim †a

Here, we investigated the translocation of biomolecules, such as DNA and protein, through a sequentially polymerized polyurea nanopore, with a thin (<10 nm) polymer membrane of uniform thickness. The polyurea membrane was synthesized by molecular layer deposition using p-phenylenediisocyanate (PDI) and p-phenylenediamine (PDA) as sequential precursors. The membrane exhibited a hydrophobic surface with a highly negative surface charge density (~51 mC m\(^{-2}\) at pH 8). It was particularly noted that the high surface charge density of the membrane resulted in a highly developed electro-osmotic flow which, in turn, strongly influenced the capture probability of biomolecules, depending on the balance between the electro-osmotic and electrophoretic forces. For instance, the capture frequency of negatively charged DNA was demonstrated to be quite low, since these two forces more or less cancelled each other, whereas that of positively charged MDM2 was much higher, since these two forces were additive. We also identified that the mean translocation time of MDM2 through the polyurea nanopore was 26.1 ± 3.7 µs while that of the SiN nanopore was 14.2 ± 2.0 µs, hence suggesting that the enhanced electrostatic interaction between positively charged MDM2 and the negatively charged pore surface affects the translocation speed.

Introduction

Nanopore sensing is an emerging technology for characterizing various biomolecules and their complexes at single-molecule resolution\(^1\) in which molecules are translocated through a nanometer-sized pore using an applied electric field and the translocations are detected by the transient changes of ionic current. The change in magnitude and dwell time of current provides information on the structural features of the analytes, such as the size and length of biomolecules, specific sequence of nucleic acids in DNA, or the conformational changes in proteins.\(^2\) There has been much progress in protein nanopores, as in α-hemolysin and MspA embedded in the lipid bilayer, with advantages of having a reproducible and uniform sized pore (<1.5 nm) and feasibility of modifying the pore functionality by genetic engineering.\(^4,5\) In contrast, progress in solid-state nanopores has been relatively slow, despite the merits of the mechanical and chemical durability of the membrane and pore-size adjustability to accommodate various-sized analytes.\(^1\)

In the solid-state nanopore analysis, one of the major difficulties is to understand the effect of surface characteristics, such as the charge density and the chemical affinity of the pore periphery, on the translocation of the biomolecule. For instance, many studies have shown that translocation of the biomolecule is affected by the surface chemistry or surface charge of the membrane, either by employing various membrane materials\(^6\) or by coating with organic species.\(^9,10\) Surface charge,\(^1,6,11\) hydrophobicity,\(^8\) and specific biological binding affinity\(^9,10\) of the membrane are known to effectively slow down the transport of molecules. Moreover, the surface charge of the membrane is well known to cause an electro-osmotic flow, which affects the molecule capture rate and translocation kinetics.\(^12,13\)

In this aspect, organic materials are highly desirable as membranes due to their wide range of surface charge densities, hydrophobicity, and chemical functionality, and can provide a suitable biomolecule-sensing platform for a specific application.\(^14-20\) Previous studies on solid-state nanopores, using organic materials, had adopted one of the following two approaches: (1) using a polymer film as membrane\(^21,22\) and (2)
coating organic molecules on an inorganic nanopore surface as a self-assembled monolayer (SAM).\textsuperscript{9,11,17,24–29} Nanopores on a polymer membrane have been fabricated by laser heating\textsuperscript{22} or the track-etch method;\textsuperscript{21,23} however, the thickness of polymer films reported so far has been >10 μm. SAM coating on the nanopore surface allows the fabrication of a sub-10 nm-thick membrane with advantages of utilizing various organic molecules for modifying its surface functional groups. However, an additional coating step complicates the fabrication process and the dimension of the nanopore is often difficult to control.

In this paper, we present a sub-10 nm-thick polyurea membrane using molecular layer deposition (MLD). MLD is considered an outstanding technique to fabricate ultrathin organic films with precisely controlled film thickness, composition, and molecular orientation.\textsuperscript{30–35} The polyurea membrane was chosen due to its high mechanical stability,\textsuperscript{30} chemical resistance,\textsuperscript{30} thermal stability,\textsuperscript{36} and its potential application in lithography.\textsuperscript{37} Moreover, a highly negative surface charge density at neutral pH is anticipated, since the isoelectric point of polyurea (pH = 2.6)\textsuperscript{38} is lower than that of the SiN nanopore (pH = 5).\textsuperscript{39} First, we fabricated uniformly aligned polyurea films through coupling reactions between \textit{p}-phenylenediisocyanate (PDI) and \textit{p}-phenylenediamine (PDA), by MLD based on self-limiting surface reactions. After transferring the polyurea film on our prepared low-noise Pyrex substrate,\textsuperscript{40} the nanopores were perforated using focused electron beams from TEM. Next, we analyzed the ionic conductance and noise characteristics of such nanopores. Finally, we investigated the translocation of dsDNA and MDM2 proteins through highly negatively charged polyurea nanopores.

**Results and discussion**

**Fabrication of polyurea nanopores**

Fig. 1a shows a schematic drawing of the measurement setup. At first, a polyurea MLD film was fabricated in a homemade MLD chamber equipped with an \textit{in situ} Fourier-transform infrared (FTIR) spectrometer. The surface reactions for the polyurea MLD film are schematically illustrated in Fig. 1b. One MLD cycle consisted of a sequential molecular reaction between the PDI and the PDA precursor, and polyurea MLD films of a desired thickness were obtained by varying the number of cycles. To identify the self-limiting surface reaction, we conducted \textit{in situ} FTIR measurements during alternative gas phase exposures of PDI and PDA on a SiO\textsubscript{2} nanoparticle substrate (Fig. S1,\textsuperscript{†} and Fig. 1c).\textsuperscript{31–33} When the PDI precursor reacted with the SiO\textsubscript{2} substrate, a N–C\textsubscript{v}O stretching vibration peak arose at 2270 cm\textsuperscript{−1} and the N–H stretching vibration appeared at 3400–3100 cm\textsuperscript{−1}. In addition, the C\textsubscript{v}O stretching (amide I) and C–N stretching vibration (amide II) peaks appeared at 1651 cm\textsuperscript{−1} and 1510 cm\textsuperscript{−1}, respectively, indicating the formation of a urethane (–NH–(C=O)–OH–) linkage. After PDA exposure, the amine group reacted with the surface-terminated N–C\textsubscript{v}O group from which the urea (–NH–(C=O)–NH–) linkage was formed. We also monitored the thickness of (PDI/PDA)_n polyurea MLD films as a function of the number of
cycles, using ex situ ellipsometry, as shown in Fig. 1d. The thickness of polyurea MLD films followed a linear growth curve with a constant growth rate of 3.9 Å per cycle.

We characterized the surface charge density of the polyurea film using a zeta potential analyzer and compared it with that of SiN (see the Methods section), deposited on the Si substrate, using low-pressure chemical vapor deposition. The measured zeta potential values of polyurea and SiN films were $-20.5 \pm 0.7$ mV and $-4.5 \pm 0.2$ mV, respectively. The relatively high negative value in the case of polyurea was also reported by Elrehim et al. ($-36.7 \pm 5.6$ mV at pH 7, 1 mM KCl), and a similar value of zeta potential was reported in the SiN membrane ($-7.9 \pm 3.6$ mV at pH 7.8, 0.4 M KCl). The highly negative surface charge density of the polyurea film is due to the interaction of the urea groups with hydroxyl anions ($\text{OH}^-$) to form the negative surface.

The polyurea films $\text{(PDA)}$, grown on the Si/SiO$_2$ substrate, were then covered with poly-methyl-methacrylate (PMMA) and immersed in diluted hydrofluoric acid to separate the PMMA-polyurea samples by etching the SiO$_2$ substrate. The floated PMMA-polyurea layers were rinsed with deionized water and transferred to the prepared Pyrex substrate with a 2 μm opening. The polyurea membrane that covered the 2 μm hole is shown in the transmission electron microscopy (TEM) image (Fig. 2b). The thickness of the transferred polyurea film (10 nm) was confirmed using an atomic force microscope (AFM), as shown in Fig. S2. Finally, a nanometer-sized pore was drilled in the polyurea membrane with a focused electron beam using 200 kV TEM (1.5 nA e-beam current and 0.12 nA nm$^{-2}$) with pore size ranging from 4 to 10 nm, as seen in Fig. 2c.

**Characterization of polyurea nanopores**

After the nanopore chip was immersed in diluted ethanol (40%) to enhance the wettability of the pores, ionic conductance was measured in 1 M KCl solution with TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) using an Axopatch.
amplifier. Fig. 2d shows the current–voltage characteristics of polyurea nanopores with 5, 7, and 10 nm diameter. The ionic currents exhibited ohmic characteristics in the voltage ranges from −300 to 300 mV and each conductance value was fitted to be 33, 54, and 112 nS, respectively. At high salt concentrations (>0.1 M), the conductance of a nanopore can be estimated by the pore dimension with the contributions from bulk ion concentration, access resistance, and electro-osmosis.\(^2\)

\[
G = \left( (\mu_{K^+} + \mu_{Cl^-}) n_{KCl} e \right) \left( \frac{4 h_{eff}}{\pi d_p^2} + \frac{1}{d_p^3} \right)^{-1} + \mu_{K^+} e \frac{\pi d_p}{h_{eff}}
\]

where \(\mu_{K^+}\) and \(\mu_{Cl^-}\) are the electrophoretic mobility of \(K^+\) \((7.62 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1})\) and \(Cl^-\) ions \((7.91 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1})\), \(n_{KCl}\) is the number density of \(K^+\) or \(Cl^-\) ions, \(e\) is the elementary charge, \(d_p\) is the diameter of the pore, \(h_{eff}\) is the effective thickness of the pore, and \(\sigma\) is the surface charge density of the pore.\(^3\)

The electro-osmosis term (the last term) in eqn (1) presents a linear dependency on the applied voltages \((\Delta \Phi)\) at applied voltages in the range 200–300 mV are shown in Fig. 3c. The number of total events was 185 (200 mV), 244 (250 mV), and 280 (300 mV) and the corresponding histograms of \(\Delta G\) fitted with the Gaussian distribution in Fig. 3d. As expected, the mean values of \(\Delta G\) present a linear dependency on the applied voltages (inset in Fig. 3d), with the blockade conductance \((\Delta G)\) being 5.53 ± 0.34 nS. \(\Delta G\) is estimated by \(\Delta G = G(d_p) - G(d_{eff})\), where \(G\) is the calculated conductance, and \(d_{eff}\) is the effective diameter of the pore with the DNA inside, calculated by \(d_{eff} = \sqrt{(d_p^2 - d_{DNA}^2)}\), taking \(d_{DNA} = 2.2 \text{ nm}\).\(^3\)

In order to explain the obtained results, namely, the noticeable increase in event frequency at 2.5 M KCl relative to that at 1 M KCl solution, we estimated the electro-kinetic forces on DNA capturing at each condition. The negatively charged polyurea surface inside the pore may induce two effects on DNA trapping: (1) electro-osmotic force (EOF) resulting from the cation flow inside the pore, acting in a direction opposite to that of the electrophoretic driving force on the DNA and (2) the electrostatic repulsion between the negatively charged DNA and negatively charged polyurea surface. The effect of electrostatic repulsion is likely to be minor, since the pore diameter

**DNA detection by polyurea nanopore**

To investigate the transport behavior of DNA molecules through polyurea nanopores, 1 kbp dsDNA was translocated through polyurea nanopores in KCl electrolyte with a TE buffer (pH 8.0), using a low pass filter of 100 kHz. At first, the transport of DNA for \(5\)–10 nm polyurea nanopores was rarely observed in 1 M KCl solution, whereas that for SiN nanopores was well observed under the same condition (Fig. S5†). Several reports have shown that the translocation frequency of DNA into the nanopore can be enhanced by altering the charge distribution of the pore,\(^52–54\) or by changing the ionic strength of the solution,\(^55,56\) mostly in the protein nanopore. For instance, Franceschini et al. demonstrated that dsDNA does not translocate through highly negatively charged C3A protein nanopores (net charge of the pore lumen; −120) at 1 M NaCl, but does so at higher ionic strengths (2.5 M NaCl).\(^56\) Inspired by Franceschini et al., we tested the translocation of DNA through a polyurea nanopore at 2.5 M KCl. Fig. 3a shows the continuous 10 s current traces at 200 mV (black), 250 mV (red) and 300 mV (blue) of applied voltages. Indeed, these results demonstrate that the DNA translocates through the polyurea nanopore at high molar concentrations; the scatterplots of blockade current (\(\Delta I\)) vs. dwell time (\(t_D\)) at applied voltages in the range 200–300 mV are shown in Fig. 3e. The number of total events was 185 (200 mV), 244 (250 mV), and 280 (300 mV) and the corresponding histograms of \(\Delta I\) fitted with the Gaussian distribution in Fig. 3d. As expected, the mean values of \(\Delta I\) present a linear dependency on the applied voltages (inset in Fig. 3d), with the blockade conductance \((\Delta G)\) being 5.53 ± 0.34 nS. \(\Delta G\) is estimated by \(\Delta G = G(d_p) - G(d_{eff})\), where \(G\) is the calculated conductance, and \(d_{eff}\) is the effective diameter of the pore with the DNA inside, calculated by \(d_{eff} = \sqrt{(d_p^2 - d_{DNA}^2)}\), taking \(d_{DNA} = 2.2 \text{ nm}\).\(^3\)

In order to explain the obtained results, namely, the noticeable increase in event frequency at 2.5 M KCl relative to that at 1 M KCl solution, we estimated the electro-kinetic forces on DNA capturing at each condition. The negatively charged polyurea surface inside the pore may induce two effects on DNA trapping: (1) electro-osmotic force (EOF) resulting from the cation flow inside the pore, acting in a direction opposite to that of the electrophoretic driving force on the DNA and (2) the electrostatic repulsion between the negatively charged DNA and negatively charged polyurea surface. The effect of electrostatic repulsion is likely to be minor, since the pore diameter

\(\mu\) atomically thin 2-D membranes such as graphene, BN, and the membrane has been reported as a source of high flicker noise in (80/120) at 1 M NaCl, but does so at higher ionic strengths (2.5 M NaCl).\(^56\) Inspired by Franceschini et al., we tested the translocation of DNA through a polyurea nanopore at 2.5 M KCl. Fig. 3a shows the continuous 10 s current traces at 200 mV (black), 250 mV (red) and 300 mV (blue) of applied voltages. Indeed, these results demonstrate that the DNA translocates through the polyurea nanopore at high molar concentrations; the scatterplots of blockade current (\(\Delta I\)) vs. dwell time (\(t_D\)) at applied voltages in the range 200–300 mV are shown in Fig. 3e. The number of total events was 185 (200 mV), 244 (250 mV), and 280 (300 mV) and the corresponding histograms of \(\Delta I\) fitted with the Gaussian distribution in Fig. 3d. As expected, the mean values of \(\Delta I\) present a linear dependency on the applied voltages (inset in Fig. 3d), with the blockade conductance \((\Delta G)\) being 5.53 ± 0.34 nS. \(\Delta G\) is estimated by \(\Delta G = G(d_p) - G(d_{eff})\), where \(G\) is the calculated conductance, and \(d_{eff}\) is the effective diameter of the pore with the DNA inside, calculated by \(d_{eff} = \sqrt{(d_p^2 - d_{DNA}^2)}\), taking \(d_{DNA} = 2.2 \text{ nm}\).\(^3\)

In order to explain the obtained results, namely, the noticeable increase in event frequency at 2.5 M KCl relative to that at 1 M KCl solution, we estimated the electro-kinetic forces on DNA capturing at each condition. The negatively charged polyurea surface inside the pore may induce two effects on DNA trapping: (1) electro-osmotic force (EOF) resulting from the cation flow inside the pore, acting in a direction opposite to that of the electrophoretic driving force on the DNA and (2) the electrostatic repulsion between the negatively charged DNA and negatively charged polyurea surface. The effect of electrostatic repulsion is likely to be minor, since the pore diameter
is considerably larger than the Debye screening length ($\lambda_D$; 0.3 nm at 1 M; 0.2 nm at 2.5 M). When $\lambda_D \ll d_p/2$, the velocity of EOF ($v_{EO}$) can be calculated by the Helmholtz–Smoluchowski equation:

$$v_{EO} = -\mu_{EO} E, \quad \mu_{EO} = \varepsilon \zeta / \eta;$$

where $\varepsilon$ is the permittivity of the electrolyte solution, $\zeta$ is the zeta potential of the pore wall, $\eta$ is the viscosity of the electrolyte solution, and $E$ is the electric field. We assumed that the surface charge density of the nanopore is constant in the salt concentration range of 1–2.5 M referring to the previous studies regarding SiN nanopores and follows the Grahame equation, $\sigma = \varepsilon \zeta / \lambda_D$ for $\zeta \ll 50$ mV (Fig. S6†). With the reported and calculated parameters ($\varepsilon = 80$ mC m$^{-2}$, $\zeta$ values as calculated from measured $\sigma = -51$ mC m$^{-2}$ and $\lambda_D = 0.303/\sqrt{\varepsilon}$; where $c$ is the salt concentration; $\eta = 1$ mPas), the calculated $\mu_{EO}$ at 1 M KCl ($1.53 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$) dropped to $0.97 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$ at 2.5 M KCl due to the decrease in $\lambda_D$. The electrophoretic velocity of DNA can be estimated by $v_{EP} = \mu_{DNA} E$, where $\mu_{DNA}$ is the mobility of DNA. The free solution mobility of 1 kbp DNA from the gel electrophoresis measurement is $3.75 \times 10^{-9}$ m$^2$ V$^{-1}$ s$^{-1}$, which is larger than the $\mu_{EO}$ within the same order of magnitudes. However, when DNA is captured into the relatively small sized pore, DNA could become less mobile, since the electrophoretic force only drags a small part of a long DNA chain. These findings suggest that the EOF on polyurea nanopores inhibits the electrophoretic capture of DNA at 1 M KCl, in addition to the entropic barrier.

**Protein detection for polyurea nanopore**

In order to confirm the EOF effect, we tested the translocation of the positively charged MDM2 protein (MW = 12.3 kDa, net charge at pH 7.4 = +2.9e, PI = 9.02, globular shape), which is opposite to the charge on the DNA. In this case, since the EOF and electrophoretic force had been acting in the same direction, we expected a higher translocation frequency. Due to the relatively low surface charge density of protein than that of DNA, EOF could be the major driving force for capture than the electrophoretic force in both solid-state nanopores and biological nanopores. For instance, Firnkes et al. observed that EOF can enhance, suppress, and even reverse electrophoretic transport, depending on the zeta potential difference between the pore surface and protein (avidin) by varying the pH at ±150 mV applied bias. In addition, the translocation frequency of streptavidin was reported to be higher in the EOF direction (36 s$^{-1}$) than in the electrophoretic direction (0.7 s$^{-1}$), despite the higher zeta potential of streptavidin (+20 mV) compared to that of the pore surface (+8 mV). Furthermore, Waduge et al. demonstrated that the capture of 10 different kinds of proteins is dominated by EOF, irrespective of the protein charge (range of −26 to +7), through negatively charged SiN and hafnium oxide nanopores.

To characterize MDM2 translocation behavior, a comparison of the current–time trace for the translocation of 100 nM...
MDM2 at −100, −125, and −150 mV, using both the polyurea nanopore \((d_p = 10 \text{ nm and } h_{\text{eff}} = 8 \text{ nm})\) and the SiN nanopore \((d_p = 9.5 \text{ nm and } h_{\text{eff}} = 9 \text{ nm})\) in 1 M KCl with 1× PBS buffer (phosphate buffered saline, pH 7.4) is shown in Fig. 4a. The most noticeable difference between the polyurea and SiN nanopores was the event frequency, plotted as a function of voltage (Fig. 4c). The MDM2 event frequency was 3.7, 4.3, and 6.4 events per s on the polyurea nanopore and 1.12, 1.20, and 1.43 events per s on the SiN nanopore at −100, −125, and −150 mV, respectively. Since the dimensions of the SiN and polyurea pores were similar, these pores exhibited comparable electrophoretic fields under the same applied voltage. Hence, the improved capture rate in the polyurea nanopore should be attributed to the drag by EOF \((v_{\text{EO}})\), exhibited by the higher surface charge density of polyurea \((-51 \text{ mC m}^{-2})\) than of SiN \((-14 \text{ mC m}^{-2})\). At the same applied voltage, the ratio of \(v_{\text{EO}}\) between polyurea and SiN nanopores \((v_{\text{EO,PU}}/v_{\text{EO,SiN}})\) in the cis-chamber is 3.64. The event frequency of MDM2 on polyurea and SiN nanopores was linearly fitted with respect to the applied voltage, using frequency = \(A \times V\) (Fig. 4c), to investigate the relation between event frequency and \(v_{\text{EO}}\). The fitting results of \(A\) were 38.81 and 9.93 for the polyurea and SiN nanopores, respectively. We found that the ratio of \(A\) \((A_{\text{PU}}/A_{\text{SiN}} = 3.91)\) was comparable to the ratio of \(v_{\text{EO}}\) (3.64). Together, the findings suggest that EOF is the primary source of MDM2 capture for nanopores. Therefore, we conclude that the high negative charge of the polyurea nanopore induces high throughput MDM2 detection by EOF.

From the current traces, MDM2 translocation events were collected to extract the mean fractional current blockade \((\Delta I/I_0)\) and the dwell time \((\Delta t)\). The scatterplots for \(\Delta I/I_0\) vs. \(\Delta t\) with applied voltages of −100, −125, and −150 mV on SiN and polyurea nanopores for 5 min are summarized in Fig. 5a. The number of total events at each applied voltage for 5 min was 336 (−100 mV), 359 (−125 mV), and 428 (−150 mV) on the SiN nanopore, and 1096 (−100 mV), 1301 (−125 mV), and 1928 (−150 mV) on the polyurea nanopore, respectively. First of all, we observed a difference in dwell times between polyurea and SiN nanopores at all applied voltages. For instance, Fig. 5b shows the dwell-time \((\Delta t)\) distribution for MDM2 both on the polyurea and SiN nanopores and these data were fitted to a 1D drift diffusion model, where

\[
P(t) = \left(\frac{h_{\text{eff}}}{4\pi D_p t^3}\right)^{1/2} e^{-\left(h_{\text{eff}} - \nu_d t\right)^2/4D_d t}
\]

where \(h_{\text{eff}}\) is the effective pore thickness, \(D_p\) is the protein diffusion coefficient inside the pore, and \(\nu_d\) is the protein drift velocity during nanopore traversing. The extracted free parameters \(D_p\) and \(\nu_d\) for the polyurea and SiN nanopores are shown in Fig. S7.† We found a slower \(\nu_d\) \((0.36 \pm 0.04 \text{ nm } \mu\text{s}^{-1})\) of MDM2 for the polyurea nanopore than that \((0.60 \pm 0.07 \text{ nm } \mu\text{s}^{-1})\) for the SiN nanopore. The slower \(\nu_d\) for the polyurea

Fig. 4 (a) Continuous 30 s ionic current traces for 100 nM MDM2 translocation at −100, −125, and −150 mV voltage through a polyurea nanopore \((d = 10 \text{ nm, 8 nm thick})\) and a SiN nanopore \((d = 9.5 \text{ nm, 9 nm thick})\) in buffered 1 M KCl (pH 7.4) (scale bar: 0.5 nA, 2 s). (b) Selected translocation events of MDM2 from (top) 150 mV current trace on SiN and polyurea pores (scale bar: 0.2 nA, 250 μs). (c) Event frequency versus voltage for SiN and polyurea pores. Each event frequency was fitted to \(y = Ax\).
nanopore suggests that the increase in EOF is not directly related to the translocation speed of biomolecules. Rather, this could be attributed to the protein–pore interaction during translocation. We suspect that the higher surface charge density of the polyurea nanopore ($-51 \text{ mC m}^{-2}$), compared to that of the SiN nanopore ($-14 \text{ mC m}^{-2}$), would promote an electrostatic interaction between the positively charged MDM2 and negatively charged pore surface. In addition, the calculated value of $D_p (0.85 \pm 0.23 \text{ nm}^2 \mu \text{s}^{-1}$ for the polyurea nanopore and $1.51 \pm 0.22 \text{ nm}^2 \mu \text{s}^{-1}$ for the SiN nanopore) indicates an additional drag force acting on MDM2 when it is translocated through the polyurea nanopore. A few studies had shown that DNA translocation through the nanopore is affected by the electrostatic interaction with a positively charged surface, such as organically coated nanopores, $^{11,67}$ Al$_2$O$_3$ (points-of-zero-charge of pH 9.1), $^{58,60}$ ZnO nanopores (points-of-zero-charge of pH 9.1), $^9$ and positive-biased nanochannels. $^{10,70,71}$ However, the effect of the electrostatic interaction has not yet been studied in the case of protein analytes, due to its fast translocation speed and heterogeneous charge profiles. $^{41,72}$

Fig. 5c shows the fractional current blockade ($\Delta I/I_0$) distribution for MDM2 both on the polyurea and SiN nanopores and these histograms were fitted into the Gaussian distribution. The mean fractional current blockade values and the geometry of the nanopore, the hydrodynamic diameter of the protein ($d_H$) was estimated by $^{41,73}$

$$d_H = [(\Delta I/I_0) / (\eta_{eff} + 0.8d_p^2)]^{1/3}. \quad (4)$$

The obtained $d_H$ of MDM2 is $3.23 \pm 0.11 \text{ nm}$ for the polyurea nanopore, and $2.81 \pm 0.03 \text{ nm}$ for the SiN nanopore. The X-ray crystallographic structure of MDM2 (2.4 $\times$ 2.6 $\times$ 4.1 nm$^3$) was provided by the Protein Data Bank (PDB code: 1YCR), which shows good correlation with the $d_H$ for polyurea nano-
pore. The findings suggest that the polyurea nanopore successfully resolved MDM2, and SiN nanopore could not sufficiently resolve the MDM2, due to the fast translocation speed. Owing to their dimension controllability and unique high negative surface charge characteristics, polyurea nanopores are applicable to high-throughput detection with greater extent of slowing down of small proteins (<20 kDa).

Conclusions

We have presented a process of fabricating a polymer membrane on a low-noise Pyrex substrate using molecular layer deposition with controllable thickness, molecular composition, and orientation. This platform has potential application in the exploration of various biomolecule/polymer interfaces. We have demonstrated the formation of polyurea nanopores with 4–10 nm diameter and sub-10 nm thickness, using a TEM perforation method that induces a highly negative surface charge. We have shown that polyurea nanopores can successfully detect single-molecule translocation events of negatively charged dsDNA at a high ionic strength of 2.5 M KCl, along with that of positively charged MDM2 protein. Comparing with SiN nanopores and by the numerical analysis of EOF, we suggest that EOF suppresses the capture of negatively charged DNA and enhances the capture of positively charged MDM2. In addition, a longer dwell time of MDM2 in the polyurea nanopore, compared to that in the SiN nanopore, was observed as a result of electrostatic interaction. The polyurea nanopore exhibits an advantage of improved detection efficiency of positively charged proteins, in terms of event frequency and the slowing down of molecular transport, relative to SiN nanopores. Our further study will be to investigate the translocation of various analytes through polyurea nanopores and we expect to demonstrate the influence of the analytes' physical and chemical properties on their translocation.

Methods

Fabrication of the polyurea MLD film

Organic polyurea MLD films were fabricated in our homemade hot wall viscous flow vacuum MLD chamber equipped with in situ Fourier-transform infrared spectroscopy (FTIR) apparatus (Fig. S1†), in the same way as in our previous report.31–33 The organic precursors, p-phenylenediisocyanate (PDI; C₈H₆(NCO)₂) and p-phenylenediamine (PDA; C₆H₄(NH₂)₂), were heated to 90 °C, and 105 °C, respectively, to achieve reasonable vapor pressure, and the MLD chamber was heated to 110 °C. After 60 s of PDI dosing with a 30 sccm flow of Ar carrier gas, the MLD chamber was maintained for 30 s to provide a sufficient opportunity for surface reaction. And, the MLD chamber was purged with Ar for 120 s at a flow rate of 400 sccm, and evacuated for 30 s to ensure that no precursors remained in the chamber. These sequences of dose, exposure, purge, and evacuation were repeated with PDA.

Ex situ characterization

Film thickness on a flat substrate was measured by ex situ ellipsometry (Gaertner Scientific Corp., L2W158380) using He–Ne laser light at a wavelength of 632.8 nm. Thickness was measured in at least four different spots on each sample to confirm the uniformity of the polyurea MLD film. Two-dimen- sional AFM images and height profiles were obtained using atomic force microscopy (AFM; Park Systems, NX-10), with a 30 nm-thick Al-coated cantilever in the noncontact mode, at a scan size of 3 µm × 3 µm. Zeta potential of the film surface was measured using an electrophoretic light scattering spectrophotometer (Delsa Nano C, ELS Z-1000). The surface of the sample was configured with the top a box-like cell. Zeta potential was calculated from the apparent electrophoretic mobility by monitoring particles at several positions inside the cell and analyzing the electro-osmotic mobility on the solid surface using the electro-osmotic profile.

Nanopore measurements

Prior to ionic current measurements, the nanopore chips were immersed in diluted ethanol (30%) for 2 h to enhance the wettability of the pores. Then, a 5 mm nanopore chip was mounted on a customized PTFE microfluidic flow cell with a PDMS gasket of 3 mm diameter, and both chambers were filled with a KCl electrolyte and TE buffer solution (pH 8) or 1× PBS (pH 7.4) buffer solution. Ag/AgCl electrodes were inserted into both chambers of the flow cell and bias voltage was applied on the trans- chamber. The electrodes were connected to an Axopatch 200B amplifier with a sampling rate of 250 kHz and low pass four-pole Bessel filter of 100 kHz. The 1 kbp NoLimits DNA fragments (Thermo Scientific) were used and 2 nM of dsDNA was inserted into the cis- chamber. A recombinant MDM2 N-terminal domain (residues 3–109) construct was overexpressed in Escherichia coli BL21 (DE3) by induction with 0.4 mM isopropyl-β-D-thiogalactoside (IPTG) at the OD600 value of 0.9. After IPTG induction, cells were grown at 20 °C for 16 hours in LB media. Protein was precipitated with ammonium sulfate and further purified using ion exchange chromatography (HiTrap™ SP and Q, GE Healthcare) and gel-filtration chromatography (HiLoad® 16/600 Superdex® 75pg, GE healthcare), as previously reported.44 Hundred nanomolar proteins were inserted into the cis- chamber. The translocation events were collected and analyzed using Clampfit, pCLAMP 10.4 software.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research was supported by the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science and ICT (MSIT) (2012–...
009563). Also, this work was supported by the BioNano Health-Guard Research Center funded by the Ministry of Science and ICT (MSIT) of Korea as the Global Frontier Project (H-GUARD_2013M3A6B2078943) and the Nanomaterial Technology Development Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (MSIT) of Korea (2015M3A7B4050454). This work was also supported by NRF grants funded by the Korean government (MSIT) (NRF-2017R1E1A1A01074403) and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2015R1A2A2A01005556, 2017R1E1A1A01075377).

References